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**The Influence of Lime Sulphur on the Quality and Sulphur Content of
Organic 'Royal Gala' and 'Braeburn' Apples**

A thesis presented in partial fulfilment of the requirements for the degree of

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in

Agribusiness

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"There is only one good, knowledge, and one evil, ignorance."

Socrates (469 BC-399 BC)

"Was man nicht aufgibt, hat man nie verloren."

Friedrich von Schiller (1759-1805)

ABSTRACT

Black spot or apple scab is a major disease in apple (*Malus domestica*) production. Its control is especially difficult in organic production systems that rely on copper- and sulphur-based fungicides which are not very effective and demand a high number of applications throughout the season. The most commonly used fungicide in organic apple production is lime sulphur, which is known to be phytotoxic, especially towards the cultivar 'Braeburn'.

The influence of different application rates of lime sulphur (1% and 2%) was evaluated when applied 11 times throughout the growing season from October to February. As varieties differ in their susceptibility to lime sulphur, the two cultivars 'Royal Gala' and 'Braeburn' were compared in this study. Black spot incidence and severity, russet development and postharvest quality parameters were evaluated. At harvest, residues of sulphur on and in the apple were determined as total sulphur, total water-soluble non-protein thiol compounds and cysteine content.

Both cultivars behaved similarly to the application of lime sulphur, but 'Braeburn' was affected to a greater extent. Lime sulphur decreased background colour, blush, firmness, soluble solids content and dry matter content in both cultivars; fruit size in 'Braeburn' and titratable acidity in 'Royal Gala'. The changes observed can possibly be attributed at least in part to the decrease in the photosynthetic rate, which was especially drastic in 'Braeburn'. Lime sulphur caused increased russet on 'Royal Gala', but not on 'Braeburn'. Significant sulphur residues were found in the skin and flesh of both cultivars and part of the lime sulphur applied was metabolized into water-soluble non-protein thiols and cysteine.

These results are of significant interest to the organic industry as the use of lime sulphur may compromise the residue-free status of organic apples and could have an influence on consumer acceptance and flavour.

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LIST OF ABBREVIATIONS

amf	acid milliequivalent factor
ANOVA	analysis of variance
Ca(OH) ₂	hydrated lime, slaked lime
DM	dry matter
DTNB	5,5'-dithiobis(2-nitrobenzoic acid)
H ₂ S	hydrogen sulphide
HCl	hydrochloric acid
ICP-OES	inductively coupled plasma optical emission spectroscopy
IFOAM	International Federation of Organic Agriculture Movements
LS1	treatment containing hydrated lime and 1% lime sulphur
LS2	treatment containing hydrated lime and 2% lime sulphur
MES	2-(N-morpholino)ethanesulfonic acid
n	sample size
NaHCO ₃	sodium bicarbonate, baking soda
NaOH	sodium hydroxide
S.E.	standard error
SH	thiol
SPI	starch pattern index
SSC	soluble solids content
TA	titratable acidity
Tris	trihydroxymethylaminomethane

Chapter 1

INTRODUCTION

A major issue in organic apple (*Malus domestica*) production in New Zealand is the dependency on lime sulphur to control the fungal disease black spot (apple scab). The use of lime sulphur has been associated with leaf injury and decreased yield, especially in the cultivar 'Braeburn'. To quantify the impact of lime sulphur, this study aimed to evaluate the postharvest quality and sulphur content of apples under different lime sulphur application rates.

1.1 ORGANICS

According to IFOAM, the International Federation of Organic Agriculture Movements, organic farming should enhance the health of the soil, plants, animals and humans; sustain living ecological systems; provide fair treatment to humans and animals; and take care to protect the well-being of current and future generations as well as the environment (IFOAM, 2005). Sustainability is an underlying concept to organic farming and denotes the ability of a system to continue or to maintain (Edwards-Jones & Howells, 2001). Social, environmental and economic sustainability are ideally achieved at the same time. That means that an organic farming system should produce enough food and be profitable while being environmentally safe and protective of its resources (Reganold et al., 1990). Sustainability also includes the maintenance of yields (Edwards-Jones & Howells, 2001).

Consumers' demand for organic food is growing worldwide, especially in developed countries (Lester, 2006). New Zealand benefits from this development with 785 ha in organic pipfruit production and the organic apple crop valued at close to NZ\$30 million in

2006 (Kerr et al., 2006). Consumers have become increasingly aware of issues surrounding health and diet, food safety and the environmental impact of food production (Lester, 2006). Subjects of a study in Italy overall felt that organic food is healthy and environmentally friendly as well as tastier and more nutritious than conventionally grown produce (Saba & Messina, 2003). Consumers agreed that pesticides are a danger to humans and food produced without them is healthier, no matter if they were consuming organic products or not (Saba & Messina, 2003). Both groups felt that pesticides are not responsibly dealt with and that the risks associated with them are underrated (Saba & Messina, 2003). Consumers are thus more willing to pay the premium that organic products attract (Lester, 2006) on the basis that they are healthier and residue-free.

There seems, however, to be some confusion if organic products are really residue-free. Many consumers would probably think they are and some companies actually claim that their products are residue-free or even spray-free (Cedenco, 2007; Fonterra, 2007). Even the certification agencies seem to be unclear about this: Organic food is either “not the same as ‘spray-free’ or ‘residue-free’” (BioGro, 2007b) or “much more than spray-free or residue-free” (BioGro, 2007a) or organic production simply “reduces the likelihood of pesticide residues” (BioGro, 2007b). In any case, organic products are in general only tested for residues from neighbouring conventional properties; they are not evaluated for residues of the sprays applied under organic certification (M. Glogau¹, personal communication).

1.2 BLACK SPOT AND LIME SULPHUR

Black spot is a fungal disease caused by the fungus *Venturia inaequalis* and occurs in all apple-producing countries. It is the most important disease in pipfruit production worldwide (Lind et al., 2003). The economic impact is enormous, as both the quality and quantity of fruit are affected (Cunningham, 1925). Black spot can decrease yield as fruit may fail to set or drop prematurely (Cunningham, 1925; Mills & LaPlante, 1954). Harvested apples can be deformed, badly affected or cracked, which reduces storage life (Cunningham, 1925). Indirect losses result from defoliation which reduces tree growth and therefore the yield for the following seasons (Mills & LaPlante, 1954). Today, there is a zero tolerance for black spot on marketed fruit (Carisse & Dewdney, 2002).

¹ Dr Michelle Glogau, BioGro New Zealand Ltd, Wellington, New Zealand, September 2007

Different apple cultivars show different susceptibility to the disease, but New Zealand mainly grows apple varieties that are highly susceptible to black spot (Beresford et al., 1995; McCarthy, 1994), such as ‘Gala’ and ‘Braeburn’ (Hampson & Kemp, 2003; Lind et al., 2003). Many older apple varieties that are no longer grown commercially in New Zealand are naturally resistant to black spot (McCarthy, 1994). The current varieties have been developed in a regime where synthetic chemicals are used to achieve near total pest and disease control (McCarthy, 1994). Although black spot is a problem in organic as well as conventional production, it is more difficult to grow these new varieties in organic production systems, which do not allow the use of synthetic fungicides.

The fungicides allowed under organic certification today are those used in horticulture in general before the emergence of synthetic ones. They are mostly copper- and sulphur-based (Beresford et al., 1991). Lime sulphur is most commonly used, but is phytotoxic upon application, especially at higher temperatures (Beresford et al., 1991; Mills & LaPlante, 1954). All fungicides available to the organic grower are less effective against black spot than synthetic fungicides (Beresford et al., 1996) and require a large number of applications, sometimes up to 40 in the season (J. Walker², personal communication). Even so, the more effective synthetic fungicides require eight to ten sprays per season (Grove et al., 2003).

1.3 PROBLEM STATEMENT

Despite the fact that the issues associated with lime sulphur, such as leaf injury, decreased photosynthesis, reduced yield and weakening of the tree are well known (Cunningham, 1935; Mills & LaPlante, 1954), lime sulphur is still the main fungicide used against black spot in organic apple production today. Different apple cultivars, e.g. ‘Braeburn’ and ‘Royal Gala’, differ in their susceptibility to lime sulphur sprays, the former being severely affected. The damage to ‘Braeburn’, namely defoliation and decreased tree growth, is obvious in production orchards where lime sulphur is used. Although the current apple varieties are not necessarily suitable for organic production practices, they are grown due to high market demand. For fruit to be free of disease symptoms, sprays have to be applied and lime sulphur is the fungicide of choice for many organic apple growers.

² Dr Jim Walker, HortResearch, Havelock North, New Zealand, August 2007

Although it is known that lime sulphur has a detrimental effect on apple trees, its impact on apple quality remains largely unexplored and the possible uptake of sulphur by the leaves or fruit has not been researched. As organic products are either perceived to be or claimed to be residue-free, it is important to ensure that products are actually residue-free. To further develop the organic industry, it is also important to quantify the influence of lime sulphur on fruit quality. Only once the true effect of lime sulphur is known, can the use of existing fungicides be optimised to minimise the damage done to trees and fruit. Moreover, other fungicides might need to be developed. The use of lime sulphur could in the long-term jeopardise the viability of growing 'Braeburn' organically. Yet, production is of importance to the New Zealand economy due to the considerable financial margin for organic apples and huge overseas demand.

1.4 RESEARCH OBJECTIVES

The main objective of this research project is to investigate if higher levels of sulphur compounds can be detected in organic 'Braeburn' and 'Royal Gala' apples treated with lime sulphur. Further, postharvest quality parameters, black spot severity and russet development are determined in relation to the application rate of lime sulphur. Russet is a rough, brownish skin and reports on the influence of lime sulphur on this condition have been mixed (Cunningham, 1935; Holb & Heijne, 2001; Palmer et al., 2003; Sanchez et al., 2001). The preharvest impact of lime sulphur on the trees is also evaluated. The apple cultivars 'Royal Gala' and 'Braeburn' are compared in the study because the trees show a different susceptibility to the application of lime sulphur. It is therefore expected that their fruit will be affected in a different way, too.

Specific research objectives are to:

- Analyse the impact of foliar application of lime sulphur on 'Braeburn' and 'Royal Gala' trees through measurement of the photosynthetic rate of leaves.
- Examine possible residues of sulphur in the apple at harvest by measuring total sulphur, total water-soluble non-protein thiol compounds and cysteine in fruit of 'Braeburn' and 'Royal Gala' treated with different levels of lime sulphur throughout the season.

-
- Evaluate postharvest quality of 'Braeburn' and 'Royal Gala' apples when treated with different application rates of lime sulphur throughout the season: black spot incidence and severity, russet development, weight, diameter, background colour, blush, firmness, soluble solids content (SSC), starch pattern index (SPI), percentage dry matter (DM) and titratable acidity (TA).
 - Discuss the results in particular with regard to their implications for the organic industry and consumer acceptability.

Chapter 2

REVIEW OF THE LITERATURE

2.1 BLACK SPOT

2.1.1 PATHOGEN

Black spot in apples is caused by the fungus *Venturia inaequalis* and since it was first reported in Sweden in 1819 has spread worldwide (Jones & Aldwinckle, 1990; MacHardy, 1996). By 1862, it was found in Australia (MacHardy, 1996). The disease was first recorded in 1894 in New Zealand (Cunningham, 1925). Black spot also occurs on pears and roses, but is caused by different fungi in these plants (Manktelow & Beresford, 1993).

The disease cycle of *V. inaequalis* is displayed in Figure 2.1. The fungus produces sexual and asexual spores, which are called ascospores and conidia, respectively (MacHardy, 1996). Ascospores develop in winter on apple leaf litter from previously infected trees and are discharged in spring when favourable conditions, combining sunlight, temperature and humidity, are met (MacHardy, 1996; Manktelow & Beresford, 1993). The establishment of ascospores on leaves is called a primary infection (MacHardy, 1996). Again, if temperature and humidity are favourable, these spores germinate on the leaves, whereby the germ tube penetrates the cuticle and hyphae grow underneath it (Cunningham, 1925; MacHardy, 1996). Younger leaves are very susceptible to infection, but they become more resistant with age (Grove et al., 2003; Lind et al., 2003; Nicholson & Rahe, 2004; Tomerlin & Jones, 1983). If the leaf becomes dry for a certain time, germination can be interrupted until it rains again, but if the dry phase is long enough, spores can die off (Lind et al., 2003). However, it was shown that 30 days at constantly low relative humidity of 65% were

necessary to prevent lesion development (Tomerlin & Jones, 1983). About two to three weeks after primary infection, the first leaf lesions appear (Lind et al., 2003; Manktelow & Beresford, 1993). These lesions produce conidia, which cause secondary infection by further spreading to leaves and fruit through wind and rain (Lind et al., 2003; MacHardy, 1996; Manktelow & Beresford, 1993). In some cases, conidia may overwinter in infected shoots and cause primary infection as soon as leaves emerge the following spring (Cunningham, 1925; Manktelow & Beresford, 1993).

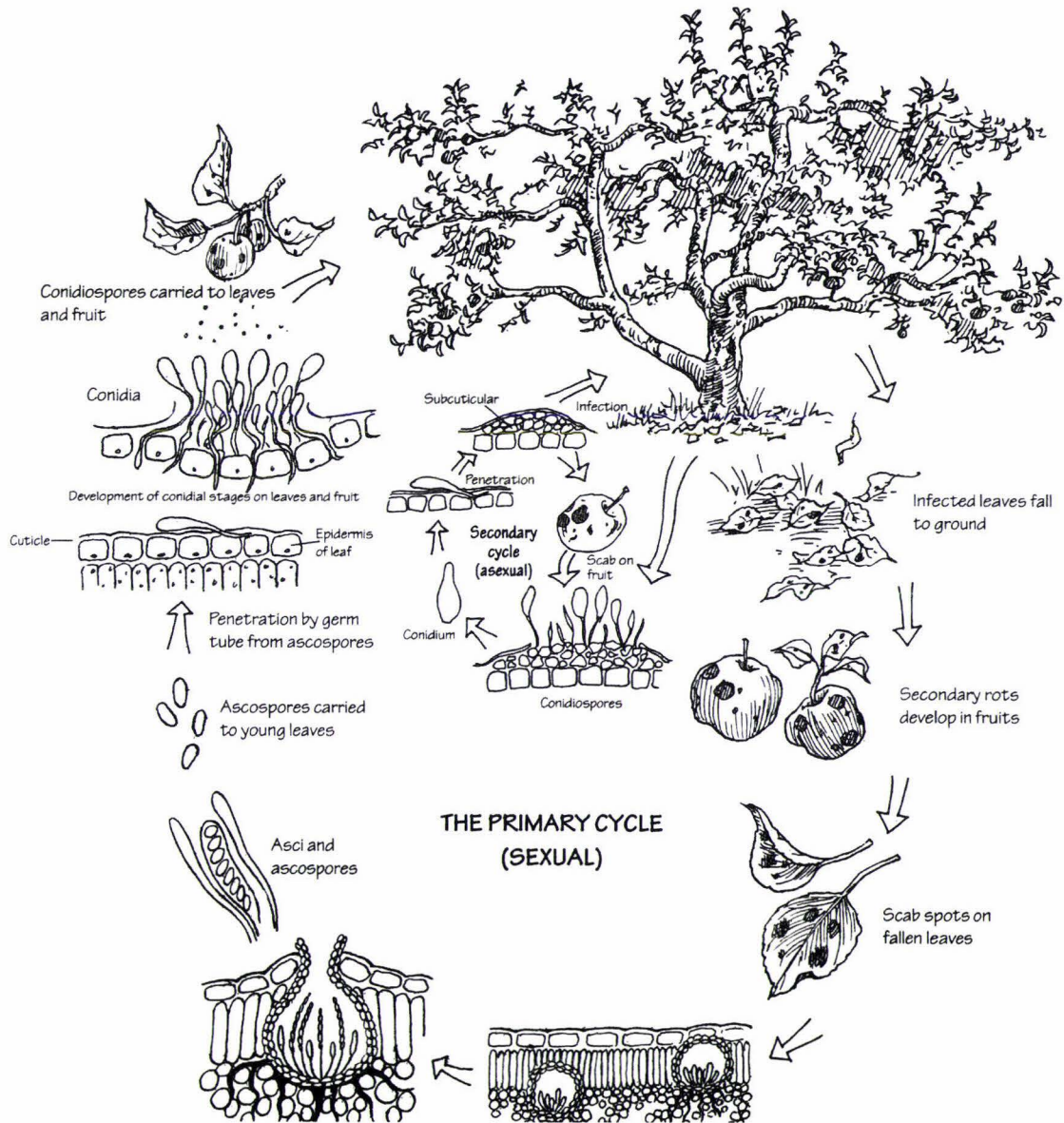


Figure 2.1 The life cycle of the black spot fungus *Venturia inaequalis* (Phillips, 1998, p.135).

2.1.2 DISEASE SYMPTOMS

Irregularly shaped lesions first develop along the veins and midrib on the underside of infected leaves (Cunningham, 1925; Grove et al., 2003; Jones & Aldwinckle, 1990; MacHardy, 1996). On the upper surface, circular velvety lesions appear that darken with time (Grove et al., 2003; MacHardy, 1996). These black spots give the disease its name. Badly affected leaves can abscise (Cunningham, 1925; Grove et al., 2003; Lind et al., 2003). Fruit are most susceptible when young and lesions mostly develop at the calyx end, later spreading over the whole apple (Cunningham, 1925; Grove et al., 2003; Jones & Aldwinckle, 1990; MacHardy, 1996). Fruit affected early in development can become deformed as growth is restricted (Cunningham, 1925; Grove et al., 2003; Lind et al., 2003). Lesions on fruit grow more slowly than those on leaves and have discrete borders (MacHardy, 1996). Older fruit lesions become corky and can crack open, allowing rot to establish (Lind et al., 2003). If fruit are affected late in the season, lesions may develop during storage causing so-called storage scab (Lind et al., 2003). Examples of black spot symptoms on leaves and fruit are shown in Figure 2.2.

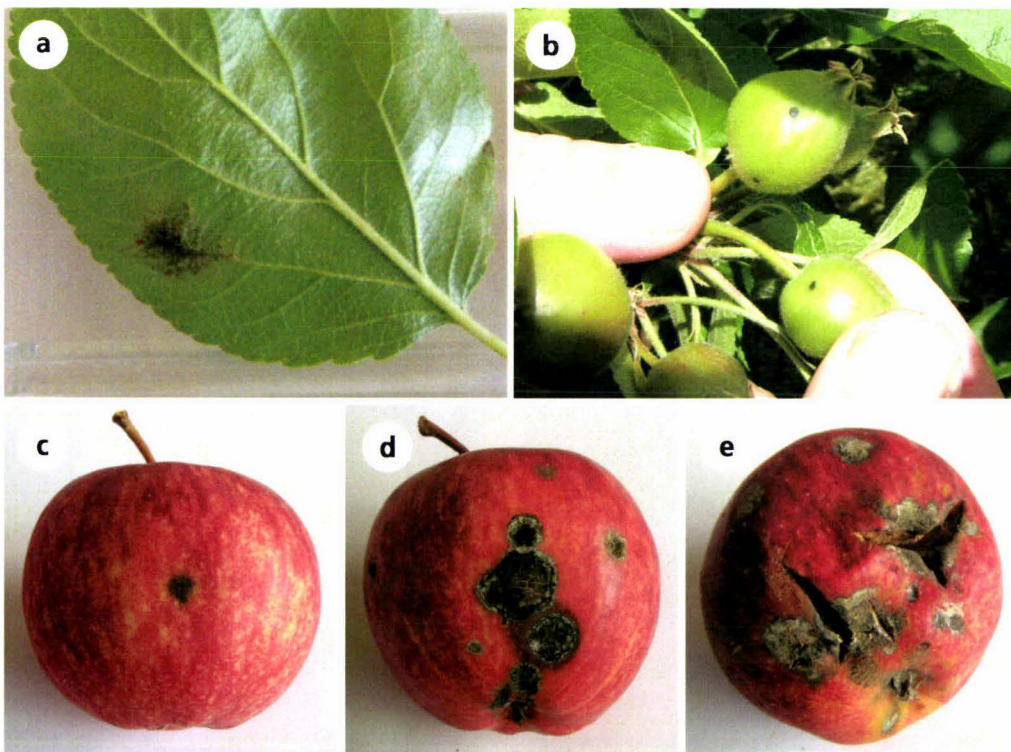


Figure 2.2: Lesions caused by black spot on apple leaves and fruit. (a) Irregularly shaped lesion on underside of leaf and (b) discrete spots on young apples in November 2006; black spot of varying severity on harvested 'Royal Gala' in March 2007: (c) single spots can (d) coalesce to form patches, (e) on badly affected apples, the skin can crack open and rot can develop.

2.1.3 CONTROL STRATEGIES

2.1.3.1 HISTORY

The first programmes for the control of black spot have been reported from the 1880s (MacHardy, 1996) and used inorganic fungicides, such as sulphur and Bordeaux mixture (Brent, 1985). The latter – a mixture of copper sulphate, lime and water – was initially developed as a control for downy mildew on grapes and also controlled black spot very well, but caused unwanted russet on apples (MacHardy, 1996). Growers were able to decrease the number of spray applications, once knowledge about *V. inaequalis* increased (MacHardy, 1996). Research in the 1920s focused on the eradication of black spot in the overwintering stage through the use of chemicals (MacHardy, 1996). This approach was abandoned with the introduction of more effective fungicides and the development of the Mills' warning system. This system is based on the Mills' period, formulated by Dr. William Mills in the 1940s due to improved understanding of the role the weather plays in the development of black spot (Mills, 1944). It describes the leaf wetness period required at different temperatures for ascospores to establish infection.

The inorganic fungicides used at the time had several drawbacks. They needed a high number of applications per season and had a toxic effect on the trees (MacHardy, 1996). Synthetic, petrochemical-based fungicides were developed in the 1930s and were more effective at lower doses without being harmful to the tree or fruit (MacHardy, 1996). The various synthetic fungicides available today are not part of this literature review as they are not permitted under organic certification. Organic growers today still rely mainly on copper and sulphur compounds as a defence against black spot despite the known phytotoxicity of these chemicals and increased spraying frequency.

2.1.3.2 BLACK SPOT CONTROL UNDER ORGANIC CERTIFICATION

The control of black spot under organic certification today is difficult due to the lack of effective fungicides. Practising hygiene to decrease spore numbers in the orchard and forecasting possible infection periods are of major importance. The main chemical control measures under organic certification are copper, lime sulphur, elemental sulphur, hydrated lime and sodium bicarbonate. Lime sulphur is the fungicide of choice for many growers as it provides good black spot control and can be applied preventatively and curatively. Black spot control is best using copper fungicides but like the remaining fungicides, it can only be

applied preventatively. The other fungicides are less effective than copper and lime sulphur. Elemental sulphur can be ground into fine powder and mixed with wetting agents to allow easy dispersion in water (Mills & LaPlante, 1954; Tweedy, 1981). It is then referred to as wetttable sulphur. The finer the particle size, the more effective is sulphur as a fungicide as the surface area is increased and more material remains on the plant (Cunningham, 1935). Hydrated lime, also known as slaked lime, is less effective in controlling black spot than the previously mentioned fungicides, but it does not result in damage to the apple tree. The ability of sodium bicarbonate, or baking soda, to control black spot is comparable to that of hydrated lime.

The effectiveness of each control method will be reviewed separately in the following sections. Most studies reviewed compared a variety of fungicides from organic and conventional apple production.

2.1.3.2.1 SANITATION

Autumn and spring are very important stages in black spot control for the apple grower (Lind et al., 2003; Manktelow & Beresford, 1993). Hygiene is crucial to prevent the build-up of high spore numbers in the orchard. Black spot control measures should not only concentrate on preventing spore discharge and germination from spring onwards, but also on reducing overwintering spores in autumn. This is especially important in organic orchards where due to lack of effective fungicides, high spore numbers can produce devastating infections. Different chemical and non-chemical sanitation treatments can be applied, e.g. removing or shredding leaf litter, mulching or applying fungicides to fallen leaves in the autumn or spring (Holb, 2006; Lind et al., 2003; Nicholson & Rahe, 2004). Shredding has been reported to reduce the risk of black spot by 50 to 90% depending on the amount of leaf litter shredded (Sutton et al., 2000). Although sanitation practices can significantly improve the organic disease management, they should be used in addition to fungicides as control is not sufficient to meet commercial fruit quality standards (Nicholson & Rahe, 2004).

2.1.3.2.2 FORECASTS

Forecasts are usually used to determine possible infection periods, so that fungicides can be applied accordingly and unnecessary spray treatments can be eliminated to improve efficiency. For many years, the Mills' system was the most important tool in black spot

control. However, Mills only took into account the influence of temperature and humidity on ascospore release and not other factors such as light, spore maturity and leaf growth to forecast possible infection periods (Lind et al., 2003; Manktelow & Beresford, 1993). Virtually all ascospores are discharged during daytime (MacHardy & D.M.Gadoury, 1989; Manktelow & Beresford, 1993); therefore, even if it rains over night, leaf wetness time is calculated from dawn onwards only (MacHardy & D.M.Gadoury, 1989). More advanced black spot warning software combining several factors is available today (Lind et al., 2003).

2.1.3.2.3 COPPER

Copper has been successfully used on its own or in various forms, such as Bordeaux mixture, since control programmes against black spot were first developed. Cupric hydroxide gave good control of black spot (Beresford et al., 1991; Beresford et al., 1995). Cupric hydroxide mixed with wettable sulphur was superior in controlling black spot than cupric hydroxide alone, with 0.6% and 8.3% of fruit affected, respectively (Palmer et al., 2003). A mixture of cupric hydroxide with sulphur gave better disease control than one containing cupric hydroxide and hydrated lime (Beresford et al., 1995; Palmer et al., 2003), but the former increased fruit russet (Beresford et al., 1995). Disease control was poor at levels low enough to prevent russet development (Beresford et al., 1995). Copper caused russet on 'Braeburn' apples, despite this cultivar generally not being sensitive to russet (Palmer et al., 2003).

The use of copper has disadvantages, which is why its use is restricted in New Zealand to 3 kg active ingredient per hectare per year (BioGro, 2001). It is toxic to people and it accumulates in the soil to levels ultimately inhibiting plant growth and soil life (Beresford et al., 1991).

2.1.3.2.4 LIME SULPHUR

Lime sulphur was first shown to be effective against black spot in apples in 1908. (Cunningham, 1935). Lime sulphur, or calcium polysulphide, is formed by boiling one part of lime (calcium oxide) and two parts of sulphur in water (Tartar, 1914; Tartar & Bradley, 1910; Thatcher, 1908). It was a popular fungicide until 1935 but was then gradually replaced because it had a negative effect on the tree and reduced yield (MacHardy, 1996).

Lime sulphur has been described as giving better control of black spot than wettable sulphur, whereby it did not matter if lime sulphur was applied curatively or preventatively

(Holb et al., 2003). However, in earlier studies under high disease pressure, the incidence of black spot was similar after treatment with lime sulphur or wettable sulphur (Holb & Heijne, 2001). In such a situation, only synthetic fungicides offered satisfactory control of black spot (Holb et al., 2003; Holb & Heijne, 2001). Under low disease pressure, however, similar levels of black spot control were achieved with lime sulphur, wettable sulphur or synthetic fungicides (Holb & Heijne, 2001). The incidence of black spot on fruit was lower when applications of lime sulphur mixed with mineral oil were made in comparison to applying lime sulphur alone (Holb & Heijne, 2001). In other studies, control of black spot on fruit by spraying lime sulphur until bloom, followed by elemental sulphur, was not satisfactory and better control was achieved when using copper sprays followed by elemental sulphur (Ellis et al., 1991). Lime sulphur resulted in significantly better black spot control than hydrated lime with 3.1% and 13.8% of fruit affected at harvest, respectively (Palmer et al., 2003).

The phytotoxic effect of lime sulphur, its impact on apple trees and fruit quality, is discussed in detail in section 2.2.

2.1.3.2.5 ELEMENTAL SULPHUR

Ground sulphur has been used in greenhouses as a control measure against fungal infections since 1892 and in the field since 1916 (Cunningham, 1935). It is inert and can therefore be used together with other fungicides and insecticides (Tweedy, 1981).

In apples, black spot control with wettable sulphur was less effective than with lime sulphur in some studies (Holb et al., 2003), whereas in others, results were comparable (Holb & Heijne, 2001; Palmer et al., 2003). Wettable sulphur resulted in good control, but high russet incidence, when mixed with cupric hydroxide (Palmer et al., 2003).

2.1.3.2.6 HYDRATED LIME

Hydrated lime ($\text{Ca}(\text{OH})_2$) is alkaline and its pH of 8.5 to 9 inhibits spore germination and infection (Beresford et al., 1996). It breaks down to calcium carbonate in the air and is safe to mammals (Beresford et al., 1996).

Hydrated lime has been shown to control black spot (Beresford et al., 1996; Beresford et al., 1995). However, when hydrated lime was used to control black spot, the percentage of fruit affected by the disease at harvest varied between orchards from just over 1% to

around 60% (Beresford et al., 1996; Beresford et al., 1995). Although the latter was commercially unacceptable, in comparison, over 95% of apples in the unsprayed control were affected (Beresford et al., 1996). Increasing the application rate of hydrated lime, improved the control of black spot without causing fruit russet and the optimum application rate was 1.6 kg/100 litre (Beresford et al., 1995). The addition of cupric hydroxide to hydrated lime greatly decreased the incidence of black spot (Beresford et al., 1995). Studies in Germany achieved complete control of black spot using 0.5 kg/100 litre hydrated lime applied from an overhead irrigation system using up to 62 treatments per season (Grimm-Wetzel & Schonherr, 2006). Whereas fruit from control trees developed black spot symptoms during storage regardless of infection at harvest, fruit treated with hydrated lime were free of black spot at harvest and after storage (Grimm-Wetzel & Schonherr, 2006). No signs of phytotoxicity were observed on either leaves or fruit (Grimm-Wetzel & Schonherr, 2006). However, hydrated lime leaves a white residue on fruit, which could have implications on sale if it is not thoroughly removed before packing (Beresford et al., 1995; Palmer et al., 2003). This deposit does not influence colour development of the apples (Beresford et al., 1995).

2.1.3.2.7 SODIUM BICARBONATE

Although hydrated lime and sodium bicarbonate have a similar pH, the fungicidal action of the latter is due to the bicarbonate ion, not due to its alkalinity (Corral et al., 1988; Marloth, 1931). Sodium bicarbonate (NaHCO_3) did inhibit spore germination and germ tube elongation of *V. inaequalis* in vitro and thus reduced the incidence of black spot in trial orchards (Ilhan et al., 2006). Effectiveness was comparable to that of hydrated lime (Beresford et al., 1996). Signs of phytotoxicity were observed at an application rate of 1% (Beresford et al., 1996) and 2% (Ilhan et al., 2006). Sodium bicarbonate did not influence firmness, soluble solids content and pH at harvest (Ilhan et al., 2006).

2.1.3.3 FUTURE PERSPECTIVES

The development of apple cultivars resistant to black spot is essential to decrease the reliance on chemicals in organic as well as conventional production systems (Beresford et al., 1991). Resistant cultivars developed in New Zealand and other countries could become commercially more acceptable as fruit quality is improved (Grove et al., 2003). Nevertheless, none of the resistant cultivars has gained commercial market share (Nicholson & Rahe, 2004). For the conventional grower, transferring the resistant gene

into commercial cultivars by genetic engineering could become an option (Nicholson & Rahe, 2004), but this possibility will not become available to the organic grower anytime in the near future because the use of genetic engineering does not align with organic principles. New means of biological control are evolving in the fight against black spot. Promising results have been achieved with fungal antagonists, e.g. *Microsphaeropsis* sp., which reduced ascospore production by approximately 80% (Carisse et al., 2000), but the method has not yet gained commercial application (Lind et al., 2003). Strobilurin (Stroby®) is the synthetic development of a natural occurring fungicide, but has not found its way into organic certification in New Zealand. Vinasse, a fermented waste product from sugar processing, has recently been reported to reduce ascospore formation by up to 95% (Anonymous, 2007). It also accelerated leaf degradation and might therefore be a valuable tool when applied in autumn (Anonymous, 2007).

2.2 PRE- AND POSTHARVEST PHYSIOLOGY

2.2.1 INFLUENCE OF LIME SULPHUR ON PLANTS

2.2.1.1 HYDROGEN SULPHIDE

Lime sulphur constantly gives off hydrogen sulphide (H_2S) and upon application to the leaf is converted to elemental sulphur (Tweedy, 1981). The characteristic “rotten egg” odour of hydrogen sulphide can be smelt at a concentration of greater 0.02 $\mu\text{l/l}$ air (Beauchamp et al., 1984) and this smell is still apparent in an orchard treated with lime sulphur even a few days after application. Hydrogen sulphide can be used as a nutrient by plants, but above certain levels it can be toxic (De Kok et al., 2002). The negative impact of lime sulphur and hydrogen sulphide on plants is very similar and is outlined below. It is therefore possible that the reason for the phytotoxicity of lime sulphur is due to its conversion to hydrogen sulphide.

2.2.1.2 PHOTOSYNTHESIS

High, long-term exposure to H_2S has been reported to reduce photosynthesis in various plants (De Kok et al., 2002; Maas et al., 1988; Olivia & Steubing, 1976; Steubing, 1979). Chlorophyll content decreased with prolonged exposure (Steubing, 1979) and electron transport as well as carbon dioxide fixation were impaired (Maas & De Kok, 1988; Maas et al., 1988).

Since the 1930s, studies have proposed that the application of lime sulphur reduces photosynthesis (Hoffman, 1935; Hyre, 1939). Application of lime sulphur led to a greater reduction in photosynthesis than wettable sulphur on 'Baldwin' and 'McIntosh' apples and the impact was greater at higher temperatures (Hyre, 1939). Application of lime sulphur as a fruit thinning agent to 'Braeburn' trees during flowering reduced photosynthesis in primary spur leaves by 16 to 47% when applied one to four times, respectively (McArtney et al., 2006). This effect was still apparent up to 51 days after the final application of lime sulphur (McArtney et al., 2006). The photosynthetic rate of basal extension shoot leaves which were not fully developed during the application of lime sulphur was not affected, indicating that those leaves, once fully expanded, may compensate for the reduced photosynthesis in spur leaves (McArtney et al., 2006). As a thinning agent, lime sulphur is applied several times during bloom and at high concentrations (Palmer et al., 2003). McArtney et al. (2006) used lime sulphur at rates of up to 4% for thinning. In the organic control of black spot, lime sulphur is applied at lower levels than for thinning and less often during flowering depending on the number of infection periods. However, the overall number of applications throughout the season is higher than when applied as a flower thinning agent, possibly decreasing the photosynthesis potential of the whole tree. In 'Braeburn', photosynthesis was 50% lower in leaves treated with any form of sulphur compared to treatments not containing sulphur (Palmer et al., 2003). The photosynthetic ability of a tree is of major importance as the nutrients produced are required for growth, especially of the fruit. Carbohydrates are in general transported in the phloem from source (leaves) to sink (growing fruit) and are incorporated into the fruit (Miranda et al., 2002; Taiz & Zeiger, 2002). Leaves of the bourse shoot play a role in that nutrient supply (Volz, 1991).

2.2.1.3 GROWTH AND YIELD

Elevated levels of hydrogen sulphide in the soil as well as the atmosphere can have a negative effect on plant growth, however, different species react in different ways and can tolerate different levels of H₂S (De Kok et al., 2002). The reason for this is not clear, but is possibly linked to differences in plant morphology and the fate of the sulphide absorbed (De Kok, 1989, 1990; De Kok et al., 1998; De Kok et al., 2002). No direct relationship has been observed between flux of H₂S to plant shoots and the sensitivity of the plant to the gas, e.g. the same uptake of H₂S led to decreased growth in spinach but not in maize (De Kok et al., 1989). Biomass production was decreased at long-term H₂S levels greater

0.03 $\mu\text{l/l}$ (De Kok et al., 1983; Krause, 1979; Maas et al., 1987a; Maas et al., 1985; Thompson & Kats, 1978).

Application of different fungicides, including lime sulphur, did not have an influence on vegetative growth of apple trees during one season as measured as spur leaf area, bourse shoot development, extension shoot leaf area, trunk cross-sectional area and total shoot growth (Palmer et al., 2003). Nevertheless, there may be a cumulative effect on growth after several years of sulphur application and associated decrease in photosynthesis (Palmer et al., 2003). Mills and LaPlante (1954) reported lower yield and lighter bloom of apple trees when using lime sulphur as opposed to elemental sulphur and this effect was cumulative and only apparent after three years.

The application of lime sulphur during bloom reduced fruit set in 'Braeburn' apples, possibly by increasing the percentage of flowers with no or fewer than 10 pollen tubes (McArtney et al., 2006). Each additional application of lime sulphur decreased fruit set by a further 10 % (McArtney et al., 2006). It has also been suggested that the application of lime sulphur results in inhibition of photosynthesis and decreased assimilate transport to the fruit which in turn leads to its abscission (McArtney et al., 2006; Noordijk & Schupp, 2003). Premature fruit drop as a result of lime sulphur application has already been reported in the first half of the 20th century (Cunningham, 1935). In 'Braeburn', black spot control with sulphur fungicides resulted in a 12% decrease in yield (fruit per tree) compared to treatments without sulphur due to reduced fruit set earlier in the season (Palmer et al., 2003). Moreover, a lower mean fruit weight and decreased total yield has been reported as a problem in organic orchards compared to conventional orchards, especially in 'Braeburn' (Walker & McArtney, 2001). Yield is an indicator of productivity and important measure for orchard profits (Heinicke, 1985).

2.2.1.4 LEAF DAMAGE

Visible injury, such as leaf lesions, leaf necrosis and defoliation, occurred in various plants at hydrogen sulphide levels greater 0.3 $\mu\text{l/l}$ (Krause, 1979; Steubing, 1979; Thompson & Kats, 1978).

From early on, lime sulphur has been reported to have a negative effect on leaves (Cunningham, 1935; Tweedy, 1981). Visible injury of leaves sprayed with lime sulphur was more pronounced the higher the temperature (Hyre, 1939; Mills & LaPlante, 1954).

Symptoms appear as burning, scorching or dwarfing of the leaves as well as premature defoliation and injury caused by lime sulphur is more severe than that caused by elemental sulphur (Tweedy, 1981). Lime sulphur caused a significantly greater phytotoxic effect, rated as leaf size and necrosis, compared to an untreated control (Holb et al., 2003; Holb & Heijne, 2001). Poor leaf condition has also been reported in organic orchards in New Zealand, especially for 'Braeburn' (Palmer et al., 2003).

2.2.2 FRUIT QUALITY PARAMETERS

2.2.2.1 COLOUR

The colour of apples is determined by its main pigments: chlorophylls, carotenoids and anthocyanins which are responsible for the green, yellow and red colour, respectively (Reay et al., 1998). The background colour of an apple changes from dark green to yellow as the chlorophyll degrades during maturation and the underlying yellow colour of the carotenoids is revealed. The production of the anthocyanins responsible for the red skin colour is dependent on a variety of internal and external factors, such as light, temperature, fertilization, thinning, nutrient status and enzymes (Saure, 1990). Shading within the canopy markedly reduces the formation of anthocyanins (Saure, 1990). Another important factor for colour development is the availability of nutrients, especially sugars (Saure, 1990).

Colour is an important consumer attribute as the first thing attracting buyers to the apples in a store is an outstanding visual appearance (Reay et al., 1998). Apart from an appealing background colour, a bright red blush is desirable. A green background reduces the intensity of red (Reay et al., 1998). A certain cultivar-specific background colour and blush percentage are not just necessary to meet consumer expectations, but also export quality standards, e.g. at least 40% of the surface area has to be blushed for export quality fruit (Mills, 1996).

2.2.2.2 FIRMNESS

Firmness is a complex parameter and depends on several factors. In general, a smaller apple size is associated with a higher firmness rating (Blanpied et al., 1978), but firmness is also dependent on cell size, shape, packing and turgor pressure as well as thickness of the cell walls (Harker et al., 1997).

High firmness ratings at harvest that can be sustained over the storage period are very important to the industry (Johnson, 1994) as texture of the apple is an important consumer attribute. It has been shown that instrumental puncture measurements are a good indicator of several sensory attributes, e.g. crispness and crunchiness (Harker et al., 2002b). The marketability of an apple decreases significantly with increasing softness (Johnson, 1992). A softer apple was associated with a mealy texture (Harker et al., 2002b) and mealiness was not acceptable to the consumer (Jaeger et al., 1998). The critical threshold for judging apples as mealy was when their firmness decreased below 50 N (Harker et al., 2002b). In sensory tests, a trained panel was able to distinguish apples according to firmness, if their puncture force differed by at least 6 N (Harker et al., 2002b). Another study showed that even untrained consumers were able to distinguish apples by firmness if they differed by as little as 5 N when presented with one apple per day (Harker et al., 2002a). When they were presented with one apple per minute, however, a difference of 10 N was necessary to detect the difference (Harker et al., 2002a). This shows that long-term memory was better than short term memory and is important as it would be more likely for consumers to eat “an apple a day”.

2.2.2.3 SOLUBLE SOLIDS CONTENT

The soluble solids content (SSC) of apples comprises sugars as well as acids. Over the storage period, sugars increase and titratable acidity decreases. Sweetness is an important consumer attribute for apples (Jaeger et al., 1998). Sweet taste was most difficult to predict from experimental measurements, but correlated well with SSC and consumers were able to distinguish between apples that differed by 1% SSC (Harker et al., 2002c).

2.2.2.4 STARCH PATTERN INDEX

Starch pattern index (SPI) is used as a maturity index in apples and every cultivar shows a characteristic starch pattern as starch is degraded. SPI is influenced by total starch concentration as well as the rate of degradation (Brookfield et al., 1997). However, it is not an exact measure of the amount of starch in the apple. Staining started to clear when starch concentrations were lower than 2-3 mg per gram fresh weight (Brookfield et al., 1997). At equal decomposition rates and low concentrations of starch in the fruit, SPI will increase quicker, whereas at a high initial concentration, the development of SPI will be delayed (Brookfield et al., 1997).

Apples with a low carbohydrate content at harvest or a high rate of degradation during storage will achieve a maximum SPI rating more quickly. This can negatively influence the fruit's storability as well flavour and textural quality (Brookfield et al., 1997). As outlined before, these attributes are very important to the consumer.

2.2.2.5 TITRATABLE ACIDITY

Titrateable acidity (TA) is not so much a postharvest quality parameter, but rather a measure for taste. TA is a very good predictor of acid taste as well as overall flavour and apple flavour (Harker et al., 2002c). Acid taste, just like sweet taste, is an important consumer attribute for apples (Jaeger et al., 1998). In sensory tests, consumers were able to distinguish apple samples when they differed by as little as 0.08% TA (Harker et al., 2002c).

2.2.2.6 DRY MATTER

The percentage dry matter greatly depends on the water content of the apple. In kiwifruit, the main components of dry matter are starch and sugars (Beever & Hopkirk, 1990). Assuming that this is also the case for apples, dry matter may give a rough estimate of the starch content.

2.2.3 INFLUENCE OF LIME SULPHUR ON FRUIT QUALITY

Studies on the influence of lime sulphur on apple quality are scarce.

Results of studies on the development of russet have been mixed. Soon after growers began to use it, lime sulphur has been reported to cause russet (Cunningham, 1935). Yet, 'Braeburn' developed increased russet when sprayed with copper, but not when treated with lime sulphur (Palmer et al., 2003). Apples of cultivar 'Golden Delicious Spur' showed less russet when treated with lime sulphur compared to an untreated control (Sanchez et al., 2001). Lime sulphur did not increase russet on 'Jonagold' compared to other treatments containing sulphur and an untreated control (Holb & Heijne, 2001).

Blush percentage was lower on 'Braeburn' treated with lime sulphur compared to hydrated lime and these differences were consistent over two harvests (Palmer et al., 2003). Blush on fruit sprayed with hydrated lime was not significantly different from fruit treated with synthetic fungicides (Palmer et al., 2003). Delayed blush development was observed in 'Braeburn' after treatment with a mixture of cupric hydroxide and wettable sulphur, but

development of background colour and starch pattern index were not affected in the same way (Palmer et al., 2003). Significant differences were observed in 'Braeburn' in flesh firmness, total soluble solids and starch pattern index between treatments with different fungicides (wetable sulphur, lime sulphur, cupric hydroxide, hydrated lime, wettable sulphur/hydrated lime, wettable sulphur/cupric hydroxide, dodine/polyram), but these differences were not consistent over two harvests (Palmer et al., 2003). Flesh firmness tended to be higher in treatments containing hydrated lime and lower in those containing sulphur compared to the control (Palmer et al., 2003). Control trees were treated with synthetic fungicides and were therefore rather a separate treatment than a true control.

2.3 UPTAKE OF ATMOSPHERIC SULPHUR BY PLANTS

High atmospheric levels of sulphur are mostly caused by pollution in highly industrialized areas or in areas with volcanic activity (De Kok et al., 2002). Agriculture plays a less important role. Research performed on the uptake of atmospheric sulphur by plants therefore refers to increased levels of hydrogen sulphide and sulphur dioxide in the air due to pollution.

Hydrogen sulphide can be taken up as well as emitted by plants through the stomata (De Kok et al., 1991; De Kok et al., 1989; Schroder, 1993). It is assumed that emission serves the regulation of sulphur content in the cell, thereby keeping cysteine concentration below toxic levels (Schroder, 1993). Nevertheless, emission of H₂S is mostly negligible and plants act as a sink for hydrogen sulphide at atmospheric levels higher than 0.001 µl/l (Cope & Spedding, 1982; De Kok et al., 1991; De Kok et al., 1989; De Kok et al., 1997; De Kok et al., 1998; De Kok et al., 2002; Poortinga & De Kok, 1997; Taylor et al., 1983).

Uptake of hydrogen sulphide by plants increased with increasing ambient temperature (De Kok et al., 1991) and was high during light and lower during darkness (De Kok et al., 1991; De Kok et al., 1989). Uptake of hydrogen sulphide by spinach, maize, pumpkin and spruce followed a linear relationship with increasing H₂S concentration up to 0.3 µl/l and thereafter reached a species-specific maximum (De Kok et al., 1989). There was no influence of H₂S concentration on transpiration (De Kok et al., 1991; De Kok et al., 1989). Therefore, closing of the stomata was not responsible for the saturation of H₂S uptake (De Kok et al., 1991; De Kok et al., 1989). It has been assumed that the low resistance to the uptake of H₂S can be attributed to the direct metabolism of the gas in the leaf (De Kok et al., 1991).

Exposure of various plant species to hydrogen sulphide led to an increase in water-soluble non-protein thiol compounds (De Kok, 1989, 1990; De Kok et al., 1989; De Kok et al., 1998; De Kok et al., 2002; De Kok et al., 1983; Maas et al., 1987a; Maas et al., 1987b; Maas et al., 1987c; Maas et al., 1985; Tausz et al., 1998), but the content of water-soluble protein thiol was unaffected (De Kok et al., 1985). Only around 2% of the organic, reduced sulphur in plants is in the form of water-soluble non-protein thiols, with glutathione being the predominant thiol (De Kok et al., 2002; Stulen & De Kok, 1993). The increase in thiol compounds upon H₂S exposure could be attributed to an increase in glutathione as well as cysteine (Buwalda et al., 1993, 1994; Buwalda et al., 1988; Buwalda et al., 1990; De Kok et al., 1998; Poortinga & De Kok, 1997). A change in the composition of the thiol pool is also possible (Buwalda et al., 1993, 1994; Buwalda et al., 1988; Buwalda et al., 1990; De Kok et al., 1998; Poortinga & De Kok, 1997; Tausz et al., 1998). The increase in water-soluble non-protein thiols was observed after a few hours of fumigation and reached a maximum after one or two days (Buwalda et al., 1994; De Kok, 1989, 1990; De Kok et al., 1985; De Kok et al., 1998; De Kok et al., 2002; Maas et al., 1987c; Poortinga & De Kok, 1997). In shoots, thiols increased up to fivefold, depending among other factors on H₂S concentration and plant species (Buwalda et al., 1993; De Kok et al., 1998; De Kok et al., 2002; Maas et al., 1987b; Poortinga & De Kok, 1997). A delayed, lower increase in thiols was also observed in the roots of fumigated plants (De Kok et al., 1997; De Kok et al., 1998; De Kok et al., 2002; Herschbach et al., 1995a, b; Poortinga & De Kok, 1997; Tausz et al., 1998; Westerman et al., 2000). Only part of the H₂S taken up by the plant could be revealed in the form of water-soluble non-protein thiols (De Kok et al., 1989; De Kok et al., 2002). When exposure to hydrogen sulphide was terminated, thiol levels decreased again rapidly and reached those of non-fumigated plants after one or two days (Buwalda et al., 1994; De Kok et al., 1985; De Kok et al., 1986; De Kok et al., 1998; Maas et al., 1987c). Thiols were metabolized and/or translocated in plants (De Kok, 1989, 1990), but sulphur was not emitted in the form of hydrogen sulphide (De Kok et al., 1986).

2.4 SUMMARY

Black spot, caused by the fungus *V. inaequalis*, is a major problem in organic apple production and thrives in the warm, humid conditions experienced in New Zealand. The fungus causes black lesions on leaves and fruit that compromise tree performance as well as fruit quality. Control of the disease under organic certification is difficult due to less effective fungicides which are mostly based on copper and sulphur. Lime sulphur is the

most commonly used fungicide in organic apple production in New Zealand, but its phytotoxicity results in visible injury to the leaves, a reduction of photosynthesis and lower yield. The cultivar 'Braeburn' is difficult to grow organically as it is severely affected by lime sulphur; the 'Royal Gala' tree, on the contrary, is not obviously damaged. Both these cultivars are of major importance to the New Zealand economy as they account for more than two thirds of its apple exports (Kerr et al., 2006).

It is not known if lime sulphur has an effect on fruit quality; a study performed on 'Braeburn' was inconclusive (Palmer et al., 2003). However, no study has compared the effect of different levels of lime sulphur on the quality parameters of apples. Moreover, to the author's knowledge, no study has looked at potential residues of sulphur on the apple as well as a possible metabolism of the sprays applied in the plant or the fruit. Hydrogen sulphide, which is emitted upon application of lime sulphur, has been shown to be taken up by various plant species and metabolized into thiol compounds. However, studies were not done on fruit-bearing plants.

Chapter 3

MATERIALS AND METHODS

3.1 ORCHARD AND EQUIPMENT

The research was performed at Massey University Plant Growth Unit (PGU) in Palmerston North, New Zealand. The PGU's small organic orchard has plantings of the cultivars 'Royal Gala' and 'Braeburn'. Trees were planted on MM.106 rootstock in 1988 at 5 x 3.5 metres in a six row block. The block was established as a systems trial, so half of each row was one cultivar and the other half the other cultivar. Trees were treated organically since planting. Three rows of centre leader trees were used in the trial.

Using a single orchard in the trial reduces experimental bias possible due to different soil types, the age of trees and overall orchard management. However, the project is limited by the fact that the experimental unit is situated in the Manawatu, whereas the Hawke's Bay is the main area of apple production in New Zealand. These two regions differ significantly in climatic factors, such as rainfall, temperature and sunshine hours (Appendix C).

3.2 TRIAL LAYOUT

The trial was arranged in three rows, i.e. six half rows, with three treatment blocks randomised over each half row (Figure 3.1). Each treatment block comprised a group of six to nine trees. Observations were only made on two evenly-sized trees in the middle of each treatment block. The experimental unit was a pair of trees. Lime sulphur sprays were also only applied to these two trees in each block. The remaining trees served as a buffer

from October 11, 2006 to February 19, 2007 at 1% and 2%. The three treatments compared in the trial were: (1) hydrated lime applied at 1.6 kg/100 litres (“Control”), (2) lime sulphur applied at 1 litre/100 litres (plus hydrated lime whenever Control was applied; “LS1”) and (3) lime sulphur applied at 2 litres/100 litres (plus hydrated lime whenever Control was applied; “LS2”). Copper, oil and hydrated lime were applied using an airblast orchard sprayer (Croplands, Cropliner 1500). Lime sulphur was applied with a hand gun (Braglia Turbo 400) connected to a sprayer (Bertolini, BA003-430EFP, 400 litre capacity), except for October 11, 2006 when lime sulphur was applied with the airblast orchard sprayer. It was then realized that the airblast sprayer was too powerful and curtains could not be kept in place. All spray treatments were applied in the morning when wind drift was minimal (except for February 19, 2007 when sprays were applied after measurement of photosynthesis had been completed). When all treatments were applied on the same day, hydrated lime was applied first, immediately followed by lime sulphur. The spray diary is outlined in Table 3.1.

Few studies have compared the effect of different fungicides suitable for organic production standards on apple quality (Holb & Heijne, 2001; Palmer et al., 2003; Sanchez et al., 2001) and only some of them used an unsprayed control (Holb & Heijne, 2001; Sanchez et al., 2001). As the disease pressure was anticipated to be high, using an unsprayed control seemed unwise. Too many spores could have been released from an unsprayed control onto neighbouring trees and the whole crop from control trees may have been lost for analysis. By applying hydrated lime to all trees as a base application, the treatment was still a true control, rather than a separate treatment.

A winter clean-up spray was necessary to decrease overwintering spore numbers. Copper was chosen as it generally achieves good control of black spot. Moreover, any chemicals containing sulphur were avoided, unless applied in the form of lime sulphur in treatments LS1 and LS2. As copper has been reported to increase russet (Palmer et al., 2003), this chemical was only applied once early in the season well before flowering. The base and control application was chosen to be hydrated lime as it does not contain sulphur, it has been shown to provide some black spot control (Beresford et al., 1996; Beresford et al., 1995; Grimm-Wetzel & Schonherr, 2006) and no phytotoxic effect has been reported (Beresford et al., 1995; Grimm-Wetzel & Schonherr, 2006). According to initial plans, the concentrations for the lime sulphur treatments were supposed to be halved later in the

season. However, due to the warm and humid weather conditions favouring black spot, the rates were kept at 1% and 2% for treatments LS1 and LS2 throughout the season.

All 'Royal Gala' trees were hand thinned in late December to similar loads. 'Braeburn' was not thinned as the orchard trees had a much lower crop load than was desired.

Table 3.1: Spray diary for 2006/07 season on 'Royal Gala' and 'Braeburn'.

<i>Date</i>	<i>Treatment</i>	<i>Water rate</i> [l/ha]	<i>Wind</i> [km]	<i>Sun</i> [hours]	<i>Rainfall</i> [mm]	T_{max} [°C]	T_{min} [°C]
5 Sep 2006	Copper/Oil	1,000	163.5	4.3	1.0	19.3	7.6
11 Oct 2006	Control	1,000	330.2	9.8	0.0	15.5	-0.5
	LS1/LS2	1,000*					
Full bloom							
25 Oct 2006	Control	1,000	230.7	4.6	0.0	14.1	6.9
	LS1/LS2	run off					
Petal fall							
13 Nov 2006	Control	1,500	320.8	6.1	0.0	16.6	8.0
17 Nov 2006	LS1/LS2	run off	198.0	2.6	15.0	22.3	15.8
22 Nov 2006	Control	1,500	385.7	1.4	1.4	17.0	10.6
	LS1/LS2	run off					
24 Nov 2006	Control	1,500	581.2	1.7	13.6	16.7	13.1
30 Nov 2006	Control	1,500	503.4	2.3	13.8	18.2	9.8
7 Dec 2007	Control	1,000	446.3	1.4	0.0	16.7	8.3
12 Dec 2007	LS1/LS2	run off	507.8	7.1	0.0	17.6	11.7
4 Jan 2007	Control	1,500	165.2	13.5	0.0	19.2	3.4
11 Jan 2007	LS1/LS2	run off	181.5	9.2	0.0	25.9	11.7
16 Jan 2007	LS1/LS2	run off	249.8	12.2	0.0	25.9	10.9
24 Jan 2007	LS1/LS2	run off	261.3	0.9	0.2	25.0	15.3
7 Feb 2007	LS1/LS2	run off	425.8	9.6	0.8	26.9	17.5
9 Feb 2007	Control	1,500	243.5	9.7	0.0	25.3	14.7
15 Feb 2007	LS1/LS2	run off	257.0	6.2	0.0	22.2	9.6
19 Feb 2007	LS1/LS2	run off	204.8	11.9	0.0	26.0	10.4

T_{max}/T_{min} = maximum/minimum temperature, Copper = copper oxychloride, Oil = DC Tron Plus, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, Control = hydrated lime. Weather data supplied per 24 h period by AgResearch, Palmerston North, New Zealand.

*LS1 and LS2 were applied using an airblast orchard sprayer on October 11, 2006.

3.4 PHOTOSYNTHESIS

Photosynthetic rate was measured on two days late in the season (February 19, 2007 and March 4, 2007) using a CIRAS-2 Portable Photosynthesis System (PP Systems, Hertfortshire, UK). Measurements were started at 9 am and completed by 1 pm. One fully expanded bourse shoot leaf was tagged per tree and the same leaf was used for measurement on both days. Environmental parameters were set as follows: (1) temperature = 25°C, (2) CO₂ = 380 ppm, (3) light intensity = 1,200 μmol m² s⁻¹, (4) leaf area = 2.5 cm², (5) chamber flow rate = 200 ml min⁻¹ and (6) humidity = 80% of ambient.

3.5 SAMPLING PROCEDURE AND SAMPLE PREPARATION

There were two harvests for each cultivar. The maturity of apples to be harvested was decided using background colour in the first harvest, which is normal industry practice ('Royal Gala' rating 4-5, 'Braeburn' rating 2-4, ENZA Ltd., Background Colour Swatch "Gala/Royal Gala" and "Braeburn", January 2003, New Zealand); in the second harvest, the remaining apples were taken off each tree. 'Royal Gala' was harvested on March 9 and 19, 2007. 'Braeburn' was harvested on April 11 and 18, 2007. One characteristic of apple growing in the Manawatu is that fruit matures much later than for example in the Hawke's Bay. Harvests were started at 8 am and apples were moved to cool storage at approximately 0°C immediately afterwards. 'Royal Gala' and 'Braeburn' were stored for five and six weeks, respectively. 'Braeburn' has a longer storage life than 'Royal Gala' (Hampson & Kemp, 2003). Analysis of stored fruit was performed on April 13 and 23, 2007 for 'Royal Gala' and on May 23 and 30, 2007 for 'Braeburn'.

Black spot and russet severity were rated on a maximum of 100 apples per tree per harvest. On some trees, there were less than 100 fruit available at harvest. All fruit on a tree were evaluated for marketability in terms of black spot infection and russet development.

Apples of a marketable size which had a low incidence of black spot were kept for quality analyses. A random sample of ten apples per tree was taken from this subgroup for analysis. These apples were washed in 10% ethanol and rinsed with water. Each apple was assigned a number and weight, diameter, background colour, blush, firmness, soluble solids content and starch pattern index were analysed for every apple. To analyse titratable acidity the juice of a piece of each of the ten apples was combined to enable one analysis per tree.

Apples for analysis of postharvest quality at harvest were taken directly from the orchard. Stored fruit were allowed to warm up to room temperature before temperature-sensitive analyses, such as measurement of firmness, were performed.

Skin and flesh samples of the first harvest of both cultivars were frozen in zip-lock bags after quality analyses were completed, and then freeze-dried. Three of the ten samples kept from each tree were used for analysis of total sulphur, total water-soluble non-protein thiols and cysteine. To avoid water absorption by the sample, the freeze-dried samples were ground up in the bag using a hammer. The bags were stored in airtight containers filled with silica gel. Percentage dry matter was measured after drying all freeze-dried flesh samples of the first harvest overnight at 105°C.

3.6 SULPHUR

3.6.1 TOTAL SULPHUR

Total sulphur was analyzed by Hill Laboratories, Hamilton, New Zealand using inductively coupled plasma optical emission spectroscopy (ICP-OES). Dried and ground fruit samples were digested at a maximum temperature of 205°C in a mixture of perchloric and nitric acids prior to analysis. It was initially planned to analyze sulphur with the LECO sulphur analyzer (CNS-2000, Leco Corporation, St. Joseph, MI), but the analysis was abandoned. The results from the LECO are compared with those of Hill Laboratories in Appendix B.

3.6.2 THIOLS AND CYSTEINE

The procedure for analysis of total thiol and cysteine content was adapted and modified from de Kok et al. (1988).

3.6.2.1 EXTRACTION

Milled, freeze-dried skin and flesh samples were homogenized in ascorbic acid solution (CAS 50-81-7, 0.15% w/v) using a pestle and mortar (1 g sample in 10 ml solution). Oxygen in the ascorbic acid solution had been previously removed by nitrogen bubbling. The mixture was transferred to centrifuge tubes and incubated for four minutes at 100°C to denature the proteins. After incubation, the tubes were centrifuged at 0°C at 30,000 g (15,840 rpm) for 15 minutes (Sorvall Instruments, RC5C, rotor SS-34). The supernatant was used for analysis of total thiols and cysteine.

3.6.2.2 TOTAL WATER-SOLUBLE NON-PROTEIN THIOLS

After centrifugation, 1 ml of the supernatant was mixed with 1 ml MES buffer (CAS 4432-31-9, 0.05 M, adjusted to pH 5.8 with NaOH) and 0.1 ml water. After incubation in a water bath at 30°C for 10 minutes, 0.1 ml DTNB solution (5,5'-dithiobis(2-nitrobenzoic acid), CAS 69-78-3, 10 mM, in potassium phosphate buffer at pH 7.0) and 1 ml Tris-HCl buffer (CAS 77-86-1, 0.2 M, adjusted to pH 8.0 with HCl) were added. The developing yellow colour was measured at 415 nm using a spectrophotometer (Philips, PU8620 UV/VIS/NIR). The mixture without the apple extract (replaced with ascorbic acid solution) was used to zero the instrument. The absorbance of each sample was further corrected for the absorbance of the mixture without DTNB (replaced by water).

According to the above procedure, a calibration curve was made using a mixture of cysteine (CAS 52-90-4) in ascorbic acid solution covering a concentration range from 1×10^{-6} to 5×10^{-4} mol/l. Using a cysteine calibration curve for the analysis of total thiols makes the assumption that all thiol compounds analysed only possess a single SH-group. This would be the case if the main compounds responsible for the rise in thiols are cysteine and glutathione, which has been shown in other plants (De Kok et al., 2002). As it was not known if there are thiol compounds with more than one SH-group in the apple, results were analyzed as sulphur in $\mu\text{mol/l}$ and recalculated to mg/100 g dry matter.

3.6.2.3 CYSTEINE

The cysteine analysis followed the procedure outlined in 3.6.2.2, but instead of water, 0.1 ml of methylglyoxal (CAS 78-98-8, 0.1 M) was added in the first step. Methylglyoxal reacted with the sulphhydryl group of cysteine during the following incubation, making it unavailable for binding by DTNB. The sulphhydryl content of a sample mixed with methylglyoxal was subtracted from the previously analysed content of total thiols to obtain the cysteine content in the sample.

3.6.2.4 REMARKS

Two measurements were taken of the absorbance of each flesh sample; when they varied, a third measurement was taken. The extraction of apple skin resulted in a bright red extract, the absorbance peak of which overlapped with that of the yellow colour to be analyzed. The blank containing the extract therefore had a high absorbance reading. As results were more variable for skin than for flesh, a third reading was taken for every skin sample.

3.7 BLACK SPOT

Black spot severity was rated on a scale from 0 to 5 (Figure 3.3). In 'Braeburn', no apple achieved a rating of greater than 3 (Figure 3.4). Only fruit with a rating of 0 are marketable.

- 0 = no black spot
- 1 = one single spot
- 2 = few spots
- 3 = spots coalesce to form patches
- 4 = one half of apple severely affected
- 5 = front and back of apple severely affected

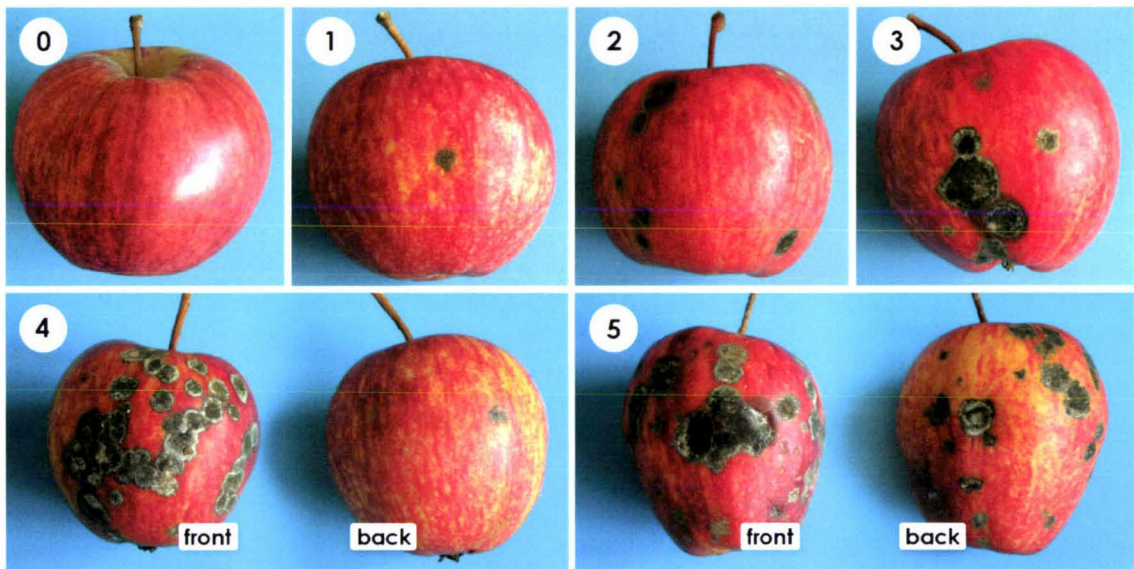


Figure 3.3: Rating scale for black spot severity on 'Royal Gala' from 0 (no black spot) to 5 (severe infection). In ratings 0 to 3, the backside is not affected.



Figure 3.4: Rating scale for black spot severity on 'Braeburn' from 0 (no black spot) to 3 (black spot patches). The backside is not affected.

3.8 RUSSET

Russet severity was rated on a scale from 0 to 4 (Figure 3.5). In 'Braeburn', no apple achieved a rating of 4 (Figure 3.6). Marketing of fruit was not possible with a rating of 3 or 4. Rating 1 is approximately equivalent to ENZA ratings 1 and 2, whereas rating 2 is equivalent to ENZA ratings 3 to 9 (ENZA Ltd, Royal Gala Stem End Russet, 1992, New Zealand).

- 0 = no russet
- 1 = minor stem end russet
- 2 = medium stem end russet
- 3 = high stem end russet
- 4 = severe russet spreading over fruit

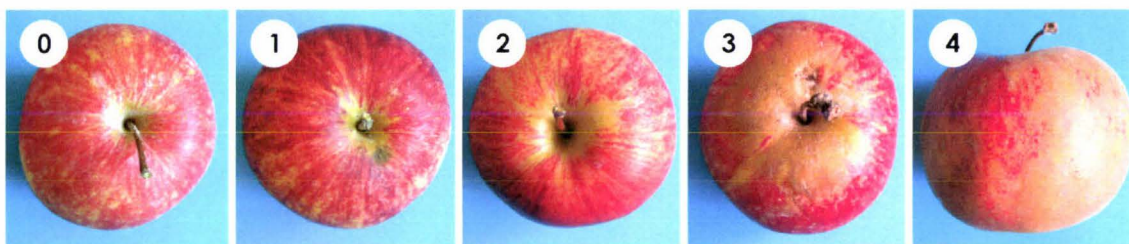


Figure 3.5: Rating scale for russet on 'Royal Gala' from 0 (no russet) to 4 (severe russet). Apples rated 3 and 4 were not suitable for export.

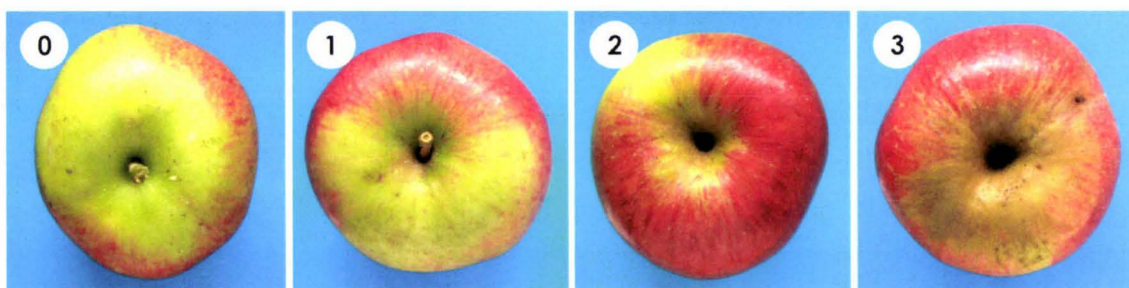


Figure 3.6: Rating scale for russet on 'Braeburn' from 0 (no russet) to 3 (high stem end russet). No apple achieved a rating of 4.

3.9 POSTHARVEST QUALITY

The following parameters were measured to determine postharvest quality of the apples and are further outlined in sections 3.9.1 to 3.9.7.

- Size: weight and diameter
- Colour: background colour and blush
- Firmness
- Soluble solids content (SSC)
- Starch pattern index (SPI)
- Titratable acidity (TA)
- Dry matter (DM)

3.9.1 SIZE

Apple size was measured as weight and diameter. Apples were weighed separately on an analytical balance (Mettler Toledo Inc., PG503-S). The maximum diameter at the equator was measured with a digital calliper (Mitutoyo Corporation, Digimatic).

3.9.2 COLOUR

Background colour was rated using colour charts (Figure 3.7; ENZA Ltd, Background Colour Swatch “Gala/Royal Gala” and “Braeburn”, January 2003, New Zealand). Possible ratings were from 1 to 10 for ‘Royal Gala’ and 1 to 7 for ‘Braeburn’. Blush was rated by estimating the percentage of surface area being red.

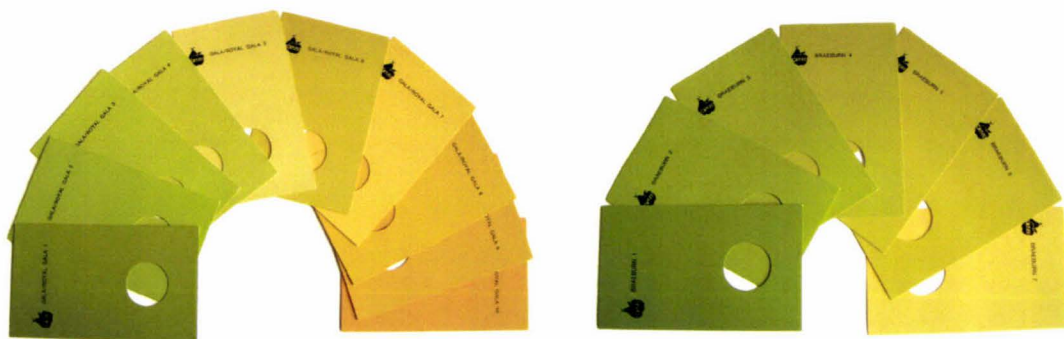


Figure 3.7: Rating scale for colour of ‘Royal Gala’ (left, from 1=green to 10=yellow) and ‘Braeburn’ (right, from 1=green to 7=yellow) using colour swatches (ENZA, New Zealand).

3.9.3 FIRMNESS

To measure firmness, a piece of skin was removed with an apple peeler on opposite sides around the equator. A hand penetrometer (R. Bryce, Fruit Tester, FT327, 11.3 mm head) was used which gives values in kilogram. Measured values were multiplied by $g = 9.80665 \text{ m s}^{-2}$ to obtain results in Newton. The penetrometer was secured in a drill press to enable consistent penetration of the apple to a depth of 8 mm. The two measurements per apple were averaged.

3.9.4 SOLUBLE SOLIDS CONTENT

A drop of juice obtained in the firmness test was analysed for SSC using a digital refractometer (Atago, Pocket PAL-1). The instrument sends a light beam through the juice sample and measures the refractive index, i.e. the light refracted by the soluble solids in the sample. Results are given in percent. SSC is sometimes also referred to as °Brix.

3.9.5 STARCH PATTERN INDEX

Starch pattern index was rated on a scale from 0 (all starch) to 6 (no starch) (Figure 3.8; ENZA Ltd, Starch Pattern Index for Apples, New Zealand), after equatorial slices had been sprayed with iodine solution (Starch Iodine Premix, Fruition Horticulture, New Zealand). The starch in the apple is stained blue-black by the iodine and starch degradation during maturation follows a characteristic pattern.

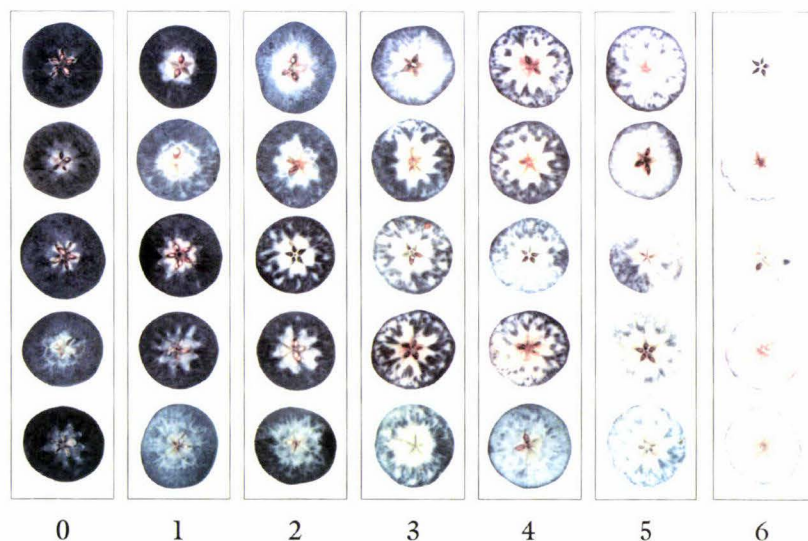


Figure 3.8: Rating scale for starch pattern index from 0 (all starch) to 6 (no starch). Adapted from ENZA, New Zealand.

3.9.6 TITRATABLE ACIDITY

A piece from each apple was juiced using a garlic press and the juice of ten apples per tree was combined for analysis. Titratable acidity (TA) was measured using an automatic titrator (Mettler, DL21). A mixture of 1 ml of juice and 50 ml of deionised water was titrated with 0.1 M NaOH to an endpoint pH of 8.2. Results were calculated in percent malic acid, the main acid in apples, according to the following formula (Mitcham et al., 2003):

$$TA[\%] = \frac{ml(NaOH) \times M(NaOH) \times amf \times 100}{ml(juice)}$$

amf = acid milliequivalent factor for malic acid = 0.067.

3.9.7 DRY MATTER

Freeze-dried apple flesh samples from the first harvest of both cultivars were oven-dried overnight at 105°C. DM was recorded in percent of fresh weight.

3.10 STATISTICAL ANALYSIS

Statistical analysis was performed using the SAS software (Version 9, SAS Institute Inc., Cary, NC, USA). All data was analysed using analysis of variance (ANOVA). The significance level for all tests was 5%. Data was transformed when plots of residuals versus predicted values indicated unstable variances across treatments. A log or an inverse transformation was used where appropriate. As all values are transformed on a common scale, the mean comparisons remain valid. Transformed means were back-transformed for display in Chapter 4. The transformed values with their standard errors are shown in Appendix A.

Chapter 4

RESULTS

4.1 PHOTOSYNTHESIS

Leaves on 'Braeburn' sprayed with lime sulphur (LS1 and LS2) had a significantly lower photosynthetic rate than leaves of control trees (Table 4.1). 'Royal Gala' showed a slight decrease in photosynthetic rate with increasing rate of application of lime sulphur, but this decrease was not significant in the first measurement. In the second measurement after 11 lime sulphur sprays, LS2 had a significantly lower photosynthetic rate than the control. The photosynthetic rate of 'Braeburn' in the treatment containing 2% lime sulphur (LS2) was only approximately 25% of that of 'Royal Gala' and only 17 to 22% of the 'Braeburn' control.

Table 4.1: Photosynthetic rate of 'Royal Gala' and 'Braeburn' on two days in late season, measured on one fully expanded bourse shoot leaf per tree. Means with the same letter are not significantly different in each column ($\alpha=0.05$, $n=6$).

<i>Treatment</i>	<i>Photosynthetic rate [$\mu\text{mol m}^2 \text{s}^{-1}$]</i>							
	<i>'Royal Gala'</i>				<i>'Braeburn'</i>			
	<i>Feb 2007</i>		<i>Mar 2007</i>		<i>Feb 2007</i>		<i>Mar 2007</i>	
Control	15.2	a	14.2	a	13.6	a	12.2	a
LS1	14.9	a	12.2	a, b	4.3	b	2.6	b
LS2	12.4	a	8.7	b	3.0	b	2.1	b
S.E.	1.5		1.1		1.5		1.1	

Control = hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, S.E. = standard error.

Two measurements of photosynthetic rate were taken on the same leaf on each tree four and 13 days after the application of lime sulphur. The first measurement was taken on February 19, 2007, four days after the 10th lime sulphur application of February 15, 2007 and before the last lime sulphur spray was applied on February 19, 2007. The second measurement was taken on March 4, 2007, 13 days after the 11th lime sulphur application and last of the season. The first photosynthesis measurement was taken 18 and 28 days before the two harvests of 'Royal Gala' and 51 and 58 days before the two harvests of 'Braeburn'. The second was taken 5 and 15 days and 38 and 45 days before the harvests, respectively.

4.2 SULPHUR

4.2.1 TOTAL SULPHUR

The total sulphur content of both flesh and skin of both apple cultivars increased with increasing application rate of lime sulphur (Table 4.2). Overall, 'Braeburn' contained more sulphur than 'Royal Gala' and in both cultivars; the skin contained approximately twice as much sulphur as the flesh despite the washing process. Apples treated with 2% lime sulphur (LS2) often contained more than three times as much sulphur as the control. Differences between all treatments were highly statistically significant.

Table 4.2: Total sulphur content of 'Royal Gala' and 'Braeburn' in the first harvest. Means with the same letter are not significantly different in each column ($\alpha=0.05$, $n=18$).

<i>Treatment</i>	<i>Sulphur [mg/100 g dry matter]</i>							
	<i>'Royal Gala'</i>				<i>'Braeburn'</i>			
	<i>Flesh</i>		<i>Skin</i>		<i>Flesh</i>		<i>Skin</i>	
Control	12.5	a	21.9	a	13.2	a	26.9	a
LS1	25.0	b	47.7	b	32.1	b	55.4	b
LS2	39.8	c	77.1	c	43.1	c	74.3	c
S.E.	2.0		2.2		2.0		2.2	

Control = Hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, S.E. = standard error.

4.2.2 THIOLS AND CYSTEINE

4.2.2.1 CALIBRATION CURVE

The calibration curve was made with cysteine in a range from 1×10^{-6} to 5×10^{-4} mol/l. A trend line was established between the concentrations of 1×10^{-6} to 1×10^{-4} mol/l as this range was relevant to the measured samples (Figure 4.1). It resulted in the following equation ($R^2=0.9994$): $y = 4241.7 x + 0.0345$.

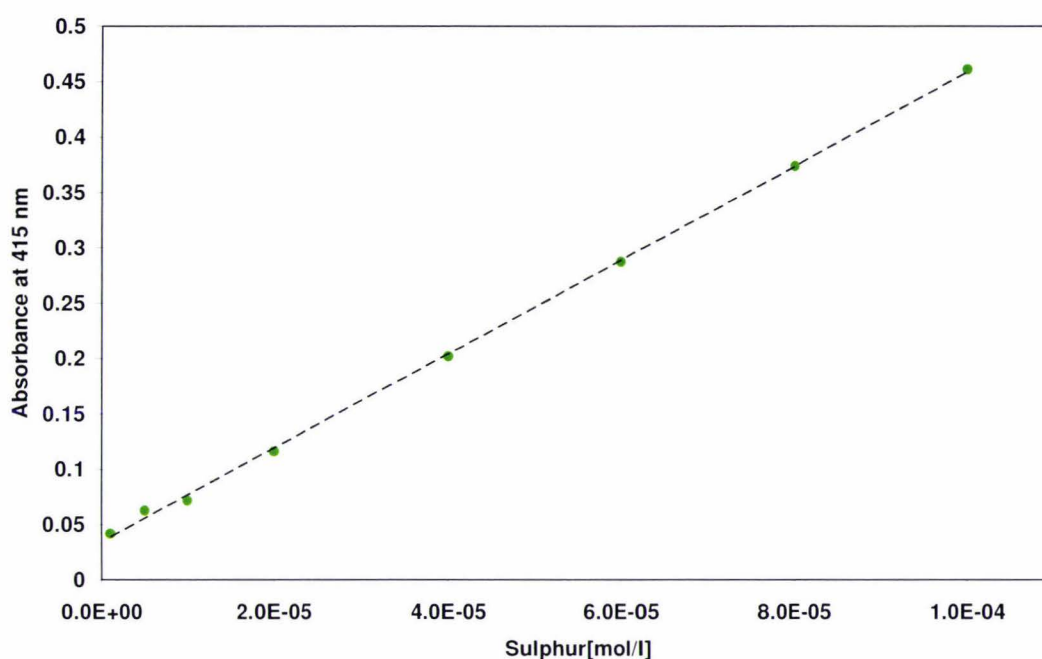


Figure 4.1: Calibration curve for analysis of total water-soluble non-protein thiols and cysteine: $y = 4241.7x + 0.0345$.

4.2.2.2 TOTAL WATER SOLUBLE NON-PROTEIN THIOLS

In both cultivars, an increased rate of application of lime sulphur led to an increase of sulphur metabolized into total water-soluble non-protein thiols in both the skin and the flesh (Table 4.3). 'Braeburn' contained more sulphur in the form of thiol compounds than 'Royal Gala' and the flesh of both cultivars always contained more than the skin. In both cultivars, the skin of apples in treatment LS2 contained significantly more sulphur in the form of thiols than both LS1 and the control. The flesh of apples from the control treatment had a significantly lower sulphur content than that of apples treated with lime sulphur.

Table 4.3: Sulphur metabolized into total water-soluble non-protein thiols of 'Royal Gala' and 'Braeburn' in the first harvest. Means with the same letter are not significantly different in each column ($\alpha=0.05$, $n=18$).

<i>Treatment</i>	<i>Sulphur [mg/100 g dry matter]</i>							
	<i>'Royal Gala'</i>				<i>'Braeburn'</i>			
	<i>Flesh</i>		<i>Skin</i>		<i>Flesh</i>		<i>Skin</i>	
Control	0.703	a	0.502	a	0.926	a	0.795	a
LS1	0.931	b	0.674	a	1.265	b	1.022	a
LS2	1.168	b	1.027	b	1.514	c	1.469	b
S.E.	0.091		0.071		0.091		0.071	

Control = Hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, S.E. = standard error.

4.2.2.3 CYSTEINE

Cysteine is part of the water-soluble non-protein thiols analyzed. The amount of sulphur in the form of cysteine in apple flesh and skin increased with increasing rate of application of lime sulphur (Table 4.4). In the skin, this increase was not statistically significant in both cultivars, although in 'Royal Gala', LS2 contained approximately twice as much sulphur in the form of cysteine than the control. In the flesh of 'Royal Gala', LS2 showed significantly higher values than both LS1 and Control; in 'Braeburn', all treatments were significantly different. In both cultivars, the skin contained more sulphur in the form of cysteine than the flesh.

Table 4.4: Sulphur metabolized into cysteine of 'Royal Gala' and 'Braeburn' in the first harvest. Means with the same letter are not significantly different in each column ($\alpha=0.05$, $n=18$).

<i>Treatment</i>	<i>Sulphur [mg/100 g dry matter]</i>							
	<i>'Royal Gala'</i>				<i>'Braeburn'</i>			
	<i>Flesh</i>		<i>Skin</i>		<i>Flesh</i>		<i>Skin</i>	
Control	0.134	a	0.165	a	0.171	a	0.268	a
LS1	0.156	a	0.225	a	0.253	b	0.275	a
LS2	0.236	b	0.339	a	0.331	c	0.385	a
S.E.	0.020		0.043		0.020		0.043	

Control = Hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, S.E. = standard error.

4.3 BLACK SPOT

Black spot incidence was very high on 'Royal Gala', especially in the control treatment in which 83% of apples were affected. Lime sulphur significantly decreased the incidence (Figure 4.1) as well as severity (Table 4.5) of black spot with increasing application rate on 'Royal Gala'. 'Braeburn' was less affected by black spot, with 24.5%, 25.3% and 32.3% of fruit showing signs of the disease in treatments LS2, LS1 and the control, respectively, but these means were not significantly different between treatments. Black spot severity was not affected by the concentration of sulphur applied (Table 4.5).

Table 4.5: Percentage of 'Royal Gala' and 'Braeburn' in each black spot category on a scale from 0 (no black spot) to 5 (severe black spot) rated on up to 100 apples per tree and harvest.

Rating	Fruit with black spot [%]					
	'Royal Gala'			'Braeburn'		
	Control	LS1	LS2	Control	LS1	LS2
0	19.7	42.0	80.5	87.2	93.3	89.9
1	12.8	19.9	10.3	6.1	2.8	4.0
2	40.2	31.3	6.5	5.7	2.6	5.2
3	17.5	5.5	1.8	1.0	1.3	1.0
4	7.1	1.2	0.7	0.0	0.0	0.0
5	2.6	0.1	0.3	0.0	0.0	0.0
<i>n</i>	1191	1113	1024	1078	925	946

Control = Hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime.

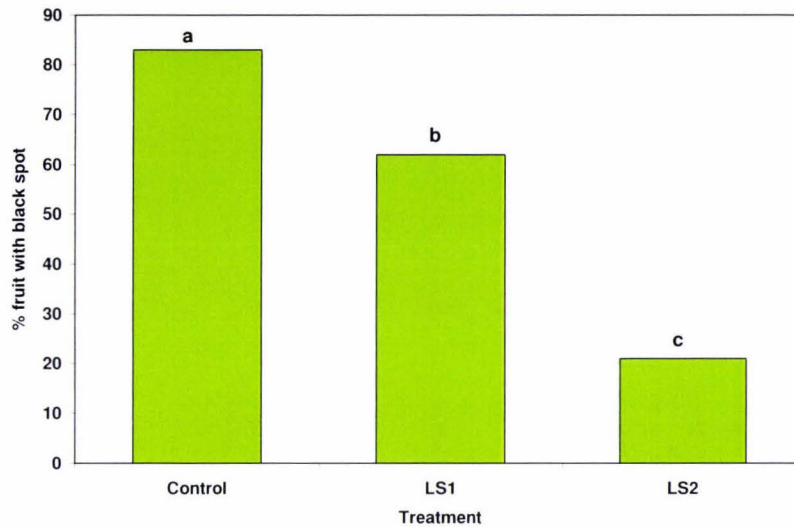


Figure 4.2: Percentage of 'Royal Gala' affected with black spot at harvest. Means are significantly different ($P < 0.0001$). Control = hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime.

4.4 RUSSET

Lime sulphur affected the incidence and severity of russet in 'Royal Gala'. The percentage of fruit with a rating of 3 or 4 nearly doubled in treatments containing lime sulphur (Table 4.6). Of all fruit at harvest, 27% were unfit for marketing due to excessive russet in both treatments LS1 and LS2. Apples from these two treatments had significantly more russet than the control treatment, in which 15% of fruit were affected (Figure 4.3). Lime sulphur did not affect the severity ratings for russet in 'Braeburn' (Table 4.6). Of all apples at harvest, 0.5%, 0.7% and 0.8% in the control, LS1 and LS2, respectively, were unfit for marketing due to increased russet, but these differences were not statistically significant.

Table 4.6: Percentage of 'Royal Gala' and 'Braeburn' in each russet category on a scale from 0 (no russet) to 4 (severe russet) rated on up to 100 apples per tree and harvest.

Rating	Fruit with excessive russet [%]					
	'Royal Gala'			'Braeburn'		
	Control	LS1	LS2	Control	LS1	LS2
0	17.1	17.6	13.4	60.8	64.8	60.3
1	22.0	14.8	14.6	36.5	32.4	37.6
2	46.9	41.4	42.8	2.6	2.6	2.0
3	10.8	20.3	21.5	0.2	0.2	0.1
4	3.1	5.8	7.8	0.0	0.0	0.0
n	1191	1113	1024	1078	925	946

Control = Hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime.

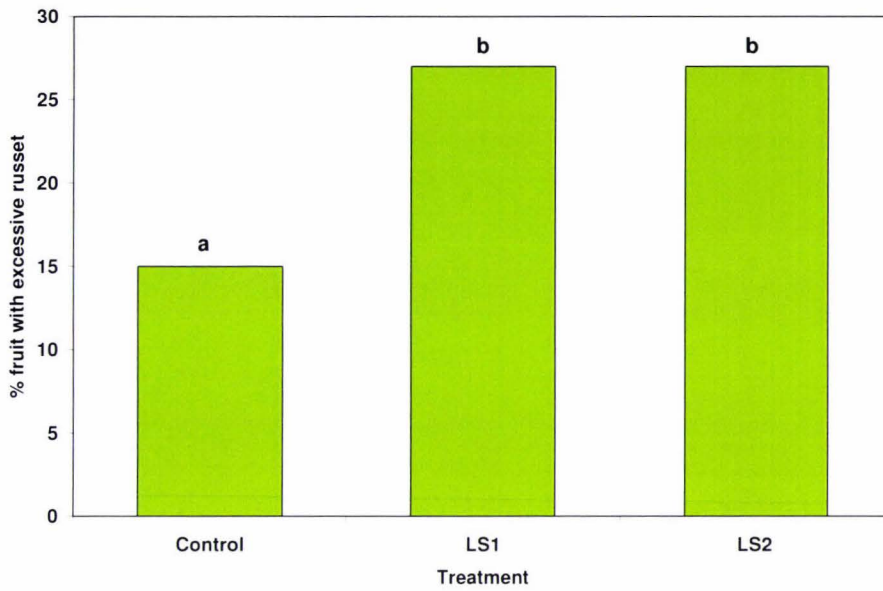


Figure 4.3: Percentage of 'Royal Gala' unfit for marketing due to excessive russet at harvest. Means with the same letter are not significantly different ($\alpha=0.05$). Control = hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime.

4.5 POSTHARVEST QUALITY

4.5.1 SIZE

A log transformation was used for weight and diameter data to stabilize the variance. The transformed values for both parameters, together with their standard errors, are displayed in Appendix A. Lime sulphur significantly affected the size of 'Braeburn' apples in terms of both weight and diameter (Table 4.7, Table 4.8). Apple size decreased with increasing rate of application of lime sulphur.

Table 4.7: Weight of 'Braeburn' at both harvests and after storage of fruit from each harvest. Means with the same letter are not significantly different in each column ($\alpha=0.05$, $n=60$). In Harvest 2, $P=0.0502$.

Treatment	Weight [g]							
	Harvest 1		Harvest 2		Storage 1		Storage 2	
Control	193.832	a	186.239	a	190.406	a	169.836	a
LS1	164.603	b	157.734	a, b	152.369	b	152.233	b
LS2	159.911	b	146.641	b	139.439	b	130.998	c

Control = Hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, Harvest 1/2 = 1st/2nd harvest, Storage 1/2 = fruit stored from 1st/2nd harvest.

Table 4.8: Diameter of 'Braeburn' at both harvests and after storage of fruit from each harvest. Means with the same letter are not significantly different in each column ($\alpha=0.05$, $n=60$).

<i>Treatment</i>	<i>Diameter [mm]</i>							
	<i>Harvest 1</i>		<i>Harvest 2</i>		<i>Storage 1</i>		<i>Storage 2</i>	
Control	74.44	a	74.38	a	75.25	a	71.63	a
LS1	70.80	b	70.09	a, b	70.02	b	69.63	a
LS2	70.35	b	68.60	b	67.71	b	66.69	b

Control = Hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, Harvest 1/2 = 1st/2nd harvest, Storage 1/2 = fruit stored from 1st/2nd harvest.

Lime sulphur did not have a consistent effect on size in 'Royal Gala' (Table 4.9, 4.10).

Table 4.9: Weight of 'Royal Gala' at both harvests and after storage of fruit from each harvest. Means with the same letter are not significantly different in each column ($\alpha=0.05$, $n=60$).

<i>Treatment</i>	<i>Weight [g]</i>							
	<i>Harvest 1</i>		<i>Harvest 2</i>		<i>Storage 1</i>		<i>Storage 2</i>	
Control	147.894	a	124.268	a	128.493	a	133.464	a
LS1	141.930	a	133.085	a	132.358	a	130.487	a
LS2	143.812	a	138.302	a	133.347	a	136.673	a

Control = Hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, Harvest 1/2 = 1st/2nd harvest, Storage 1/2 = fruit stored from 1st/2nd harvest.

Table 4.10: Diameter of 'Royal Gala' at both harvests and after storage of fruit from each harvest. Means with the same letter are not significantly different in each column ($\alpha=0.05$, $n=60$).

<i>Treatment</i>	<i>Diameter [mm]</i>							
	<i>Harvest 1</i>		<i>Harvest 2</i>		<i>Storage 1</i>		<i>Storage 2</i>	
Control	69.37	a	65.96	a	66.68	a	67.71	a
LS1	68.32	a	67.37	a	67.30	a	64.97	b
LS2	68.68	a	68.30	a	67.82	a	67.34	a

Control = Hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, Harvest 1/2 = 1st/2nd harvest, Storage 1/2 = fruit stored from 1st/2nd harvest.

4.5.2 COLOUR

4.5.2.1 BACKGROUND COLOUR

The data for both varieties was not pooled for colour analysis as different rating scales were used for each cultivar. Lime sulphur negatively affected background colour development in both cultivars (Table 4.11, 4.12). Background colour further developed during storage, but differences were still apparent after storage. At harvest as well as after storage, differences were more pronounced in 'Braeburn' than in 'Royal Gala'. During storage, the initially lower background colour of lime sulphur treated 'Royal Gala' seemed to catch up with that of the control.

Table 4.11: Background colour rating in 'Royal Gala' on a scale from 1 (green) to 10 (yellow) at both harvests and after storage of fruit from each harvest. Means with the same letter are not significantly different in each column ($\alpha=0.05$, $n=60$).

<i>Treatment</i>	<i>Background colour rating</i>							
	<i>Harvest 1</i>		<i>Harvest 2</i>		<i>Storage 1</i>		<i>Storage 2</i>	
Control	4.7	a	5.1	a	7.3	a	7.2	a
LS1	4.4	a, b	4.6	b	7.2	a	7.2	a
LS2	4.0	b	4.0	c	7.1	a	6.8	b
S.E.	0.12		0.13		0.09		0.06	

Control = Hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, Harvest 1/2 = 1st/2nd harvest, Storage 1/2 = fruit stored from 1st/2nd harvest, S.E. = standard error.

Table 4.12: Background colour rating in 'Braeburn' on a scale from 1 (green) to 7 (yellow) at both harvests and after storage of fruit from each harvest. Means with the same letter are not significantly different in each column ($\alpha=0.05$, $n=60$).

<i>Treatment</i>	<i>Background colour rating</i>							
	<i>Harvest 1</i>		<i>Harvest 2</i>		<i>Storage 1</i>		<i>Storage 2</i>	
Control	3.9	a	4.6	a	5.1	a	5.3	a
LS1	2.8	b	3.5	b	4.2	a	4.4	a, b
LS2	2.0	b	2.7	b	3.1	b	3.4	b
S.E.	0.26		0.27		0.26		0.33	

Control = Hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, Harvest 1/2 = 1st/2nd harvest, Storage 1/2 = fruit stored from 1st/2nd harvest, S.E. = standard error.

4.5.2.2 BLUSH

To stabilise the variance, data for blush percentage was transformed on a log scale. The transformed values together with their standard errors are displayed in Appendix A. In both cultivars, blush was consistently lower on fruit treated with lime sulphur compared to the control treatment (Table 4.13, 4.14). Nevertheless, blush of the control fruit was only significantly higher than LS1 and LS2 in the second harvest of ‘Braeburn’.

Table 4.13: Percentage of surface area blushed on ‘Royal Gala’ at both harvests and after storage of fruit from each harvest. Means with the same letter are not significantly different in each column ($\alpha=0.05$, $n=60$).

<i>Treatment</i>	<i>Blush [%]</i>							
	<i>Harvest 1</i>		<i>Harvest 2</i>		<i>Storage 1</i>		<i>Storage 2</i>	
Control	74	a	74	a	78	a	75	a
LS1	66	a	71	a	73	a	72	a
LS2	64	a	69	a	71	a	69	a

Control = Hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, Harvest 1/2 = 1st/2nd harvest, Storage 1/2 = fruit stored from 1st/2nd harvest.

Table 4.14: Percentage of surface area blushed on ‘Braeburn’ at both harvests and after storage of fruit from each harvest. Means with the same letter are no significantly different in each column ($\alpha=0.05$, $n=60$).

<i>Treatment</i>	<i>Blush [%]</i>							
	<i>Harvest 1</i>		<i>Harvest 2</i>		<i>Storage 1</i>		<i>Storage 2</i>	
Control	57	a	59	a	58	a	61	a
LS1	44	a	46	b	49	a	51	a
LS2	50	a	46	b	42	a	39	a

Control = Hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, Harvest 1/2 = 1st/2nd harvest, Storage 1/2 = fruit stored from 1st/2nd harvest.

4.5.3 FIRMNESS

To stabilise the variance, an inverse transformation was used for the firmness data. The transformed values together with their standard errors are displayed in Appendix A. Apples of both cultivars showed a trend towards lower flesh firmness at harvest with increasing rate of application of lime sulphur (Table 4.15, 4.16). These differences were only statistically significant in the second harvest (In the first harvest, $P=0.106$ and 0.0843 for 'Royal Gala' and 'Braeburn', respectively). After storage, this trend remained in 'Braeburn', but differences were not statistically significant. Results for stored 'Royal Gala' were inconclusive. The trend towards lower flesh firmness was actually reversed with lime sulphur treated fruit being firmer than the control in Storage 1, whereas results were not significantly different in Storage 2.

Table 4.15: Flesh firmness in 'Royal Gala' at both harvests and after storage of fruit from each harvest. Means with the same letter are not significantly different in each column ($\alpha=0.05$, $n=60$).

<i>Treatment</i>	<i>Flesh firmness [N]</i>							
	<i>Harvest 1</i>		<i>Harvest 2</i>		<i>Storage 1</i>		<i>Storage 2</i>	
Control	89.7	a	81.9	a	51.5	a	48.9	a
LS1	88.6	a	78.2	a	53.6	a, b	50.6	a
LS2	83.5	a	74.3	b	55.4	b	49.0	a

Control = Hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, Harvest 1/2 = 1st/2nd harvest, Storage 1/2 = fruit stored from 1st/2nd harvest.

Table 4.16: Flesh firmness in 'Braeburn' at both harvests and after storage of fruit from each harvest. Means with the same letter are not significantly different in each column ($\alpha=0.05$, $n=60$).

<i>Treatment</i>	<i>Flesh firmness [N]</i>							
	<i>Harvest 1</i>		<i>Harvest 2</i>		<i>Storage 1</i>		<i>Storage 2</i>	
Control	77.9	a	74.7	a	74.0	a	75.2	a
LS1	77.7	a	71.7	a, b	73.9	a	73.1	a
LS2	73.2	a	69.0	b	72.9	a	71.5	a

Control = Hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, Harvest 1/2 = 1st/2nd harvest, Storage 1/2 = fruit stored from 1st/2nd harvest.

4.5.4 SOLUBLE SOLIDS CONTENT

In treatments containing lime sulphur, soluble solids content (SSC) was consistently lower in both cultivars at harvest and after storage (Table 4.17, Table 4.18). Differences between treatments were less pronounced in 'Royal Gala' than in 'Braeburn' and were in most cases not statistically significant.

Table 4.17: Soluble solids content in 'Royal Gala' at both harvests and after storage of fruit from each harvest. Means with the same letter are not significantly different in each column ($\alpha=0.05$, $n=60$).

<i>Treatment</i>	<i>Soluble solids content [%]</i>							
	<i>Harvest 1</i>		<i>Harvest 2</i>		<i>Storage 1</i>		<i>Storage 2</i>	
Control	10.4	a	10.5	a	12.1	a	12.3	a
LS1	10.4	a	10.3	a	11.7	a	12.0	a, b
LS2	9.8	a	10.1	a	11.4	a	10.9	b
S.E.	0.19		0.31		0.24		0.34	

Control = Hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, Harvest 1/2 = 1st/2nd harvest, Storage 1/2 = fruit stored from 1st/2nd harvest, S.E. = standard error.

Table 4.18: Soluble solids content in 'Braeburn' at both harvests and after storage of fruit from each harvest. Means with the same letter are not significantly different in each column ($\alpha=0.05$, $n=60$).

<i>Treatment</i>	<i>Soluble solids content [%]</i>							
	<i>Harvest 1</i>		<i>Harvest 2</i>		<i>Storage 1</i>		<i>Storage 2</i>	
Control	10.0	a	10.7	a	11.5	a	11.4	a
LS1	9.6	a	9.7	a	10.9	a	10.7	a, b
LS2	8.9	b	9.3	a	10.1	b	9.9	b
S.E.	0.19		0.31		0.24		0.34	

Control = Hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, Harvest 1/2 = 1st/2nd harvest, Storage 1/2 = fruit stored from 1st/2nd harvest, S.E. = standard error.

4.5.5 STARCH PATTERN INDEX

In 'Royal Gala', starch pattern index (SPI) was significantly higher at harvest in fruit treated with lime sulphur compared to the control (Table 4.19). After storage, the trend towards a higher SPI in sulphur-treated fruit remained, but differences between treatments were not statistically significant (Table 4.19). In 'Braeburn', fruit at harvest tended towards a lower SPI when treated with lime sulphur, but differences were not statistically significant (Table 4.20). However, after storage this trend was reversed and LS2 had a significantly higher SPI than LS1 and the control (Table 4.20).

Table 4.19: Starch pattern index in 'Royal Gala' on a scale from 0 to 6 at both harvests and after storage of fruit from each harvest. Means with the same letter are not significantly different in each column ($\alpha=0.05$, $n=60$).

<i>Treatment</i>	<i>Starch pattern index</i>							
	<i>Harvest 1</i>		<i>Harvest 2</i>		<i>Storage 1</i>		<i>Storage 2</i>	
Control	0.7	a	1.6	a	5.8	a	5.6	a
LS1	1.1	a	2.2	b	5.9	a	5.7	a
LS2	2.3	b	3.2	c	6.0	a	5.9	a
S.E.	0.23		0.14		0.13		0.14	

Control = Hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, Harvest 1/2 = 1st/2nd harvest, Storage 1/2 = fruit stored from 1st/2nd harvest, S.E. = standard error.

Table 4.20: Starch pattern index in 'Braeburn' on a scale from 0 to 6 at both harvests and after storage of fruit from each harvest. Means with the same letter are not significantly different in each column ($\alpha=0.05$, $n=60$).

<i>Treatment</i>	<i>Starch pattern index</i>							
	<i>Harvest 1</i>		<i>Harvest 2</i>		<i>Storage 1</i>		<i>Storage 2</i>	
Control	2.7	a	2.5	a	4.9	a	4.7	a
LS1	2.2	a	2.3	a	5.0	a	5.0	a
LS2	2.2	a	2.1	a	5.6	b	5.5	b
S.E.	0.23		0.14		0.13		0.14	

Control = Hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, Harvest 1/2 = 1st/2nd harvest, Storage 1/2 = fruit stored from 1st/2nd harvest, S.E. = standard error.

4.5.6 TITRATABLE ACIDITY

A log transformation was used for the acidity data to stabilise the variance. The transformed values together with their standard errors are displayed in Appendix A. The first harvest of 'Royal Gala' could not be analysed for titratable acidity (TA) due to equipment failure. In 'Royal Gala', there was a consistent trend at harvest and after storage towards lower TA in fruit treated with lime sulphur, but only in the second harvest, was LS2 significantly lower than the control (Table 4.21). Lime sulphur did not affect TA in 'Braeburn' (Table 4.22).

Table 4.21: Titratable acidity in 'Royal Gala' at both harvests and after storage of fruit from each harvest. Means with the same letter are not significantly different in each column ($\alpha=0.05$, $n=60$). In Storage 1, $P = 0.0561$, in Storage 2, $P = 0.0518$.

<i>Treatment</i>	<i>Titratable acidity [%]</i>							
	<i>Harvest 1</i>		<i>Harvest 2</i>		<i>Storage 1</i>		<i>Storage 2</i>	
Control	n/a		0.435	a	0.361	a	0.355	a
LS1	n/a		0.400	a, b	0.335	a, b	0.320	a, b
LS2	n/a		0.376	b	0.313	b	0.303	b

Control = Hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, Harvest 1/2 = 1st/2nd harvest, Storage 1/2 = fruit stored from 1st/2nd harvest.

Table 4.22: Titratable acidity in 'Braeburn' at both harvests and after storage of fruit from each harvest. Means with the same letter are not significantly different in each column ($\alpha=0.05$, $n=60$).

<i>Treatment</i>	<i>Titratable acidity [%]</i>							
	<i>Harvest 1</i>		<i>Harvest 2</i>		<i>Storage 1</i>		<i>Storage 2</i>	
Control	0.583	a	0.574	a	0.590	a	0.600	a
LS1	0.621	a	0.592	a	0.599	a	0.611	a
LS2	0.605	a	0.574	a	0.606	a	0.620	a

Control = Hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, Harvest 1/2 = 1st/2nd harvest, Storage 1/2 = fruit stored from 1st/2nd harvest.

4.5.7 DRY MATTER

Data for percent dry matter (DM) was transformed on a log scale to stabilise the variance. The transformed values together with their standard errors are displayed in Appendix A. In both cultivars, DM decreased with increasing application rate of lime sulphur (Table 4.23). Values were only available for the first harvest. In 'Braeburn', all treatments were significantly different; in 'Royal Gala', DM of apples in LS2 was significantly lower than that of LS1 and the control.

Table 4.23: Dry matter content of 'Royal Gala' and 'Braeburn' at the first harvest. Means with the same letter are not significantly different in each column ($\alpha=0.05$, $n=60$).

<i>Treatment</i>	<i>Dry matter [%]</i>			
	<i>'Royal Gala'</i>		<i>'Braeburn'</i>	
Control	12.65	a	12.11	a
LS1	12.29	a	11.14	b
LS2	11.56	b	10.40	c

Control = Hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime.

Chapter 5

DISCUSSION

The results obtained in this study confirmed earlier findings on the influence of lime sulphur on the photosynthetic rate of apple trees. More importantly, this is the first study to show that the application of lime sulphur leads to residues of sulphur in the fruit, which in part are metabolites of sulphur in the form of thiol compounds. In addition, different rates of lime sulphur influenced the postharvest quality of apples to varying degrees. It is crucial to note that both cultivars investigated, 'Royal Gala' and 'Braeburn', were affected in the same way. So far, the organic industry claims that only 'Braeburn' is difficult to grow organically. The fact that the quality of 'Royal Gala' is affected by lime sulphur as well and that both cultivars contain high residues of sulphur is important as it means that any excessive use of lime sulphur in organic apple production is questionable. These results are discussed in detail in the following sections.

5.1 PHOTOSYNTHESIS

The application of lime sulphur led to a sharp, statistically significant decrease in the photosynthetic rate of 'Braeburn'. The reduction in the photosynthesis of 'Royal Gala' was less and mostly not statistically significant.

Earlier studies have already reported a reduction of photosynthesis in 'Braeburn' after lime sulphur was applied (McArtney et al., 2006; Palmer et al., 2003). The application of lime sulphur also led to a reduction in the photosynthesis of other apple cultivars ('Royal Gala', 'Pacific Rose' and 'Fuji'), but photosynthesis recovered the next day in all cultivars except

'Braeburn' (J. Wuensche¹, personal communication). A decrease in photosynthesis of apple trees due to the application of lime sulphur was first reported in the 1930s (Hoffman, 1935; Hyre, 1939). Hydrogen sulphide is emitted with the application of lime sulphur and is possibly the agent responsible for inhibiting photosynthesis. This has been shown for various other plant species (De Kok et al., 2002; Maas et al., 1988; Olivia & Steubing, 1976; Steubing, 1979). Several reasons for a decrease in the photosynthetic rate have been proposed. It has been suggested that the presence of hydrogen sulphide leads to a reduction in chlorophyll (Steubing, 1979) as well as inhibition of electron transport and CO₂ fixation (Maas & De Kok, 1988; Maas et al., 1988). Moreover, it has been suggested that an accumulation of starch in 'Braeburn' leaves may lead to a feedback inhibition of photosynthesis (J. Wuensche², personal communication).

The application of lime sulphur or hydrogen sulphide can also result in visible leaf damage and leaf necrosis (Cunningham, 1935; De Kok et al., 2002; Holb et al., 2003; Holb & Heijne, 2001; Maas et al., 1988; Steubing, 1979; Tweedy, 1981). In the current trial, damage to the leaves was not obvious until the start of the year 2007, although spraying had started in October of the previous year and five applications of lime sulphur had been made. Leaves of 'Braeburn' trees treated with lime sulphur showed obvious browning (Figure 5.1), but 'Royal Gala' leaves were not affected. Poor leaf condition of organically-treated 'Braeburn' has been reported previously (Palmer et al., 2003). This leaf damage may also be the reason for the depressed photosynthetic rate in 'Braeburn'.

Doubling the dose of lime sulphur did not double the impact on the photosynthetic rate. Although LS2 had a lower photosynthetic rate than LS1, these differences were not statistically significant. A 50% decrease in photosynthesis has been reported earlier for 'Braeburn' treated with any form of sulphur compared to fungicide treatments not containing sulphur (Palmer et al., 2003). In the current study, an even greater decrease in photosynthesis to less than 20% of the control value was observed on 'Braeburn' trees treated with the highest rate of lime sulphur (2%, LS2).

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² Prof. Dr. J. Wuensche, University of Hohenheim, Stuttgart, Germany, July 2006



Figure 5.1: Damage caused by lime sulphur to 'Braeburn' leaves: Control (hydrated lime, left), LS1 (1% lime sulphur + hydrated lime, middle) and LS2 (2% lime sulphur + hydrated lime, right).

The study only evaluated the impact of different doses of lime sulphur in 11 applications throughout the season from October to February, but this research was not designed to explore the impact of varying numbers of spray applications as well as different timing (e.g. only early in the season vs. throughout the season). Harvest was approximately one and two months after the last spray treatment in 'Royal Gala' and 'Braeburn', respectively. Due to labour, budget and time constraints, spray treatments were started later than planned and overall fewer lime sulphur sprays were applied than was desirable and is common industry practice. The average number of sprays of lime sulphur in the Hawke's Bay growing region in the 2006/07 season was 15 for 'Royal Gala' and 'Braeburn', but up to 40 spray applications were observed on some properties on 'Royal Gala' and up to 24 on 'Braeburn' (J. Walker³, personal communication). In the Hawke's Bay, lime sulphur is often applied many times early in the season before the actual fruit development and less late in the season (J. Walker⁴, personal communication). In the Manawatu, where rain is common later in the season, further applications of lime sulphur are necessary during apple development. Long periods of rain can also occur in the Hawke's Bay in January and February in some years which would mean that further sprays are necessary. The application of lime sulphur early in the season, during active leaf growth only, might mean that newly emerging leaves can compensate for the lost photosynthetic rate of the ones treated with lime sulphur. It

³ Dr. J. Walker, HortResearch, Havelock North, New Zealand, August 2007

⁴ Dr. J. Walker, HortResearch, Havelock North, New Zealand, August 2007

has also been reported that 'Braeburn' needed up to 51 days for its photosynthetic rate to recover after the application of lime sulphur (McArtney et al., 2006). A recovery would therefore not be possible if lime sulphur was used as a fungicide throughout the season and this constant decrease of photosynthesis will have a major impact on tree and fruit growth.

Reduced tree growth has been reported when lime sulphur was applied, but was only apparent after several years (Mills & LaPlante, 1954) and not after one season (Palmer et al., 2003). The trees in the trial orchard at Massey University were all planted at the same time on the same rootstock and have been treated with lime sulphur extensively for black spot control ever since. There are obvious differences in the trunk cross sectional area and tree size of both cultivars with 'Braeburn' trees being a lot smaller than 'Royal Gala'. Sustainability is an underlying concept of organic farming and will become increasingly important in the future. Sustaining and increasing yields will be necessary to feed the growing population of the world and a more immediate question for the grower is the economic sustainability of the organic orchard. It is questionable if the use of lime sulphur can be deemed sustainable if it leads to a decrease in yield and severe damage to the trees, especially of the 'Braeburn' variety.

5.2 SULPHUR

Results from this study show that residues of the applied lime sulphur can be found both in the fruit and on the skin of the apple. In addition, part of the sulphur was metabolized, resulting in higher concentrations of thiol compounds in the fruit. Despite the number of lime sulphur sprays applied being lower than what is commercial practice, sulphur residues were highly significant. In addition, on commercial orchards, lime sulphur use on 'Royal Gala' is generally higher than on 'Braeburn' because the negative effects on the 'Braeburn' tree are known. That would mean that residues on 'Royal Gala' could be even higher.

The apple skin and flesh were analysed at harvest for total sulphur and for total water-soluble non-protein thiols and cysteine, a sulphur-containing amino acid. The application of lime sulphur led to a highly significant increase in the total sulphur content of both skin and flesh in both cultivars. Apples were washed before peeling and analysis. Commercially, apples are usually washed in floatation tanks prior to packing, but on the one hand, this may not remove the excess sulphur and on the other hand, commercial washing may not be employed by small scale organic producers whose products are destined for the local market. Due to consumers' fear of pesticides in conventional production, they may wash or

peel fresh produce before consumption. However, the behaviour of consumers of organic products may be different due to the spray-free or residue-free image that these products enjoy. Anecdotal evidence suggests that consumers either wash or peel an apple before eating it, or may simply rub the fruit to remove dirt and make it shiny. Furthermore, briefly washing an apple under running water is unlikely to remove all sulphur residues. Despite thorough washing before analysis, the complete removal of sulphur deposits could not be guaranteed, so skin increases may be attributed at least in part to spray residues. When peeling the apple, some remaining sulphur residues may have been transferred onto the flesh, creating the potential for contamination. However, the increase in flesh sulphur content is too high to be attributed to contamination only.

The analysis of sulphur in the form of total water-soluble non-protein thiols and cysteine showed an increase in both these compounds in the flesh and the skin of both apple cultivars at harvest, which proves the assimilation of the applied sulphur by the plant. Therefore, the increase in the total sulphur content of apples treated with lime sulphur cannot solely be attributed to contamination by spray residues. As mentioned earlier, hydrogen sulphide is emitted when lime sulphur is applied and at the time of spraying and in the days afterwards, levels of H_2S in the air were possibly significant, at least above the detectable threshold of $0.02\mu\text{l/l}$ air (Beauchamp et al., 1984). It is likely that the lime sulphur was taken up in the form of H_2S , which would support proposals by De Kok et al. (1991; 1989; 1998). They showed that hydrogen sulphide can be taken up by plants through the stomata. Studies have not included fruit-bearing plants, so it may be possible that hydrogen sulphide is taken up by the apple tree either by the leaves or by the apple itself. The uptake of H_2S by other plants led to increased levels of water-soluble non-protein thiol compounds, namely cysteine and glutathione (De Kok et al., 1991; De Kok et al., 1989; De Kok et al., 1998). Glutathione is a tripeptide of cysteine, glutamate and glycine. The greater part of the total thiol pool in plants is glutathione, whereas cysteine is only a minor part (De Kok et al., 2002). Glutathione was not analyzed in the current study due to time constraints, but it is most likely that the greater part of total thiols in apples is glutathione, too. Research on the uptake of H_2S by plants has shown that upon termination of H_2S exposure, thiols were either metabolized or transported to other parts of the plant (De Kok, 1989, 1990), e.g. the roots (De Kok et al., 1997; De Kok et al., 1998; De Kok et al., 2002; Herschbach et al., 1995a, b; Poortinga & De Kok, 1997; Tausz et al., 1998; Westerman et al., 2000), but no information was found in the literature on fruit-bearing plants. If lime sulphur was taken up in the form of hydrogen sulphide by the leaves of

apple trees, thiol compounds could have been transported in the phloem from there to the fruit together with other nutrients. If sulphur was taken up by the fruit itself, it would be unlikely for its metabolites to be transported out of the fruit during active cell growth as the main flow of nutrients is from source (leaves) to sink (growing fruit) and not backwards, as main nutrients, i.e. carbohydrates are incorporated into the fruit (Miranda et al., 2002; Taiz & Zeiger, 2002).

As has been mentioned in the previous section, the decrease in the photosynthetic rate of 'Braeburn' may be attributed to starch accumulating in the leaves (J. Wuensche⁵ – personal communication). If there is a problem with phloem loading or transport, sulphur compounds from the leaf are unlikely to be transported to the fruit in 'Braeburn', which means 'Royal Gala' apples should have a higher sulphur content. However, this hypothesis was not verified in the current trial as the increase of total water-soluble non-protein thiols and cysteine followed similar patterns in both cultivars. Figure 5.2 and Figure 5.3 show the concentrations of total thiols and cysteine in the skin and flesh of 'Royal Gala' and 'Braeburn', respectively.

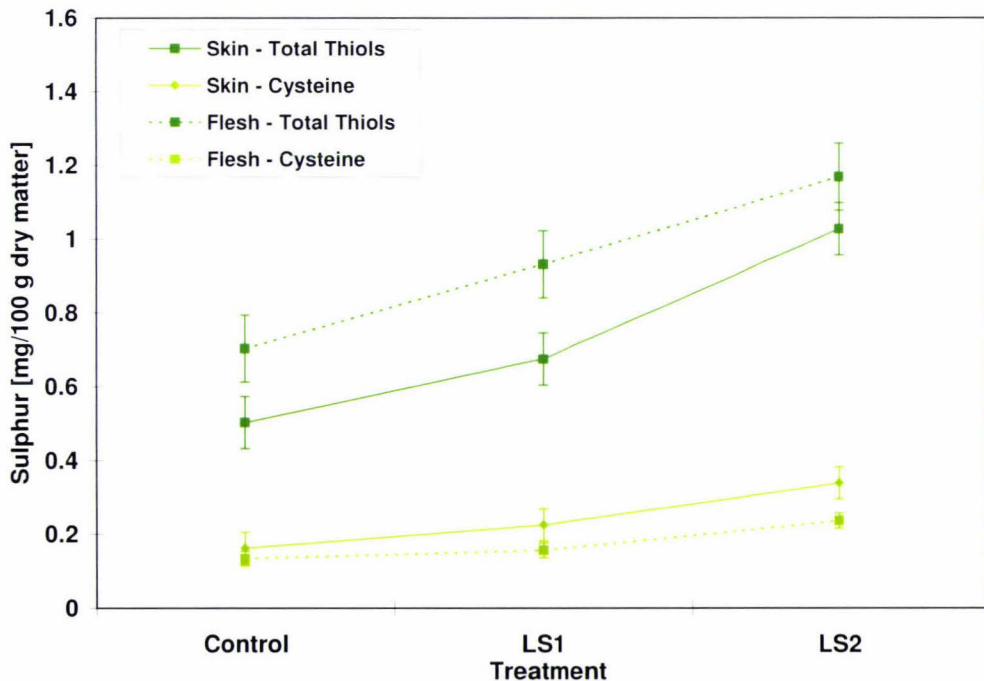


Figure 5.2: Sulphur metabolized into total water-soluble non-protein thiols and cysteine in skin and flesh of 'Royal Gala' at harvest (Control = hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime).

⁵ Prof. Dr. J. Wuensche, University of Hohenheim, Stuttgart, Germany, July 2006

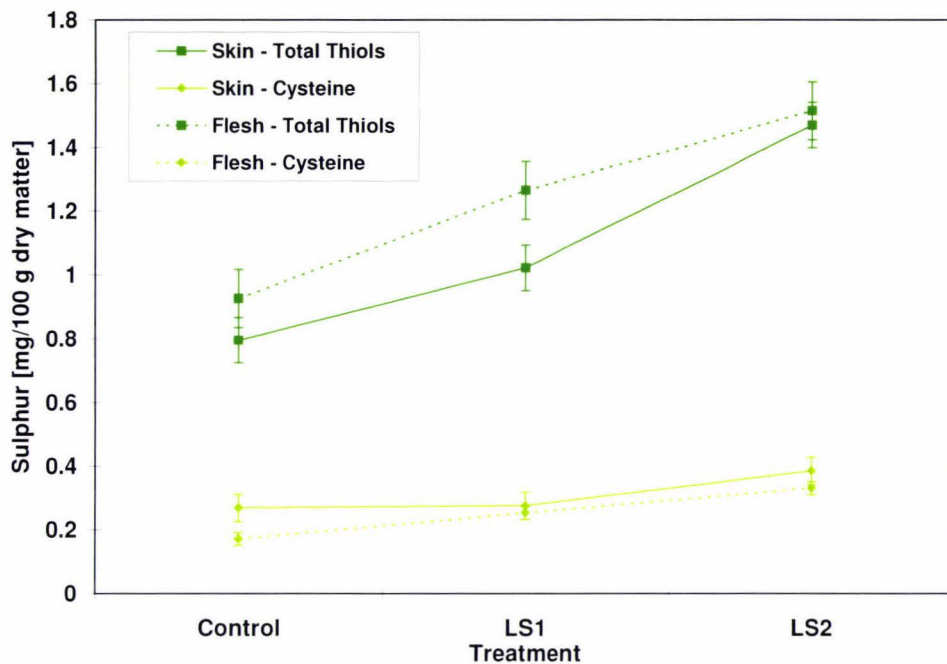


Figure 5.3: Sulphur metabolized into total water-soluble non-protein thiols and cysteine in skin and flesh of 'Braeburn' at harvest (Control = hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime).

The graphs show the concentrations of cysteine and thiols in terms of the amount of sulphur they contain. This approach was taken because cysteine was used to produce the calibration curve. Cysteine has a single SH group available for binding to DTNB. However, it is not known which molecules made up the total thiols analyzed and how many SH groups each molecule had available for binding. The cysteine calibration curve (Figure 4.1 on page 37) indicates how many moles of SH-groups were binding to produce a certain colour depth and it is therefore possible to calculate how many milligrams of sulphur were in the solution.

'Braeburn' contained slightly more total thiols and cysteine in both skin and flesh than 'Royal Gala'. Doubling the application rate of lime sulphur approximately doubled the assimilation of thiols in the flesh of both cultivars. Cysteine levels increased to a similar extent in both flesh and skin. Sulphur assimilation into total thiols approximately doubled again in the skin when an additional 1% of lime sulphur was applied in LS2. This may indicate that at a high application rate of 2% lime sulphur and therefore presumably high atmospheric concentration of H_2S , there may be an additional uptake of hydrogen sulphide through the apple skin. As only three data points are available, further research is necessary to determine if this conclusion is legitimate. The high sulphur value in treatment LS2 may have occurred by chance and the relationship between lime sulphur application rate and thiol content may actually be linear. Possible ways to confirm if sulphur is taken up by the

fruit or the leaf could involve spraying separate parts of the tree with lime sulphur while covering other parts. A radioactive sulphur isotope (S^{35}) could be used to make lime sulphur to track the applied sulphur in the plant. This type of research should be carried out in a glass house on potted trees to exclude environmental influences and contain the radioactive material.

The percentage of total sulphur in the form of thiols varied from 1.3 to 3% in the skin and from 2.9 to 7% in the flesh. The increase in total sulphur could only to a minor part be attributed to the increase in thiol compounds. So there was a large amount of sulphur in apples treated with lime sulphur that was unaccounted for. In the flesh, 11.6 to 28.4 mg/100 g dry matter and in the skin, 25.1 to 54.2 mg/100 g dry matter were either spray residues of lime sulphur or other sulphur compounds not analyzed in this study. Whereas sulphur on the skin could be attributed to spray residues, it is unlikely that these high amounts of sulphur in the flesh were due to contamination from the skin while peeling. Further research is necessary to evaluate the nature of the sulphur residues.

These results are of great significance to the industry. Organic products are not spray-free, as although not synthetic, a lot of sprays are applied in organic pest and disease management. Nevertheless, products from organic production should be residue-free. The current study showed that there were considerable sulphur residues as well as metabolites of sulphur in the apple. These results compromise the residue-free status of organic apples and need urgent attention. Furthermore, research is necessary to evaluate the nature of the sulphur residues that were not thiol compounds.

Many consumers perceive organic products to be healthier than the conventional alternative (Baker et al., 2004; Roth et al., 2004; Saba & Messina, 2003). Increased levels of total water-soluble non-protein thiol compounds are unlikely to have a negative health effect. Earlier studies suggest that glutathione is the major compound responsible for the increase in total thiols (De Kok et al., 2002). Glutathione is an antioxidant and therefore may be nutritionally valuable. However, glutathione is normally synthesized by the body and it has been shown that oral supplementation is not effective in increasing glutathione levels in humans (Witschi et al., 1992). Moreover, not all of the sulphur had been metabolized into antioxidants. A large amount was unaccounted for and may therefore either be attributed to spray residues or other sulphur compounds. The impact of increased uptake of those sulphur residues needs to be evaluated especially considering the perceived health benefits of organic products.

Consumers believe that organic food tastes better (Baker et al., 2004; Saba & Messina, 2003). The way in which increased levels of sulphur compounds may influence taste is unknown. Residues of elemental sulphur on the skin may, however, have a significant impact on flavour. As no taste panels were performed in the current trial due to time and budget constraints, the true effect of increased lime sulphur application on flavour could not be evaluated. However, the possibility of a significant impact on consumer acceptance should not be ignored and needs further study.

The current study was not designed to evaluate differences between the treatments after long-term commercial storage conditions. It would therefore be pure speculation that the application of lime sulphur would impact on storage life or that the residues of possibly antioxidants would have a positive effect. As apples are commercially stored for a considerable time frame, this issue needs further attention.

It would be in the best interest of the organic industry to find an alternative to lime sulphur in the long-term, i.e. develop new fungicides or disease-resistant apple cultivars. As these developments take time, a short-term solution could be the modification of spray schedules. In this study, more residues were found in apples subject to higher application rates. Decreasing the total amount of lime sulphur used in the season will therefore decrease residues. As outlined in section 5.1, commercial practice sometimes differs in the amount and timing of sprays from what was applied in the current trial. Despite a higher number of applications throughout early season in commercial orchards, there may be fewer residues in the apple at harvest as less sulphur is applied directly to the fruit and the time between the last sulphur application and harvest is longer. This does not mean, however, that there are no sprays applied at all. Although long periods of rain in January and February are less common in the Hawke's Bay than in the Manawatu, they do occur in some years and there may be a need for applying sprays directly to the developing fruit. Some orchards now switch completely to copper in the second half of the season, but there are still many that apply lime sulphur throughout the season (M. Glogau⁶, J. Walker⁷, personal communication). It should also be considered here, that often more sprays are applied to 'Royal Gala' than to 'Braeburn' in total (refer to section 5.1), because 'Royal Gala' is not thought to suffer from the lime sulphur application. Sulphur residues could

⁶ Dr Michelle Glogau, BioGro New Zealand Ltd, Wellington, New Zealand, September 2007

⁷ Dr Jim Walker, HortResearch, Havelock North, New Zealand, August 2007

therefore be considerable on 'Royal Gala' or other cultivars not considered sulphur-sensitive. Even if lime sulphur is not applied directly to the fruit, if hydrogen sulphide is taken up by the apple leaves, a transport to the growing apple may still take place after early season spraying. Sulphur residues on the skin could possibly be removed by a suitable washing procedure, for example a high pressure apple washer, but the increased sulphur content in the flesh obviously cannot be removed.

The use of copper as a control measure for black spot was not directly part of this study, but it should be mentioned that although copper is very effective in controlling black spot it cannot be seen as a solution to the problems encountered with the use of lime sulphur. Copper is a heavy metal and its use is restricted in New Zealand to 3 kg per hectare per year (BioGro, 2001). However, considering that copper accumulates in the soil, this is a large amount to be applied every year and will in the long-term lead to severe copper toxicity problems. High levels of copper ultimately inhibit plant growth and soil life (Beresford et al., 1991). Moreover, it has not been tested yet if copper causes residues in or on the apple (M. Glogau⁸, personal communication).

5.3 BLACK SPOT

Hydrated lime was not successful in controlling black spot on 'Royal Gala', but lime sulphur decreased the incidence and severity of black spot significantly. Nevertheless, in treatment LS2 at harvest, 21% of fruit were not marketable. The extent to which black spot would have developed during storage was not evaluated. 'Royal Gala' trees in the trial orchard suffered heavy disease pressure, both because neighbouring trees were treated with hydrated lime which did not achieve satisfactory control and because the 2006/07 season produced a large number of infection periods with long periods of warm and humid weather. Moreover, an issue was that spray treatments were not started earlier in the season as they were withheld until the trial design was confirmed. Fewer sprays were applied in total due to labour constraints.

Although 'Braeburn' sprayed with lime sulphur showed less black spot than the control, these differences were not statistically significant. Between treatments, 24.5% to 32.3% of fruit were affected at harvest. Again, the possible development of storage scab was not

⁸ Dr Michelle Glogau, BioGro New Zealand Ltd, Wellington, New Zealand, September 2007

evaluated. 'Braeburn' and 'Royal Gala' are both cultivars susceptible to black spot (Hampson & Kemp, 2003; Quamme et al., 2005; Washington et al., 1998). 'Braeburn' is highly susceptible to leaf scab, but has a lower incidence of fruit scab (Washington et al., 1998) and has been reported to have partial disease resistance (McCarthy, 1994). Trees in the trial orchard are also an early clone of 'Braeburn' and possibly have some resistance against black spot as trees were always affected to a lesser extent than the 'Royal Gala' trees at this site. It is interesting though, that lime sulphur did not significantly decrease the incidence of black spot on 'Braeburn', and this result has not been explained. Copper was only applied once at the beginning of September 2006. The timing of application plays an important factor in disease control as most fungicides have only protective action, i.e. they cannot cure black spot once established (Atkinson et al., 1956). The developmental stages are slightly shifted in both cultivars with bud break occurring earlier in 'Braeburn'. As copper was only applied once, the timing may have been suitable for 'Braeburn' for protecting newly emerging leaves, but buds were still closed on 'Royal Gala' trees.

The trial should not be seen as a study on the effectiveness of hydrated lime and lime sulphur per se. Black spot was a major problem due to late start and inadequate number of sprays despite several infection periods during the season. Fruit free of disease would have been desirable to eliminate any confounding factors and therefore apples with a low incidence of black spot were chosen for postharvest quality analysis.

5.4 RUSSET

Lime sulphur did not increase russet development on 'Braeburn'. This was expected as the cultivar 'Braeburn' is generally not prone to russet (Palmer et al., 2003). Palmer et al. (2003) reported increased russet on 'Braeburn' apples when treated with copper, but not when sprayed with lime sulphur. In the current trial, the application of lime sulphur significantly increased russet occurrence in 'Royal Gala', but the concentration of lime sulphur did not make a difference. Russet is an issue because above a certain level the fruit is not marketable for export. On some apples treated with lime sulphur, nearly the complete surface was covered with russet (Figure 5.4). These fruit were also too small for market standards. In the current trial, 27% of 'Royal Gala' were lost due to russetting in treatments containing lime sulphur compared to 15% in the control, when no other factors (e.g. black spot, other diseases and size) were considered. However, the application of lime sulphur was necessary to control black spot and although there was less russet in the control

treatment, a lot more apples were lost due to black spot infection. This trade-off has to be considered when applying lime sulphur. Changes in the spray schedule and avoiding spraying from pink tip to one month after petal fall when the fruit is sensitive to russetting (Beresford, 1996) could be measures to decrease russet despite the use of lime sulphur. However, black spot disease pressure is highest during that time and other effective fungicides would have to be applied. As outlined earlier, the fungicides available to the organic apple grower are in general not very effective in controlling the disease, except for copper and lime sulphur and copper is already known to cause russet. Sanitation practices, such as removing or shredding leaf litter, should be employed. Although they cannot eliminate spores completely, they can at least decrease disease pressure.

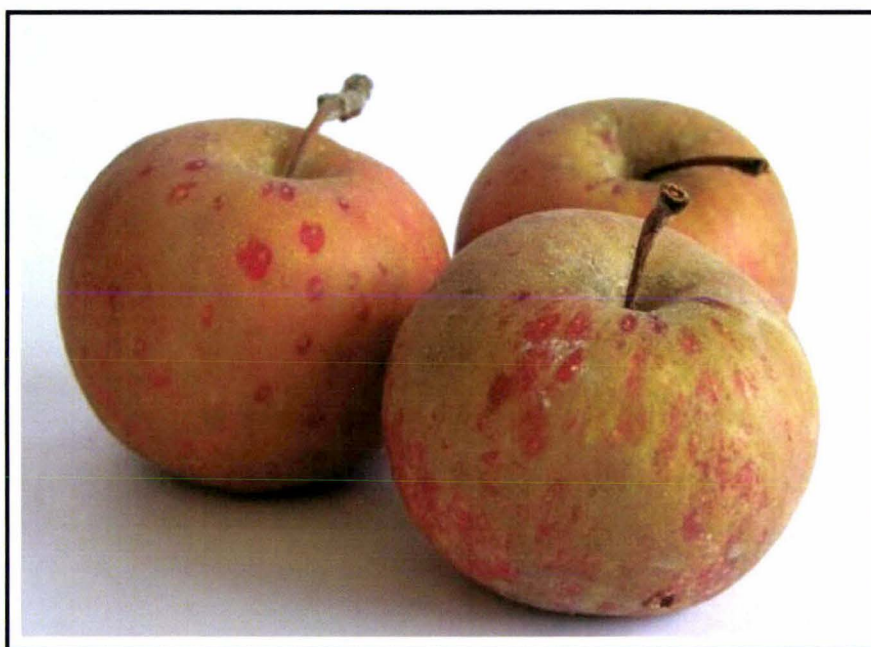


Figure 5.4: 'Royal Gala' apples severely affected by russet in treatment LS2 (2% lime sulphur + hydrated lime).

5.5 POSTHARVEST QUALITY

5.5.1 SIZE

Lime sulphur led to a decrease in fruit size in terms of weight and diameter in 'Braeburn' (Figure 5.5 in Section 5.5.2), but not in 'Royal Gala'. 'Braeburn' apples of the control treatment also seemed to vary in shape from those of treatments containing lime sulphur. Shape was not analyzed explicitly, but the apples from sulphur treatments were round whereas apples treated with hydrated lime had a more elongated shape (Figure 5.5 in Section 5.5.2). In 'Braeburn', the application of lime sulphur caused a significant decrease in

the photosynthetic rate of fully expanded bourse shoot leaves. These leaves play a role in supplying nutrients to the fruit (Volz, 1991). The photosynthetic rate is related to the amount of nutrients produced and transported to the apple, so a decrease in photosynthesis can result in fewer nutrients and in turn lead to a smaller fruit size. Wuensche (J. Wuensche⁹ - personal communication) reported an accumulation of starch in the leaves of 'Braeburn'. This could indicate a problem with phloem loading and transport, which in turn hinders nutrients from reaching the apple.

In 'Royal Gala', there was no decrease in weight and diameter observed when lime sulphur was applied. However, the interaction between treatments and their effects on the measured attributes mean that these results could be confounded. There were significant differences in russet and black spot ratings between the treatments. Therefore, apples in the control treatment might have been bigger if they had not been severely affected by black spot. In addition, the photosynthetic rate in 'Royal Gala' was slightly decreased by the application of lime sulphur, although not significantly. If there was no black spot, a size difference between treatments could possibly have been due to differing photosynthetic rates, but it is unlikely to be significant.

Lower mean fruit weight and decreased total yield has been reported to be a problem in organic orchards, especially for 'Braeburn' (Walker & McArtney, 2001). A lower mean fruit weight could influence the fruit's marketability as size standards are in place for export quality produce. The industry wants high yields at a specific fruit size to receive high profits. In this way, the application of lime sulphur is counterproductive, but currently necessary to control black spot as long as no effective substitutes are available. Without alternatives, the trade-off between black spot infection and decreased fruit size currently favours the application of lime sulphur to eliminate disease symptoms.

5.5.2 COLOUR

Lime sulphur negatively affected the background colour in both cultivars. After six weeks of storage of 'Braeburn', colour ratings had increased in all treatments to approximately the same extent and apples treated with lime sulphur were therefore still significantly greener than the control. In 'Royal Gala', however, treatment differences at harvest decreased over the 5-week storage period, because the colour of apples treated with lime sulphur

⁹ Prof Dr. J. Wuensche, University of Hohenheim, Stuttgart, Germany, July 2006

developed faster and caught up to the control. The storage period for 'Royal Gala' was possibly too long as apples were past optimum eating quality and colour had developed to near maximum.

Chlorophylls, carotenoids and anthocyanins are responsible for the green, yellow and red colour of an apple, respectively (Reay et al., 1998). The background colour of an apple changes from dark green to yellow as the chlorophyll degrades. As apples treated with lime sulphur were greener, lime sulphur sprays may have caused a problem with the degradation of chlorophyll and/or the production of carotenoid. The chlorophylls may have degraded at a slower rate or the onset of degradation may have been delayed. The production of carotenoids may also have been lower or retarded which means that a yellow colour of a lower intensity was revealed as the chlorophyll degraded. Both these factors might have come into play in the current trial. In 'Royal Gala', differences between treatments diminished over the storage period, i.e. the degradation of chlorophyll may have been delayed. As colour differences were still apparent after the storage of 'Braeburn', the degradation of chlorophyll may have been slower or the carotenoids content decreased in this cultivar. However, 'Braeburn' stored very well, i.e. maturity advanced only slowly, and the trial did not evaluate if colour differences between treatments may have diminished after long-term storage.

The red blush on both cultivars decreased with increasing application rate of lime sulphur, but due to a high variability between apples, this decrease was not statistically significant. However, blush was only measured on ten apples per tree at each point in time and a larger sample size would have been advantageous. Moreover, these results confirm those of an earlier study by Palmer et al. (2003) which showed that lime sulphur decreased blush percentage in 'Braeburn' whereas blush in treatments containing hydrated lime or synthetic fungicides did not differ.

The production of the anthocyanins responsible for the red skin colour could have been influenced by a variety of factors, for example enzymes, light and nutrient status (Saure, 1990). The uptake as well as the deposit of sulphur on the outside of the apple could have influenced the production of red pigments by for example inhibiting the enzymes responsible for the formation of anthocyanins. Spray deposits on the skin could have reduced the intensity of available sunlight. The availability of nutrients, especially sugars, is important for anthocyanins production (Saure, 1990). A lowered photosynthetic rate and a

decrease in the soluble solids content were observed in both cultivars when treated with lime sulphur and may be responsible for a poorer pigment formation.

Although the results in the current trial were not statistically significant, they may be of importance for the industry as colour is an important consumer attribute and certain colour standards have to be met for export quality fruit (Mills, 1996; Reay et al., 1998). Excessive use of lime sulphur may lead to fewer apples reaching export colour standards, which is accompanied with a negative impact on growers' returns. An appealing background colour and bright red is desirable by the consumer and a green background reduces the intensity of red (Reay et al., 1998). This was also observed in the current trial (Figure 5.5). A dissatisfied consumer could be attracted to other apple cultivars or other types of fruit (Harker et al., 2002a). Colour is also important at harvest time. Harvest maturity is determined using certain parameters, such as starch pattern index, soluble solids content and firmness, and fruit are then harvested according to colour and blush (Brookfield et al., 1997). So there could be problems with picking apples at the right time if maturity differs from what was expected according to the colour rating.

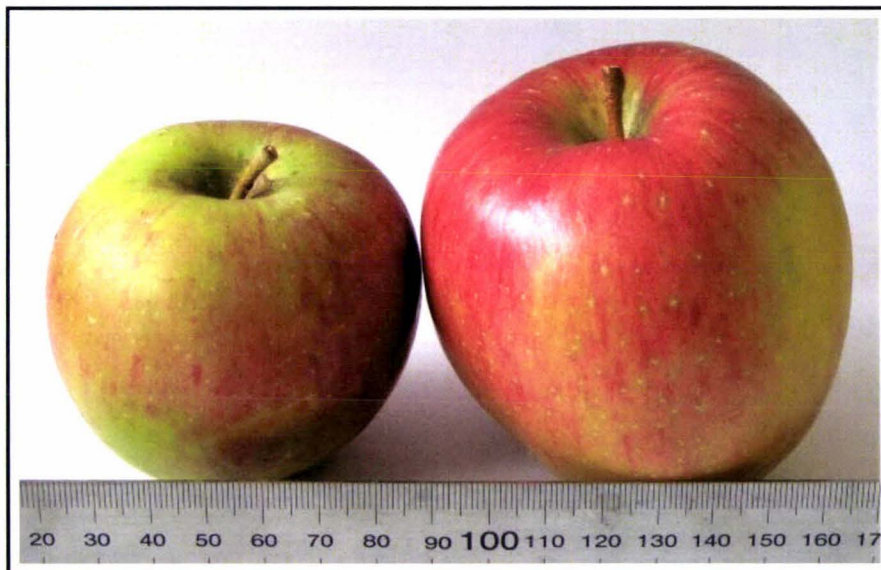


Figure 5.5: In 'Braeburn', a high application of 2% lime sulphur (LS2, left) led to a decreased fruit size, different shape, lower background colour, reduced blush percentage and a less intense red compared to the control (right).

5.5.3 FIRMNESS

There was a trend towards lower flesh firmness in both cultivars at harvest when apples were treated with lime sulphur. In 'Braeburn', LS2 was significantly softer than the control and in 'Royal Gala', LS2 was significantly softer than the control and LS1. In 'Royal Gala' results were inconclusive after storage which could be due to the length of the storage period. Flesh firmness of stored 'Royal Gala' was low overall and apples had exceeded their optimum quality. In 'Braeburn', the trend towards lower flesh firmness in apples treated with lime sulphur remained after storage, but to a lesser extent. This confirms results by Palmer et al. (2003) showing a trend towards lower flesh firmness in treatments containing sulphur and higher flesh firmness in treatments with hydrated lime.

In general, a smaller apple size is associated with a higher firmness rating (Blanpied et al., 1978). In the current study, 'Braeburn' apples were smaller in size when treated with lime sulphur, but were also softer. Differences in other parameters responsible for the texture of apples, such as cell size, shape and packing and thickness of the cell walls (Harker et al., 1997), may therefore have caused the firmness differences between treatments. Although no measurements were taken during the development of the apple, fruit on the tree did not seem to differ between treatments earlier in the development, i.e. during the stages of cell division. Afterwards, cell enlargement takes place and only later in the season, were there apparent visual size differences of 'Braeburn' fruit between treatments. That means parameters influencing firmness, other than size, could have been affected during cell enlargement.

As apples are stored for several months, it is important to the industry that high firmness ratings at harvest can be sustained over the storage period (Johnson, 1994). 'Braeburn' had a lower firmness than 'Royal Gala' at harvest, but kept well over storage, whereas 'Royal Gala' was firmer at harvest, but levels could not be sustained in any treatment. However, the trial was not set up as a long term storage experiment and also did not involve controlled atmosphere.

The texture of an apple is also very important to consumers as they do not like soft and mealy apples (Harker et al., 2002b; Jaeger et al., 1998). In the current trial, 'Royal Gala' scored firmness ratings below 50 N after five weeks of storage. Instrumental measurements are a good indicator of crispness and crunchiness of an apple and the critical threshold for

judging apples as mealy was when their firmness decreased below this threshold of 50 N (Harker et al., 2002b). In addition, both cultivars showed differences in firmness between the treatments at harvest that exceeded 5 N and were therefore great enough to be distinguished by both trained and untrained sensory panels (Harker et al., 2002a; Harker et al., 2002b). There are always variations between apples on the market, even between those from the same tree, and especially when coming from different orchards. However, the results of the current study show that lime sulphur did influence the texture of 'Braeburn' and 'Royal Gala' apples to an extent possibly significant to the consumer. The differences in lime sulphur use between different apple orchards may actually in part be responsible for the differences in apple quality between orchards.

5.5.4 SOLUBLE SOLIDS CONTENT

In both cultivars, a decrease in the soluble solids content (SSC) was observed with increasing application rate of lime sulphur. Although this trend was consistent in harvested and stored fruit, differences were not always statistically significant. SSC did increase in the fruit over the storage period as would be expected during maturation.

The decrease in SSC in apples treated with lime sulphur is possibly linked to its impact on the photosynthetic rate. A reduced photosynthetic ability of the leaves decreases the amount of nutrients produced and therefore available for transport to the fruit. Measurements in the current trial showed a sharp decrease in the photosynthetic rate of 'Braeburn' when lime sulphur was applied and a lesser, non-significant reduction in 'Royal Gala'. Under these circumstances, SSC behaved as expected and the drop was greater in 'Braeburn' than in 'Royal Gala'. SSC comprises sugars as well as acids. The influence of lime sulphur on titratable acidity is further discussed in section 5.5.6.

In the current trial, lime sulphur caused a drop of 1.1 to 1.5% SSC in 'Braeburn' between the control and LS2 and in three out of four instances this drop was significant. In 'Royal Gala', treatment differences were lower and only exceeded 1% after storage of the second harvest. Sweetness correlates well with SSC (Harker et al., 2002c) and is an important consumer attribute for apples (Jaeger et al., 1998). The differences in SSC observed in the current trial could have an impact on consumer acceptance as they exceeded the taste threshold of 1% SSC at which taste panels could detect a difference between apples (Harker et al., 2002c). So there is some evidence that lime sulphur could affect the taste and therefore consumer acceptance of organic 'Braeburn'. Sensory panels are still advised to

confirm the true impact of different quality parameters on consumer preference (Harker et al., 2002c).

5.5.5 STARCH PATTERN INDEX

Whereas both cultivars behaved similarly in terms of colour, firmness and SSC, their starch pattern index (SPI) was affected differently. At harvest and after storage, 'Royal Gala' showed a consistent trend towards a higher SPI as more sulphur was applied. However, results after storage were not as clear cut; most 'Royal Gala' reached the maximum SPI score and differences were therefore minute. In 'Braeburn', the trend was reversed at harvest, in a way that apples treated with lime sulphur had a lower SPI than the control. However, these differences were not statistically significant. After storage, 'Braeburn' followed the trend of 'Royal Gala' and LS2 showed a significantly higher SPI than LS1 and the control.

As SPI is dependent on maturity, it may simply be that 'Braeburn' treated with lime sulphur were less mature. However, the differences in SPI between 'Braeburn' and 'Royal Gala' in the current trial may also be attributed to the different effects lime sulphur had on the photosynthetic rate of the two cultivars. A decreased photosynthetic rate can restrict nutrients from reaching the developing fruit, which was possibly the case with SSC. That means, due to a lower photosynthetic ability, 'Braeburn' apples treated with lime sulphur may have contained less starch than apples from the control treatment. If this was the case, the decomposition of starch in 'Braeburn' treated with lime sulphur would have been completed in a shorter time (Brookfield et al., 1997), leading to a more advanced SPI after storage than the control. If apples from different treatments had the same starch content, the rate of degradation in 'Braeburn' treated with lime sulphur might have been faster during the storage period than in the control. To obtain an estimate of the starch content, percentage dry matter of apples in the first harvest was measured and is discussed in section 5.5.7.

Differences in SPI development can negatively influence the fruit's storability as well flavour and textural quality (Brookfield et al., 1997). These attributes are very important to the industry and the consumer. SPI is also a maturity marker and changes in this rating could have implications for determining proper harvest time.

5.5.6 TITRATABLE ACIDITY

Lime sulphur did not have a consistent effect on the titratable acidity (TA) of 'Braeburn'. In 'Royal Gala', however, fruit showed consistently lower TA when treated with increasing amounts of lime sulphur. Differences were significant between the control and LS2 in all measurements (measurements from the 1st harvest were not available). These results have to be considered with caution though, as juice was obtained by combining a small piece of each of the ten apples per tree. This procedure was necessary due to time constraints and also because the remainder of each fruit was used for other analyses. Ten separate analyses or combining the juice from each whole apple would have possibly been more accurate.

Sugars as well as acids contribute to SSC in fruit juice. In 'Braeburn' there was no difference between treatments in terms of TA, the decrease in SSC could therefore be attributed to decreased sugar levels. In 'Royal Gala', SSC decrease was lower than in 'Braeburn', but greater than expected, considering photosynthesis was only slightly affected. As acidity was decreased in 'Royal Gala', the SSC observed could have been due to acids as well as sugars. The decrease of sugars might play a lesser role in the overall decrease of SSC in 'Royal Gala'.

Acid taste is an important consumer attribute for apples (Jaeger et al., 1998) and TA is a very good predictor of acid taste as well as overall flavour and apple flavour (Harker et al., 2002c). The differences in TA between the control and LS2 were statistically significant in 'Royal Gala', but possibly not high enough to have an impact on flavour and therefore consumer acceptance as the proposed taste threshold is 0.08% TA (Harker et al., 2002c).

5.5.7 DRY MATTER

Dry matter (DM) decreased significantly with increasing application rate of lime sulphur in both cultivars, but especially in 'Braeburn'. The reason for this is most likely a reduced photosynthetic rate in lime sulphur treatments and in turn decreased amount of nutrients available to the fruit. DM may give a rough estimate of the starch content. In 'Braeburn', the decrease in photosynthesis, DM and SSC combined with a faster development of SPI, may indicate that there is a lower starch content at harvest in apples treated with lime sulphur.

The parameters outlined in sections 5.5.1 to 5.5.7 all work together to achieve optimum maturity, storability and quality of the apple and no single one could be denoted as most important. Again, considering that commercial orchards may apply more sprays than was the case in this trial means that the impact on all quality parameters could be a lot higher.

5.6 SUMMARY

In this study, the application of lime sulphur had a significant impact on the quality as well as sulphur content of both cultivars, 'Braeburn' and 'Royal Gala'. It is known that 'Braeburn' is very sulphur-sensitive and this study has confirmed that the photosynthetic rate of this cultivar is severely decreased after lime sulphur application, whereas 'Royal Gala' is only mildly affected. Because of the obvious differences in sulphur sensitivity of both cultivars, it would have been expected that sulphur content and quality parameters would not be affected in similar ways. However, both cultivars showed comparable residues of sulphur and a metabolism of the applied lime sulphur into water-soluble non-protein thiols appears to have taken place. In addition, the quality of apples from both cultivars was negatively affected with increasing application rate of lime sulphur, but this effect was greater in 'Braeburn' than in 'Royal Gala'. In both cultivars, background colour ratings, blush percentage, firmness, SSC and DM were decreased. In addition, 'Braeburn' apples treated with lime sulphur were smaller in size than the control. The TA of 'Royal Gala' apples treated with lime sulphur was lower than that of the control. The two cultivars behaved differently in terms of SPI, probably due to a greatly decreased photosynthetic rate in 'Braeburn' and associated differences in initial starch content. In this study, 'Royal Gala' was affected to a lesser extent than 'Braeburn' when treated with the same number of sprays. However, in commercial orchards, 'Braeburn' is sprayed less with lime sulphur than other cultivars and therefore the impact on the quality of 'Royal Gala', and possibly other cultivars not considered sulphur-sensitive, could be significant.

The implications of these results for the organic industry have been discussed. Of major importance is the detection of sulphur residues in an organic apple that is perceived as residue-free, as at least part of these residues were metabolites of sulphur which cannot be removed by a washing procedure. The shift in quality parameters may complicate the determination of optimum harvest maturity and in turn export quality and storage life may be affected. Visual appearance and taste are important attributes to the consumer. The use of lime sulphur in organic apple production may have an impact on consumer acceptance

as firmness, SSC and TA are important sensory parameters and the former two were affected to an extent that could be perceived by taste panels. Residues of sulphur may also impact on flavour. The nature of the sulphur residues that were not thiol compounds and the possible health issues associated with these sulphur residues need to be evaluated.

Suggestions were given to decrease the negative impact of lime sulphur sprays. It may be advantageous to apply sprays only early in the season before actual fruit development to reduce the effects on fruit quality. There may, however, still be sulphur metabolites reaching the growing fruit. The research into alternatives to lime sulphur for the control of black spot as well as the development of apple cultivars resistant to the disease should be of top priority for the organic industry.

Chapter 6

CONCLUSIONS AND FUTURE WORK

6.1 FUTURE RESEARCH POSSIBILITIES

Given that no previous research has looked at a metabolism of lime sulphur in organic apples or evaluated the impact of different doses of this fungicide on fruit quality, the results from the current project have raised many questions to be addressed in further studies.

6.1.1 ALTERNATIVES TO LIME SULPHUR

Priority should be given to research into finding alternative control measures against black spot that fit with the organic philosophy. There are two possibilities around the problems associated with lime sulphur: the use of different fungicides or the development of cultivars resistant to black spot. The latter is obviously the favourable option as it can significantly decrease the use of sprays, in organic as well as conventional apple production. However, consumers often have their favourite apple variety and may be hard to convert to a new one. As all these developments take time, they should rather be started earlier than later.

6.1.2 FRUIT QUALITY

The current study only evaluated the influence of lime sulphur on the most important quality parameters of apples at harvest and after short-term storage. Future studies should evaluate how the shift in quality parameters influences the determination of optimum harvest maturity, export quality and long-term storage life. Harvesting at the right maturity is an important determinant for quality and storage life and the latter is especially important

for apples which are stored for a prolonged period of time. Moreover, many of New Zealand's apples reach distant export markets and have to meet certain quality standards, such as colour and size.

Sensory attributes important for the consumer, such as size, colour, firmness, SSC and TA, were influenced by the application of lime sulphur. The first two visual attributes may attract buyers to other products or cultivars. Firmness, SSC and TA are major factors determining apple flavour and SSC and firmness have been influenced in a way that may be detectable by consumers. In addition, it is not known if the residue of sulphur in the apple or on the skin influences taste. Taste panels should be performed to find out if the change in apple quality caused by the application of lime sulphur is great enough to impact on consumer acceptability. The increased uptake of spray residues may also represent a health issue and needs further investigation.

6.1.3 SPRAY SCHEDULES

It has been discussed in the previous chapter that the current study only evaluated the influence of lime sulphur when applied 11 times throughout the season and showed that a higher concentration has a greater effect on both quality and sulphur residues. How a different number of spray applications or a restriction of lime sulphur use to early in the season only would influence the results is not known. This, however, should be the purpose of future studies. Applying lime sulphur only before the actual fruit development may significantly decrease the negative effects on fruit quality. The overall photosynthetic ability of the tree may be compromised to a lesser extent and give the trees a chance to recover their lost photosynthetic activity. The metabolism of lime sulphur or hydrogen sulphide by the apple tree needs to be evaluated. If the sulphur is taken up by the apple, applying lime sulphur before fruit development would decrease residues in the fruit. However, in other plants hydrogen sulphide is taken up by the leaves and if this is the case in apple trees, the sulphur applied early in the season may still be eventually transported into the fruit.

6.2 CONCLUDING REMARKS

In New Zealand, organic apple production relies heavily on the use of lime sulphur as a defence against black spot. The application of lime sulphur on 'Braeburn' trees results in necrotic leaves and a reduced photosynthetic rate. This problem is recognized, but the reasons for it remain unknown. 'Royal Gala' is not obviously sensitive to lime sulphur. This research aimed to evaluate the influence of lime sulphur on the postharvest quality and sulphur content of organic 'Royal Gala' and 'Braeburn' apples. Typical postharvest quality parameters (weight, diameter, background colour, blush, firmness, SSC, SPI, TA and DM) were measured at two harvests and after storing fruit from these harvests for five to six weeks. For the first time in apples, the levels of total sulphur and the metabolites water-soluble non-protein thiols and cysteine were examined. As the trees of the two cultivars are affected in very different ways, it was expected that fruit quality is also affected differently. However this was not the case.

The results obtained showed that sulphur residues and uptake were similar in both cultivars. This is a major issue for a product that is perceived and often claimed to be residue-free. Both cultivars showed a decrease in background colour, blush, firmness, SSC and DM, but 'Braeburn' was affected more strongly. TA was decreased with increasing application rate of lime sulphur in 'Royal Gala', but not in 'Braeburn'. Fruit size was greatly reduced in 'Braeburn', but not in 'Royal Gala'. It may be that a size reduction in the latter cultivar was masked by high black spot infection. The photosynthetic rate was affected in both cultivars; in 'Braeburn' it dropped to less than 20% of the control value, whereas 'Royal Gala' was only slightly affected. It is most likely this drop in photosynthesis causing the shift in the postharvest quality of apples treated with lime sulphur compared to the control. Some parameters (firmness and SSC) were affected to an extent that could have an impact on flavour and consumer acceptance.

These results are of great significance to the organic industry and have added to the increasing amount of information that lime sulphur is not a suitable fungicide for organic production standards. Many of the issues associated with lime sulphur were known in the 1930s before the development of synthetic fungicides. As synthetic compounds are not allowed under organic certification standards, organic apple growers today are still relying on compounds that were mostly abandoned by conventional growers 70 years ago because of their phytotoxicity and low efficacy. Moreover, it has already been questioned if the use

of lime sulphur aligns with the organic principle of sustainability as it leads to decreased photosynthesis, tree growth and yield. It is known that the sulphur-sensitive cultivar 'Braeburn' is difficult to grow under organic certification guidelines; nevertheless, it continues to be grown due to strong market demand. The findings of the current study have found further problems associated with the use of lime sulphur and show that the apple cultivar 'Royal Gala', that has previously been thought to be unaffected by sprays, is actually influenced in a similar manner to 'Braeburn' in terms of postharvest quality and sulphur content. The use of lime sulphur needs to be regulated to minimise its impact on apple quality in any cultivar grown organically. Further research needs to look at how lime sulphur influences fruit quality and sulphur content, so that spray schedules can be optimised. The only real solution to the problem, however, is the use of different fungicides or the development of disease resistant cultivars. The latter would be the favourable option as it may actually be a way to make organic apples what they should be: residue-free and spray-free.

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APPENDIX

A TRANSFORMED POSTHARVEST QUALITY DATA

A.1 WEIGHT

Table A.1: Transformed weight data for 'Royal Gala' at both harvests and after storage of fruit from each harvest. Means with the same letter are not significantly different in each column ($\alpha = 0.05$, $n = 60$).

Treatment	<i>log(weight) [log(g)]</i>							
	Harvest 1		Harvest 2		Storage 1		Storage 2	
Control	2.170	a	2.094	a	2.109	a	2.125	a
LS1	2.152	a	2.124	a	2.122	a	2.136	a
LS2	2.158	a	2.141	a	2.125	a	2.116	a
S.E.	0.017		0.025		0.017		0.013	

Control = hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, Harvest 1/2 = 1st/2nd harvest, Storage 1/2 = fruit stored from 1st/2nd harvest, S.E. = standard error.

Table A.2: Transformed weight data for 'Braeburn' at both harvests and after storage of fruit from each harvest. Means with the same letter are not significantly different in each column ($\alpha = 0.05$, $n = 60$). In Harvest 2, $P = 0.0502$.

Treatment	<i>log(weight) [log(g)]</i>							
	Harvest 1		Harvest 2		Storage 1		Storage 2	
Control	2.287	a	2.270	a	2.280	a	2.230	a
LS1	2.216	b	2.198	a, b	2.183	b	2.183	b
LS2	2.204	b	2.166	b	2.144	b	2.117	c
S.E.	0.017		0.025		0.017		0.013	

Control = hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, Harvest 1/2 = 1st/2nd harvest, Storage 1/2 = fruit stored from 1st/2nd harvest, S.E. = standard error.

A.2 DIAMETER

Table A.3: Transformed diameter data for 'Royal Gala' at both harvests and after storage of fruit from each harvest. Means with the same letter are not significantly different in each column ($\alpha = 0.05$, $n = 60$).

<i>Treatment</i>	<i>log(diameter) [log(mm)]</i>							
	<i>Harvest 1</i>		<i>Harvest 2</i>		<i>Storage 1</i>		<i>Storage 2</i>	
Control	1.84	a	1.82	a	1.82	a	1.83	a
LS1	1.83	a	1.83	a	1.83	a	1.81	b
LS2	1.84	a	1.83	a	1.83	a	1.83	a
S.E.	0.005		0.008		0.005		0.005	

Control = hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, Harvest 1/2 = 1st/2nd harvest, Storage 1/2 = fruit stored from 1st/2nd harvest, S.E. = standard error.

Table A.4: Transformed diameter data for 'Braeburn' at both harvests and after storage of fruit from each harvest. Means with the same letter are not significantly different in each column ($\alpha = 0.05$, $n = 60$).

<i>Treatment</i>	<i>log(diameter) [log(mm)]</i>							
	<i>Harvest 1</i>		<i>Harvest 2</i>		<i>Storage 1</i>		<i>Storage 2</i>	
Control	1.87	a	1.87	a	1.88	a	1.86	a
LS1	1.85	b	1.85	a, b	1.85	b	1.84	a
LS2	1.85	b	1.84	b	1.83	b	1.82	b
S.E.	0.005		0.008		0.005		0.005	

Control = hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, Harvest 1/2 = 1st/2nd harvest, Storage 1/2 = fruit stored from 1st/2nd harvest, S.E. = standard error.

A.3 BLUSH

Table A.5: Transformed blush data for 'Royal Gala' at both harvests and after storage of fruit from each harvest. Means with the same letter are not significantly different in each column ($\alpha = 0.05$, $n = 60$).

<i>Treatment</i>	<i>log(blush) [log(%)]</i>							
	<i>Harvest 1</i>		<i>Harvest 2</i>		<i>Storage 1</i>		<i>Storage 2</i>	
Control	1.9	a	1.9	a	1.9	a	1.9	a
LS1	1.8	a	1.9	a	1.9	a	1.9	a
LS2	1.8	a	1.8	a	1.8	a	1.8	a
S.E.	0.04		0.02		0.04		0.04	

Control = hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, Harvest 1/2 = 1st/2nd harvest, Storage 1/2 = fruit stored from 1st/2nd harvest, S.E. = standard error.

Table A.6: Transformed bluish data for 'Braeburn' at both harvests and after storage of fruit from each harvest. Means with the same letter are not significantly different in each column ($\alpha = 0.05$, $n = 60$).

<i>Treatment</i>	<i>log(bluish) [log(%)]</i>							
	<i>Harvest 1</i>		<i>Harvest 2</i>		<i>Storage 1</i>		<i>Storage 2</i>	
Control	1.8	a	1.8	a	1.8	a	1.8	a
LS1	1.6	a	1.7	b	1.7	a	1.7	a
LS2	1.7	a	1.7	b	1.6	a	1.6	a
S.E.	0.04		0.02		0.04		0.04	

Control = hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, Harvest 1/2 = 1st/2nd harvest, Storage 1/2 = fruit stored from 1st/2nd harvest, S.E. = standard error.

A.4 FIRMNESS

Table A.7: Transformed firmness data for 'Royal Gala' at both harvests and after storage of fruit from each harvest. Means with the same letter are not significantly different in each column ($\alpha = 0.05$, $n = 60$).

<i>Treatment</i>	<i>1/Firmness [1/N]</i>							
	<i>Harvest 1</i>		<i>Harvest 2</i>		<i>Storage 1</i>		<i>Storage 2</i>	
Control	0.0111	a	0.0122	a	0.0194	a	0.0204	a
LS1	0.0113	a	0.0128	a	0.0187	a, b	0.0198	a
LS2	0.0120	a	0.0135	b	0.0180	b	0.0204	a
S.E.	0.0003		0.0002		0.0003		0.0003	

Control = hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, Harvest 1/2 = 1st/2nd harvest, Storage 1/2 = fruit stored from 1st/2nd harvest, S.E. = standard error.

Table A.8: Transformed firmness data for 'Braeburn' at both harvests and after storage of fruit from each harvest. Means with the same letter are not significantly different in each column ($\alpha = 0.05$, $n = 60$).

<i>Treatment</i>	<i>1/Firmness [1/N]</i>							
	<i>Harvest 1</i>		<i>Harvest 2</i>		<i>Storage 1</i>		<i>Storage 2</i>	
Control	0.0128	a	0.0134	a	0.0135	a	0.0133	a
LS1	0.0129	a	0.0140	a, b	0.0135	a	0.0137	a
LS2	0.0137	a	0.0145	b	0.0137	a	0.0140	a
S.E.	0.0003		0.0002		0.0003		0.0003	

Control = hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, Harvest 1/2 = 1st/2nd harvest, Storage 1/2 = fruit stored from 1st/2nd harvest, S.E. = standard error.

A.5 TITRATABLE ACIDITY

Table A.9: Transformed titratable acidity data for 'Royal Gala' at both harvests and after storage of fruit from each harvest. Means with the same letter are not significantly different in each column ($\alpha = 0.05$, $n = 60$). In Storage 1, $P = 0.0561$, in Storage 2, $P = 0.0518$.

Treatment	<i>log(acidity) [log(%)]</i>							
	Harvest 1		Harvest 2		Storage 1		Storage 2	
Control	n/a		-0.361	a	-0.443	a	-0.450	a
LS1	n/a		-0.398	a, b	-0.475	a, b	-0.495	a, b
LS2	n/a		-0.425	b	-0.505	b	-0.518	b
S.E.	n/a		0.012		0.015		0.017	

Control = hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, Harvest 1/2 = 1st/2nd harvest, Storage 1/2 = fruit stored from 1st/2nd harvest, S.E. = standard error.

Table A.10: Transformed titratable acidity data for 'Braeburn' at both harvests and after storage of fruit from each harvest. Means with the same letter are not significantly different in each column ($\alpha = 0.05$, $n = 60$).

Treatment	<i>log(acidity) [log(%)]</i>							
	Harvest 1		Harvest 2		Storage 1		Storage 2	
Control	-0.234	a	-0.241	a	-0.229	a	-0.222	a
LS1	-0.207	a	-0.228	a	-0.223	a	-0.214	a
LS2	-0.218	a	-0.241	a	-0.218	a	-0.208	a
S.E.	0.010		0.012		0.015		0.017	

Control = hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, Harvest 1/2 = 1st/2nd harvest, Storage 1/2 = fruit stored from 1st/2nd harvest, S.E. = standard error.

A.6 DRY MATTER

Table A.11: Transformed dry matter data for 'Royal Gala' and 'Braeburn' at the first harvest. Means with the same letter are not significantly different in each column ($\alpha = 0.05$, $n = 60$).

Treatment	<i>log (dry matter) [log(%)]</i>			
	'Royal Gala'		'Braeburn'	
Control	1.102	a	1.083	a
LS1	1.089	a	1.047	b
LS2	1.063	b	1.017	c
S.E.	0.008		0.008	

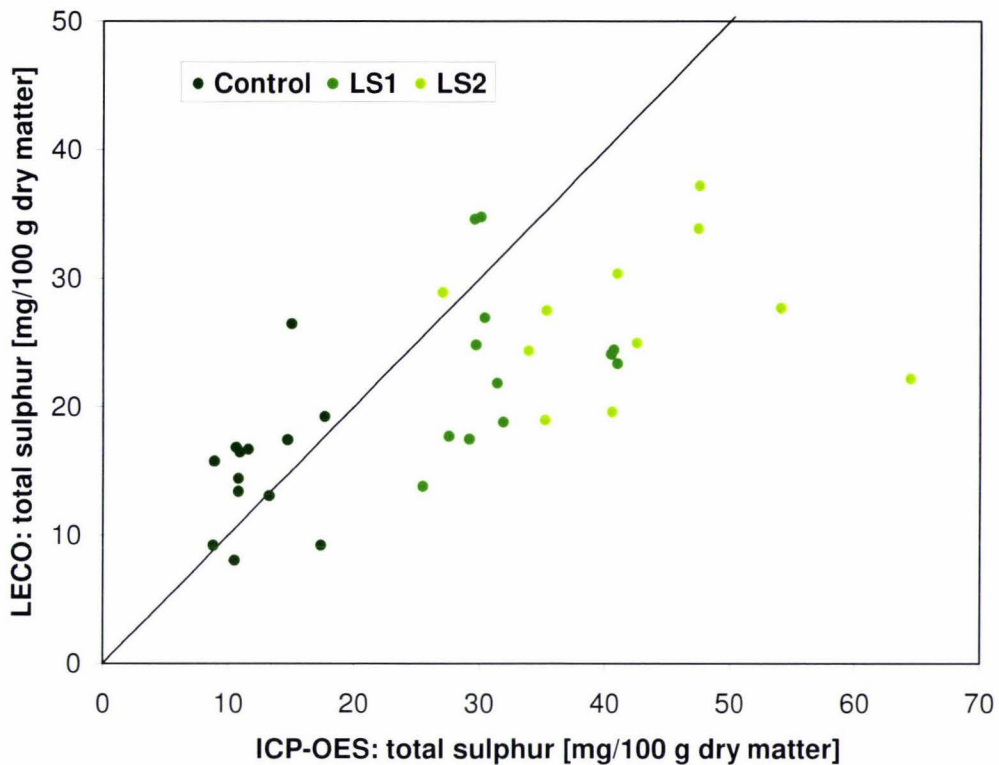
Control = hydrated lime, LS1 = 1% lime sulphur + hydrated lime, LS2 = 2% lime sulphur + hydrated lime, S.E. = standard error.

APPENDIX

B TOTAL SULPHUR ANALYSIS

Total sulphur in skin and flesh samples was initially meant to be analysed using the LECO sulphur analyzer (CNS-2000, Leco Corporation, St. Joseph, MI, herein LECO). In this procedure, the sample is weighed into a ceramic boat which is pushed into a furnace at 1050°C for combustion. Water released by combustion is trapped in columns of magnesium perchlorate to prevent interference with the carbon and sulphur detectors. All carbon and sulphur is converted to carbon dioxide and sulphur dioxide, respectively, and analyzed by infrared detectors. Nitrogen was not analyzed in the apple samples. The LECO has been reported to give accurate and precise results for total sulphur in plant material (Kowalenko, 2000; Soon et al., 1996).

Analysis on the LECO was abandoned because results were very variable and replicates analyzed did not match. It was decided to outsource the total sulphur analysis to Hill Laboratories, Hamilton, New Zealand, who used inductively coupled plasma optical emission spectroscopy (ICP-OES) to determine total sulphur following acid digestion. Altogether, only 36 samples of all treatments for 'Braeburn' flesh samples in the first harvest were analyzed with the LECO. The 36 results obtained with the LECO were plotted against the corresponding values supplied by Hill Laboratories and are displayed in Figure B.1.



APPENDIX

C CLIMATIC DIFFERENCES: MANAWATU AND HAWKE'S BAY

The Hawke's Bay is the major apple growing region of New Zealand. The Manawatu, where the current study was performed, is not a commercial apple growing area. There are climatic differences between these two regions. Although the mean annual temperature is similar (Figure C.1), summers in the Hawke's Bay are warmer than in the Manawatu (Figure C.4 and Figure C.5). Annual sunshine hours again seem similar on average (Figure C.2), but it is often cloudy inland in Palmerston North (NIWA, 2007b) and generally sunny in the Hawke's Bay (NIWA, 2007a). Although mean annual rainfall is only slightly lower in some areas of the Hawke's Bay than in the Manawatu (Figure C.3), the summer is a lot dryer in Napier (Figure C.5) than in Palmerston North (Figure C.4). For this reason, black spot was a major problem in the trial orchard in the Manawatu and sprays had to be applied throughout the season. In the Hawke's Bay, the use of fungicides can sometimes be decreased later in the apple season as the weather improves.

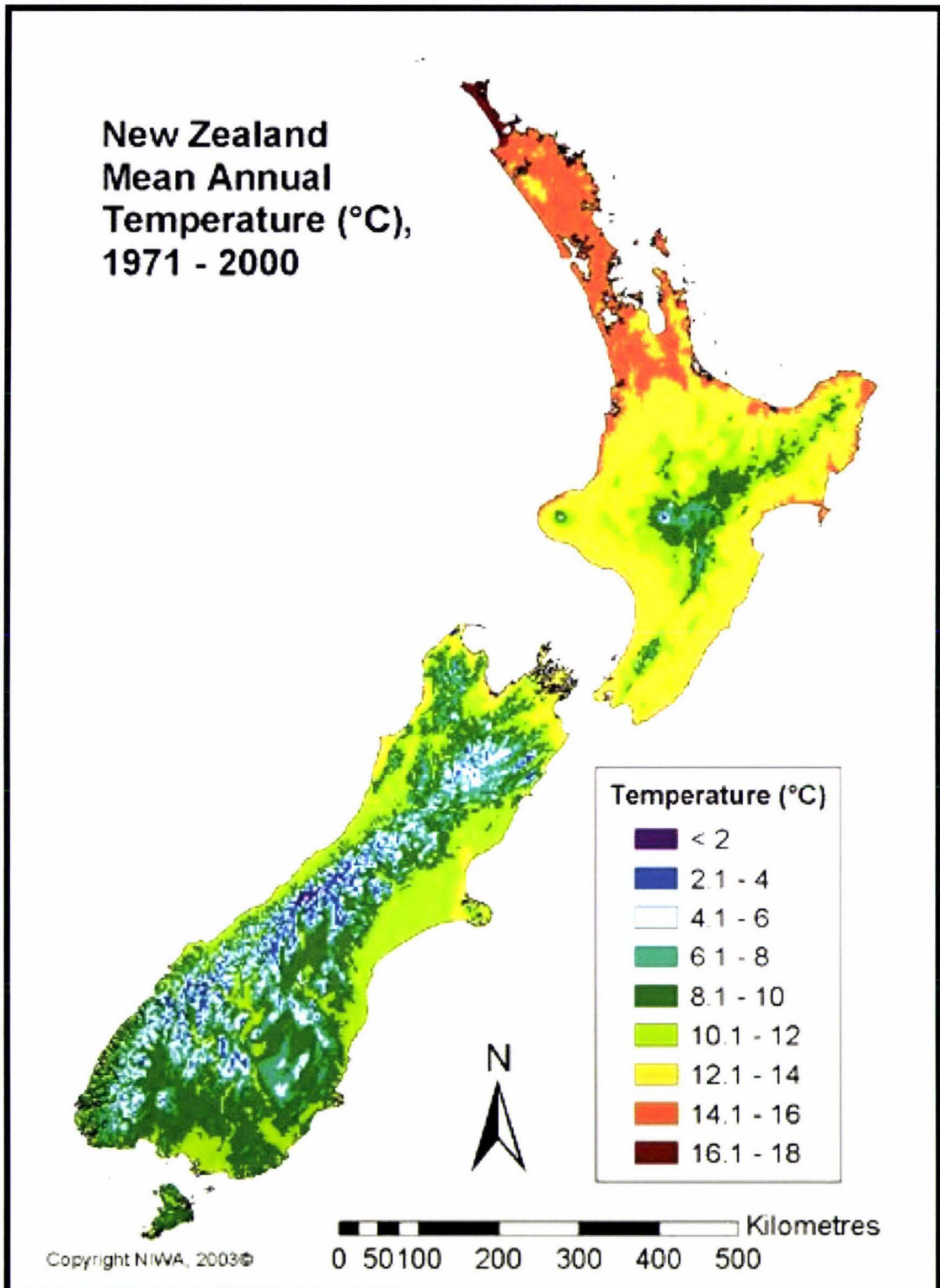


Figure C.1: Mean annual temperature in New Zealand (NIWA, 2003c).

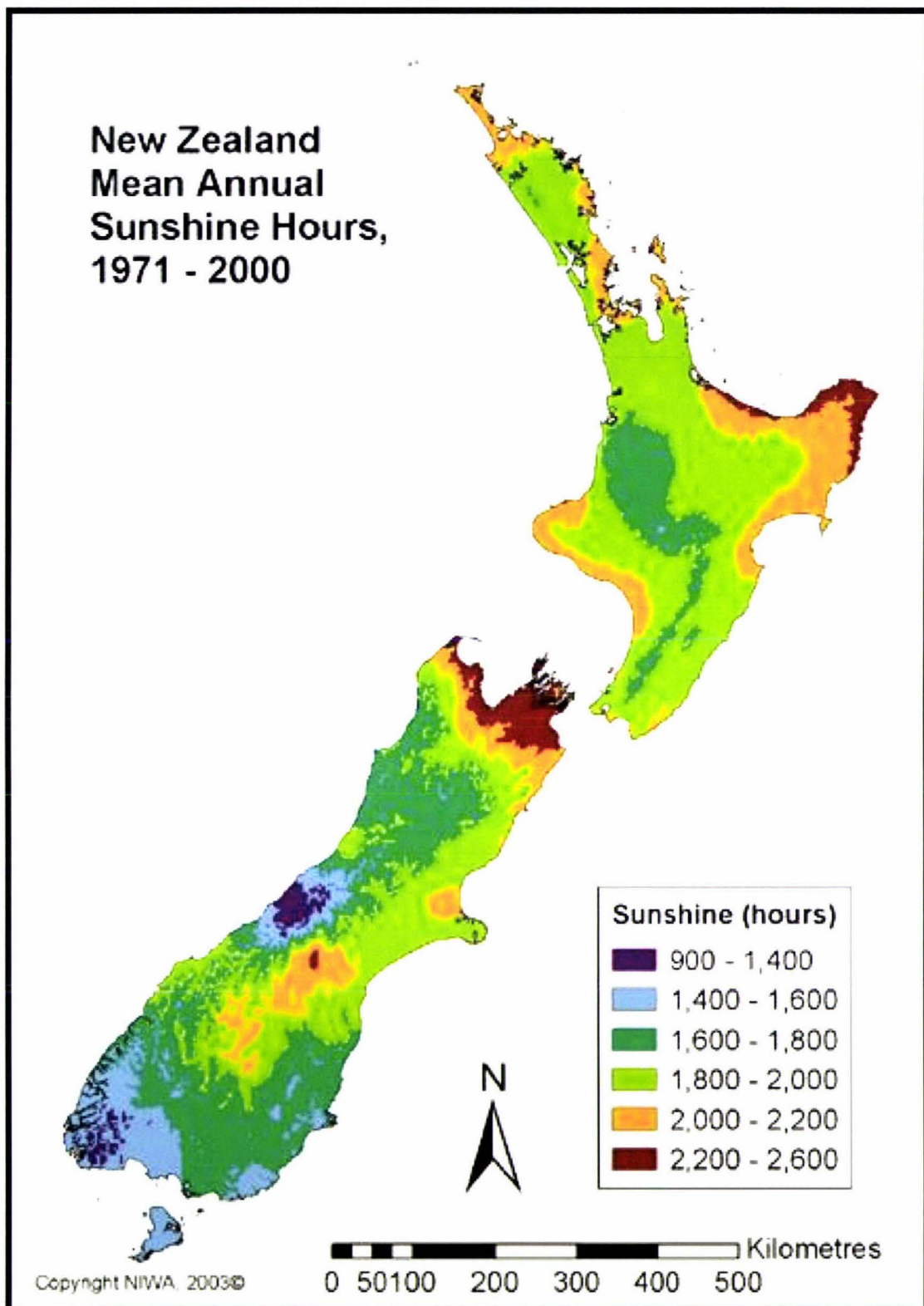


Figure C.2: Mean annual sunshine hours in New Zealand (NIWA, 2003b).

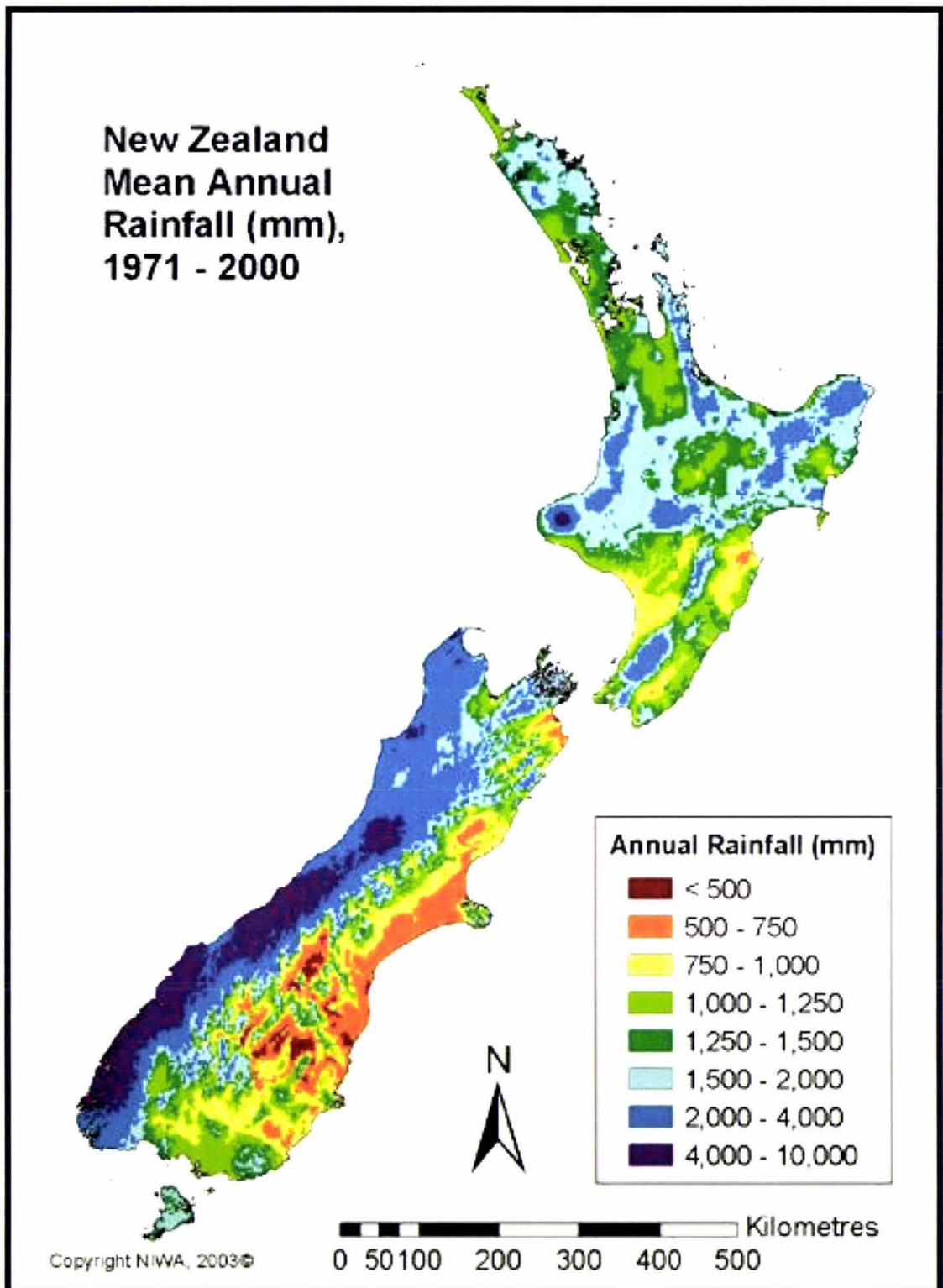


Figure C.3: Mean annual rainfall in New Zealand (NIWA, 2003a).

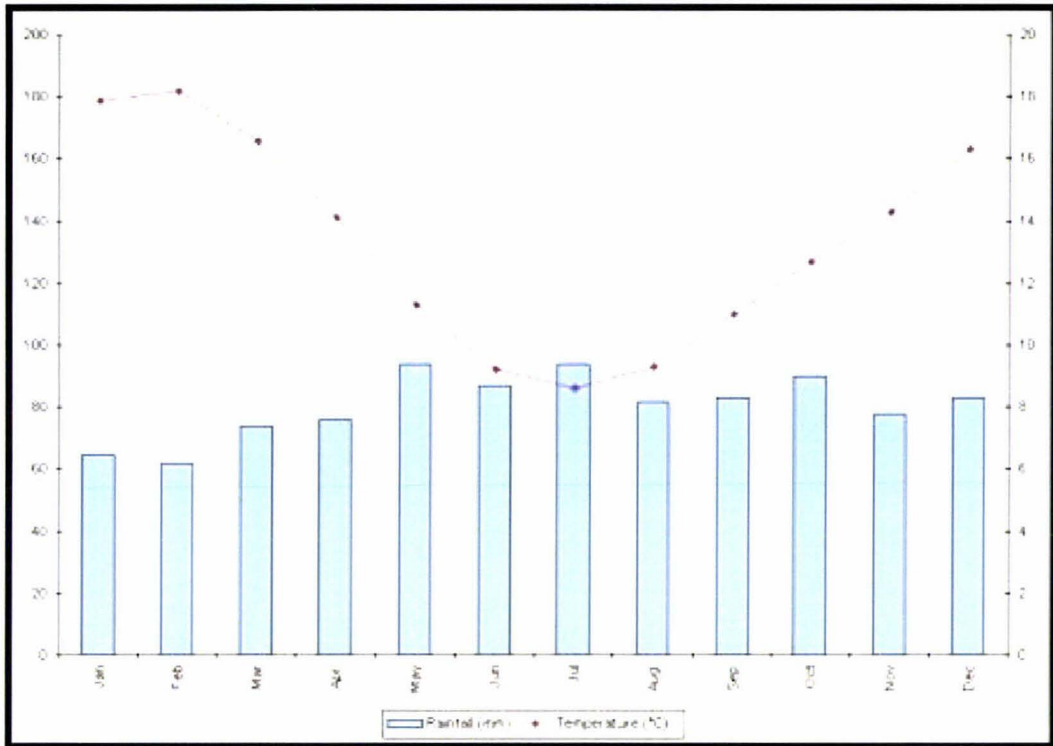


Figure C.4: Mean monthly rainfall and temperature in Palmerston North, Manawatu, New Zealand (NIWA, 2007d).

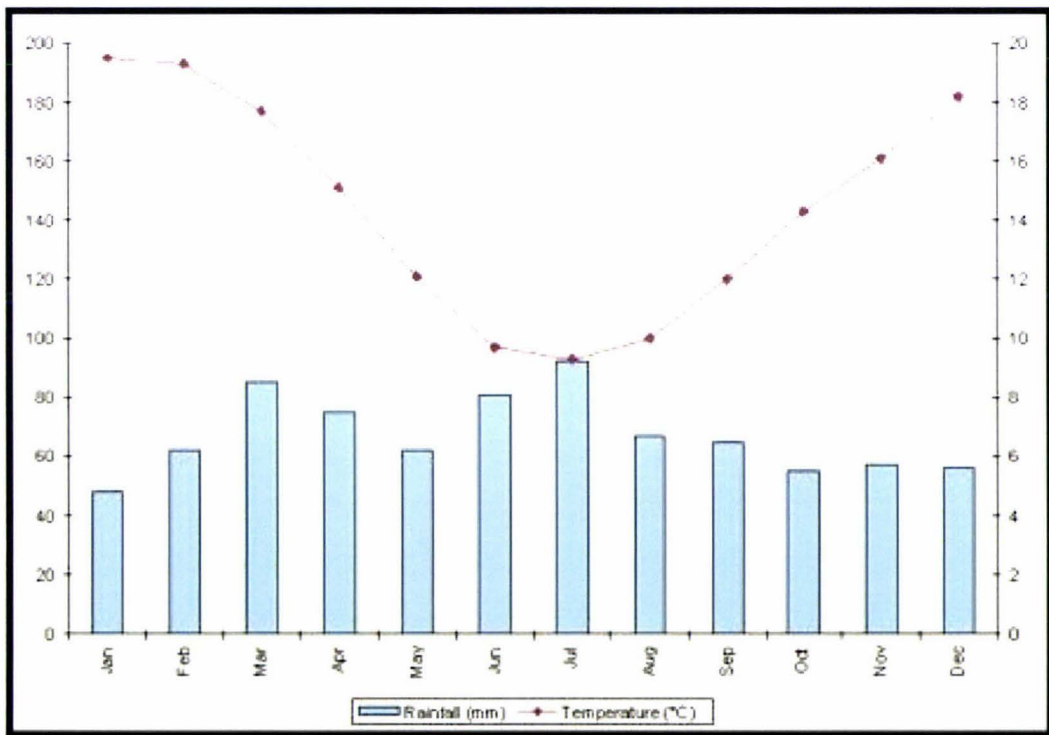


Figure C.5: Mean monthly rainfall and temperature in Napier, Hawke's Bay, New Zealand (NIWA, 2007c).