# Kiwifruit (*Actinidia* spp.) vine and fruit responses to nitrogen fertiliser applied to the soil or leaves

A thesis presented in partial fulfilment of the requirements

for the degree of

Doctor of Philosophy

in Fruit Science

at

Massey University

Palmerston North, New Zealand

**MASSEY UNIVERSITY**
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**UNIVERSITY OF NEW ZEALAND**

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**2013**

# Abstract

Dry matter concentration (DM%) of the fruit is a primary indicator of quality for kiwifruit (*Actinidia* spp.), lower levels being associated with inferior tasting fruit. Carbohydrates and particularly starch, are the main component of dry matter in the fruit of *Actinidia* spp. In plants, N fertilisation can reduce carbohydrate levels and increase succulence. Therefore high levels of N fertilisation could reduce fruit DM% by reducing its dry matter accumulation and increasing its water content. High rates of N fertiliser applied to kiwifruit vines (*A. deliciosa*) over four seasons tended to produce larger fruit (5% heavier on average over the four seasons) mainly due to increased water content with less effect on total dry matter contents. Consequently DM% was reduced from an average over the four seasons of 16.1% in the unfertilised (control vines) to 15.6% in fruit from the N fertilised vines. However, vegetative vigour in terms of the weight of shoots was increased by up to 150% by N fertiliser. Biostimulants applied as foliar sprays and surplus water supplied to the soil appeared to alter the balance between dry matter and water accumulation in the fruit in a similar way to soil-applied N fertiliser. It is concluded that increases in fruit size induced by N fertilisation, biostimulants, surplus water, and even girdling are at least partly due to the creation of increased hydraulic gradients between the vine and fruit leading to increased water uptake by the fruit. Other effects on fruit of high rates of soil-applied N fertiliser included reduced ascorbic acid, oxalate, and epidermal phenolics. Reductions in levels of these compounds and the generally increased succulence of N fertilised vines may increase the susceptibility of the vines to pests and diseases. In contrast to soil-applied N, foliar sprays of N applied during early fruit development stages increased fruit growth with no apparent effect on vegetative vigour. Aqueous solutions (1% w/v) of both urea and potassium nitrate were effective forms of N for foliar application and could increase fruit fresh weight by between 6 and 10% depending on the season and number of applications. It is estimated that the use of foliar-applied N during early fruit development could represent an increase in crop value of between $3600 and $15,000 per hectare depending on size and yield. Foliar-applied N shows promise as an alternative way to manage the N nutrition of kiwifruit with favourable effects on fruit quality since dry matter accumulation in fruit tended to increase proportionately with increased water uptake. Foliar application of N can also avoid some of the adverse environmental effects associated with the soil application of soluble N fertilisers.

# Acknowledgements

# List of Abbreviations

| | |
|---|---|
| BK | Benefit Kiwi® |
| CPPU | N-(2-chloro-4-pyridyl)-N'-phenylurea |
| CV% | coefficient of variation |
| DAFB | days after full bloom |
| DPBB | days 'post' or after bud break |
| DM% | dry matter concentration |
| DW | dry weight |
| EC | electrical conductivity |
| FC | field capacity |
| FN1, FN2 | foliar-applied N at time 1 or time 2, abbreviation used in Experiment 1 in Chapter 6. |
| FW | fresh weight |
| HATS | high affinity transport system |
| HN | high nitrogen (high rates of N fertiliser) treatment |
| HN+SF | high nitrogen plus spring-applied fertiliser treatment |
| LN | low nitrogen (nil N fertiliser) treatment |
| LN+SF | low nitrogen plus spring-applied fertiliser (Chapter 3) |
| LNF | low nitrogen plus foliar urea treatment (Chapter 7) |
| MN | moderate rates of N fertiliser treatment (Chapter 7) |
| MNF | moderate rates of N fertiliser plus foliar urea treatment (Chapter 7) |
| N | nitrogen |
| N1-N4 | potassium nitrate (foliar treatments at times 1 to 4) |
| NAA | 1-Naphthaleneacetic acid |
| $NO_3^-$ | nitrate |
| NR | nitrate reduction |
| NUE | nitrogen use efficiency |
| | New Zealand daylight saving time |

| | |
|---|---|
| NZDST | photosynthesis |
| Pn | soluble solids content |
| SSC | soluble solids as percentage of DM% |
| SSFDM% | soil organic matter |
| SOM | surplus water treatment (Chapter 8) |
| SW | titratable acidity |
| TA | urea (foliar treatments at times 1 to 4) |
| U1 – U4 | un-watered (control) treatment (Chapter 8) |
| UW | water |
| W | fruit water potential |
| $\Psi_{fruit}$ | leaf water potential |
| $\Psi_{leaf}$ | osmotic potential |
| $\Psi_{s}$ | vine water potential |
| $\Psi_{vine}$ | |

# Table of Contents

# Contents

## List of Tables

## List of Figures

# 1. Introduction and Literature review

## 1.1 Introduction

Kiwifruit (*Actinidia* spp.) is New Zealand's highest earning fresh fruit export and is second only to wine in area planted (Aiken and Hewett 2012). Most of the kiwifruit produced in New Zealand is exported to markets around the world to increasingly discerning customers who have expectations of high fruit quality (Jaeger et al. 2003). Therefore, fruit quality is of major importance to the kiwifruit industry. The important attributes of quality in kiwifruit include size, appearance, taste, and resistance to postharvest deterioration. Increasingly, taste, qualified in terms of fruit dry matter concentration (DM%) has become an important marketing criterion (Richardson et al. 1997b). Fruit size is an important component of yield and a primary focus of most orchard management systems.

High yields are dependent on a relatively high level of plant vigour being preferentially directed into reproductive rather than vegetative growth (Elfving 1988). To promote vigour and obtain heavy crops of large fruit, relatively large fertiliser inputs are often recommended for kiwifruit orchards (Sale 1997a,b). However, if applied in excess, fertilisers can adversely affect fruit quality and the environment (Weinbaum et al. 1992). Nitrogen (N) is an important determinant of yield, is involved in a wide range of fruit quality attributes and issues, and more than any other mineral nutrient changes the composition of plants (Marschner 2002). To understand the relationships between fruit quality and fertilisation it is necessary to understand firstly how the kiwifruit berry develops and how the accumulation of dry matter and water is regulated. This is reviewed in Section 1.2 of this chapter. In Section 1.3 the effects of N on fruit quality and vine vigour will be discussed, and evidence is presented from the literature in support of the hypothesis that nitrogen fertiliser increases vine and fruit water content. Section 1.4 reviews the potential of foliar-applied N as an alternative for soil-applied N fertiliser and its potential to ameliorate some of the problems identified in Section 1.3. In the final section of this first chapter a summary conclusions, problem statement, and objectives of the research project will be presented.

Buxton (2005) correctly identified some inconsistencies and ambiguousness in the terminology used to discuss the kiwifruit's dry matter component of the whole fresh fruit. In particular this is to do with the use of abbreviations that allow confusion between the fruit's dry weight (DW) and the DW or 'dry matter' concentration in the fruit. The latter is normally given as the ratio between a fruit's DW and its fresh weight (FW) and expressed as a percentage (DM%). However, other abbreviations have been used instead of DM% including 'DM' or 'DMC' standing for 'dry matter contents'. Either expression could lead to confusion since 'DM' could refer to a fruit's DW and not mean its concentration, and 'DMC' standing for 'dry matter content' could also refer to DW since the word 'content' can mean either total amount or concentration (Collins 2007). Therefore, throughout this work, DM% is used to refer to the dry matter concentration of the fruit (DM% = (DW/FW) × 100), while DW refers to the fresh weight of the fruit minus its water (W) contents. The fully written term 'dry matter accumulation' is also used interchangeably with DW to better convey a sense of the movement of assimilates to the fruit and their accumulation there over time. By not abbreviating this term for example to 'DM' it is hoped to avoid any confusion with the similar term 'DM%'. At times changes in DM% have also been referred to as percentage changes. This has been deemed necessary to best display in tables how DM% has been affected by proportionate changes in the fruit's DW and W contents and resulting FW. Care has been taken in the construction and explanation of such tables to avoid any ambiguity in respect to describing a change in DM% as a percentage.

## 1.2 The regulation of dry matter and water contents in the kiwifruit (*Actinidia* spp.) berry during its growth

### 1.2.1 Introduction

Fruit from several species of *Actinidia* have been developed as horticultural crops in New Zealand and are now known and grown widely in different parts of the world. The cultivar 'Hayward' (*A. deliciosa* A.Chev. var. deliciosa) produces a green-fleshed fruit, while 'Hort16A' (*Actinidia chinensis* Planch. var. chinensis) has a yellow-fleshed fruit (Ferguson 2011). Fruit quality is a primary concern of the kiwifruit industry since it is widely acknowledged that this is essential for the long term success of the industry (Wright 2005). Some markets have expressed dissatisfaction with the taste of the kiwifruit fruit in some seasons, so understanding factors that influence taste has become a more recent focus of research. The concentration of dry matter (DM%) in the kiwifruit can be a quality predictor because the main components of dry matter at harvest are starch and soluble sugars (Richardson et al. 1997b). The starch is converted to soluble sugars as the fruit ripens so that fruit DM% at harvest is closely correlated to soluble solids concentration in the ripened fruit, which in turn contributes much to its taste (Rossiter et al. 2000; Hall et al. 2006). The DM% of kiwifruit harvested from New Zealand orchards typically ranges between about 14 and 17% for Hayward (Burdon et al. 2004). Consumer evaluation of ripe kiwifruit shows a general increase in liking as DM% measured at harvest increases to about 16% (Harker 2004). Although kiwifruit can develop dry matter contents above 20%, consumers are generally less receptive to values above about 16% in fruit from either *A. deliciosa* or *A. chinensis* (Burdon et al. 2004; Harker 2004).

Apart from the fruit's dry matter content, the other determinant of DM% is its water content. Sink strength is a function of sink activity and sink size (Wardlaw 1990; Pavel and DeJong 1993). The capacity of a fruit to utilise assimilates corresponds to its activity as a sink or in other words its capacity to accumulate dry matter. However, its sink size is corresponds more to its capacity for cell expansion and hence its capacity for water import. Therefore the stimulation of sink strength needs to favour sink activity rather than size if a dilution of DM% is to be avoided. Since the roles of carbon and water in the control of fruit development are different, their import may be regulated independently of each other (Coombe 1976; Han and Kawabata 2002).

Therefore in the following discussion the relationship of the two fruit constituents in relation to kiwifruit DM% have been examined separately in sections 1.2.3 and 1.2.4 respectively.

Before these two sections, Section 1.2.2 describes the phenology of fruit development and patterns of dry matter and water accumulation. The final section 1.2.5 combines a brief summary with the main conclusions.

### 1.2.2 Fruit development

The development of a kiwifruit berry has been divided (arbitrarily) into three main stages (Figure 1.1): Stage 1 (anthesis to about 58 days later) in which fruit size increases rapidly; Stage 2 (from about 58 to about 76 days after flowering) – a period of slow growth; and Stage 3 (from about 76 to about 160 days after flowering) when fruit grows more rapidly again until maturity and harvest (Hopping 1976; Richardson and Currie 2007). In *A. chinensis* a longer period of rapid cell division may extend the duration of Stage 1 to between 70 and 80 days from anthesis (Richardson and Currie 2007).

According to this model Stage 1 of kiwifruit growth is characterised firstly by cell division and then later by both cell division and cell expansion. Coincident with the peak in cell division during Stage 1, kiwifruit enters its most rapid growth phase during which time cell expansion in the inner pericarp is a major contributing factor to growth. Stage 2 of kiwifruit development is of shorter duration than either Stage 1 or Stage 3 and coincides with the main starch accumulation phase. Its duration could therefore have an important influence on fruit DM% at harvest. Nevertheless, it is unclear what factors are involved in determining the duration of starch accumulation in kiwifruit. Stage 3 covers the period of fruit maturation and is characterised by increasing accumulation of soluble sugars due both to continued sucrose import and to increasing starch turnover and net degradation of starch to soluble sugars (Hopping 1976; Richardson and Currie 2007).

6

Other fruits show similar developmental patterns, although the delineation of stages varies. Thomas et al. (2006) describes grape berry growth in similar three stages of growth as for kiwifruit. However, more often Stage 1 has been limited to the period of rapid cell division, Stage 2 is then initially marked by rapid growth as cells enter the main period of expansion but growth then slows, which is where the typical sigmoidal growth pattern is established (Coombe 1976; Bain 1958; Hartman 1948; Janssen et al. 2008). This demonstrates the extremely arbitrary nature of such delineations of fruit growth since the definitions given by Hopping (1976) and Richardson and Currie (2007) could create the false impression that kiwifruit differs in developmental pattern to other fruit such as apples, citrus, olives, grapes, or stonefruit (Coombe 1976). In reality such stages overlap so that cell division, albeit at a reduced rate, extends further into Stage 2 and similarly cell expansion continues through Stage 3 (Morandi et al. 2012). The peak period of cell division in kiwifruit is from 0-30 DAFB (Hopping 1976) similar to apples (Devoghalaere et al. 2012), citrus (Bain 1958), and olives (Hartman 1948). This would advance the delineation between Stage 1 and 2 depicted in Figure 1.1 and extend the duration of Stage 2 and would make the model more in conformance with the descriptions given for other fruits and more useful in distinguishing the characteristic physiological activities found at different times during fruit development. However, the definitions given by Hopping (1976) and Richardson and Currie (2007) will be adhered through the rest of this thesis (see Figure 1.1).

The growth curve of kiwifruit can be quite variable with both single and triple sigmoid curves being reported by different researchers (Richardson and Currie 2007; Beever and Hopkirk 1990). Environmental factors may explain some of this irregularity and why growth curve patterns differ between different growing regions and seasons. More numerous and precise measurements of fruit growth can also increase the irregularity of growth curves (Coombe 1976).

Dry matter accumulation in kiwifruit follows a more linear pattern than FW (Richardson et al. 1997a; Han and Kawabata 2002; Richardson and Currie 2007) (Figure 1.1A). The more linear rate of dry matter accumulation suggests that the potential for dry matter accumulation is set by conditions before fruit set (Richardson et al. 1997a). However, DM% is a function of both DW and FW (DM% = [FW/DW]×100). The more variable and erratic pattern of FW development, and the

similarity of these patterns to corresponding patterns of water accumulation, implies that cultural and environmental conditions after anthesis are also important determinants of DM%. Furthermore, dry matter accumulation is not always linear. For example, a double sigmoidal pattern reported for *A. chinensis* (Boldingh et al. 2000) and *A. deliciosa* (Greer et al. 2003) fruit DW suggests post-anthesis factors can also influence dry matter accumulation. For example, Greer et al. (2003) reported that elevated temperatures produced a more curvilinear DW line than low temperatures did.
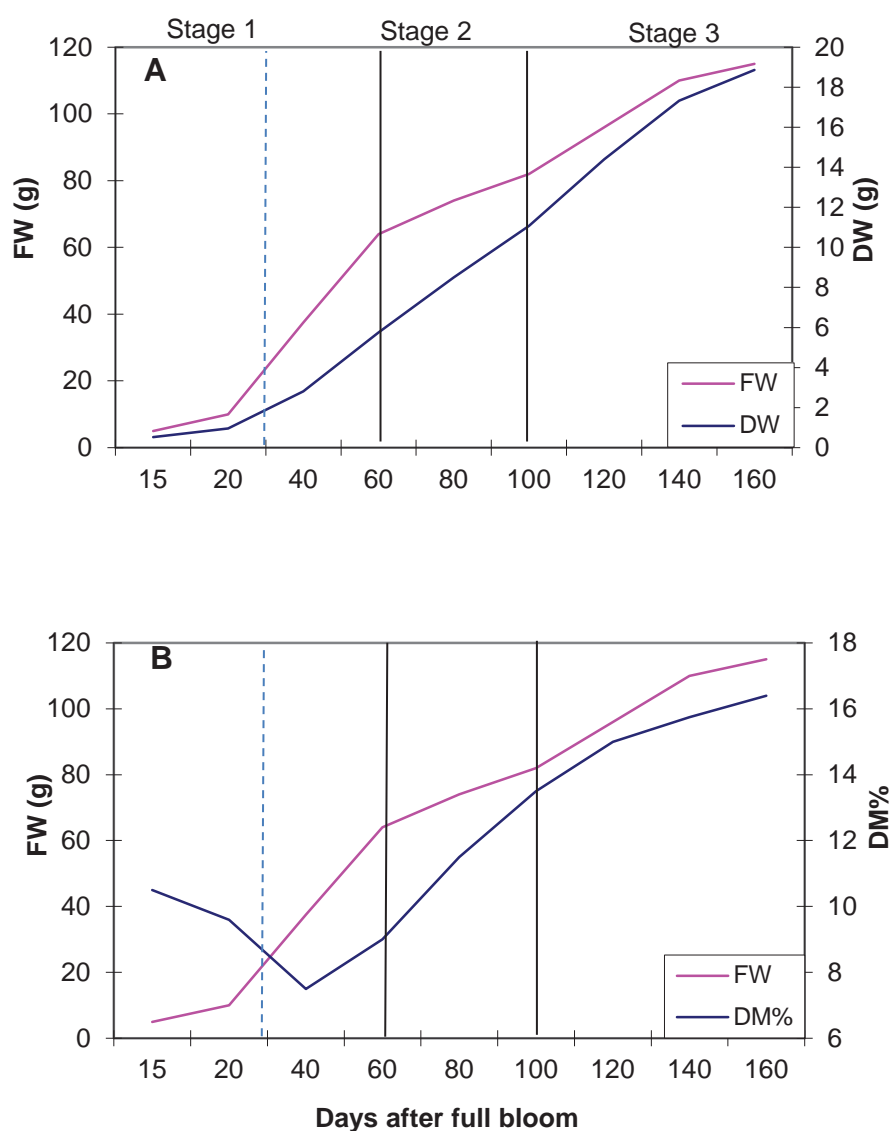
Figure 1.1 Changes during fruit development in (A) fresh weight (FW, g), dry weight (DW, g), and (B) FW and DM% of *Actinidia deliciosa*, with fruit developmental stages (solid vertical divisions) and suggested revised delineation of Stage 1 and 2 (dashed vertical line) (adapted from Richardson and Currie (2007) and modified according to various sources as given in text).

8

Dry matter concentration increases during ontogeny but drops sharply during early fruit development at about 30-40 DAFB coincident with the start of rapid fruit growth and cell expansion (Richardson et al. 1997a; Figure 1.1B). Some researchers have found a second drop in DM% at the beginning of Stage 3 of kiwifruit berry growth and, in some seasons, a third decrease close to harvest (Han and Kawabata 2002), while Richardson et al. (1997a) found an increase in DM% close to harvest. The more linear pattern of kiwifruit DW compared to FW increase indicates that decreases in DM% during ontology are due to increased water influx at those times rather than to decreases in the rate of dry matter accumulation. A somewhat variable and erratic growth pattern, decreased DM% during rapid growth phases, and increased water influx immediately preceding increases in the rate of dry matter accumulation are also found in other fruits (Coombe 1976).

When the growth curves for kiwifruit reported by different authors are compared, the largest variation between studies, seasons, or orchards in fruit FW and dry matter accumulation often occurs in the final stages of development. For example, Han and Kawabata (2002) reported that DM% of *A. deliciosa* fruit decreased during the last week before harvest in 1995 but increased during the same period in 1996. Increased variability in FW, DW, or DM% during Stage 3 compared to earlier fruit developmental stages are also found in the data of Clark and Smith (1988) and Walton and de Jong (1990). This might reflect the effect of increased solute accumulation or relaxation of cell walls on lowering fruit water potential ($\Psi_{fruit}$) that increases the capacity for maturing fruit to attract water if vine water potential ($\Psi_{vine}$) is increased by rain or irrigation (Han and Kawabata 2002; Rose and Bennett 1999). Nevertheless, there is a general tendency for FW growth to slow while the rate of dry matter accumulation continues during the final days or weeks before harvest (Snelgar et al. 2005). This suggests that at least some of the DM% variation between fruit lines is linked to maturity, in which case, the easiest way to improve kiwifruit DM% would be to allow fruit a longer developmental time (Beever and Hopkirk 1990). The ability of the maturing fruit to accumulate solutes against a concentration gradient and without inducing excessive water influx has a large influence on the fruit DM% at harvest.

### *1.2.2.1 Cell division and differentiation*

In *A. deliciosa*, cell division in fruit peaks at about 15 days after full bloom (DAFB) and declines to a low plateau by 30 DAFB (Lewis et al. 1996). While cell division and the total number of cells is a major factor determining final fruit size (Coombe 1976), the effect of cell number on DM% depends on the relationship between cell expansion (water uptake) and dry matter accumulation. Furthermore, fruit dry matter includes structural components such as cell walls that while making a contribution to fruit DM%, do not contribute to its soluble sugar content. Thus stimulation of cell division could conceivably increase fruit DM% without improving fruit quality in terms of starch or soluble solids concentration.

The relationship between cell division and DM% in terms of fruit quality depends on the way cells develop after division. Kiwifruit pericarp tissue includes large juice containing cells and smaller starch containing cells (Patterson et al. 1993). Fruit DM% might be increased if the proportion of starch containing cells was increased relative to juice cells. However, there was little difference between *A. deliciosa* genotypes in the proportion of these cell types despite large differences in DM% and starch (Nardozza et al. 2008). Increasing the size of the small parenchyma cells might also increase fruit starch content. However, although the size of the small parenchyma cells was increased by 31% by treatment with the synthetic cytokinin CPPU, starch content of the fruit was only increased by 10% by the time of peak starch content (approximately 120 DAFB; Antognozzi et al. 1997).

Starch is synthesised and accumulated within specialised organelles (amyloplasts) in kiwifruit cells, which are formed from their own division within the dividing cells (Lopez-Juez and Pyke 2005). The number contained within cells is related to cell size so that proliferation of amyloplasts is likely to continue as cells expand (Lopez-Juez and Pyke 2005). Cell division precedes amyloplast division and it may take some time for the cell's full complement of amyloplasts to develop. This might explain why rapid starch synthesis in kiwifruit lags behind cell division and expansion during early fruit development. It remains still unclear how the size or number of amyloplasts within the kiwifruit fruit is affected by cell division, cell expansion, or by cultural and environmental conditions. Nevertheless, variables such as light, temperature, and nutrition are thought to be influential in the division of plastids (Lopez-Juez and Pyke

2005). Amyloplasts and chloroplasts share the same limited zone pressed between the vacuole and the cell wall (Possingham 1980), therefore there might be some competition for space between the two plastid types. This might explain the relatively small increase in starch content in CPPU treated fruit despite the larger size of the starch containing cells noted in the previous paragraph. Chloroplast size and development can be increased by low light and CPPU (Possingham 1980; Antognozzi et al. 1996) but whether kiwifruit developing in shaded canopies or after being treated with CPPU have reduced amyloplasts as a result of increased chloroplast size is unclear.

In tobacco cells the formation of amyloplasts was stimulated by cytokinins, including CPPU (N-(2-chloro-4-pyridyl)-N'-phenylurea), but inhibited by auxin (2,4-D), although 2,4-D stimulated cell division (Miyazawa et al. 2002). The same conditions that promoted amyloplast differentiation appeared to also promote starch synthesis. The synthetic cytokinin CPPU and auxin containing compounds (pruning gel) can be used to stimulate kiwifruit berry size or reduce shoot re-growth following summer pruning, respectively. However, both these plant growth regulators are associated with reduced kiwifruit DM% (Currie et al. 2005). CPPU stimulated both cell division and cell expansion in kiwifruit (Woolley et al. 1991) and in the fruit of other crops (Quan Yu et al. 2001), but no effects of pruning gels on fruit cell division and expansion, or on starch synthesis have been reported. Increased understanding of how cultural and environmental variables influence development of fruit amyloplasts could improve our ability to increase fruit DM% and starch content.

### 1.2.3 Fruit water content

Water moves to the kiwifruit via both xylem and phloem vascular systems, with phloem transport becoming comparatively more important during fruit development (Dichio et al. 2003; Clark and Smith 1988). The driving force for water influx is a water potential gradient between the vine and the fruit generated by low turgor ($\Psi_p$) and osmotic ($\Psi_s$) potentials in the fruit (Patrick 1988). Turgor potential is regulated by cell wall expandability and growth, and transpiration from the fruit surface; while $\Psi_s$ is regulated by the accumulation of solutes in the fruit, such as hexose sugars and organic acids. In kiwifruit a peak in the concentration of hexoses coincides with the

rapid growth phase of Stage 1 (Boldingh et al. 2000). However, organic acids are thought to be more important osmotica in kiwifruit particularly during early fruit development (Nardozza et al. 2010). The rapid growth is mainly due to cell expansion in the inner pericarp (Hopping 1976) and it seems likely that the accumulation of organic acids generates the gradient for the increased water influx driving cell expansion at this time (Nardozza et al. 2010).

During Stage 1 of fruit development the potential for an uncoupling of dry matter and water import is high because (1) dry matter utilisation by the fruit remains at a relatively low level and (2) the potential for water import is high due to high extensibility of fruit cell walls and high rates of fruit transpiration that creates a steepened hydraulic gradient to the fruit (Clark and Smith 1988; Morandi et al. 2012). Furthermore, at this stage fruit xylem connections are apparently more active (Dichio et al. 2003), coupling the fruit more directly to the main water conduits of the vine and to vine water status (Thomas et al. 2006; Morandi et al. 2010).

Stimulation of fruit growth by CPPU during Stage 1 of fruit growth stimulates cell expansion in the pericarp resulting in larger fruit (Patterson et al. 1993; Lewis et al. 1996). The stimulation of fruit growth by CPPU is associated with an increased accumulation of hexose sugars in the fruit (Antognozzi et al. 1996). Such an accumulation would further lower $\Psi_{fruit}$ and the resulting increased water influx could account for the effect of CPPU on cell expansion. Although CPPU also increases fruit dry matter accumulation and starch synthesis (Antognozzi et al. 1996), fruit DM% is lower in treated fruit from soon after its treatment right up to harvest (Patterson et al. 1993; Famiani et al. 1997; Antogonozzi et al. 1996). For example, in the study of Patterson et al. (1993) a 44% increase in fruit size of CPPU treated fruit was mostly due to a 47% increase in water content, compared to a 27% increase in DW and an 11% decrease in DM% (a 1.7 percentage point decrease). Patterson et al. (1993) also reported that CPPU increased the size of small but not large parenchyma cells in kiwifruit pericarp tissue, indicating that interactions with CPPU are complex.

Similar effects are found with other plant growth regulators such as the proprietary biostimulant Benefit Kiwi® (Currie et al. 2005) and early season phloem girdling (Currie and Richardson 2007). Most plant growth regulators and early season girdling may have similar modes of action, i.e., they increase the transport of carbohydrate to

the fruit before starch synthesis has gathered pace, resulting in an accumulation of osmotically active solutes. This accumulation coincides with a high capacity for cell wall expansion in the young fruit and these conditions are the main reasons for the increased fruit growth and the increased ratio of water to dry matter accumulation (Table 1.1; Figure 1.2).

Table 1.1 Effect of plant growth regulators applied during Stage 1 of fruit growth on fruit FW (g), DM%, DW (g), and water (g) at harvest on two kiwifruit cultivars.

|  | FW (g) | DM% | DW (g) | Water (g) |
| --- | --- | --- | --- | --- |
| [1]Control | 95 | 17.5 | 17 | 78 |
| Benefit® + NAA | 131 | 16.7 | 22 | 109 |
| % difference | 27.5 | -4.8 | 24.0 | 28.2 |
|  |  |  |  |  |
| [2]Control | 110 | 15.4 | 17 | 93 |
| CPPU | 146 | 14.1 | 21 | 126 |
| % difference | 32.7 | -9.0 | 20.8 | 34.9 |

[1] Benefit Kiwi® plus 1-Naphthaleneacetic acid (NAA) on *A. chinensis*; Currie et al. (2005). [2] CPPU on *A. deliciosa*; Patterson et al. (1993).

The water potential gradient between vine and fruit will also be influenced by the water status of the vine. Kiwifruit berry growth remains highly sensitive to vine water status throughout its development (Judd et al. 1989; Miller et al. 1998). When vine water status is reduced, fruit growth slows. In the study by Miller et al. (1998) DM% was increased concurrently with the reduction in FW, suggesting that the growth reduction was due to decreased water influx. Water deficits during Stage 1 of fruit development suppressed fruit growth more than water deficits during Stage 3 (Miller et al. 1998). These results are consistent with those already discussed in relation to the effects of PGRs and girdling, and give further evidence for the importance of vine-fruit water potential gradients in determining growth and DM% during Stage 1 of fruit development. Water deficits have the potential to increase fruit DM% throughout fruit development since the flow of assimilates to the fruit may be less affected by reduced plant's water status than that of water (Chalmers et al. 1984) and because of the greater sensitivity of cell turgor and growth to water deficits than photosynthesis (Hsaio 1973). Indeed, phloem loading and transport of assimilates to sink tissues can be stimulated by water deficits and lower sink turgor (Patrick 1988; Wolswinkel 1985).

The importance of $\Psi_{vine}$ for fruit water influx is supported by the greater inter- and intra-seasonal variation in the patterns of FW and DM% development compared to DW (Han and Kawabata 2002; Fig 1). Such variability is consistent with seasonal and environmental conditions altering the rates of water flux to the fruit as the vine's water status changes in concert with fruit transpiration rates (Morandi et al. 2012). This is also supported by studies of the effect of water deficits, irrigation, and rainfall on kiwifruit. Individual fruit from fully irrigated vines accumulated 11.8% more water but only 0.4% more dry matter (a 1.6 percentage point decrease in DM%) than fruit from vines receiving one quarter of the full irrigation volume (Currie et al. 2008a). Miller et al. (1998) also found fruit growth and water content responded rapidly to soil water availability with fruit DM% increasing during periods of vine water stress. Experiments with other crops also show the same pattern of increased fruit water content and decreased DM% in fruit from fully irrigated crops compared to fruit of the same crop exposed to a water deficit (Table 1.2). Rain near harvest was reported to increase fruit water uptake and depress DM% (Han and Kawabata 2002) and dilute fruit SS% (Pailly et al. 1995). Han and Kawabata (2002) suggested that fruit become more liable to take up excess water close to harvest because the accumulation of soluble solids during fruit maturation lowers $\Psi_{fruit}$.

Table 1.2 Relative change (%) in fruit dry matter concentration (DM%), water and dry weight (DW) content between fully irrigated and deficit irrigation treatments (see text for details) in apples and peppers.

| Crop | DM% | Water | DW | Source |
|------|-----|-------|-----|--------|
| Apples | -3.2 | 13.3 | 9.1 | Mpelasoka et al. 2001 |
| Apples | -13.3 | 15.8 | -2.1 | Kilili et al., 1996 |
| Peppers | -47 | 62 | 3.5 | Dorj et al., 2005 |

High vine water status would also be associated with high vegetative vigour because of the close dependence of growth and general metabolic activity on plant water status (McIntyre 1987). For example, fully irrigated grapevines had a leaf area of 6.3 m$^2$ vine$^{-1}$, while non-irrigated vines had a leaf area of only 3.6 m$^2$ vine$^{-1}$ (Chaves et al. 2007).

Since water potential gradients in trees are basically linear with increasing distance from the ground (Hellkvist et al. 1974), it follows that the gradient could be greater in vigorous canopies due to greater total growth and transpiration. Since in a kiwifruit

14

vine fruit is positioned in the basal zones of the shoots the apoplastic water potential close to the fruit e.g. in subtending leaves, would be higher in a well-watered vigorous vine than in a less vigorous vine in the same conditions. Furthermore, in a vigorous vine competition for assimilates from growing shoots and their leaves would be increased. Thus in a vigorous vine, fruit DM% could be reduced both by an increase in water potential gradients between vine and fruit and a decrease in partitioning of carbohydrates to the fruit. Support for this relationship is found in the observation that fruit on canes originating from close to the trunk can have lower DM% than fruit from more distally positioned canes (Smith et al. 1994; Max 2004). The extent to which vine water status and vigour would influence or alter the DM% of mature kiwifruit probably depends on both on their degree and the duration of the effect.

### 1.2.3.1 Cell expansion

The increase in fruit size during fruit development is largely due to cell expansion with final fruit size being a function of cell size and cell number (Coombe 1976). Cell expansion occurs as the influx of water into the cell vacuoles presses against the cell wall causing it to deform and expand. The expansion of the cell wall prevents increases in $\Psi_p$ and thereby maintains the hydraulic gradient from vine to fruit. Accumulation of solutes in the fruit also helps in maintaining the gradient. Cell wall expansion involves both cell wall loosening and the synthesis of new structural material, processes regulated by complex enzymatic and hormonal interactions (Rose and Bennett 1999). Given the major role of cell expansion in regulating fruit water influx, factors governing cell wall extensibility would also be important in determining fruit water content. Such factors include the threshold turgor at which cell walls yield (yield threshold turgor), the deformability of the wall (Boyer 1988), and rates of wall synthesis. It seems likely that the capacity for wall extension and/or softening is genetically regulated so that the fruit cells possess an increased potential for expansion/softening during Stage 1 of fruit development and during maturation in Stage 3. However, whether plant growth regulators or biostimulants that stimulate fruit growth, such as CPPU or Benefit, alter the capacity for wall extension/softening is not clear. McIntyre (1987) suggested that the signal for many growth and metabolic processes may be through changes in water potential including subtle alterations to cell turgor. Hence, water status of the vine might in effect orchestrate some of the physiological changes that mark the different stages of fruit development.

### 1.2.3.2 Xylem functionality

Several studies in kiwifruit have shown that xylem contributes less to fruit water uptake during later fruit development than during the earlier stages (Dichio et al. 2003; Montanaro et al. 2006; Morandi et al. 2010). According to Hall et al. (2006) the ratio between xylem and phloem flow to the fruit changes from approximately 5:1 during early fruit development to about 1:1 by harvest. A similar reduction in xylem flux has been found in other fruits including apple and grape, and has been attributed to increasing xylem dysfunction due to the breakage of xylem vessels by rapid expansive fruit growth (Drazeta et al. 2004; During et al. 1987; Dichio et al. 2003). However, others have provided evidence that in grape the switch to predominantly phloem supply is due to increased water potential in the fruit apoplast and that the fruit xylem vascular system remains functionally connected throughout fruit development (Bondada et al. 2005; Keller et al. 2006). In kiwifruit the main reduction in xylem flux reported by Dichio et al. (2003) was coincident with a decline in *A. chinensis* fruit growth rate from about 75 DAFB rather than an increase as would be expected if xylem rupture was the cause. This change in xylem function may have had been the effect of physiological changes that marked the commencement of the main period for starch accumulation in Stage 2 of fruit development. Furthermore, the changes in xylem inflow reported by Dichio et al. (2003) show relatively large rapid fluctuations that are also more suggestive of physiological rather than morphological changes. The reduction in fruit xylem activity in kiwifruit might therefore reflect a change in apoplastic water potential of fruit cells due to uptake of solutes (i.e., sugars) and active efflux of surplus phloem derived water from fruit cell cytoplasm.

Light may promote xylem differentiation in fruit and pedicel (Biasi and Altamura 1996). However, it is unclear what effect this might have on kiwifruit water accumulation. For example, some researchers have found increased water accumulation in light exposed fruit (Montanaro et al. 2006), while others have found the opposite (Tombesi et al. 1993). Although exposed fruit may have higher transpiration rate and xylem flux (Montanaro et al. 2006), water following a transpiration induced gradient through the fruit apoplast will not directly affect the accumulation of water by fruit tissues since this is governed by the gradients to fruit cytoplasm.

## 1.2.4 Dry matter

Although fruit dry matter includes numerous diverse constituents (Beever and Hopkirk 1990), it is the starch and sugar component that is mostly referred to in regards to kiwifruit dry matter. Carbohydrates produced in the source leaves are transported almost exclusively to the kiwifruit via the phloem. Carbon imported in the xylem and fruit photosynthesis itself are considered to make insignificant contributions to kiwifruit DW (Clark and Smith 1988; Biasi and Altamura, 1996). As with water, the flux of assimilates to the fruit is due to pressure gradients between leaf and fruit maintained variously during fruit development by cell expansion, and solute polymerisation, accumulation, and assimilation.

### 1.2.4.1 Carbohydrate supply

Fruit development and dry matter accumulation depend firstly on the supply of assimilates from the canopy leaves. Carbohydrate supply from the canopy leaves can be reduced by factors such as shading, leaf damage, and dull weather that reduce photosynthetic rates. Vine phenology changes the patterns of carbohydrate partitioning so that the potential competitive strengths of different organs such as shoots and roots changes during fruit development (Piller et al. 1998; Blattman et al. 2008). For example, shoot growth peaks during Stage 1 of fruit development and root growth peaks during Stage 2 (Buwalda and Hutton 1988). Excessive stimulation of the growth of these competing sinks for carbohydrates could result in reduced dry matter accumulation by the fruit and, depending on concurrent fruit water relations, could also reduce DM% (see Section 1.2.3). The potential for an uncoupling of fruit dry matter and water import appears high during Stage 2 due to increased vine water uptake as an effect of increased root growth and concurrently decreased availability of assimilates due to increased partitioning to the roots. The duration of Stage 2 of fruit development might even be linked to root phenology as regulated by climate and the effect of temperature and moisture on root growth. Regrowth of shoots following summer pruning can also increase shoot competitiveness and reduce fruit DM% (Blattmann et al. 2008). An increased number of active meristems in vigorous canopies might also increase the translocation of auxins to developing fruit, delaying amyloplast differentiation and starch synthesis (see Section 1.2.1 above).

The increased dry matter accumulation in fruit associated with girdling during Stage 2 of fruit development has been attributed to the diversion of dry matter away from roots to the fruit (Currie and Richardson 2007). The associated increase in fruit DM% may be because active starch synthesis keeps pace with the increased assimilate supply, avoiding the accumulation of sugars and the associated risk of increased water influx as seems to occur when assimilate availability is increased during Stage 1. Generally, girdling increases fruit dry matter accumulation by approximately the same amount regardless of the time the girdle is applied, but the later the girdle is applied during Stage 2, the smaller is the effect on fruit water content (Figure 1.2). However, if the time of girdling was moved into Stage 3 of fruit growth, when starch synthesis has slowed and soluble sugars once again accumulate, perhaps there would be an increase in fruit water uptake relative to dry matter. Such an experiment would improve our understanding of fruit development (see Chapter 7).
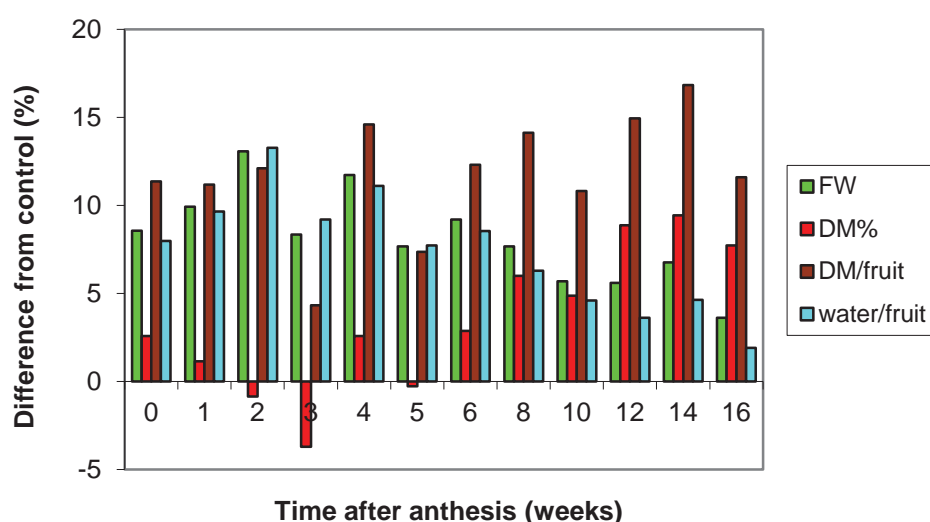


Figure 1.2 Percentage difference compared to control (no girdle) in FW, DM%, DW and water contents in fruit (water/fruit) at harvest from vines trunk girdled at different times during Stage 1 (weeks 0- 6) or Stage 2 (weeks 8 to 16) of fruit growth (adapted from Currie and Richardson 2007).

### 1.2.4.2 Dry matter import

Carbohydrates imported to the fruit through the phloem system must move to fruit parenchyma cells to be converted to starch, utilised for growth or synthesis of other fruit constituents, or be accumulated as soluble sugars. Phloem unloading and post-phloem transport can involve symplastic or apoplastic pathways. Symplastic pathways are probably prevalent during Stage 1 and Stage 2 of fruit development; with an

apoplastic step intervening during Stage 3 to allow solute accumulation during fruit ripening (Patrick 1988; Lalonde et al. 2003). Fruit parenchyma cells are connected to the phloem sieve cell complex by plasmodesmata whose conductance may be adjusted according to the needs and developmental stage of the fruit (Lalonde et al. 2003). Plasmodesmata are formed in the cell wall as it expands (Maule 2003). Interestingly their formation is strongly enhanced by cytokinins (Maule 2003), suggesting a possible mechanism to explain the accumulation of sugars after CPPU application to kiwifruit (see Section 1.2.3). Solute accumulation followed by water influx can also occur with treatment of plant tissues with auxins and gibberellins (McIntyre 1987), however, it is not certain if these effects involve an increase in plasmodesmatal activity.

### 1.2.4.3 Dry matter accumulation

The rate of starch synthesis probably regulates the flux of carbohydrate to the fruit (Patrick, 1988). The rate and duration of starch synthesis determines the final starch content of the fruit but whether it is also important for determining final DM% is uncertain. Regardless of the fruit's starch content at the end of Stage 2, DM% might be more influenced by water relations during Stage 1 and Stage 3. Evidence for this is found in the persistence of elevated water content in CPPU treated fruit despite increased dry matter accumulation during Stage 2 (Antognozzi et al. 1996). Despite an increased rate and duration of starch synthesis and increased starch concentration in CPPU treated fruit during Stage 2, untreated fruit still had nearly equal starch concentration on a fresh weight basis by harvest. The CPPU treated fruit also had higher soluble solids concentration (SSC) at harvest suggesting that the rate of starch hydrolysis and fruit maturation is linked to starch concentration. This in turn suggests starch concentration and perhaps also DM% is under genetic control and therefore limited within a genetically determined range. Indeed the largest differences in fruit DM% and starch concentration were found between different *A. deliciosa* genotypes (Nardozza et al. 2008). *A. deliciosa* genotypes with high DM% had elevated expression of a sucrose synthase gene and a greater rate of starch accumulation (Nardozza et al. 2008). Patterson et al. (1993) reported that CPPU increased the size of small but not large parenchyma cells in kiwifruit pericarp tissue. It would be useful to know if increased water uptake and expansion of these small cells affected starch synthesis within the amyloplasts.

Starch synthesis is decreased at both high and low temperatures (Mohabir and John 1988). The direct effect of temperature on kiwifruit DM% is unclear because temperature also changes fruit maturation and developmental rates (Currie and Richardson 2007). Earlier cell maturation during Stage 1 should give earlier commencement of starch synthesis, which might explain the increase in fruit DM% associated with warm spring conditions. Warm temperatures during Stage 3 can slow starch hydrolysis so that fruit maturation is delayed. This could allow an extension in dry matter accumulation resulting in higher DM% (Currie and Richardson 2007). Enclosing kiwifruits in aluminium foil reduced the fruit temperatures by 4º to 5ºC and resulted in approximately a 23% reduction in DM% by harvest (Tombesi et al. 1993), suggesting a low temperature inhibition of starch synthesis. The enclosed fruits also accumulated more water, consistent with an accumulation of unassimilated solutes causing a lower $\Psi_{fruit}$.

The accumulation of soluble solids during Stage 3 decreases $\Psi_s$ presumably within parenchyma symplastic domains. While the general principals are understood, the exact pathways and mechanisms of fruit solute accumulation during fruit maturation are not certain (Lalonde et al. 2003; Bondada et al. 2005). In particular, how does the fruit avoid excessive water influx in response to the lowered $\Psi_s$ of the solute accumulating cells? Interruption of the symplastic route by an apoplastic step and active unloading from the phloem has been suggested for tomato, grapes, and apples (Lalonde et al. 2003) and kiwifruit (Morandi et al. 2007). However, water would still tend to diffuse into the sugar loaded cells suggesting a mechanism of active water efflux back into the fruit apoplast. This in turn suggests a continued role for the xylem in removal of such surplus water back to the vine. The ability of the fruit to increase DM% during this stage must depend therefore both on physiological processes regulating sugar uptake and water efflux and on vine:fruit water gradients. Experiments need to be carefully designed to unravel these interactions. Of particular interest would be the measurement of fruit symplast and apoplast water potentials during fruit maturation under differing vine water statuses. The similarity between cell wall softening during early fruit growth and during fruit maturation suggests that similar reductions in cell turgor may occur during Stage 3 of fruit development (Rose and Bennett 1999). Thus dynamics of wall metabolism and factors affecting these processes might influence DM% during the final stages of fruit growth.

## 1.2.5 Summary and conclusions

Although fruit development and DM% characteristics are largely determined by the genome of the particular cultivar, environmental and cultural conditions may influence DM% by altering the relative uptake of dry matter and water during the course of fruit development. Increased vine:fruit water potential gradients that favour fruit water import appear to be the most likely and common cause of low DM% fruit. The potential for an uncoupling of dry matter and water import may be greatest at certain times during the developmental cycle of the fruit. Three such times corresponding to the three stages of fruit development as defined in Figure 1.1 were identified:

a. Stage 1 of fruit development. Fruit pericarp cells have a large capacity for individual and collective expansion but the development of 'machinery' for starch synthesis lags behind cell division and expansion. Solutes can accumulate in the fruit if their import exceeds the capacity of the cells to utilise them. The combination of low osmotic and turgor potentials increases water influx and lowers DM%.

b. Stage 2 of fruit development. Increased competition for assimilates from vigorous root or shoot growth may decrease the rate or duration of starch synthesis. If fruit water relations favour continued water import then fruit DM% is likely to be lowered.

c. Stage 3 of fruit development. Accumulation of solutes as part of the maturation process decreases fruit osmotic potential. If environmental conditions favour vine water uptake at this time then increased fruit water import in response to the increased vine:fruit water potential gradient could result in lower fruit DM%.

The gradual slowing of FW accumulation coincident with the maintenance of dry matter accumulation rates in the final fruit growth period leading up to harvest, as shown in growth curves, makes delaying the time of kiwifruit harvest the simplest management tactic for increasing fruit dry matter concentration.

21

Although excessive nutrient uptake especially of N and P could directly affect kiwifruit DM%, for example, by interfering with starch synthesis, the indirect effect of excess nutrients or water availability on vine vigour may be more important. Excessively vigorous vines are likely to have increased water content and this together with the stronger sink capacity of root and shoot sinks is likely to result in decreased kiwifruit DM%. The central role of N in all aspects of plant metabolism and particularly its effect on vine vigour justifies attention being given to this nutrient to further understand the regulation of dry matter and water uptake by the fruit. In the next section the effects of N availability on the vine and fruit is reviewed.

## 1.3 Effects of nitrogen fertiliser on kiwifruit vegetative and fruit growth, and fruit quality

### 1.3.1 Introduction

The replacement of nutrients removed in harvested products is often used as the basis for rational fertiliser use (Huett and Dirou 2000). Judged against this standard, fertiliser inputs to kiwifruit orchards often exceed theoretical fertiliser requirements and therefore can be considered excessive (Table 1.3). The balance of inputs and outputs is the accepted method for judging whether inputs of fertiliser are excessive and are fundamental to the guidelines for sustainable fertiliser use being produced by various environmental regulatory bodies (Edmeades et al. 2011; Anon 2007).

Table 1.3 Examples of annual fertiliser nutrient inputs for different orchards with estimated quantities removed with harvested crop (in parenthesis).

| Orchard type | Yield (t/ha) | Nutrient applied (kg/ha) | | | | |
|---|---|---|---|---|---|---|
| | | N | P | K | S | Mg |
| 'Hayward'[1] | 30 | 100 (55) | 32 (7) | 129 (96) | 60 (6) | 16 (5) |
| 'Hayward'[1] | 44 | 126 (80) | 59 (10) | 305 (140) | 212 (9) | 136 (7) |
| 'Hort16A'[1] | 59 | 211 (108) | 14 (14) | 226 (187) | 121 (12) | 29 (9) |
| 'Hayward'[2] | 45 | 226 (83) | 72 (11) | 405 (144) | 162 (9) | 83 (7) |
| Apples[3] | 70 | 30 (29) | 10 (6) | 30 (168) | - | 2 (6) |
| Grapes[4] | 13 | 5 (18) | 0 (4) | 0 (33) | - | 2 (1) |

[1] NZ Kiwifruit Journal (2005) Jan/Feb: 21-23. [2] Courtesy Colin Jenkins, Ngai Tukairangi Orchards. [3] Hawkes Bay Focus Orchard. [4] Courtesy Carla Emms, AgFirst Ltd, Hastings; applied as foliar sprays.

Application of excessive quantities of nitrogen (N) and phosphorus (P) fertilisers are of particular concern because of their association with environmental degradation (Matson et al. 1997; Tilman et al. 2002; Cantarella et al. 2003; Dymond et al. 2013). There is also evidence that supports the possibility for high fertiliser rates, especially of N and K, to have adverse effects on fruit quality. Excessive K fertilisation is associated with loss of acidity, increased pH, less stability during storage, and reduced colour of grape juice (Morris et al. 1983); and excessive N fertilisation with reduced polyphenol content and stability of oil extracted from olives (Fernandez-Escobar et al. 2002), and reduced colour, firmness, and increased storage disorders in apples,

apricots, pears, and citrus (Sanchez et al. 1995). In the case of kiwifruit, there are no reported studies of the effect of excessive K fertilisation on fruit quality, but there are reports of excessive N fertilisation having adverse effects on fruit quality, including reduced firmness and increased incidence of botrytis storage rots (Prasad and Spiers 1992; Sher and Yates 1992).

An excess or deficiency of any nutrient could be detrimental to fruit quality (Marschner 2002). However, the focus in the following discourse is on N for the following reasons:

1. Nitrate ($NO_3^-$) leaching from fertiliser inputs can be rapid and is a potential environmental pollutant (Mills et al. 2005; Anon 2013a);
2. High N availability is linked to excessive vegetative vigour, and management of excessive vegetative growth represents a major operating cost for orchards. (Elfving 1988; Woodward and Patterson 2009);
3. N is an important determinant of yield, is implicated in a wide range of fruit quality attributes and issues, and more than any other mineral nutrient changes the biochemical composition of plants (Marschner 2002); and finally,
4. It remains unclear how much N fertiliser is necessary to maintain kiwifruit orchard productivity (Mills et al. 2009).

Nitrogen is the dominant element in plant nutrition and generally contributes about 80% of the total cation and anion uptake of plants (Marschner 2002). The function of N and its assimilates in forming structural, cellular, and biochemical components of plants explains its central role in the growth and development of crops (Lawlor 2002). Nitrogen interacts with plant carbon metabolism by directly affecting the photosynthetic response to light intensity and by altering the shoot:root ratio (Gastel and Lemaire 2002). A linear relationship between N and plant growth rates exists over a wide range of plant N content (Hirose 1988). Furthermore, there may be competition between carbon and N for assimilation substrates (Lawlor 2002). Hence, N has an important role in determining the productivity of crops (Chapin et al. 1987).

Determining the fertiliser requirements of perennial crops is made difficult by the large buffering capacity of trees and uncultivated orchard soils, which can often disguise the effects of treatments (Helyar and Price 1999). This can be avoided to some extent if trials are continued over successive seasons. However, even long term trials of crop response to N fertiliser can be inconclusive because of the large number of variables involved. Another problem is the large size of orchard trees or vines that makes many measurements such as nutrient partitioning or seasonal uptake rates physically difficult to make. For this reason, many orchard nutrition and fertiliser studies use young plants. Young plants might not accurately represent much larger mature plants. For example, young plants have smaller nutrient reserves able to support early season growth (less buffering capacity), and have relatively high nutrient demands necessary to support high developmental growth rates. Nevertheless, there is abundant research that shows fertilisers are often applied far in excess of actual crop requirements and that in many cases there is no yield response to fertiliser applications (Huett 1996; Weinbaum et al. 1992) (Table 1.4).

Table 1.4 Effect of the amount of urea-N applied annually on yield and vegetative growth of olives (average of 1994-1999) (from Fernandez-Escobar et al. 2002).

| N applied (kg/tree) | Average yield (kg/tree) | Average shoot length (cm) | Average fruit weight (g) | Average oil content (%FW.) |
|---|---|---|---|---|
| 0 | 27.6 | 6.2 | 3.9 | 24.2 |
| 0.12 | 32.2 | 6.1 | 3.7 | 23.8 |
| 0.25 | 28.9 | 5.8 | 3.8 | 25.1 |
| 0.50 | 30.9 | 5.9 | 3.4 | 25.0 |
| 1 | 30.0 | 6.1 | 3.9 | 24.8 |
| Significance | NS | NS | NS | NS |

Although considerable research has already been undertaken into kiwifruit nutrition, much of this work was done more than two decades ago (Sale 1997a,b). Since then production goals in orchards have increased emphasis on fruit quality, although yield and fruit size remain important (Richardson et al. 1997).

## 1.3.2 Nitrogen supply

Although the amount of N fertiliser applied to kiwifruit orchards varies widely, rates in excess of 150 kg N ha$^{-1}$ yr$^{-1}$ are commonly used (Table 1.1) and recommended (Smith et al., 1987; Buwalda et al. 1990; Sher 1992; Sale 1997a,b). Often the contribution to the N supply from the mineralisation of soil organic matter (SOM) is hardly recognised and N rates are based on the assumption that only about 50% of applied fertiliser N will be recovered by the vine (Sale 1997b; Buwalda and Smith 1990). For example, according to a mathematical model developed to predict fertiliser requirements of kiwifruit, if the total uptake of the vine was 218 kg N ha$^{-1}$, the vine's fertiliser N requirement was estimated to be 295 kg N ha$^{-1}$ (Buwalda and Smith 1990).

How much of the seasonal demand for N needs to be supplied as fertiliser depends on the amount of N contained in vine reserves and how much is already available in the soil or made available during the season by mineralization of SOM. The amount of N available in soil solution varies seasonally but will generally be quickly immobilised and depleted by soil microbes or plant uptake. Therefore, availability in the absence of fertiliser inputs depends mainly on the replenishment of soil solution N by the mineralization of N out of SOM. Accurate estimation of fertiliser requirements is dependent on realistic predictions of the supply of N from existing soil reserves and in particular from the mineralization of SOM (Murphy et al. 1998).

Many soils used for kiwifruit are well supplied with organic matter and can be expected to generate a significant quantity of plant-available N (Sparling et al. 2001). Annual mineralization rates of between 1.5 and 3.5% of SOM-N are considered normal for temperate soils (Seiter and Horwath 2004). For soils with an SOM content of 7.5% this would equate to between 50 and 130 kg N ha$^{-1}$ becoming available for plant uptake annually. Mineralisable N in the top 10 cm of Te Puke kiwifruit orchard soils (SOM content >7%) was measured in laboratory incubations as equivalent to about 150 kg N ha$^{-1}$ yr$^{-1}$ (Sparling et al. 2001). The potential for N mineralization in Bay of Plenty (BOP) soils might be even greater where there are higher SOM levels and if a greater depth of topsoil was included. Significant recovery of N from deeper soil levels seems likely given the deep rooting habit of kiwifruit in some soils and the accumulation of fertiliser ions at depth in such soils (Greaves 1985; Sher 2004).

Direct measurement of leaching losses of N from kiwifruit have not been made but estimates using the SPASMO computer simulation estimated annual losses of between 40 and 75 kg N ha$^{-1}$ with fertiliser inputs of 120 kg N ha$^{-1}$ yr$^{-1}$ (Mills and Clearwater 2007). However, using the Overseer Nutrient Budget computer simulation gave a much lower estimate for the same N rate of just 6 kg N leached (Overseer 2009). In reality the variables that determine rates of $NO_3^-$ leaching might be too complex for current computer models to accurately simulate. Leaching of significant quantities of $NO_3^-$ is likely if heavy rainfall follows soon after application of high rates of soluble N fertilisers, but the risk of leaching is greatly reduced with modest N input rates spread over two or more split applications over the growing season (Lamb et al. 1999).

The factors that regulate N mineralization and temporal availability from SOM in kiwifruit orchard soils are complex and make it difficult to predict actual mineralization rates from SOM and more research is needed to improve our understanding. For example, soil moisture is a major factor influencing SOM mineralization of N (Paul et al. 2003), and rhizodeposition and interactions with soil microbes by groundcover plants can increase SOM mineralization (Paterson 2003) and influence N cycling (Patriquin 1986). Accumulation of SOM in orchard environments compared to pastoral ones may occur due to enhanced herbaceous productivity and increased N inputs, or reduced decomposition rates of woody litter inputs (Hudak et al. 2003). The high total microbial biomass-N and its rapid turnover rate in pasture soils compared to cultivated soils suggests large amounts of N may become available in the orchard during the season especially if stimulated by climatic or anthropic disturbances (Murphy et al. 1998; Patriquin 1986). Environmental disturbances stimulate the cycling of nutrients and disturbances by orchard personae and their equipment and materials combine to create a level of continuous disturbance that maintains a corresponding level of nutrient mineralization and cycling. Low level disturbance of the soil with mowers, mulches, fertilisers, weeding operations, herbicides, stimulate mineralization of N from SOM, but unlike with cultivation for arable or vegetable crops, the mineralization is much less and combined with maintenance of plant root and residue inputs means that SOM levels are well maintained (Carey et al. 2009). Research to increase our understanding of these

processes would allow more certainty to be had in fertiliser use and improve our management of orchard nutrition generally.

Although symbiotic N fixation is probably limited in shaded pergola type orchards (Sale 1997; Goh and Ridgen 1997), associative or diazotrophic N fixation could be stimulated by the abundance of carbon substrate from mulched prunings or compost applications (Kennedy et al. 2004; Unkovich and Baldock 2008). This aspect of N cycling also deserves attention since it may contribute to the orchard's N balance, and may be a factor in the comparable performance sometimes reported from orchards receiving low fertiliser N inputs (Sher and Yates 1992; Mills et al. 2009).

### 1.3.3 Vine demand (uptake)

Total N uptake will be determined both by N availability and vine demand (Devienne-Barret et al. 2000; Schenk 1996). Plant N demand fluctuates during the growing season according the number and strength of vegetative and reproductive sinks (Youssefi et al. 1999). Total seasonal demand is determined by vine size and vigour, and is an outcome of the interaction between the vine and its environment. Factors involved in this outcome include climate, nutrient availability, and vine management. Demand and total uptake are not equivalent because plants can continue to accumulate N above the critical concentrations necessary for normal metabolic function and maximum growth rates if availability permits (Devienne-Barret et al. 2000; Gastal and Lemaire 2002). Nitrogen availability, especially the overall supply over successive seasons, has a large influence on the growth of the vine (vine size and vigour) and hence on the creation of demand (Mills et al. 2008; Burns et al. 1997). Nitrogen availability depends on the regulation by soil moisture and temperature of SOM mineralization and on the form, rate, and timing of additional fertiliser N inputs.

The rate of N uptake of kiwifruit orchards is similar to that of other agricultural crops but more than double that of most natural ecosystems (Haynes 1986). Compared to other fruit crops, kiwifruit N uptake is high because of the large canopy area that needs to be reformed each season; current season's growth accounted for 70 – 79% of total canopy dry weight (Buwalda and Smith 1987). In four-year-old apple trees on M9 rootstock the annual growth accounted for only 10% of total tree dry weight

(Neilsen et al. 2001). In fruits such as apples and citrus a much smaller re-vegetation takes place in mature plants each season, and in wine grapes a much smaller and less vigorous canopy is maintained in order to improve the organoleptic properties of the fruit.

Estimates of seasonal uptake of nutrients by fruit trees or vines can be based on complete destructive analysis of excavated whole plants done at various times during the season. In one such study, Clark and Smith (1992) reported seasonal N accumulation by five-year-old orchard vines at 143 kg N ha$^{-1}$. In a similar study, six-year-old kiwifruit vines were estimated to absorb up to 165 kg N ha$^{-1}$ yr$^{-1}$ depending on crop load (Smith et al. 1988). Such studies are more useful for determining the distribution of nutrients and seasonal uptake trends than for indicating overall demand. For example, in these two studies, vines received 220 and 172 kg N ha$^{-1}$ at the start of the season before excavation respectively, so it is unclear whether the total uptake was more demand or supply driven.

Young vines have a higher net accumulation as they increase their biomass and fill their allotted spaces and may take up 50% more N per year than older vines with similar crop loads (Smith et al. 1988). Nevertheless, total uptake and fertiliser recovery efficiency will often be less for young vines because of their smaller biomass, less extensive root systems, and smaller crop loads (Sanchez et al. 1995; Smith et al. 1988).

The efficiency of N fertiliser recovery (NUE) by kiwifruit was estimated to be between 48 and 53% of applied N in 15N tracer studies (Ledgard et al. 1992). Although these results are in conformance to similar studies with other fruit crops (e.g., Feigenbaum et al. 1987), the tracer method is likely to underestimate the actual recovery because of factors such as pool substitution and the 'priming' effect (Woods et al. 1987); and because N can continue to cycle through organic matter for years after its application (Gratton and Denno 2003). Pool substitution refers to the situation where there is an immobilisation-mineralisation exchange between applied N and SOM-N, and the priming effect refers to the stimulatory effect of applied N on soil microbial activity, which can lead to an increase in mineralised N additional to that which has been applied. Differences in how NUE is calculated make comparisons

between studies difficult. For example, a low NUE reported by Narioa et al. (2005) of between 8 and 12% for peach trees ignored fertiliser N remaining in the soil. However, Hajrasuliha et al. (1998) claimed a 100% NUE for grapevines, but included significant residual fertiliser N to depths of 240 cm. Nitrogen moving within the short experimental period (5 months) to this depth would probably be on its way out of the rootzone (Pratt 1984). An earlier study of NUE in lysimeter-grown citrus found moderately fertilised citrus trees (58 kg N ha$^{-1}$) recovered up to 83% of applied $NH_4NO_3 - N$, although the recovery was much reduced when rain caused excessive drainage and leaching (Syvertsen and Smith 1996).

Ledgard et al.'s (1992) study may have underestimated the NUE of kiwifruit vines by not accounting for 15N immobilised into the willow shelter trees that encircled the small 0.3ha experimental plot, or into groundcovers that covered 40% of the treatment area. Although sometimes considered as lost (Ledgard et al. 1992; Sale 1997), fertiliser N incorporated into SOM will in the long term become available, especially as the system reaches equilibrium in respect to SOM accumulation. This applies also to fertiliser N recycled in prunings, which although somewhat recalcitrant (Ledgard et al. 1992), will be eventually be mineralised and made available for crop uptake (Loganathan et al. 2000; Rowe et al. 2005). Nitrogen use efficiency may be much reduced by leaching and denitrification losses if N fertilisers are placed in single applications in early spring as recommended by Sale (1997). The numerous factors influencing NUE, including the timing of applications and form of N, need more research if improved efficiencies and environmental outcomes are to be realised in horticultural production systems.

The estimates of N removal in fruit range from about 35 kg N ha$^{-1}$ for a 6000 tray yield (21 tonne ha$^{-1}$) to about 83 kg N ha$^{-1}$ for a 12,000 tray crop. Removal rates can vary according to fruit N% and yield. Beever and Hopkirk (1990) reported fruit N content to range from about 0.093% to 0.163% (FW) so the range for a 6000 tray crop would be between 20 and 34 kg N ha$^{-1}$. But a larger crop of 12,000 trays could remove up to about 70 kg N ha$^{-1}$. Higher fruit N contents of up to 1.42% (DW) were reported by Smith et al. (1991). Using this value would give a N removal rate in a 12,000 tray crop of about 95 kg ha$^{-1}$. It is unclear how crop load affects fruit N concentration but some high yielding orchards also have high N inputs (Anon 2005),

which may result in high fruit N% depending on the vigour of the canopy and how strongly it competes with the fruit for N (Mills et al. 2008).

Nitrogen fertilisation is necessary to sustain these N loss rates. Nevertheless, high levels of fertility built up after many years of fertilisation offer a valuable buffer that provides an opportunity to minimise the use of inefficient soluble N forms in favour of slow release amendments such as compost or foliar applications. Slow release forms of N lower the quantity of $NO_3^-$ present at any one time in the soil solution, thereby reducing the risk of significant $NO_3^-$ leaching

### 1.3.4 Nitrate

In well-aerated agricultural soils, $NO_3^-$ is the main form of N in the soil solution taken up by crops (von Wiren et al. 1997). Because of its high mobility in the soil, movement to the roots and replenishing of the rhizosphere is mainly by mass flow regulated by plant transpiration, with diffusion making a smaller contribution (Miller and Cramer 2004). Uptake by the roots is mostly active via proton coupled symporters ($2H^+$: $1NO_3^-$) and has a relatively low energy demand calculated to represent about 10% of the total N nutritional costs (Miller and Cramer 2004; Raven 1985). At very low endogenous $NO_3^-$ concentrations (<200 µM) uptake is by a saturable high affinity transport system (HATS) in root hairs (von Wiren et al. 1997). At higher concentrations (>200 µM) uptake is by non-saturable and inducible low affinity transport system, also concentrated in root hairs (von Wiren et al. 1997). Because of the effectiveness of the HATS plants can achieve optimum growth at very low concentrations of $NO_3^-$ in the soil solution. For example, 90% of optimum growth could be achieved in some plant species at $NO_3^-$ concentrations in the soil solution of only 14 µM $NO_3^-$-N (Clement et al. 1978). However, at low levels of N availability, plants typically respond with increased assimilate partitioning to the roots. In kiwifruit the peak of root growth overlaps with that of fruit dry matter accumulation during Stage 2 of fruit development, which suggests that under conditions of low N availability fruit growth might be reduced. Although girdling during Stage 2 could counter this effect it is unclear what the effects of sustained low N availability (e.g., if N was omitted from annual fertiliser inputs) in combination with girdling would have on vine health.

Nitrate is chemically very reactive and its reduction and assimilation bears a high energy cost and may compete for energy resources with reduction of $CO_2$ in fruiting plants or in low light situations, leading to reduced carbohydrate synthesis (Marschner 2002). Within the plant $NO_3^-$ is considered to be the primary signal molecule involved in N assimilation and can induce multiple gene responses in tissues within minutes of exposure (Crawford 1995). For example, in *Arabidopsis* shoots 183 genes were identified as responding within 20 minutes of exposure to $NO_3^-$, while in tomato roots over 1200 genes responded to $NO_3^-$ exposure within 96 hours (Wang et al. 2001). Nitrate uptake increases the synthesis of organic acids, decreases starch synthesis, changes plant hormone levels, and alters shoot:root allocation and root morphology (Stitt 1999). Nitrate also leads to wide ranging and rapid changes in enzyme transcription involved in carbon and N metabolism (Stitt 1999). The capacity of $NO_3^-$ to increase plant water uptake has been noted in a wide range of species (McIntyre 1997; Cardenas-Navarro et al. 1999). This might involve the increased activation or induction of aquaporins by $NO_3^-$ (Guo et al. 2007; Wang et al. 2001) and its role as an osmoticum lowering the $\Psi p$ (McIntyre 1997).

There are few published reports of $NO_3^-$ levels in kiwifruit berries. In two commercial orchards $NO_3^-$ concentration on a dry weight basis in fruit ranged from 60 to 80 ppm (Pickston et al. 1980). The levels of $NO_3^-$ found were high compared to other fruit included in the survey such as, citrus, grapes, and peaches, although no details of N fertiliser inputs to the studied orchards were included (Pickston et al. 1980). Walton and De Jong (1990) reported $NO_3^-$ levels in 'Hayward' fruit of 140 ppm (dry weight) 52 days after flowering but by harvest no $NO_3^-$ was detected. Nitrate accumulation in other fruits increases with increasing N availability and $NO_3^-$ uptake (Chairidchai 2000; Menary and Jones 1972; Hoff and Wilcox 1970), and it is therefore reasonable to expect this would also be the case in kiwifruit. Although in respect to human food safety, levels of $NO_3^-$ are unlikely to accumulate to toxic levels in fruit, as they can in some vegetables where concentrations >2500 ppm can be found (Blom-Zandstra 1989). Nevertheless, considering the activity of $NO_3^-$ in initiating wide ranging physiological responses and effects, even small amounts in developing fruit could have an effect on fruit quality.

Most plant tissues, including fruit, possess the capacity for $NO_3^-$ reduction, although $NO_3^-$ in excess of the tissue's reduction capacity can be stored in cell vacuoles, and this might even be a preferred storage form due to the low energy cost involved (Schroeder 2006). Because of the limited mobility of $NO_3^-$ in the phloem, the persistence of detectable levels in kiwifruit at maturity could indicate that higher concentrations were present at earlier times during the fruit's development (e.g.,Walton and De Jong 1990). With the high transpiration rates of the fruit during early developmental stages, the supply of $NO_3^-$ in the xylem flow would be increased depending on its concentration in the xylem. The concentration of $NO_3^-$ in the xylem is increased with N fertilisation. Given the potential for $NO_3^-$ to interact with vine and fruit metabolism the effect of elevated $NO_3^-$ uptake on fruit quality deserves further study.

### 1.3.4.1 Nitrate reduction

Nitrate taken up by the plant can be stored in vacuoles of roots, shoots, and storage organs, or assimilated into organic compounds, in which case it must first be reduced to $NH_3$ (Marschner 2002). Nitrate uptake, assimilation, and particularly $NO_3^-$ reduction (NR) are energy intensive processes. In barley 15% of the energy from root respiration is used for NR, compared to 5% for uptake and 3% for assimilation (Marschner 2002). In leaves, NR may alleviate the effects of excessive light (photo-inhibition and photo-oxidation). Although kiwifruit leaves require a high light level to be light-saturated and photosynthesis (Pn) is light limited for much of the season, photoinhibition could result from exposure of previously shaded leaves to high light (Buwalda and Smith 1990). However, NR also competes with the plant's energy reserves with reduction of $CO_2$ in fruiting plants or low light situations so that elevated NR can result in reduced carbohydrate synthesis.

Nitrate reduction can occur in roots or shoots depending on the level of $NO_3^-$ supply, and the age and species of the plant. At low levels of availability most reduction takes place in roots, but as supply increases, root capacity becomes saturated and $NO_3^-$ is translocated to shoots. However, subtropical and tropical perennials tend to reduce more in the shoots even at low external supply (Marschner 2002). Metge (1980) found the $NO_3^-$ content of kiwifruit xylem sap ranged between 10 and 50% of the total N content and concluded that compared to apple (most reduction in roots) and cocklebur

(most reduction in shoots), kiwifruit was intermediate in the distribution of NR between roots and shoots.

For a given species, NR in roots increases with temperature and plant age (Marschner 2002). This suggests that NR in kiwifruit leaves may decrease during the summer relative to the spring and also decrease with older plants. Metge (1980) also found increased NR in roots with increasing air temperature but this effect was only found in one district of the two included in his investigation, and considered NR to occur mostly in the leaves of young kiwifruit plants based on the distribution of NR between different organs. The accompanying cation also influences the distribution of $NO_3^-$ reduction. For example, when $K^+$ is the accompanying cation, translocation of both $K^+$ and $NO_3^-$ to shoots is more rapid than if $Ca^{+2}$ or $Na^+$ is the cation (Marschner 2002). Thus NR rates in leaves or N content within the vine generally is likely to be positively affected by high K fertiliser rates and availability.

Nitrogen fertilisation is likely to increase the ratio of NR shoot:root and this is confirmed by studies that show increased sap flux (Peuke 2000; Hubbard et al. 2004) and sap $NO_3^-$ concentration in eucalyptus (Hubbard et al. 2004), broccoli (Belec et al. 2001), tomato (Anderson et al. 1999), and grapevine (Roubelakis-Angelakis and Kliewer 1979) in response to N fertilisation. No published research discusses the effect of increasing $NO_3^-$ availability or N-fertilisation on kiwifruit xylem sap composition or NR distribution or intensity. Measurements of the N composition of xylem sap in kiwifruit and NR have been done on vines receiving conservative N fertiliser rates compared to those sometimes found in New Zealand orchards (Metge 1980; Ferguson et al. 1983) (Table 1.3). Nevertheless, high $NO_3^-$ concentrations and $NO_3^-$:reduced-N ratio in xylem during late December (Ferguson et al. 1983; Metge 1980; Clark and Smith 1991), together with high transpiration rates at this time, supports the idea that NR could be at high levels during this critical period of fruit development (Beever and Hopkirk 1990). In the study by Metge (1980), $NO_3^-$ content of the xylem sap fell during the month between bud-break and leafing out, but then rose again coincident with canopy filling. This pattern, and that recorded later in the season, roughly coincides with the rainfall distribution and fertiliser applications for both sites used in the study. An increase in $NO_3^-$ content of the xylem sap later in the

34

season is also consistent with increased root growth at this time (Buwalda and Hutton 1988).

### 1.3.5 Uptake and vegetative response

Nitrogen stimulates the vegetative growth of many plants, including kiwifruit, due to its dominant role in most aspects of plant nutrition and physiology (Mills et al. 2008; Marschner 2002; McIntyre 2001). A linear relationship between N and plant growth rates exists over a wide range of plant N contents (Hirose 1988). However, $NO_3^-$ uptake stimulates growth more strongly than $NH_4^+$, which tends to reduce the growth of plants if they are supplied only with this N form (Miller and Cramer 2004). To maintain optimum growth in typical edaphic environments where N availability fluctuates widely, plants can accumulate N in excess of immediate metabolic requirements and store it for later use. For example, in maize, shoot N% can be 65% higher than the level required to achieve maximum above-ground biomass (Devienne-Barret et al. 2000). Nitrate is stored in cell vacuoles with a homeostatic regulation of the concentration being maintained (Cardenas-Navarro et al. 1999). Thus the quantity of $NO_3^-$ that can be stored in vacuoles is related to the water content and size of the vacuoles, plus their number. The indeterminate growth habit of a liana such as kiwifruit includes a large capacity for vacuolar $NO_3^-$ accumulation as well as an expanding vegetative sink, which increases the total vacuolar storage capacity. The expanding photosynthetic canopy also increases the energy supply for NR and provides a sink for the products of N assimilation. This allows the vine to monopolise soil resources by immobilising them within its expandable vegetative sink so they become inaccessible to other plants (Dillenburg et al. 1993). In slow growing plants that lack a vegetative response to N and a large capacity for cell expansion, $NO_3^-$ taken up in excess is effluxed rather than accumulated (Miller and Cramer 2004). The root morphology of the kiwifruit supports its capacity for high rates of nutrient uptake with its high root densities (high compared to other fruit species such as apples or grapes) and variable root distribution patterns that might follow the high spatial heterogeneity of soil and nutrient rich patches (Gandar and Hughes 1988; Cain et al. 1999). The capacity of kiwifruit to accumulate large quantities of $NO_3^-$ and to thrive in conditions of high N availability places it in the category of nitrophilous plant species (Bharucha and Dubash 1951).

A close coupling between $NO_3^-$ and water uptake has been noted in a wide range of species (McIntyre 1997; Cardenas-Navarro et al. 1999) and has been attributed to the osmotic effect of $NO_3^-$ and its reduction products on water uptake combined with its nutritional effect on protein synthesis (Fricke et al. 1997; McIntyre 1997; Guo et al. 2007). The combination of protein synthesis and water drives the expansion of plant tissues. Because it is usually the dominant anion in the soil solution of fertilised soils, $NO_3^-$ regulates the soil solution concentration of cations and thereby also tends to govern the uptake of many other nutrient cations (Okajima 1977; Yanai et al. 1998; Guo et al. 2007). This promotes the effect of $NO_3^-$ and water on vegetative vigour by contributing further to tissue osmotic potentials and by increasing the supply of other potentially limiting nutrients (McIntyre 2001). An increasing availability of $NO_3^-$ in the soil is associated with a proliferation of fine roots increasing the surface area for water absorption leading to increased vine water uptake (Green and Clothier 1995). Associated with the increased proliferation of small roots in response to $NO_3^-$ is an increase in the synthesis of cytokinins, which are transported up into the canopy where they stimulate meristemic cell division (van der Werf and Nagel 1996; Gebler et al. 2004; Sakakibara et al. 2006). Cytokinins are also associated with activation of genes in the cell wall that regulate the activity of expansins – proteins that regulate softening of the cell wall and its capacity for expansion (Downes and Crowell 1998). With these combined effects of $NO_3^-$ and water driving canopy expansion, transpiration is correspondingly increased which drives further increases in water and $NO_3^-$ uptake resulting in an exponential vigour – $NO_3^-$ relationship until some limitation to growth intervenes, whether environmental, nutritional, or genetic. This is particularly true for kiwifruit which, like other woody lianas, has a more indeterminate growth habit than most other fruit species and will respond to increased soil N availability with rapid extension growth (Dillenburg et al. 1993).

### 1.3.6 Yield

Generally increasing N application rates have been associated with only small or nil yield gains in kiwifruit (Buwalda et al. 1990; Costa et al. 1997; Vizzotto et al. 1999; Clark et al. 1992). Some studies were limited by including only a single season and lacking a nil-N control (Smith and Miller 1992; Testoni et al. 1990). The effect of different fertiliser rates on vine yield need to be investigated over many seasons

because the buffering capacity of mature vines. Soils can reduce treatment effects and make single season trials difficult to interpret correctly. Where yield responses did occur they were due to increased bud break and flower numbers rather than increased fruit size (Smith and Miller 1992; Buwalda et al. 1990). Increased bud-break and flower numbers following very high fertiliser rates was probably more due to osmotic effects of the fertiliser ions generating increased sap flow rather than being a nutritional effect (Smith and Miller 1992). Increased sap flow with fertiliser applications has been observed in grapevines (Peuke 2000). In another study with kiwifruit there were increased fruit numbers in one out of two seasons with high N rates of 750 kg N ha$^{-1}$ (Buwalda and Meekings 1993), which might also have been an osmotic effect and not a nutritional one as suggested by the authors, since even the nil-N vines had very high leaf N% measured six weeks after bud-break (4.1% N on a dry weight basis). The levels reported for the nil-N vines by Buwalda and Meeking (1993) are high in relation to those given as the normal range for either *A. deliciosa* or *A. chinensis* at a similar phenological stage, of 2.4% to 4.0% (Hills 2010a,b) or for 'Hort16A' vines given nil-N or 295 kg N ha$^{-1}$ yr$^{-1}$, where levels between 2.4% and 2.7% respectively were found (Mills et al. 2008). The high leaf N% in Buwalda and Meeking's (1993) trial might reflect low vine vigour; in a vigorous plant the concentration of elements in leaves can be diluted due to the so-called 'growth dilution' effect (Smith et al. 1987). In respect to the osmotic effects of fertiliser, the relationships between vine N status, root pressure, soil EC, and bud development in kiwifruit need further investigation. The early studies showing increased flower and fruit numbers with high levels of fertiliser were done prior to the introduction of the use the plant growth regulator hydrogen cyanamide (Hi-Cane®), which also has this effect (McPherson et al. 2001). However, with the imminent withdrawal of Hi-Cane®, from horticultural use in NZ (Chamberlain 2010) and the possibility of reduced winter chilling due to climate change (Kenny 2012), the osmotic effects of fertiliser on bud-break might become more important once again. It would be useful to find possible other solutes which could be substituted for $NO_3^-$ and allow a beneficial effect on bud-break without the inducement of unwanted vegetative growth.

In a trial by Buwalda et al. (1990) four N rates from 0 to 200 kg N ha$^{-1}$ yr$^{-1}$ were applied to four-year-old kiwifruit vines at two planting densities. Only the high

density planting (8.33 $m^2$ $vine^{-1}$) had consistently higher yield at the highest N rate (Figure 1.3). High planting densities have been reported to reduce root development in kiwifruit (Massai et al. 1997) and would have made the vines more dependent on N inputs compared to the low density vines since N uptake is positively correlated to root volume (Bar-Yosef et al. 1988). A similar N yield response was reported in young close planted kiwifruit spaced at 7.6 $m^2$ $vine^{-1}$ and 10 $m^2$ $vine^{-1}$ by Testoni et al. (1990) and Smith and Miller (1992) respectively. In Buwalda et al.'s (1990) trial, the low density planting (25 $m^2$ $vine^{-1}$) only recorded a yield response to increasing N rates in one out four years (Figure 1.3). In the other three seasons the N rate of 50 kg $ha^{-1}$ had the highest yield and yields declined with increasing rates above this.
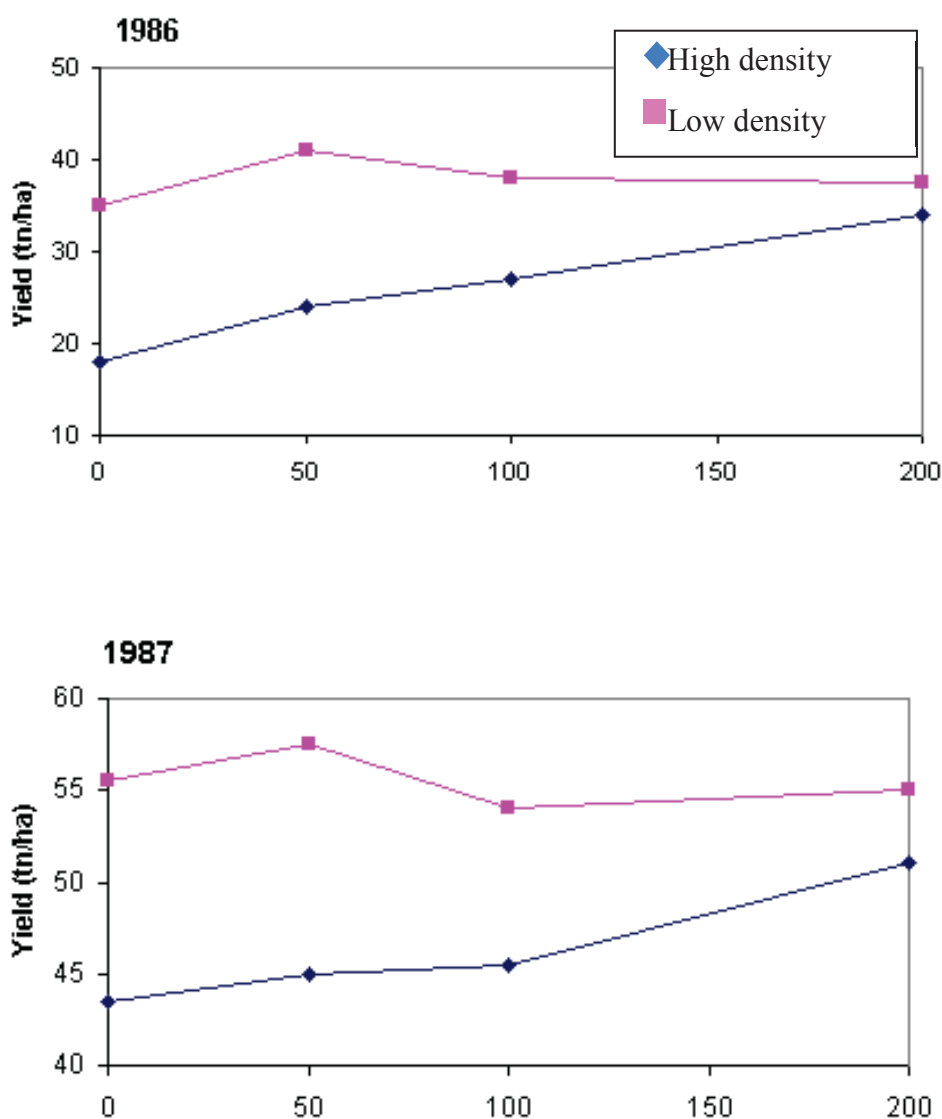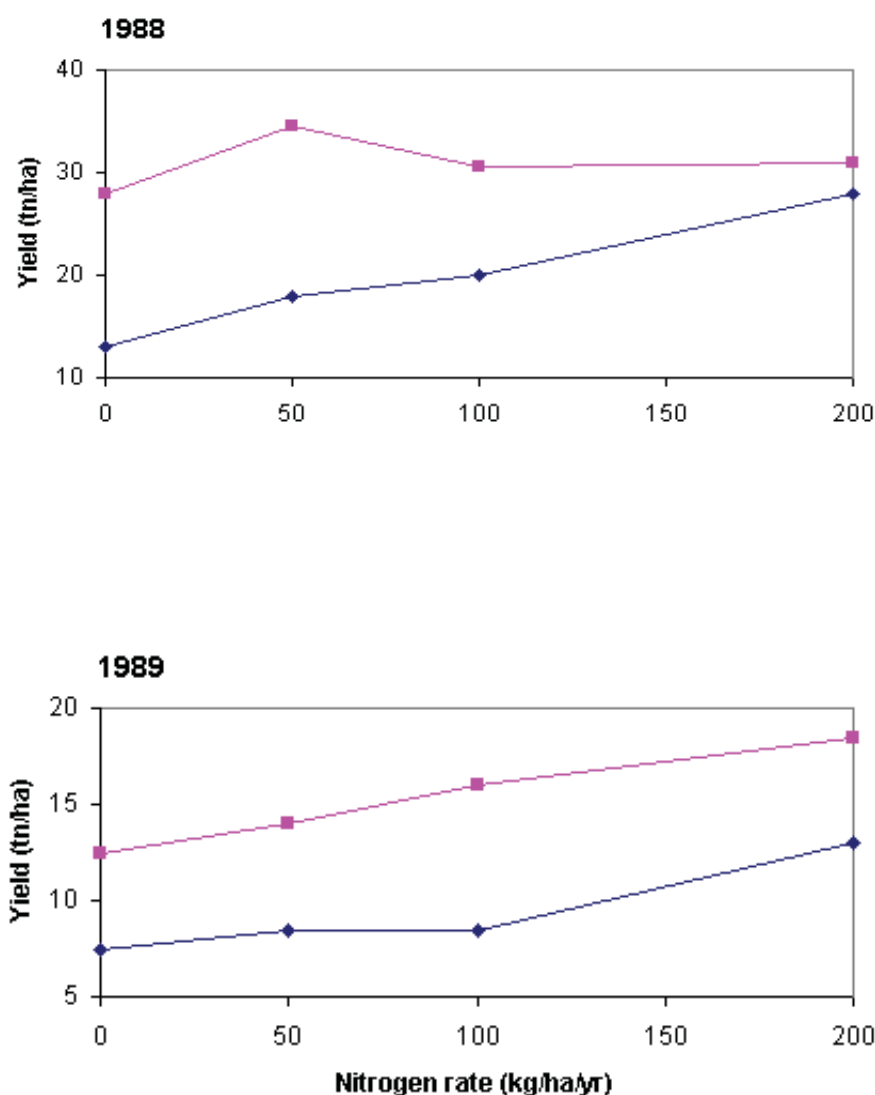
Figure 1.3 Effects of different nitrogen fertiliser rates on kiwifruit yield at high and low vine planting densities and in four seasons (after Buwalda et al. 1990).

Climatic variability and biennial bearing patterns further complicate the results of fertiliser trials. For example, Buwalda et al. (1990) claimed the yield decline observed during the last three years of their trial was due to declining vine N status indicative of insufficient N even at the higher rate of 200 kg N ha^{-1}. However, examination of the data and climatic records suggests it was more due to a biennial bearing trend (Figure 1.4) and adverse climatic conditions (low winter chilling and cyclonic summer storms) during the last two seasons of the trial (Wilson 1989; McAneney et al. 1989). There were widespread vine deaths in the region due to summer waterlogging during this period (McAneney et al. 1989). Kiwifruit are extremely susceptible to root anoxia

(Smith et al. 1990) and the symptoms of leaf necrosis and N deficiency reported by Buwalda et al. (1990) in the low N vines of their trial during the 1989 season are consistent with this effect. Waterlogging and root death is associated with reduced N uptake and increased response to N fertilisation (Ashraf and Rehman 1999; Russell 1973, p 418). The higher yields recorded for all treatments in 1987 (Figure 1.3) followed an exceptionally good winter with high chilling and good fruit set (Wilson 1989).
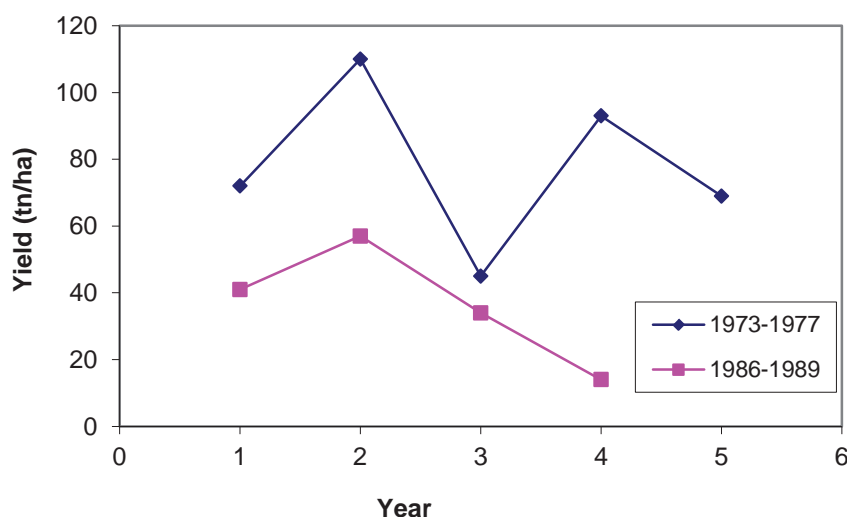


Figure 1.4 Biennial trends in kiwifruit yields; 1973-1977 from Davison (1990); 1986-1989 from Buwalda et al. 1990).

In the 1989 season, the increased yield (Figure 1.3) and leaf N concentration (%) with higher N fertiliser rates, and the development of N deficiency symptoms at the lower N rate as reported by Buwalda et al. (1990) is consistent with the effects of a wet season. In this situation, there would be less available N in the lower N rate treatments because of leaching and denitrification. However, the amount of $NO_3^-$ lost to leaching and denitrification would have also been higher with increasing N rates and therefore the higher rates would have lower NUE and be less sustainable from the environmental perspective.

In 1989 all treatments including the nil-N treatment had leaf N% at four weeks after bud-break above the optimum range for kiwifruit at this time (Sale and Lyford 1990). Although by 20 weeks after budbreak, leaf N% in the nil-N treatment (2.0%) had declined to below the optimum (2.2 –2.8%) it was still well above the deficiency range (<1.5%) as given by Sale and Lyford (1990). Leaf N% for the 100 kg N ha$^{-1}$

rate (2.5%) was within the optimum range and barely, if at all, significantly different from the 200 kg N ha$^{-1}$ rate (2.7%). Neither the leaf N% or yield data from this trial seem to support the author's claim for N rates for kiwifruit needing to be in excess of 200 kg N ha$^{-1}$ in order to maintain yields and vine N status, or their contention that vine and orchard N status during the period of the trial was declining. Rather it seems that both yield and leaf N% were strongly affected by seasonal climatic fluctuations during the trial period.

The effect of N fertiliser on leaf N% is complex because of soil and climatic factors that affect plant recovery of fertiliser and plant factors such as the dilution effect (when growth is stimulated by increased N availability. For example, a reduction in leaf N% with increasing N rates was noted in kiwifruit by Testoni et al. (1990). Management practices that affect the size of the soil pool of N would also affect the long-term response to fertiliser-N. The '…normal commercial management…' of the vines in Buwalda et al.'s trial would have included a wide herbicide strip (Sale and Lyford 1990). This practice can cause a decline in SOM content as well as reducing soil pH (Hogue and Neilsen 1987). Reduced SOM content could be expected to reduce the supply of indigenous soil N, and a lower pH could increase the $NH_4^+:NO_3^-$ ratio, thereby reducing the uptake of fertiliser-$NO_3^-$ (Peuke 2000). The interplay between fertiliser inputs, crop load, climate, and vine N content needs more elucidation.

Yield reductions with increasing N rates or compared to nil-N treatments have been found in at least three studies. Testoni et al. (1990) reported a 20% yield reduction when N was increased from 200 to 300 kg N ha$^{-1}$ and Vizzotto et al. (1999) reported yield reductions ($p<0.05$) with N rates of 350 kg ha$^{-1}$ and above 150 kg in two seasons respectively (Figure 1.5). Yield reductions at N rates above 50 kg ha$^{-1}$ yr$^{-1}$ were also reported by Buwalda et al. (1990), although these were statistically significant ($p<0.05$) in only one out of four seasons. Yield depression with excessive N fertilisation also occurs in other crops (Marschner 2002). In the study by Testoni et al. (1990) leaf N% declined as N was increased from 100 to 300 kg N ha$^{-1}$, which is suggestive of a growth dilution effect. Stimulation of vegetative growth by N fertilisers is a common effect and excessive vegetative growth could reduce fruit yield by shading fruit and fruit buds, or by shifting vine phenology and increasing the risk of frost damage, and in addition yield was reduced with the higher N rate (Testoni et

al. 1990). In the study by Vizzotto et al. (1999), the largest yield reduction in the last year of the trial was due to a greater level of frost damage to buds of high N vines. The recovery of yields after the third season by the nil-N vines suggests that vines can adapt well to changing soil conditions (Vizzotto et al. 1999).
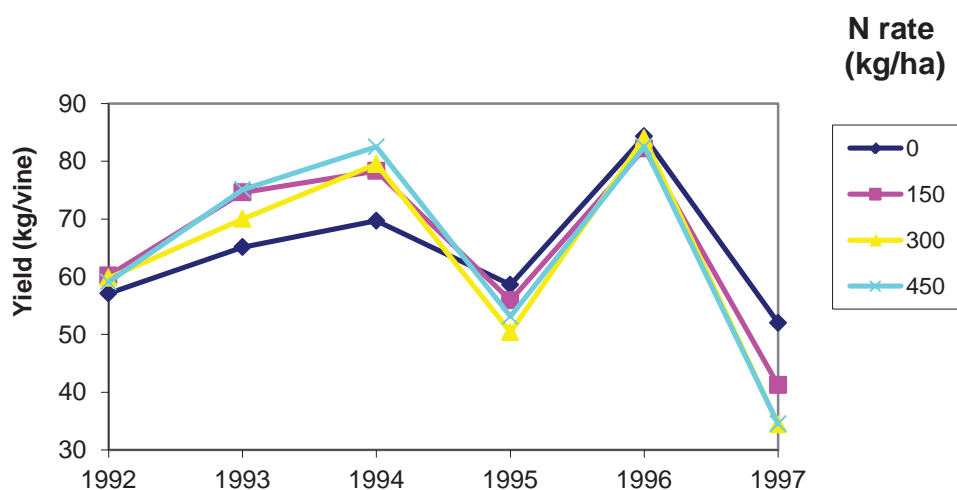


Figure 1.5 Kiwifruit yields (kg/vine) over 6 years at 4 different nitrogen fertilisation rates (from Vizzotto et al. 1999).

Especially in view of the ambivalent evidence for N effects on kiwifruit yield, a better understanding of how N affects vine physiology and productivity would assist in determining efficient N application strategies. Nevertheless, the existing data suggests that high N application rates (>150 kg N ha$^{-1}$ yr$^{-1}$) do not increase kiwifruit yield.

### 1.3.7 Fruit size

Fruit size determined by fresh weight (FW) is an important component of yield and the larger size grades of kiwifruit receive higher returns (Sale 1998). Excessive N fertilisation can increase or decrease fruit FW according to different reports (Weinbaum et al. 1992). Generally, increases in kiwifruit FW in response to N fertilisation have occurred infrequently or have only occurred at the lowest rates with no further increase as the N rate was increased. Buwalda et al. (1990) found N fertilisation increased FW in only one out of six years, in which case average FW was significantly increased by 5.6% when N was increased from 0 to 200 kg ha$^{-1}$. Costa et al. (1997) reported that the lowest N rate of 150 kg ha$^{-1}$ yr$^{-1}$ increased average FW by 10% compared to the nil-N control, but there was no further increase with higher rates

of 300 and 450 kg N ha$^{-1}$ yr$^{-1}$. Using the same range of N rates, Johnson et al. (1997) claimed no effect on FW over two successive years. Similarly, Buwalda and Meekings (1993) found N application up to 750 kg N ha$^{-1}$ yr$^{-1}$ in two seasons had no effect on FW compared to a nil-N control (section 1.3.6). In 'Hort16A' FW was increased by N applications of 145 or 295 kg ha$^{-1}$ yr$^{-1}$ compared to the nil-N control, but only in one out of three years (Mills et al. 2008).

Similar inconsistent or minor FW responses to N fertilisation have been reported for apples (Hipps and Perring, 1989; Wargo et al. 2003), and avocado (Broadbent et al. 1989). However, when apples were grouped into commercial size categories, there were increased numbers of larger sized fruit in response to N fertilisation (Wargo et al. 2003). This approach also revealed size differences between N treatments in kiwifruit. Nitrogen applications at rates equivalent to 50 or 122 kg N ha$^{-1}$ increased the number of fruits within the optimum size range (80-120 g) compared to fruit from unfertilised vines Prasad et al. (1986). Similarly, Tagliavini et al. (1995) in a two year study found unfertilised vines had a smaller proportion of kiwifruit in the preferred size range (80-120 g) than fruit from vines fertilised at 100 or 200 kg N ha$^{-1}$yr$^{-1}$ but increasing the rate from 100 to 200 kg N ha$^{-1}$ had no further significant effect on the size distribution. Lack of a FW response to N fertilisation might also be due to the trees or vines being already adequately supplied with N from previous fertiliser applications (e.g., Vizzotto et al. 1999), from N contained in irrigation water (e.g., Buwalda et al. 1990), or from mineralization of soil organic matter (Sparling 2001).

In 'Hort16A', average fruit size was 5.6% and 9.6% smaller from vines receiving 0 and 145 kg N ha$^{-1}$ respectively, compared to fruit from vines given 295 kg N ha$^{-1}$, but only in one out of two seasons (Mills et al. 2008). In the third season there were no significant differences between the three N treatments in fruit size. Decreased FW in kiwifruit has also been observed in fruit growing in shaded conditions (Grant and Ryugo 1984), which may occur in canopies whose growth has been stimulated by N fertilisation. In other crops, decreases in FW were reported in some seasons for tomato receiving high rates of N (Everett 1976) and blueberries at various rates of N (Austin and Bondari 1989).

## 1.3.8 Dry matter

In kiwifruit at harvest about 35% of the dry matter of the fruit is starch, nearly all of which will be converted to sugar by the time the fruit is ready to eat (Richardson et al. 1997a). The concentration of sugar in the ripe fruit is a major factor in consumer satisfaction. Thus the dry matter concentration (DM%) of the fruit at harvest is an important predictor of quality at eating ripeness (Richardson et al. 1997a). Interactions between N nutrition and carbohydrate metabolism are well known in many crops (Chapin et al. 1987). However, there is little published research on the effect of N fertilisation on DM% in kiwifruit. Boyd and Barnett (2007) reported a negative correlation between fruit N concentration and DM% in 'Hort16A'. In Mills et al.'s (2008) trial with 'Hort16A' fruit DM% was slightly higher in the nil-N treatment than in fruit from 145 and 295 kg N ha$^{-1}$ treatments in the third year of the trial but there were no significant differences in the previous two seasons. However, Smith et al. (1994) reported a positive correlation ($r^2 = 0.37$ and $r^2 = 0.54$ for pergola and T-bar grown fruit respectively) between 'Hayward' fruit N and DM% measured after 12 weeks cool storage. Lower fruit DM% has also been associated with low fruit Ca concentration (Ferguson et al. 2003), and reduced Ca uptake following N fertilisation was found in 'Hort16A' (Mills et al. 2008).

There are likely to be a large number of factors other than N that could contribute to reduced DM% in kiwifruit. For example, both winter and summer pruning affect vine physiology. Following pruning the vine responds by growing to restore the root:shoot balance. Buxton (2005) found fruit on long un-pruned shoots had higher DM% than the fruit on pruned laterals. It is likely that this relates to the induced re-growth and consequent diversion of carbohydrates to the re-growing shoots (Snelgar et al. 2012; Stevenson et al. 2006). Higher DM% is typical of fruit from vines less than 4 years old and could be due to the smaller number of meristems and lower vigour of young vines (Stevenson et al. 2006). Other factors that have been associated with effects on DM% in kiwifruit include canopy density (Patterson et al. 2006), plant growth regulators (PGRs) (Burdon and Lallu 2006), girdling (Currie et al. 2008b), water uptake (Currie et al. 2008a), canopy position (Smith et al. 1994), and climate (Snelgar et al. 2007). Nevertheless, N could also be interacting with these effects. For example, $NO_3^-$ content of plant tissue can increase in shade due to the dependence of nitrate reductase activity (NRA) on light (Stitt et al. 2002); there are close relationships

between PGRs and N and $NO_3^-$ (Sakakibara et al. 2006); girdling could interrupt phloem transported signals involved in the regulation of N and $NO_3^-$ uptake (Goren et al. 2004); plant water content might be related to $NO_3^-$ content as the plant strives to maintain a constant $NO_3^-$ concentration (McIntyre 1997); spatial variation in $NO_3^-$ and N in the canopy (Ferguson et al. 1981) is similar to the spatial distribution of fruit DM% (Thorp et al. 2003); and finally, the dependence of $NO_3^-$ availability, uptake, and assimilation on soil moisture and temperature and therefore on climate is well known (Miller and Cramer 2004).

### 1.3.8.1 Water accumulation

Nitrate uptake could affect vine and fruit water content by acting as an osmoticum (McIntyre 1997; section 1.3.5). Evidence suggests that $NO_3^-$ can substitute osmotically for carbohydrates in conditions of low-light or defoliation (McIntyre 1997). Such an effect would have important implications for kiwifruit where canopies become too dense or are excessively defoliated during summer pruning. Increased synthesis of soluble N forms with increased $NO_3^-$ uptake could also contribute to the osmotic effect of $NO_3^-$ on vine water uptake (Zhang et al. 2012) and protein synthesis and hydration could further lower osmotic potentials to increase cell water uptake (McIntyre 1997).

Gloser et al. (2007) found root hydraulic resistance was rapidly decreased by 20% in sunflower roots exposed to increased soil $NO_3^-$ availability and attributed this to increased aquaporin activity. The expression of water channels or aquaporins in roots and cell membranes can be increased in response to increased levels of $NO_3^-$ (Guo et al. 2007; Wang et al. 2001). Aquaporins could also play a role in water relations and phloem unloading processes within the fruit (Zhou et al. 2007). Aquaporins have been implicated in this role in ripening grape berries (Picaud et al. 2003). They are the most abundant proteins in pear fruit cell tonoplasts and are thought to play an important role in fruit cell expansion and growth (Katsuhara et al. 2008). Interestingly the reduced growth and lower shoot water potential of plants supplied solely with $NH_4^+$-N appears to be due to the rapid suppression of aquaporin activity in roots by $NH_4^+$ (Guo et al. 2007). Increased gene expression of aquaporins will not necessarily result in increases in net fruit water uptake since this will still depend on the gradients established by solute accumulation, transpiration, and cell wall expandability.

However, increased aquaporin expression may increase short term water fluxes allowing more rapid responses to any temporary or diurnal variation lowering of $\Psi_{fruit}$. Also aquaporins regulate membrane permeability affecting water flow in both directions so efflux of water from fruit could prevent a net increase in water uptake.

### 1.3.8.2 Dry matter accumulation

Nitrogen deficiency is likely to result in a decreased leaf area, reduced photosynthesis, and a reduced flow of assimilates to the fruit (Buwalda and Meekings 1993; Stitt et al. 2002; Manter et al. 2005). Based on measured increases in quantum efficiency and saturated photosynthetic rate Buwalda and Meekings (1993) predicted increased photosynthetic rates for vines receiving up to 750 kg N $ha^{-1}$ $yr^{-1}$. Unfortunately such increases in photosynthetic rate may not benefit fruit dry matter accumulation because of increased competition for assimilates from N stimulated shoot growth (Minchin et al. 2010; Snelgar et al. 2012). Furthermore, the high energy requirement for $NO_3^-$ reduction and assimilation consumes carbon and carbon skeletons for organic acid synthesis, which especially under low light conditions and supra-optimal N supply could reduce the flow of carbohydrates to the fruit (Chapin et al. 1987; Stitt et al. 2002). Smith and Miller (1992) reported increases in leaf chlorophyll with N fertiliser applied at bud-break at rates between 480 and 1200 kg N $ha^{-1}$ compared to a control receiving 200 kg N $ha^{-1}$. However, although chlorophyll content increases with increasing N supply, the photosynthetic rate can be less if N levels are supra-optimal, due to lower efficiency of the chloroplasts in which the chlorophyll accumulates (Jia et al. 1999; Bondada and Syvertsen 2003). Similarly, although leaf Rubisco content increases linearly with increasing N supply the ratio of active to inactive forms of the enzyme decreases (Manter et al. 2005). Thus the relationship between N supply and photosynthesis is curvilinear and maximum photosynthetic rates are found at an immediate leaf N concentrations (Bondada and Syverstsen 2003; Manter et al. 2005). Respirational losses of C may also increase with N supply due to energy consumption for protein turnover and maintenance (Manter et al. 2005). Consistent with some of these effects, Clark et al. (1992) reported fruit from vines receiving no N fertiliser accumulated significantly more dry matter than those from vines given 250 kg N in a single application at bud-break. Thus there is the potential for both supra- and sub-optimal N supply to adversely affect fruit dry matter supply.

46

### 1.3.9 Starch and soluble solids

Starch begins to accumulate in 'Hayward' fruit from about 50 DAFB until it accounts for about 50% of total fruit dry matter at about 126 DAFB (approx. one month before harvest). Between about 119 DAFB and 140 DAFB there is a marked change in the physiology of the fruit leading to a rapid decrease in starch and increase in sugars (Beever and Hopkirk, 1990). By harvest, which is usually delayed until the soluble solids content (SSC) reaches a concentration of 6.2% ($^{\circ}$ Brix), starch comprises about 35% of total fruit dry matter (Richardson et al. 1997a).

Because starch makes such an important contribution to ripe fruit quality it is also important to understand the factors influencing its synthesis and accumulation in the fruit. Nitrogen and $NO_3^-$ might affect starch accumulation in the fruit by interaction with photosynthesis in the canopy, with the potential to either increase or decrease the supply of carbohydrates as discussed in the previous paragraph. There are numerous reports of reduced carbohydrate synthesis being attributed to increased N uptake (e.g., Marschner 2002; Stitt 1999; Scheible et al. 1997; Aloni et al. 1991; Table 1.5).

Table 1.5 Effect of increasing nitrogen supply ($NH_4NO_3$) on dry matter production and composition of ryegrass (from Marschner 2002).

| | Nitrogen supply (g/pot) | | | |
|---|---|---|---|---|
| | 0.5 | 1.0 | 1.5 | 2.0 |
| Dry matter (g/pot) | 14.9 | 23.2 | 26.2 | 26.0 |
| Composition (% DW) | | | | |
| Total N | 2.0 | 2.8 | 3.6 | 4.2 |
| Sucrose | 7.7 | 7.3 | 7.1 | 6.3 |
| Polyfructosans | 10.0 | 4.3 | 1.8 | 1.1 |
| Starch | 6.1 | 3.4 | 2.1 | 1.4 |
| Cellulose | 14.4 | 13.9 | 13.9 | 17.6 |

In tobacco leaf tissue $NO_3^-$ was shown to be a signal for the regulation of starch synthesis (Scheible et al. 1997). The expression and activity of key enzymes for starch synthesis were inhibited by increasing $NO_3^-$ concentrations. Starch degradation may

also have been stimulated by $NO_3^-$ (Scheible et al. 1997). Nevertheless, the concentrations of $NO_3^-$ involved (27.9 mM $g^{-1}$ FW) were much higher than were reported for kiwifruit fruit tissue (2.3 mM $g^{-1}$ FW; Walton and DeJong 1990).

Nitrate is also directly involved in the regulation of cytokinins (Sakakibara et al. 2006) and in cell culture studies a depletion of auxin and exposure to cytokinin triggered the formation of amyloplasts (Lopez-Juez and Pyke 2005). The differentiation of amyloplasts (starch storing plastids) early in fruit development might determine the potential capacity of the fruit for starch accumulation.

Reduced starch concentration but no differences in SSC (P>0.05) in 'Hayward' kiwifruit at harvest were associated with N fertilisation in the long term study reported by Vizzotto et al. (1999). Mills et al. (2008) reported 'Hort16A' fruit from nil-N vines had lower starch concentration in one out of two years compared to fruit from vines receiving 295 kg N $ha^{-1}$.

### 1.3.10 Oxalate

Kiwifruit contain relatively high levels of oxalate (Beever and Hopkirk 1990). Oxalate crystals in kiwifruit are considered an 'anti-nutrient' (Rassam and Laing 2005) because of their potential to cause irritation in the mouths and throats of some people eating the fresh fruit (Perera et al. 1990). The total oxalate content of kiwifruit is comparatively low and is in an insoluble form making absorption unlikely (Ritter and Savage 2007). The insoluble oxalate in kiwifruit is precipitated as crystals of calcium oxalate, which represents an immobilisation of Ca. In kiwifruit leaves 79% of leaf Ca was in the form of calcium oxalate crystals (Clark et al. 1987). This might have implications for the occurrence of Ca related disorders in the fruit (Hopkirk et al. 1990).

#### 1.3.10.1 Oxalate and $NO_3^-$ reduction

About half the N in kiwifruit xylem sap by flowering is $NO_3^-$ (Davison 1990). The presence of $NO_3^-$ in xylem sap and leaf petioles indicates that leaves are active in $NO_3^-$ reduction. The reduction of $NO_3^-$ in shoots is a common characteristic of subtropical plants but also increases in other plants when uptake of $NO_3^-$ exceeds the capacity of roots for $NO_3^-$ reduction (Marschner 2002). The assimilation of $NO_3^-$

produces an excess of $OH^-$, which can be neutralised by the synthesis of organic acids as counter-ions. Both may then be stored in cell vacuoles. In species where $NO_3^-$ assimilation occurs in the shoots, oxalic acid can be the charge balancing acid and has the advantage that it can be precipitated as calcium oxalate in the vacuoles thereby maintaining osmotic potentials (Raven and Smith 1976). Surplus $NO_3^-$ can also be stored directly in the vacuole using oxalate as a balancing ion (Granstedt and Huffaker 1982; Raven and Smith 1976). This might explain the increased oxalate levels found in some shaded plants (Morita and Tuji 2002). This suggests the oxalate content of kiwifruit might be increased with increased levels of $NO_3^-$ fertiliser. Rinallo and Modi (2002) reported oxalate in kiwifruit varied with different N sources and was increased by $NO_3^-$ compared to $NH_4^+$, which is consistent with other studies. For example, oxalate content in tea increased with increasing leaf $NO_3^-$ content and concentration in the nutrient solution (Morita et al. 1999). Increased oxalate content in association with $NO_3^-$ fertilisation was also found in sugar beet (Joy 1964), tomato, and spinach (Libert and Franceschi 1987).

### 1.3.11 Acidity

The acidity of ripe kiwifruit plays a major role in determining consumer satisfaction (McMath et al. 1992). Different organic acids vary in their influence on taste and the perception of acidity in kiwifruit (Marsh et al. 2006). The main acids in kiwifruit are citric, malic, and quinic, although numerous other acids such as ascorbic acid (Vitamin C; Dawes and Keene 1999) and amino acids (Lobit et al. 2002) also contribute to total acidity of the fruit. The composition of organic acids, their concentration and relative amounts, in kiwifruit may be affected by N fertilisation, as is the case in peaches (Jia et al. 1999), grapes (Renquist and Reid 2001), sour cherry (Hansen 1997), and citrus (Albertini et al. 2006; Smith 1966). Stimulation of organic acid synthesis by N fertilisation and $NO_3^-$ has been widely reported. For example, in tobacco leaf tissue increasing $NO_3^-$ fertilisation stimulated the synthesis of organic acids which are used in the assimilation of N or as counter-anions for $NO_3^-$ reduction (Scheible et al. 1997).

There are few reports of N fertilisation effects on kiwifruit acidity. In two Italian studies there was no significant effect on fruit titratable acidity (TA) when kiwifruit vines were fertilised with 0, 150, 300, or 450 kg N $ha^{-1}yr^{-1}$ over two years (Vizzotto et

al. 1999), or at N rates between 100 and 300 kg ha$^{-1}$ yr$^{-1}$ in a single season study (Testoni et al. 1990). However, fruit from New Zealand orchards may be more acid than equivalent fruit from elsewhere (MacRae et al. 1989). High fruit acid levels in New Zealand kiwifruit may be caused by lower autumn temperatures or dense canopies as was suggested in respect to New Zealand citrus (Richardson 1996). Clark et al. (1992) found increased levels of the amino acid arginine in fruit from N fertilised vines, although fruit TA was not recorded. The relationship between TA and °Brix is more important than TA alone in determining fruit taste quality so both these variables need to be measured and assessed (Rossiter et al. 1999; Woodward and Clearwater 2007). The close correlation between DM% at harvest and °Brix suggests that the ratio between TA and DM% at harvest would also indicate fruit organoleptic quality (Woodward and Clearwater 2007).

### 1.3.11.1 Amino acids

Amino acid synthesis is increased by $NO_3^-$ fertilisation (Stitt 1999), and increased amino acid content in fruit can have an adverse effect on fruit flavour (Jia et al. 2000). Increasing the rate of N fertilisation increased the total free amino acid content of kiwifruit by up to 360% at harvest (Clark et al. 1992). Measurement of individual amino acids showed that, at the time of harvest the dominant species, arginine, had increased from 4 µM g$^{-1}$ dry weight in the nil-N fruit to 25 µM g$^{-1}$ dry weight in fruit from vines receiving 250 kg N ha$^{-1}$. Jia et al. (2000) found that N fertilisation at 250 kg N ha$^{-1}$ also increased the amino acid content of peaches (Jia et al. 2000). Furthermore, using taste panels they determined that increased contents of asparagine and arginine in high N fruit were responsible for the unsavoury and poor flavour of this fruit. Kiwifruit from nil-N vines had higher proportions of amino acids such as glutamate (Clark et al. 1992), which in peaches were associated with improved flavour (Jia et al. 2000). The strong correlation between fruit arginine and N content (Clark et al. 1992) and the increase in fruit N with increasing N fertilisation rates (Buwalda et al. 1990; Costa et al. 1997) indicates not only a decrease in fruit flavour but also an increase in TA (Lobit et al. 2002) are possible negative outcomes of high levels of N fertilisation.

### 1.3.11.2 Ascorbic acid

Ascorbic acid or Vitamin C is an important fruit quality attribute affecting not only fruit acidity but also its nutritional value. Kiwifruit contain relatively large amounts of ascorbic acid, an attribute used to promote the fruit as being particularly healthy.

Vitamin C is consistently reduced in fruit as N fertiliser rates increase (Benbrook 2005). This effect has been found in many fruit and vegetable crops. For example, in black currants increasing N fertilisation rates reduced Vitamin C and sugar content. In this case DM% content (measured by specific gravity) was also reduced although seasonal and cultivar effects on these quality attributes were greater (Ljones 1966).

### 1.3.12 Phenolics

Phenolic compounds are a wide range of secondary metabolites derived from the shikimate pathway and phenylpropanoid metabolism and these compounds are involved in many aspects of fruit quality including flavour, taste, disease and stress resistance, maturation, and the rate of softening (Macheix et al. 1990; Antolovich et al. 2000; Robards et al. 1999). Evidence exists that protein and phenolic synthesis are competitive metabolic pathways, and increasing N fertilization causes increased protein synthesis at the expense of phenolics or secondary metabolites. There are no published reports on the effect of N fertiliser on the phenolic content or levels of aroma volatiles in kiwifruit. However, reduced phenolic content in fruit with increasing N fertilisation rates has been reported for grapes (Pirie and Mullins 1976; Delgado et al. 2004), blueberries (Witzell and Shevtsova 2004) and strawberries (Anttonen et al. 2006). The reduction of phenolic content in plant tissues might explain the increased disease susceptibility of crops as N fertiliser rates are increased (Kiraly 1964). Increasing N rates, by increasing the reducing power of tissues, can lower the activity of enzymes important in phenolic synthesis. Increasing N rates can also alter some of the active properties of phenolic compounds (Kiraly 1964). Although kiwifruit have low levels of phenolics compared to some other fruits, these compounds are generally important for the flavour and taste of fruit (Dawes and Keene 1999). A major flavour compound in kiwifruit is the ester, ethyl butanoate (Marsh et al. 2003). Esters are included in the range of phenolic compounds whose synthesis depends on availability of phenylalanine, the substrate also is needed for protein synthesis. This dependency is the basis of the suggested competition between protein and phenol synthesis in plant tissues (Jones and Hartley 1999). Plant demand for protein therefore increases with increasing growth rates and hence the correlation between phenolic content and plant vigour.

Phenolic compounds are involved in the delay of fruit softening (Macheix et al. 1990). Loss of firmness in N fertilised kiwifruit has been consistently reported (see section 1.3.14 below) and could be due to reduced phenolic content. Furthermore, phenolic compounds found in kiwifruit such as quercetin glycosides and chlorogenic acid have been shown to inhibit softening in apples (Dawes and Keene, 1999; Macheix et al. 1990). Phenolic compounds are also important in auxin regulation. Since the degradation and loss of auxin can promote amyloplast differentiation, alteration of phenolic metabolism due to excessive $NO_3^-$ uptake could also influence starch accumulation. Understanding the complex interaction between $NO_3^-$, cytokinins, auxins, and phenolics would add to our knowledge of kiwifruit starch accumulation and other aspects of fruit quality.

### 1.3.13 Variability

Consistency of product quality is important for most industries. Variability between kiwifruit in quality attributes especially DM% and postharvest disorders such as premature softening and loss of firmness, has been recognised as a problem for the kiwifruit industry (Praat et al. 2001). About 60% of the variability found in fruit dry matter (%) of kiwifruit crops can be found within single vines (Max 2004). This is also the case for fruit size and mineral content (Ferguson et al. 2003). Therefore sampling and analysis of individual fruit is an appropriate strategy for investigating the effects of $NO_3^-$ on fruit quality.

Most studies have found a tendency for fruit on the distal ends of canes and from short terminated shoots to have lower DM% than fruit closer to the cordon or carried on long non-terminated shoots (Boyd et al. 2004; King et al. 2006; Wakefield and Max 2007). There are no studies relating such spatial variation in fruit quality attributes to N fertilisation. However, Thorp et al. (2003) found fruit from short terminated shoots in outer canopy positions had lower DM, Ca, Mg, and Mn, and higher P, S, and Na contents than fruit from inner canopy shoots.

Since $NO_3^-$ is highly mobile within the plant differences between shoot types and canopy positions in fruit quality attributes might be due to higher or lower concentrations of $NO_3^-$ at those sites. A gradient of increasing $NO_3^-$ content from roots to youngest shoots was found in xylem sap of kiwifruit and this gradient was

opposite to that of most other nutrients (Ferguson et al. 1981). It is of interest that lower ascorbic acid (Remorini et al. 2007) and SSC (Zang et al. 2004) have also been found in shaded shoots. Nitrate is likely to accumulate in shaded shoots because of reduced nitrate-reductase activity and the effect of $NO_3^-$ on ascorbic acid content in fruit has already been shown (Table 7). Another zone of low DM% fruit has been identified close to the main trunk (King et al. 2006). In this part of the canopy there is likely to be increased shading from strong 'water' shoots and replacement canes overgrowing the fruiting laterals (King et al. 2006). A similar effect might be involved in other canopy positions where short terminated shoots are more likely to be shaded by longer un-terminated growth. Therefore the mineral nutrient and DW differences found in fruit from short shoots may reflect lower xylem/phloem fluxes as a result of lower transpiration and photosynthetic rates. Nevertheless, higher leaf:fruit ratios on large diameter canes compared to small diameter canes did not result in different elemental concentrations (Thorp et al. 2003). This suggests meristematic activity and increased hormone synthesis in un-terminated shoots leads in turn to increased fruit metabolic activity and related sink strength.

Between and within vine variability needs to be taken into account during research so that an appropriate sampling methodology is used (Ferguson et al. 2003).

### 1.3.14 Fruit firmness

Increasing N fertiliser rate in kiwifruit is likely to result in decreased fruit firmness at harvest (Buwalda et al. 1990; Vizzotto et al., 1999; Testoni et al., 1990) or after storage (Vizzotto et al., 1999; Testoni et al. 1990; Prasad and Spiers 1992; Johnson et al. 1997; Smith et al. 1994). However, others have claimed N fertiliser has no effect on postharvest fruit firmness and found no simple direct relationship between fruit N% and fruit firmness (Smith and Miller 1991; Buwalda et al. 1990). Nevertheless, Smith and Miller (1991) did not include a nil-N control the lowest N rate and the results of Buwalda et al. (1991) were strongly affected by climatic extremes (section 1.3.6) and therefore cannot be considered representative.

In brambles, increasing N fertilisation was associated with softer fruit (Ljones 1966). As with kiwifruit softer fruit can also develop with wet weather. Both wet weather

and increased N are likely to lead to increased water uptake which suggests that both N and wet weather have similar effect mechanisms in respect to fruit firmness.

Prasad et al. (1986) found negative correlations between sap $NO_3^-$ concentration in leaf petioles of kiwifruit in December and postharvest fruit firmness. In later trials, they found both leaf petiole sap $NO_3^-$ and fruit N concentrations at harvest to be correlated with the rate of postharvest fruit softening (King et al. 1987; Prasad et al. 1988). Johnson et al. (1997) reported similar findings and found N to be more strongly and consistently associated with fruit firmness than K, Ca, or the N:Ca ratio. Costa et al. (1997) found no difference in firmness at harvest of fruit from fertilised and non-fertilised vines, but following 4 months of storage, fruit firmness declined with increasing N fertiliser rate. These results were repeated in the subsequent years of the trial (Vizzotto et al. 1999). Finally, Hasey at al. (1997) found organically grown kiwifruit had lower leaf N% and equal or greater firmness than conventionally grown fruit.

### 1.3.15 Conclusions

Despite considerable research over many years, it remains unclear how much fertiliser N should be applied to kiwifruit orchards. High levels of available N may induce excessive vigour in the vines and could be detrimental to fruit quality. However, maintaining adequate levels of N within the vines is essential to maintain their productivity and fruit quality. An alternative way of supplying the necessary N might avoid some of these problems. Foliar applications instead of soil applications might offer such an alternative and will be reviewed in the next section.

## 1.4 Foliar-applied nitrogen

### 1.4.1 Introduction

The capacity of plants to absorb nutrients through aerial tissues has long been recognised (Wittwer and Teubner 1959; Fernandez and Eichert 2009). It may even be a deliberate adaptive strategy in some species (Burkhardt 2010). Foliar nutrition has a long history of use in horticulture and published research dealing with the foliar nutrition of fruit trees, particularly apples and citrus, is abundant (Swietlik and Faust 1984; Fernandez and Eichert 2009). The main environmental risk associated with soil-applied N fertiliser is leaching of $NO_3^-$, the levels of which can increase dramatically in the soil following N fertiliser application (Marschner 2012; Parfitt 1991). Foliar applications of N can supplement the soil supply and thereby reduce the quantity of N fertiliser that needs to be applied to the soil (El-Otmani et al. 2004; Alexander and Schroeder 1987).

Foliar nutrient application also has significant advantages in terms of nutrient use efficiency (NUE) with higher proportions of the applied nutrient being recovered by the crop compared to soil-applied nutrients (Weinbaum et al. 2002). For example, the average recovery (i.e. uptake) of N from foliar applications is about 40% higher than that of soil-applied N (Weinbaum et al. 1992), although estimates of N recovery from fertilisers might underestimate the NUE of soil applications since N can continue to cycle through organic matter for years after its application (sections 1.3.2 and 1.3.3; Gratton and Denno 2003). Nevertheless, generally the greater efficiency of foliar-applied N does offer an opportunity to reduce the amount of nutrients applied to a crop. Thus foliar-applied N can reduce the risk of $NO_3^-$ leaching and is an important technique for efficient nutrient management in sustainable fruit production systems (Dong et al. 2005; Alexander and Schroeder 1987).

Foliar-applied N can also allow supplementary N to be supplied at specific times during the season when there is an increased demand for N (Klein 2002; Mengel 2002). Competition for carbohydrates by vegetative and reproductive sinks reduces root growth and N uptake during the early part of the season (Marschner 2002). This is reflected in the phenological patterns of root, shoot and fruit growth which typically sees root growth peaking later in the season after the main vegetative and reproductive

growth has slowed (Smith et al. 1988). The direct application of N to parts of the plant where it is currently needed avoids a delay associated with soil-applied N due to the dependence of root uptake on variables affecting root activity and N uptake such as soil moisture and temperature (Weinbaum 1988; Miller and Cramer 2004). The efficiency of foliar-applied N is supported by the high mobility of N forms within the plant that allows rapid translocation to sink tissues (Porro et al. 2010). Foliar-applied N might also avoid unwanted stimulation of vegetative vigour that is often associated with soil-applied N (Dong et al. 2005; Klein 2002).

Foliar-applied N is usually supplementary rather than alternative to the soil N supply because of the difficulty in supplying sufficient quantities of a macronutrient such as N via the leaves to completely satisfy a crop's N demand. This would require either excessively concentrated spray solutions likely to result in foliar damage (i.e., phytotoxicity) or an unpractical number of separate dilute applications. Autumn pre-senescence applications could allow stronger solutions to be used since leaf damage has less import due to imminent natural defoliation. Furthermore, N absorbed by leaves from autumn foliar applications is efficiently translocated from leaves into perennial organs and storage tissues (Swietlik and Faust 1984; Xia and Cheng 2004 and references therein). Nevertheless, autumn applications of foliar urea above 5% caused bud death in apple (Wood and Beresford 2000).

According to Weinbaum et al. (2002) foliar-applied N is not effective if plant is adequately supplied with N but only if the supply and uptake is insufficient to meet the crop's N demand. However, evidence for this view appears to be limited to the effects of post-harvest sprays on fruit trees (e.g., Weinbaum 1988). In studies of foliar N (urea) applied to citrus over the fruit set period there was significant uptake even in plants with leaf N already above 3% (Albrigo 2002) and other studies are presented below that suggest N status of the plant is not the only factor that determines the effectiveness of foliar N. Foliar N can also be complementary to the soil supply because if foliar nutrient applications are timed to coincide with specific phenological stages they can increase yield, fruit size, or quality even in trees with no nutrient deficiency in terms of accepted optimum leaf nutrient levels (Gonzalez et al. 2010).

## 1.4.2 Forms of foliar N

Urea is a common foliar treatment in perennial fruit and arable crops (Mengel 2002) and is the most effectively absorbed form of N for foliar application (Furuya and Umemiya 2002). Although only known as a fertiliser since the invention of the Haber-Bosch manufacturing process early in the twentieth century, it is ubiquitous in nature and plants are well adapted to its assimilation as a N source (McLaren and Cameron 1996; Wang et al. 2008). Urea also dominates the experimental literature reporting on foliar-applied N in fruit and other crops. According to Jones (1954) urea was first used as a foliar spray in apples in 1943. Inorganic forms of N can also be efficiently absorbed by plant leaves. Potassium nitrate ($KNO_3$) is widely used in crops to improve fruit quality and yield and can be an effective source of both K (Sing and McNeil 1992; Coker et al. 2009) and N (Weinbaum 1978). It has been widely used for foliar K nutrition where K uptake is limited by soil conditions (Calvert 1969; Howard et al. 1998; Nelson et al. 2005).

Other forms of inorganic N have also been used for foliar application, although mainly for experimental purposes. These include ammonium nitrate (Guvenc and Badem 2002; Pares et al. 2010; Schreiber et al. 2002), ammonium sulphate (Bowman and Paul 1992; Tan et al. 1999), ammonium chloride (Furuya and Umemiya 2002), and sodium nitrate (Furuya and Umemiya 2002; Tan et al. 1999). Sodium nitrate was more effectively absorbed by peach leaves than other nitrate or amino forms of N (Furuya and Unemiya 2002). Calcium nitrate causes russetting of kiwifruit even at low concentrations (Hopkirk 1990).

Various proprietary products containing a variety of N forms and meant for foliar application are available. For example, a slow release form of urea, Trizone-urea has lower volatilisation properties and by remaining on the leaf longer acts as a slow release (2-3 weeks) source of N (Clapp and Parham 1991). The product is claimed to have lower phytotoxicity than urea (Clapp and Parham 1991) although some reports are inconclusive (Widders 1991) or contradictory (Wedenfeld 2009) in this regard. Trizone-urea resulted in higher leaf N% after two months than urea, and might be a means of reducing the number of foliar applications needed (Bondada et al., 2001). Amino acids can also be absorbed by leaves but the efficiency of uptake is less than

for inorganic N forms (Furuya and Unemiya 2002). Foliar-applied N has also been combined with PGRs. For example, $KNO_3$ when combined with 2,4-D increased citrus fruit size (Erner et al. 1993).

Although tolerance to foliar-applied urea or $KNO_3$ varies between different species, concentrations above about 1.2% are generally only suitable for postharvest or pre-leaf fall applications because of the risk of leaf damage (Weinbaum 1978).

### 1.4.3 Use of foliar nitrogen in kiwifruit

There is scant published research regarding the use of foliar-applied N in kiwifruit. Two trials on 'Hayward', the first with a commercial foliar fertiliser product containing N applied 10 times during mid and late season and the second using urea (4% w/v) applied twice postharvest failed to find any beneficial effects (Mulligan 2007; Boyd et al. 2007). In the first study, fruit DM% was significantly reduced by the foliar treatments apparently because of reduced dry matter accumulation, although insufficient detail was provided in the report to understand this effect. In grapevines however, a significant reduction in non-structural carbohydrates including glucose and fructose followed autumn foliar application of urea (Xia and Cheng 2004). Approximately 60% of the carbon reduction in non-structural carbohydrates was found in proteins and amino acids reflecting the increased demand for carbohydrates for carbon skeletons and energy associated with N assimilation (Xia and Cheng 2004). Phytotoxic effects of the foliar treatments is another possible explanation for the reduction of DM% found by Mulligan (2007). Foliar sprays of a seaweed extract were reported to reduce kiwifruit shoot growth, which might indicate a phytotoxic effect as well (Perham 1999).

In the second study, the urea was applied to the leaves after harvest, a practice which has been reported to increase shoot growth (Han et al. 1989; Shim et al. 1972) and fruit growth (Johnson et al. 2001) in the following season in apples. Nevertheless, the effect of autumn foliar applications of N probably depends on the N status of the plant (Swietlik and Faust 1984) and phenology of the species involved. In the study of Boyd et al. (2007) the vines were apparently well supplied with N from soil mineralisation, soil-applied N fertiliser, and accumulated reserves. No yield or fruit

set response to autumn-applied urea was found in apples having a high N status according to Swietlik and Faust (1984). In respect to phenology, kiwifruit fruit growth commences later in the season than temperate deciduous fruits such as apples, pears, and peaches. In these other species, fruit growth commences more or less simultaneously with shoot growth in the spring so that competition between developing fruit and shoots for a limited supply of N remobilised from storage pools may make responses to autumn applied N more likely.

Apart from N, trials have been done with foliar Ca, Fe, and extracts of seaweed. Foliar Ca is applied to kiwifruit to improve storage quality and reduce the incidence of physiological disorders (Boyd et al. 2004; Rouhana 2004). Iron sulphate sprays can be successful for the amelioration of lime-induced iron chlorosis in kiwifruit (Rombola et al. 2002). Although seaweed extracts are commonly used in kiwifruit orchards (John Evans personal comment) there is only one published report showing beneficial effects on fruit from seaweed foliar sprays. In this study one to two sprays of a 1-2% seaweed extract applied to selected canes increased average fruit FW by up to 23% and advanced maturity of the fruit by about 10 days (Chouliaras et al. 1997). However, a similar trial in New Zealand with foliar application of three different commercial seaweed extracts found no significant effects on kiwifruit FW (Snelgar et al. 2006).

Kiwifruit leaves have numerous anomocytic stomata on the abaxial surface of leaves and many trichomes, features likely to facilitate uptake of foliar-applied substances (Haynes and Goh, 1977; Ferguson, 1990). Such a dense indumentum probably also assists spray retention (Hall et al. 1997). When kiwifruit are grown on a pergola training system, the underside of leaves and the fruit hanging below the canopy are well exposed to ground applied foliar applications. Furthermore, kiwifruit have a thin epidermal layer and high rates of surface conductance, compared to some other fruit such as apple, making efficient absorption of foliar N likely, especially during early fruit development (Smith et al., 1995). Thus kiwifruit appear to be well suited to foliar nutrition. However, the abaxial leaf surfaces and young fruitlets of both main cultivars of kiwifruit are very difficult to wet and repellent to spray droplets (Pathan and Gaskin 2008). Therefore surfactants able to reduce the surface tension of spray

droplets may be necessary to achieve effective uptake of foliar-applied N, especially during the early season (Pathan and Gaskin 2008).

### 1.4.4 Effect of foliar N on other fruits

Fruit size can be increased by foliar-applied N, particularly with applications during early fruit development. Foliar-applied urea during the early season increased the size of apples (Dong et al., 2005), citrus (Lovatt, 1999), and olive (Inglese et al. 2002).

In apples, fruit size, fruit number, and yield were significantly increased by a series of seven 0.5% urea foliar sprays applied fortnightly over the period of fruit growth (Dong et al. 2005). The effect of the foliar-applied urea on fruit size and yield was almost the same as the effects of the same amount of urea applied to the soil at the same times. In an earlier study with apples, three or six sprays of 0.5% urea increased fruit size by 11 and 15% respectively compared to an unfertilised control but there was no increase in yield, perhaps because of a thinning effect of the urea, some of which was applied just before or at petal fall (Fisher et al. 1948). Urea applied at this time is known to have a thinning effect in apples (Handschack and Alexander 2002) and other fruit species (Curretti et al. 2013). Reduced fruit number following application of foliar urea at full bloom probably also explains why Fallahi et al. (2002) found foliar urea had a negative effect on yield of young dwarf apples. Although phenological details were not given in the paper by Dong et al. (2005), their foliar program began in mid-May, which might have been several weeks after petal fall in an early flowering Gala apple crop used for their experiment and thus they may have avoided fruit being thinned (Anon 2013b). Foliar urea applied at monthly intervals during the growing season increased the yield of adequately fertilised apple trees by increasing fruit set and their resistance to thinning treatments but did not affect fruit size (Wargo et al. 2003).

In pear, strong solutions of urea (up to 7.5% w/w) applied during or close to full bloom reduced fruit set, which probably accounted for the associated increase in fruit size (Sanchez et al. 2008). However, Curetti et al. (2013) reported that an application at full bloom of 5% urea increased fruit size of 'Bon Cretien' pears with only a slight

thinning effect. Fruit cell number but not cell size was significantly increased by the foliar urea in this study.

In citrus, pre-blossom (period of flower initiation) foliar urea applications (1% w/v) increased yield mainly by improving fruit set rather than fruit size (Rabe 1994; Ali and Lovatt 1994; Otmani et al. 2004). Similar results were reported by Albrigo (1999) and Lovatt (1999). When applied post-blossom at the end of the cell division stage (Stage 1 of fruit growth; see Fig 1.1) and beginning of the cell expansion stage (Stage 2 of fruit growth; see Fig 1.1) foliar-applied urea was more effective at increasing fruit size without reducing yield (Otmani et al. 2004; Lovatt 1999). It has been suggested that increased fruit set following even a single pre-blossom foliar urea spray is due to a transient increase in $NH_3$-$NH_4^+$ status in the treated tissues since the effect of foliar-applied urea on citrus fruit set did not appear to depend on the overall N status of the tree and therefore was not just due to the alleviation of N deficiency (Ali and Lovatt 1994; Lovatt 1999). Nevertheless, the effects of pre-blossom foliar urea on citrus are not consistent, with no effect on yield or fruit size being found by Chao and Lovatt (2006). Such contrasting results may be due to seasonal, environmental, or genetic differences between reported trials. For example, although two studies with contrasting results were with the same mandarin cultivar, one was a two year study in Morocco (Otmani et al. 2004) while the other was a single season trial in California (Chao and Lovatt (2006).

Urea is not the only form of foliar-applied N that has been successfully used in citrus. Potassium nitrate (4% w/v) and a proprietary product based on $KNO_3$ sprayed on young fruitlets increased yield and average fruit size of three citrus varieties (Achilea et al. 2002). A similar result was found in citrus with 5% $KNO_3$ by Erner et al. (1993). Relating the application dates given in these Israeli studies with phenological stages for citrus in the same growing region indicates that the applications coincided with the period of cell division (Stage 1 of fruit growth; Shalom et al. 2012). Less consistent results were reported by Calvert (1969) in a two season trial with citrus in Florida where $KNO_3$ was applied at concentrations between 2.4% and 7.0%. Yield was increased by $KNO_3$ in 'Valencia' oranges in both seasons, in 'Hamlin' oranges in one of the seasons, but not significantly in 'Temple' oranges in either season. Comparing the application dates given by Calvert (1969) with phenological details for

citrus in the same growing region indicates that the foliar applications were made during a later stage of fruit development (Stage 2 and 3 of fruit growth), which might explain the inconsistent results of Calvert's study (Albrigo 2013). Fruit size of tangerine was increased with foliar $KNO_3$ applied at dormancy (February), post-bloom (~April) and at exponential fruit growth stage (July-August) (Boman 2002).

In olive, $KNO_3$ (3%) applied during the middle and towards the end of fruit development increased average fruit weight, but yield was increased most with foliar urea (2%) and $K_2SO_4$ (4%) applied at the same times due to increased fruit retention (Inglese et al. 2002). In another trial with olives, urea (1.5%) was applied twice weekly over the growing season to individual shoots, which treatment increased fruit set and weight, and shoot growth (Cimanto et al. 1990). The success of foliar N application to citrus and olives might be related to the greater importance of the leaves in evergreen trees as potential nutrient storage pools (Weinbaum 1988).

Grapes present a challenge in respect to N nutrition although low levels of N in the berries inhibit the fermentation process; very low levels in the vine are usually maintained in order to avoid unwanted vegetative vigour (Jackson and Lombard 1993). Therefore, grapes would seem to be an ideal crop for foliar applications of N, but the literature reporting research with foliar-applied N on grapes is relatively scarce (Porro et al. 2006). Two mid-season dilute foliar applications of urea had no effect on yield or bunch weight but did significantly increase yeast available N in the juice (Lacroux et al. 2008). In another study with two grape cultivars, two mid-season applications of foliar-applied $KNO_3$ (1%, 2%, and 3%) significantly increased yield and berry weight, and had significant effects on must chemistry leading to positive effects on organoleptic quality of the wine (Altindisli et al. 1999; Kalkan et al. 1999). Other studies have focused on maintaining vine N status with autumn applications or increasing N content of the grape must (Xia and Cheng 2004; Schreiber et al. 2002).

### 1.4.5 Effect of foliar N on vegetative vigour

Foliar-applied N is generally less stimulatory to vegetative growth than soil-applied N (Klein 2002). The stimulation of vegetative growth by soil-applied fertiliser can occur at even quite low rates of fertiliser N for example 50 kg N ha$^{-1}$ increased shoot

biomass by 70% in grapes (Conradie 2001) and 56 kg N ha$^{-1}$ increased leaf area of apples by about 55% (Cheng et al. 2007). Johnson et al. (2001) reported reduced vegetative vigour with autumn-applied foliar urea in peach compared to spring or autumn soil-applied nitrogen. Also in moderately N deficient peach trees foliar urea (1.2%) and $KNO_3$ (1.8% and 4%) had no significant effect on shoot growth but an equivalent amount of N supplied to the soil did (Leece and Kenworthy 1971). Dong et al. (2005) found shoot growth was increased slightly by a series of foliar urea sprays (0.5% w/v) but not as much as the same amount of urea-N applied to the soil. In this experiment 12-15% of the spray solutions landed onto the ground beneath the trees, which may explain the increased shoot growth compared to unfertilised trees. A similar effect on apple shoot growth with three to six applications of 0.5% urea commencing in the early season was reported by Fisher et al. (1948). Lacroux et al. (2008) applied foliar urea twice close to veraison with little effect on vegetative vigour of the grapevines in the same season.

Marks and Clarke (1995) also reported shoot growth of apple to be increased by seven sprays of urea similarly to an equivalent application of N to the soil but did not specify what amount of the foliar-applied N might have reached the soil as runoff from the foliar applications. The effectiveness of the foliar urea in this study at increasing fruit N content suggests that coverage of the trees was thorough therefore significant runoff seems likely. Forshey (1963) found vegetative growth of apples dependent on foliar-applied urea as a N source was reduced compared to soil-fertilised trees. Growth of individual olive shoots treated with foliar-applied urea (1.5%) applied twice weekly from about the middle of the first growth flush in spring (10 May) through to late autumn (22 October) was increased by nearly 40% compared to unsprayed shoots on the same trees (Cimato et al. 1990). However, the N status of the trees used was not reported. A vegetative response to foliar N seems more likely if trees are significantly N deficient (Weinbaum et al. 2002)

### 1.4.6 Absorption of foliar-applied N

Application techniques particularly spray volumes and droplet size will have a large influence on the amount of the applied N retained on the plant surfaces and therefore also determine the amount taken up (Bukovac et al. 2002). Low volume spraying, by reducing runoff, can increase the amount of applied nutrient retained on leaves and

fruitlets (Bukovac et al. 2002; Manktelow 2011). The contact angle between spray droplets and the target plant surface is theoretically important for spray retention although in practice it shows poor correlation with spray adhesion (Gaskin et al. 2005).

The principle barrier to the absorption of foliar-applied N is the leaf cuticle and particularly associated wax layers (Weinbaum 1988). The properties of the cuticle and therefore the uptake of foliar-applied N will vary greatly between species, with leaf age, and with environmental factors, such as nutrition, light, humidity, and temperature (Weinbaum 1988). Passage through the cuticle is driven by passive diffusion and the electro-chemical gradient formed by a negative charge increase across the cuticular membrane with the rate of absorption being much influenced by temperature and the concentration gradient (Swietlik and Faust 1984; Wojcik 2004).

Penetration often parallels stomatal density, but this might be more due to the greater capacity for absorption by guard and subsidiary cells (Haynes and Goh 1977). Direct stomatal uptake is limited by surface tension, but might be increased by the use of surfactants (Haynes and Goh 1977). Nevertheless there is evidence for continuous liquid water connections between the leaf apoplast and the leaf surface via the stomata which would facilitate the stomatal uptake of foliar-applied nutrients (Peuke et al. 1998; Burkhardt 2010). Uptake can also be through trichomes, especially in the basal parts of glandular trichomes that have an abundance of ectodesmata and less cuticular development (Haynes and Goh, 1977). Spray retention and, presumably uptake, has a positive correlation with leaf hair density (Hall et al., 1997). The thin walled epidermis of kiwifruit fruitlets contains trichomes and small pores which probably facilitate uptake of foliar-applied N (Hallet and Sutherland 2005). During the period of rapid fruit growth in Stage 1 the newly formed periderm can tear, forming lenticels which probably improve gas exchange and presumably would also promote uptake of externally deposited solutes by the young developing kiwifruit (Hallet and Sutherland 2005). Other fruits show similar morphology amenable to direct uptake of foliar-applied N, for example, apples (Sweitlik and Faust 1986) and grapes (Blanke and Leyhe 1988). Foliar urea was particularly effective at raising apple fruit N% (Marks and Clarke 1995).

64

Although uptake of urea was faster through abaxial than adaxial apple leaf surfaces during the first 24 hours, by the seventh day after application uptake was similar for both leaf surfaces (Boynton et al. 1953). Faster uptake from abaxial than adaxial leaf surfaces probably reflects the distribution of stomata and ectodesmata, which are generally more numerous on abaxial leaf surfaces, and the thinner cuticular membrane (Wojcik 2004). Greater uptake of foliar-applied N in apple from postharvest applications might be due to deterioration of cuticle and epidermal waxes late in the season as leaves enter the early stages of senescence (Fernandez and Eichert 2009). In grapes, increased uptake of foliar-applied N late in the season compared to spring applications may have been related to the early applications being partly washed off by rain (Porro et al. 2010). However, generally uptake is higher in young developing leaves than in more mature leaves (Toselli et al. 2002; Bondada et al. 2001). Bondada et al. (2001) attributed this to increased accumulation of epicuticular wax as leaves aged. The thickness of cuticles might also explain interspecies variation in the efficiency of foliar-applied N uptake, although foliar-applied N can still be effective in species such as apples and oranges that have particularly thick cuticles (Weinbaum 1988). Soil fertilisation, particularly with N, can reduce the thickness and density, and alter the morphological characteristics of the wax component of the cuticle leading to increased permeability (Chui et al. 1992; Prior et al. 1997; Bondada et al. 2001).

Urea is particularly suitable for foliar application because it is water-soluble, and the molecule is electrically neutral and therefore less restricted in its movement through the epicuticular wax and cutin layer. The cuticular membrane is 10 to 20 times more permeable to urea than to inorganic ions (Swietlik and Faust 1984), suggesting its initial uptake is not by simple diffusion but rather by 'facilitated diffusion' (Kannan 1980). In this respect there is evidence for urea being involved in a chemical interaction with the membrane that loosens its bonds (Wojlik 2004; Yamada et al. 1965). This might explain the observation that urea can promote the uptake of other compounds applied with it (Swietlik and Faust 1986; Weinbaum 1988). Because foliar urea has inherent surfactant properties it is often used without adjuvants (Swietlik and Faust 1984). Urea may also be able to penetrate the plasma membrane directly without dependence on active transport channels (Mengel 2002). Rapid metabolic assimilation would maintain a gradient for further absorption of urea from the leaf or fruit surface (Mengel 2002). Urea might also move out of the recipient

tissues before reduction or assimilation since the molecule is phloem mobile (Swietlik and Faust 1984).

Efficiency of uptake of foliar-applied urea has been reported as being between 48 and 69%, which is 40% higher than recovery of soil-applied N (Weinbaum et al. 2002). Uptake of urea can be rapid with up to 70% of the applied urea absorbed by the leaves within 24 hours (Albrigo 2002). Toselli et al. (2002) reported in apple maximum uptake of urea in the first hour after application and least between 48 and 120 hours. Spray carrier volume did not affect final uptake but higher volume and low concentration had faster initial uptake in the first 48hrs.

Uptake of urea and corresponding increases of leaf N appear to be less dependent on the existing N status of the plant than is the case for other foliar-applied nutrients such as P and K. Urea N was still taken up when leaf N was high in citrus whereas P and K uptake depended on the existing concentrations of these nutrients in the leaf (Albrigo 2002). It was suggested that this might be because urea and ammonium are not naturally present in the leaf and therefore do not have to move against a diffusion gradient. Bondada et al. (2001) also found that N-sufficient citrus leaves absorbed more foliar-applied urea than leaves from N deficient trees. Apart from the absence of a concentration gradient for urea and ammonium, increased uptake of foliar-applied N in trees of low N status might be due to thicker cuticles and wax layers in such trees (Bondada et al. 2001). Perhaps for similar reasons young apical leaves of apple trees absorbed more urea than basal leaves (Toselli et al. 2002). Nevertheless a higher concentration gradient for absorption of foliar-applied N in trees of lower N status could also lead to greater uptake compared to trees of higher N status in citrus as was suggested by the results of Lea-Cox and Syvertsen (1995). Different results in relative uptake by trees of different N status could be due to different interpretations of 'deficiency'. Optimum levels of N% in leaves are often based on levels that are normally found during routine analysis of commercial crops rather than on levels shown experimentally to be necessary for optimum performance of the crop. Therefore N% below the 'normal range' does not necessarily mean a crop is physiologically N-deficient to the point that yield or growth has been adversely affected. The effect of N status on foliar-applied N uptake is therefore likely to depend on how deficient the low status plants really are.

66

Some studies show foliar uptake of $NO_3^-$ to be equal to urea (Furuya and Umemiya 2002; Bowman and Paul 1992). Like urea, $KNO_3$ is able to rapidly diffuse through the cuticle and the free space of the cell wall, although uptake into the cytosol depends on the efficiency of $NO_3^-$ transporters in the plasma membrane (Mengel 2002). Uptake of $NO_3^-$ from foliar calcium nitrate may be slower since the $Ca^{+2}$ tends to be bound to cell wall anions (Mengel 2002). Although Bowman and Paul (1992) found urea and $KNO_3$ were absorbed equally, the high rates used in their study were very phytotoxic so their conclusions as to the accumulation of $NO_3^-$ and subsequent translocation patterns might not apply to situations where phytotoxic effects are limited.

Factors such as the pH of the spray solution, the period of time allowed for uptake, the use of surfactants, spray concentration, and the method for determining uptake may affect the outcome of experiments comparing the uptake of different forms of foliar-applied N (Fernandez and Eichhert 2009). For example, in tomatoes when the pH of the spray solution was reduced the uptake of $NO_3^-$ from $NaNO_3$ was increased, that of $NH_4$ from $(NH_4)_2SO_4$ was reduced, but uptake of urea was not affected (Tan et al. 1999). Nevertheless, there are also reports for increased foliar uptake of acidified solutions of urea. For example, El-Omani et al. (2004) reported significantly increased citrus leaf uptake of foliar urea when pH of the spraying solution was lowered from 7.6 in the un-buffered solution to between 5.5 and 6.0. Similarly, Faust and Swietlik (1984) reported studies that found highest rates of urea uptake by apple leaves was between pH 5.4 and 6.6, lowest at pH 7.3, and intermediate at pH 8.0. In cotton, foliar-applied $KNO_3$ and $K_2SO_4$ gave increased yields only when pH was reduced to pH4 (Howard et al. 1998). Mengel (2002) suggested this effect might be due to $H^+$ ions from the buffered solutions being taken up and lowering pH in the apoplast, which favoured uptake of $K^+$ and its accompanying anion. The pH of solutions made from fertiliser grade $KNO_3$ can be highly alkaline (pH 8 to 9) due to impurities such as $K_2O$ (MacDonald 1960).

Uptake is also influenced by environmental conditions such as humidity, temperature, and light (Wojcik 2004). These factors can: delay drying; alter physical properties on the leaf surface, e.g. thickness or composition of wax layers and permeability of

ectodesmata; or influence physiological processes related to active uptake mechanisms (Fernandez and Eichert 2009; Schönherr 2002; Swietlik and Faust 1984; Wojcik 2004). Rapid drying and increased volatilisation of urea also means that studies done in different environments might not be comparable. Absorption is greater during dull (low light levels) and humid conditions, at night, or early morning, than in bright sunny afternoon conditions (Bondada et al. 2001). The relationship between uptake and ambient relative humidity is variable because although cuticular permeability is increased as RH approaches 100%, at low levels of humidity the driving force for diffusive uptake is increased by the high concentration of the foliar-applied solutes on the leaf surface (Fernandez and Eichert 2009). Differences in leaf morphology between species, and even between cultivars, are also likely to result in different uptake behaviour from foliar-applied N (Swietlik and Faust 1984; Wojcik 2004).

### 1.4.7 Surfactants

The outer surface of leaves and fruitlets are covered with cuticles and wax layers that repel water and reduce retention of waterborne nutrients (Fernandez and Eichert 2009). Adjuvants are therefore commonly included in spray formulations to reduce surface tension, increase spreading and retention, delay drying, and as a result promote uptake of foliar-applied chemicals (Fernandez and Eichert 2009). However, the complexity of interactions between surfactant, the nutrient and/or chemical being applied, and leaf surface characteristics makes it difficult to predict the uptake behaviour of any particular combination of adjuvant, main spray ingredient, crop species, and environment (Fernandez and Eichert 2009). For example, surfactants increased runoff and reduced the amount of foliar-applied urea retained on the leaves of prune trees (Leece and Dirou 1979). Surfactants work by altering the hydrophobic properties of the cuticle but their effect can extend to the cell membranes causing disruption and breakdown (Orbovic et al. 2001; Fernandez and Eichert 2009). Surfactant-induced ethylene production with accompanying symptoms of phytotoxicity was induced by a range of non-ionic, anionic, and cationic surfactants when applied to various annual and perennial species (Lowndes and Bukovac 1989).

68

Orbovic et al. (2001) found a non-ionic surfactant X-77 was equally effective as the organo-silicon surfactant L-77 in foliar urea treatment of grapefruit but suggested there might be more likelihood of a direct physiological effect due the surfactant itself in the case of L-77 since it appears to be absorbed through the cuticle more than X-77. Both surfactants increased the initial penetration of urea through the cuticle but after about 2 hours the rate of penetration was not different than for urea alone (Orbovic et al. 2001). After 96 hours approximately 88% of applied urea with X-77 had penetrated isolated grapefruit leaf cuticles compared to approximately 72% of urea applied without surfactant. However, the use of isolated astomatous cuticles to study barrier properties of leaf surfaces may not accurately reproduce uptake of agrichemicals *in vivo*, especially because it is difficult to ensure the cuticle is not damaged or its barrier properties altered during its removal and preparation (Fernandez and Eichert 2009). In prune trees, high volume spraying of urea with L-77 resulted in excessive runoff and reduced uptake compared to X-77 (Leece and Dirou 1979). Surfactants might be more useful for low volume spraying to ensure adequate wetting of the foliar surfaces (Leece and Dirou 1979; Bukovac et al. 2002). Organosilicon surfactants are thought to increase direct stomatal uptake of foliar-applied chemicals (Fernandez and Eichert 2009) and might therefore be useful where uptake of foliar-applied N is limited by the increased thickness of leave cuticles and wax layers in N deficient plants.

Studies on the effect on grape berries of a range of commonly used surfactants revealed that most of the product formulations impaired the barrier properties of the cuticular wax layer leading to significant increases in the development of fungal diseases (Marois et al. 1987; Rogiers et al. 2005). The possibility of such unintended effects needs to be considered in the selection of adjuvants added to foliar N treatments. Such effects could conceivably stem from the foliar nutrient itself either from it interfering with cuticular waxes or a nutritional interaction with the leaf's epidermal microflora leading to increased virulence of pathogenic species. Increased multiplication of a phytopathogenic virus was reported by Rao et al. (1995) with applications of 0.5 and 1.0% foliar urea. Nevertheless, both urea and $KNO_3$ also have potential for the suppression of fungal diseases in crops (Frageria et al. 2009; Wood and Beresford 2000; Bhuiyan et al. 2007).

## 1.4.8 Effect of foliar-applied N on leaf N content

Leaf analysis does not always show the expected increase in N concentration following foliar N application, which can be due to poor uptake; or to excellent uptake and efficient translocation to tissues not analysed, especially fruit (Gonzalez et al. 2010). Leaf analysis can also give a false indication of uptake if the applied N is immobilised in the cuticle and the leaves are not washed properly (Gonzalez et al. 2010).

Various methods for washing leaves before analysis have been used. Bondada et al. (2001) studying urea uptake by citrus leaves harvested seven days after the last urea application simply washed the leaves in distilled water to remove any residual N. Use of distilled water to wash leaves following foliar applications was also reported by (Chamel 1988). Olive leaves were washed three days after a foliar urea application with the organo-silicon surfactant L-77 (Klein and Weinbaum 1984). Leece and Dirou (1979) washed leaves sampled at various times after foliar urea applications with acidified detergent (0.1N HCl with 0.1% w/v sodium lauryl sulphate). To differentiate between foliar-applied N immobilised in the cuticle and that more completely absorbed, Lea-Cox and Syvertsen (1995) sprayed treated citrus leaves with a 5% w/v cellulose acetate in acetone solution, which solidified and could be peeled off.

Even when uptake is more or less demonstrated by leaf analysis benefits to the crop are often presumed (Gonzalez et al. 2010). For example, Porro et al. (2006) concluded that $NH_4$-N was more effective than $NO_3^-$ -N for foliar N use in grapevines but this was only based on increased leaf N% with $NH_4$-N with no significant effects being found in shoot growth, which was the only other reported response.

Dong et al. (2005) maintained apple leaf N% with seven applications of 0.5% foliar urea, and Leece and Dirou (1979) maintained prune leaf N% with 0.5% foliar urea. In peach, three sprays of urea (1.2%) or $KNO_3$ (4%) in autumn followed by another three in the spring significantly increased leaf N% in moderately N deficient trees, an effect that persisted until the following autumn (Leece and Kenworthy 1971). Urea raised leaf N by up to 54% 48 hours after application but it had declined by 15 days to the level of the untreated control level; but repeated applications had a cumulative

effect and gradually raised leaf N (El-Otmani 2002). Maximum leaf N was recorded two days after foliar application of urea in citrus and declined thereafter (El-Otmani et al. 2004). Three consecutive foliar urea (1.2%) applications in summer raised peach leaf N concentration by about 5-8% (Klein 2002).

### 1.4.9 Assimilation and translocation

The metabolism of foliar-applied N once absorbed into the leaf is not believed to be different from that taken up by roots (Swietlik and Faust 1984; Wojcik 2004). Following uptake through the cuticle, there may be active or passive transport through the cell wall and plasmalemma into the cytoplast where the foliar-applied N is assimilated into phloem mobile forms or moved into vacuoles for storage (Swietlik and Faust 1984; Wojcik 2004). The assimilation of foliar-applied urea and $NO_3^-$ differs mainly in the initial stages, although neither nitrate reductase or urease are likely to be rate limiting (Swietlik and Faust 1984). Urea is hydrolysed by the ubiquitous urease enzyme and $NO_3^-$ induces and is reduced by nitrate reductase (Swietlik and Faust 1984). Following this, $NH_4/NH_3$ compounds are incorporated into amino acids (Guo et al. 2007). Both urea and $NO_3^-$ can be stored before assimilation in cell vacuoles or in the case of urea, can be transported in the phloem as preferred route before hydrolysis (Tan et al. 1999; Wang et al. 2008; Witte et al. 2011). The rate of hydrolysis of urea can be considerably less than that theoretically possible based on the concentration of urease within the tissues alone suggesting active storage in vacuoles (Witte 2011). The storage of foliar-applied N in cell vacuoles could prolong or delay the time course of its action (Bowman and Paul 1992). The persistence of urea within plant tissues after its application is attributed to vacuolar storage (Witte 2011), as were some of the effects of foliar-applied $NO_3^-$ (Bowman and Paul 1992).

Prior to assimilation, foliar-applied N could move through the apoplast towards vascular tissues with subsequent phloem loading and transport away from the site of uptake (Haynes and Goh 1977). But movement of $NO_3^-$ along this pathway might be limited due to repellent properties (negative charges) of the cell walls and the very limited mobility of $NO_3^-$ within the phloem (Haynes and Goh 1977; Peuke et al. 1998). Urea is more likely to move out of the recipient tissues before reduction or

assimilation since the molecule is phloem mobile and plants are equipped with a variety of transporter proteins (Swietlik and Faust, 1984; Wang et al. 2008).

N is highly mobile within plants so foliar-applied N is readily translocated elsewhere following uptake (Porro et al. 2010). The amount of foliar-applied N translocated from the leaves depends on the physiological stage of growth, and translocation can be extensive and rapid especially when applied towards the end of the season (Swietlik and Faust 1984). But translocation may also be dependent on the presence of sink activity (Klein 2002). Translocation of foliar-applied urea-N from young leaves may be less than from older leaves or from leaves later in the season perhaps because N absorbed from foliar applications by leaves that are still expanding is immobilised into structural components of the leaf cells (Khemira et al. 1999).

In peaches, the best absorption rates were from foliar-applied urea and sodium nitrate but translocation within the plant was similar for these two compounds (Furuya and Umemiya 2002). Widders (1991) found that when applied as foliar sprays to tomato, absorption was similar for both urea and triazone-N, but triazone-N was preferentially translocated to untreated vegetative tissue while urea-N was preferentially translocated to developing fruit. This effect might give triazone an advantage on foliage crops while urea would have an advantage on fruiting crops (Wedenfeld 2009).

Cimato et al. (1990) suggested N absorbed by olive leaves moves to reproductive tissues following a concentration gradient. Klein and Weinbaum (1984) also working with olives found 57% of $^{15}$N-enriched urea applied to the leaves ended up in the fruit and the rate of translocation was determined both by the N status of the trees and the size of the fruit sink (crop load). In grapevines, Schreiber et al. (2002) found about 30% of the N from 0.6% w/v $NH_4NO_3$ foliar-applied fortnightly from soon after fruit set, had accumulated in the fruit clusters by harvest at the end of the season. Also in grapevines, Porro et al. (2010) reported that N absorbed from two foliar applications of 0.5% $(NH_4)_2SO_4$ applied early in the vegetative season (spring or beginning of summer) was recovered in leaves or in fruits, while that absorbed from a late application (veraison, pre-harvest) was reallocated to storage sinks within perennial tissues.

## 1.4.10 Phytotoxicity

Foliar application of nutrients can cause phytotoxicity, the most common symptoms of which are 'burning' or 'scorching' of the leaves, i.e., leaf necrosis (Krogmeier 1989; Burkhardt 2010). Although tolerance to foliar-applied urea or $KNO_3$ varies between different species, concentrations above about 1.2% are generally only suitable for postharvest or pre-leaf fall applications because of the risk of leaf damage (Weinbaum 1978). Typically young leaves are more susceptible to damage from foliar-applied chemicals so that the risk of foliar damage is greater with early season applications (Weinbaum 1988). Foliar damage is usually related to localised desiccation of the leaf tissues due to the hydroscopic properties of the foliar-applied solutes and the relatively high concentrations that remain on the leaf surface (compared to those around the roots in the soil solution) following evaporation of the spray carrier (usually water) (Burkhardt 2010; Fernandez and Eichert 2009; Wojcik 2004). Such phytotoxic effects are related to the salt index of the foliar solute, which could be a practical measure for predicting safe concentrations (Lea-Cox and Syvertsen 1995). Nevertheless, the range of optimal rates reported is too broad to allow definitive recommendations for optimal concentrations to be made for any particular nutrient form (Fernandez and Eichert 2009). Symptoms of phytotoxicity usually involve marginal and tip chlorosis or necrosis which might reflect the accumulation of the spray solutions at these parts of the leaf which results in much higher residual concentrations following evaporation of the water carrier (Albrigo 2002). Therefore, application techniques and use of adjuvants that reduce runoff and allow more uniform distribution of the spray on the leaves could help to minimise phytotoxic effects. Early morning applications when dew is still present on leaves might be more phytotoxic than those made at midday or during the late afternoon but this probably depends on the prevailing atmospheric conditions (Frageria et al. 2009). 'Leaf tip yellowing' with foliar urea applied to citrus during hot weather was attributed to rapid drying and high concentrations of residual urea remaining on the leaf surface (Kiang 1982).

Urea is generally believed to be less phytotoxic than other N forms used for foliar N sprays (Weinbaum 1978; Klein and Weinbaum 1984; Swietlik and Faust 1984). However, at the same compound concentration, rather than N concentration, the phytotoxicity of urea and inorganic N salts, such as $KNO_3$, is similar. For example,

although Furuya and Umemiya (2002) considered $KNO_3$ more phytotoxic than urea, both compounds have a very similar salt index (73.6 and 75.4 units, respectively), and if solutions of comparable osmotic concentration are used the risk of leaf damage is probably similar for both compounds (Lea-Cox and Syvertsen 1995).

### 1.4.10.1 Biuret phytotoxicity

Urea can contain biuret, a potentially phytotoxic compound present in fertiliser grade urea. It is created in the high temperatures occurring during urea manufacture by the fusion of two urea molecules (Mikkelsen 2007; Figure 1.6). Modern urea manufacture has been improved to reduce the content of biuret to between 1 and 2% by weight (Mikkelsen 2007).

$$\underset{\text{Biuret}}{H_2N - \overset{\overset{\displaystyle O}{\|}}{C} - NH - \overset{\overset{\displaystyle O}{\|}}{C} - NH_2} \qquad \underset{\text{Urea}}{H_2H - \overset{\overset{\displaystyle O}{\|}}{C} - NH_2}$$
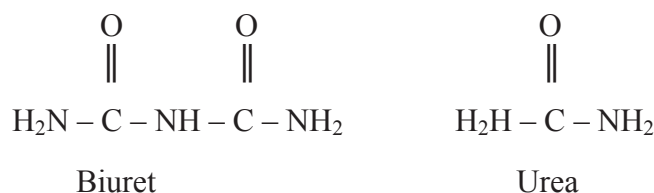
Figure 1.6 Representations of the molecular structure of biuret (left) and urea (right).

Biuret is not quickly metabolised in the plant and its toxicity has been attributed to disruption of nitrogen metabolism and irreversible damage to chloroplasts (Mikkelsen 2007; Albrigo 2002). Typical foliar symptoms of biuret toxicity include leaf tip chlorosis and necrotic leaf margins and were first reported on pineapple (Sanford et al. 1954). In practice these symptoms are difficult to distinguish from those caused by urea (El-Zeftawi 1974). For use in foliar applications, urea with a biuret content between 0.25 and 0.80% (low biuret urea) is generally recommended to reduce the risk of leaf damage. There are several crops that are exceptionally sensitive to biuret, including citrus, pineapple, potato, and avocado. However, its toxicity to crops in general may have been exaggerated. For example, applications of biuret alone at concentrations considerably above those likely to be encountered in the foliar use of fertiliser grade urea failed to cause phytotoxic symptoms in apple (Khemira et al. 2000). However, there was some phytotoxicity when biuret was combined with urea at concentrations of 2% and higher. Biuret by itself did damage the leaves of young

74

citrus trees (Jones 1954). Obviously sensitivity to biuret varies greatly between species and might reflect the capacity of a species to metabolise it. For example, one study found biuret persisted in orange leaves eight months after foliar urea application (Mikkelsen 2007). Despite their low tolerance to biuret, citrus are relatively tolerant of urea with application rates up to 34 kg N ha$^{-1}$ being reported without damage (Albrigo 2002).

Biuret can also have beneficial effects on crops. For example, small amounts of biuret applied to the soil increased the growth of Douglas fir seedlings (Xue et al. 2004). According to Xue et al. biuret acts like a plant growth regulator, while also having a priming effect on the mineralisation of nitrogen by soil microorganisms. However, higher rates of biuret applied to the soil resulted in seedling mortality and reduced nitrification. Its relatively slow mineralisation in soil compared to urea gives it some potential for use as a slow release nitrogen fertiliser (Xue et al. 2004).

Because biuret tends to accumulate in plant tissues, repeated applications may have a cumulative effect with subsequent appearance of symptoms of toxicity (Mikkelsen 2007). Albrigo (2002) suggested a biuret limit of 0.34% in urea used for foliar application to avoid phytotoxicity in citrus, but a higher content of up to 0.5% was probably suitable for occasional use providing application rates were about 1168 L ha$^{-1}$ and supplied not more than 15.7 kg N ha$^{-1}$.

The pattern of chlorosis and leaf damage at the leaf tips is consistent with spray solution runoff and accumulation at the tips. Therefore, the higher tolerance to biuret found by Albrigo (2002) than was reported in earlier studies might be related to the 4-8 fold decrease in spray volumes since the time of the earlier studies.

Singh et al. (1979) found that three consecutive urea (2%) foliar sprays increased potato yields by between 15 and 31% in three seasons, but when the urea contained biuret at levels above 0.8%, the yield advantage was steadily decreased with increasing biuret content. Biuret at 0.35% had no adverse effects on potato yield although symptoms of leaf damage were not reported.

### 1.4.10.2 Urea phytotoxicity

Apart from biuret, the formation of ammonium cyanate in urea solutions might contribute to phytotoxicity (El-Zeftawi 1974). The formation of this compound in urea solutions increases with time and in conditions of high pH and temperature. Therefore the use of freshly prepared urea solutions would be prudent. Within the plant the enzymatic hydrolysis of urea by urease generates $NH_3$, and both $NH_3$ and $NH_4^+$ are phytotoxic and capable of disrupting a large range of metabolic processes within plant cells (Vines and Wedding 1960; Britto and Kronsuker 2002; Fernandes and Rossiello 2011). Effects of foliar-applied urea on increasing flowering and fruit yields of citrus may be due to the slight phytotoxicity of urea and its breakdown product ammonium (Albrigo 2002). Lovatt (1999) also suggested that the increased fruit set following even a single pre-blossom foliar urea application is due to a transient increase in $NH_3$-$NH_4^+$ content in the treated tissues. Orbovic et al. (2001) concluded that temporary toxication symptoms caused by foliar urea may be due to ammonia toxicity. Nevertheless, urea itself is considered more phytotoxic than $NH_3$ or $NH_4^+$ (Orbovic et al. 2001).

In soya beans, foliar urea (presumably laboratory grade) applied at concentrations of 2.5% (w/v) and higher caused leaf necrosis and the damage was associated with the accumulation of urea within the necrotic tissues (Krogmeier et al. 1989). Accumulation of urea and/or biuret might result when tissues have been severely damaged halting further metabolic activity. Plasmolysis of mesophyll cells in citrus leaves following foliar urea application have been observed (Orbovic et al. 2001) and the chlorotic symptoms of biuret toxicity being characteristically irreversible are indicative of senescence (Achor and Albrigo 2005). In tomatoes, daily foliar urea applications (as the only N source) at concentrations above 0.2% caused phytotoxicity, also apparently due to urea accumulation in the treated tissues (Nicoulaud and Bloom 1996). El-Zeftawi (1974) recommended an interval of at least 4 weeks between foliar urea applications to avoid excessive accumulation of urea in the leaf tissue. The accumulation of urea in treated tissues might be a phenomenon peculiar to certain species because generally its high mobility through membranes and within the phloem, and also the likelihood of its rapid hydrolysis by urease makes accumulation less likely (Wang et al. 2008; Swietlik and Faust 1984).

Nickle (Ni) is an micronutrient essential for the urease activity and accumulation of urea and associated symptoms of phytotoxicity can be caused by Ni deficiency (Wood et al. 2006). Inclusion of Ni with foliar urea can reduce phytotoxicity of foliar urea sprays (Kutman et al. 2013). Nickle deficiency could be induced in fruit trees by excessive or unbalanced fertilisation practices that lead to the accumulation of elements such as Zn, Cu, Mn, Mg, Fe, and Ca that compete or otherwise inhibit Ni uptake (Wood et al. 2006). Foliar symptoms of Ni deficiency were induced in citrus growing on soils high in Ca and Mg by foliar urea applications (Wood et al. 2006).

Also working with tomato Tan et al. (1999) found slight marginal scorching two days after a single application of urea at 2.2% but not with concentrations of 1.1% or lower. Phytotoxicity symptoms appeared with solutions of 1% $(NH_4)_2SO_4$ but not at 0.5% and with 3% $NaNO_3$ but did not 1.2% or lower. Raising the solution pH lessened the severity of the symptoms in the case of $NaNO_3$ but also reduced its uptake; higher pH had no effect on phytotoxicity caused by urea or on its uptake; but worsened the symptoms in the case of $(NH_4)_2SO_4$ and increased its uptake. The non-ionic hydrocarbon surfactant 'Tween' (0.1% v/v) was used in this experiment (Tan et al. 1999). Guvenc and Badem (2002) reported some foliar damage following five applications of 1.1% $KNO_3$ on greenhouse grown tomato but no phytotoxic symptoms apparently appeared with the same number of applications of fertiliser grade urea (biuret content unknown) at concentrations up to 0.8% .

In 'Golden Delicious' apples, two foliar sprays of urea at concentrations between 0.4 and 4% in early summer caused no leaf damage or fruit russetting but the quality of the urea used (e.g., its biuret content) was not reported (Thalheimer and Paoli 2002). Weinbaum (1988) suggests phytotoxicity occurs in apples with springtime application of urea at >0.5% concentration but concentrations of 4 to 5% in autumn were possible because leaf damage at this time would not adversely affect tree productivity. However, Wood and Beresford (2000) reported severe bud damage in apples with autumn applications of foliar urea at concentrations above 5%.

In citrus, single applications of urea (biuret content 0.01% w/w) at concentrations above 1.8% w/v caused foliar burn on young grapefruit leaves and leaf abscission on older leaves but older leaves were undamaged by three applications at 1.3% (Lea-Cox

and Syvertsen 1995). No foliar burn was found in young or old leaves with urea concentrations between 0.5 to 0.9% urea (Lea-Cox and Syvertsen 1995). The sprays were applied with the non-ionic hydrocarbon surfactant Triton X-77 (0.1% v/v). Page et al. (1963) found only minor foliar damage on orange trees sprayed up to six times with $KNO_3$ solutions between 2.5 and 5%. The alkyl resin adhesive surfactant Triton B1956 (0.25% v/v) was used in the sprays. In prune, six high volume spray applications during spring of 1.35% w/v urea (<0.4% biuret) with organosilicon or hydrocarbon surfactants and caused slight marginal and tip necrosis on leaves (Leece and Dirou 1979). 'Partial blights of the leaf margins' were reported with 3% $KNO_3$ on grapes (Altindisli et al. 1999). Calvert (1969) found only slight leaf burn in oranges following four applications of 2.4% $KNO_3$, but damage was more severe with concentrations of 4.8% and 7.2%. Triton B1956 (0.25% v/v) was added to the foliar treatments.

### 1.4.11 Conclusion

The foliar application of N has been successfully used in many fruit crops. This method of applying nitrogen is likely to be less environmentally harmful than the conventional soil application of N fertilisers and might also confer advantages in being able to supply supplemental N at particular times during the season when the demand or response to N is increased. Both morphological characteristics of kiwifruit leaves and fruit, and the overhead training systems commonly used for commercial production appear to be well suited for foliar N application. However, little research has hitherto been undertaken to investigate the utility of foliar-applied N in kiwifruit.

## 1.5 Overall conclusions

The balance between the fruit's water and dry matter contents can be highly influential in determining the quality of the fruit at harvest. The accumulation of water and dry matter by the fruit is separately regulated and therefore there is potential for their accumulation to become uncoupled by factors acting more or less independently on one or the other. The potential for such uncoupling leading to increased fruit water uptake and/or reduced dry matter accumulation appears to increase during early and late stages of fruit development. Soil-applied N fertiliser could influence both water and dry matter accumulation by increasing vine water uptake and increasing the competitive strength of vegetative growth component. Foliar-applied N might offer an alternative or be complementary to a reduced rate of soil-applied N and thereby allowing the advantages of adequate N nutrition while at the same time avoiding some of the disadvantages associated with soil-applied N.

## 1.6 Problem statement

To maintain the competitiveness of kiwifruit as a traded commodity demands that high levels of productivity are accompanied by consistent and high standards of fruit quality while also meeting societal expectations that environmental standards are not unduly compromised by the production process. Although N fertilisation is needed for optimum yields to be maintained, excessive N could result in reduced fruit quality by increasing fruit water content and reducing its dry matter contents both of which contribute to reduced DM%, which is the accepted general measure of fruit quality for kiwifruit.

## 1.7 Objectives

- To investigate relationships between N fertilisation and fruit and vine growth.

Further objectives were:

- Improve understanding of the regulation of water and dry matter uptake by developing fruit.
- To investigate effects of soil-applied nitrogen fertilisation on some secondary metabolites having importance for fruit quality.
- Compare the use of foliar application of nitrogen as a possible alternative to soil applications both in respect to fruit quality and for its effects on vegetative growth.
- Define the optimum timing of foliar-applied nitrogen in terms of fruit size and dry matter accumulation under conditions typically used on commercial properties such as girdling and the use of the stimulant 'Benefit Kiwi'.

Chapter 2 summarises some method development for $NO_3^-$ determination in dried fruit tissues. Included in this chapter are details of the procedures used for treatment allocation and sampling to allow for the quite large natural variability in fruit FW and DM% both within and between vines.

80

Chapter 3 and 4 summarise the results of experiments applying high rates of $NO_3^-$ fertiliser during the period of fruit development. These experiments were continued over four seasons to 'Hayward' vines growing in the Massey University experimental orchard at Palmerston North, NZ; but also includes results of a pot trial with 'Hort16A' vines within a greenhouse.

Chapter 5 introduces and reports results from experiments with the foliar application of N during early fruit development conducted in two seasons using 'Hort16A' vines in a commercial orchard.

Chapter 6 reports the effects of similar foliar treatments applied to 'Hayward' vines over two seasons. In the first season, the foliar N treatments were applied in combination with high or nil rates of N fertiliser, while in the second season they were applied to 'Hayward' vines in the same commercial orchard as was used in Chapter 5. Within this chapter is included the results of a small additional experiment that compares foliar applications of urea and a seaweed extract during early fruit development alone or in combination.

Chapter 7 reports the results of an experiment on Hayward vines in the Massey orchard where foliar application was compared to soil application of equivalent amounts of N. The chapter is an expanded version of a paper presented at a soil-fertiliser conference at Massey University (Appendix 1). Some of the key findings from this chapter and Chapters 5 and 6 were published as an article in the NZ Kiwifruit Journal (Appendix 1).

Chapter 8 is an expanded version of a paper presented at the 7th International Kiwifruit Symposium (Appendix 1) and details the interactive effects of differences in soil water availability at particular times during fruit development with girdling, CPPU, and foliar N.

Finally, in Chapter 9 the various results and conclusions are integrated into a general discussion.

Specific experimental questions addressed in each of the six experimental chapters were as follows:

Chapter 3. Does maintaining a high supply of $NO_3^-$-N in the soil solution result in increased water uptake and reduced dry matter accumulation in the fruit? Does $NO_3^-$ accumulate in the fruit? What are the effects of an elevated $NO_3^-$ supply on vine growth?

Chapter 4. What are the effects of $NO_3^-$ supply on fruit quality parameters, like fruit firmness, soluble sugars and titratable acid levels? Is the oxalate content of the fruit increased by high levels of $NO_3^-$ consistent with its increased synthesis in response to higher rates of $NO_3^-$ reduction? Given the possible competitive relationship between phenolic and protein synthesis, is the phenolic content of the fruit reduced by high levels of $NO_3^-$ consistent with increased protein synthesis?

Chapter 5. What are the effects of N applied as a foliar spray during early fruit development on 'Hort16A' fruit FW and DM% and how does the time of application influence these effects? Are these effects similar when fruit is treated with a proprietary biostimulant?

Chapter 6. Can foliar N applied during early fruit development increase fruit FW of 'Hayward'? Are the effects of urea and $KNO_3$ applied at the same concentration similar? Are the responses to foliar-applied N in vines given high rates of soil-applied N fertiliser different than in vines given no soil applications of nitrogen? How do these effects change in fruit also treated with CPPU and, in the case of urea, also treated with a seaweed extract?

Chapter 7. In terms of vine vigour how do the effects of foliar-applied N compare to soil-applied N at equivalent application rates? What is the effect on fruit FW and DM% of a moderate rate of soil-applied N compared to the same amount foliar-applied?

Chapter 8. How does increasing the availability of water to the vine during early and late stages of fruit development affect fruit water uptake? How are the effects of surplus water modified by girdling, treatment with CPPU, wrapping to prevent transpiration, and/or with nitrogen applied as foliar sprays of urea during early fruit development?

82

# Contents

## List of Figures

# 2. Materials and methods

## 2.1 Introduction

The methods included in this chapter were used in two or more chapters of the study. Methodologies specific to experiments reported in each chapter are given in the Materials and Methods section of that chapter. The experiments described in this work were conducted between 2006 and 2011 at one of three sites. Additional fruit and leaf samples were collected from a similar long-term N fertilisation trial in Te Puke (Mills et al. 2008) and used for oxalate and ascorbate analysis (Chapter 4). Otherwise, the three experimental sites were:

1. A pot trial reported in Chapter 3 and 4 used 'Hort16A' vines growing in pots at the HortResearch site at Palmerston North (now Plant and Food Research) with calcium nitrate fertigated at high and low rates.

2. A long term N fertilisation trial with soil-applied nitrate fertiliser over four successive seasons using mature *A.deliciosa* cv.Hayward vines trained to T-bar support structures (Sale and Lyford 1990) in the Massey University experimental orchard (Fruit Crops Unit) at Palmerston North (Chapters 3, 4, & 6). Some of these same vines and additional ones in the same orchard were also used for experiments with foliar-applied N in one season (Chapters 7 & 8).

3. Experiments over three seasons using mature 'Hort16A' (two seasons) and 'Hayward' (one season) in a commercial orchard near Hastings in Hawkes Bay (Chapters 5 & 6).

The Massey University orchard had been managed according to 'organic' principles (Reganold et al. 2001) since the year 2000 when it gained 'in conversion' status prior to full BioGro registration (BioGro 2004). The orchard had been given no N fertiliser applications for at least six years prior to the start of the experiments in 2006. Soil type is a Manawatu sandy silt loam Manawatu fine sandy loam (Cowie and Rijkse 1977; Green and Clothier 1995).

| Experimental bay | Buffer bay | Experimental bay | Buffer bay | Experimental bay | |
|---|---|---|---|---|---|
| **F    F** | **F  M  F** | **F    F** | **F  M  F** | **F    F** | Vine gender |
| **O    O** | **O  O  O** | **O    O** | **O  O  O** | **O    O** | Row centre |
| | | | | | |

2.5 m          5 m          5 m
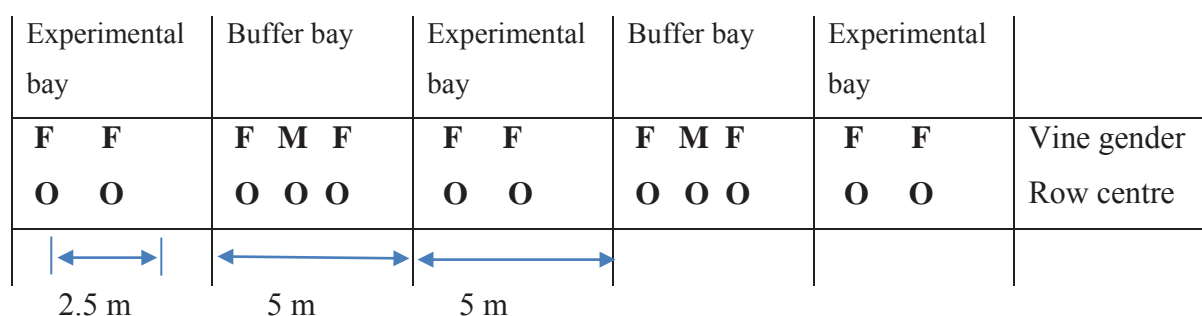
Figure 2.6 Aerial representation of the vine planting system in the Massey University orchard showing male and female vines, planting distance between vines, and the pattern of experimental and buffer bays. **O** vine stem aerial view, **F**: female vine, **M**: male vine.

## 2.2 Vine and cane uniformity

Where whole vines were used as replicates, care was taken to include bays containing two uniform healthy vines. On two occasions during trials in the Massey University orchard obvious and serious declines in vine health affecting single bays forced exclusion of the affected vines from the data analysis (details are given in Chapter 6 and 8).

Where foliar treatments were applied to individual canes on vines care was taken to select canes of uniform size, usually full length one-year-old replacement canes, rather than spurred canes.

Row variation: Effects of variation between rows are reported in Chapter 3 section 3.3.7 The source of this variation might be due to soil spatial heterogeneity. Although the centres of the two rows were only 10 metres apart being separated by a single buffer row, there were noticeably more stones present throughout the profile in Row 2 than in Row 1.

## 2.3 Sampling

The large variation in fruit characteristics such as FW and DM% that exists between fruit from the same vine makes sampling methodology an important factor in obtaining an unbiased sample (Miles et al. 1996).

Fruit samples were taken from the same shoot type and location in the canopy. In the case of T-bar vines the fruit was taken from between the second and third wire and from either both sides equally or from the same single side. In the pergola experiments, fruit samples were collected from a 50 cm wide strip centred by the second wire out from the cordon leader.

Fruit was picked into large snap-lock plastic bags containing about 20 fruit each, or larger volumes were harvested directly into 20kg capacity plastic crates. Fruit was either processed the same day or was placed in a cool-store and processed within two days of the harvest. The only exception was where the post-harvest storage properties of the fruit was being studied (Chapter 4). In this case fruit was removed from the cool-store and allowed to reach room temperature by standing in the laboratory for 24 hours before measurements and samples were taken.

## 2.4 Fruit measurements

### 2.4.1 Weighing and drying

Fruit from each replicate was sorted into groups containing four to five fruit of approximately equal sized fruit. The weight of each group was recorded and the result divided by the number in the group to give an average weight. One fruit from each group was taken for measurement of firmness and soluble solids content, and then returned to the group. Two equatorial slices were then taken from each fruit in the group. A slice from each fruit was pooled to make a single sample and placed immediately into a small zip-lock plastic bag, weighed, and frozen for later chemical analysis (each bag containing four or five slices). The other slices from the group were pooled into a second sample, put into a paper bag of known weight, weighed, and placed in a forced draft oven at 65°C. A set of empty bags were weighed and placed in the oven with the samples to give a value for bag moisture content. Generally variation between bags and their moisture contents was shown to be uniform and this allowed a single average bag weight to be used in the final calculations. After about a week in the oven, a sample of bags was removed and re-weighed every second day until their weights over four successive weighings showed no further change. For the final weighing, four bags were removed at a time and placed one at a time on a set of scales. Initially the bags gained weight as the paper bags absorbed atmospheric moisture (empty bags removed from the oven showed the same pattern), but the weight change then plateaued for a sufficient period to allow each of the four bags to be weighed. After this short period of stability the

bags began again to increase in weight presumably as the moisture content of the dried fruit equilibrated to the atmosphere. All weights (FW and DW) were made to two decimal points (g) but these data and the derived values for DM% and water content used in the text and tables for each chapter are rounded to one decimal place for clarity.

### 2.4.2 Firmness

The method for measuring fruit firmness was based on the procedure of Pike et al. (1996). After removing a thin layer of skin with a sharp craft knife on two opposite sides of the fruit, firmness was measured using a Effegi Fruit Pressure Tester model FT 327 fitted with a 7.9 mm diameter tip (Hopkirk et al. 1990). The two readings were averaged.

### 2.4.3 Soluble solids

Measurement of soluble solids followed the procedure of Hopkirk et al. (1986). An Atago Hand Refractometer model N-20 was used to record °Brix of juice manually expressed from caps cut from each end of the same fruit used for firmness. The refractometer was regularly re-calibrated with distilled water. The two readings were averaged.

## 2.5 Sap nitrate

The method used for measurement of petiole sap $NO_3^-$ was modified from that of (Prasad and Spiers 1984; Prasad et al. 1986). Petioles were removed from 20 youngest fully expanded leaves from shoots emerging between the leader and the second wire of the T-bar support structure from the selected vines. No differentiation was made between fruiting and non-fruiting canes. The petioles were placed in zip-lock plastic bags and frozen. After thawing the petioles were cut in half and the sap was manually squeezed from the cut ends of each petiole into a small beaker placed on a set of zeroed scales to give 1 g of sap. Pre-freezing softened the petioles for easy expression of the sap and prevented the red pigmentation typical of kiwifruit petioles from discolouring the sap as occurred with fresh or ground petioles. Fifteen mls of deionized water was then added to the sap. The diluted sample was then placed in a 35ml screw top plastic container and re-frozen. The containers were then removed from the freezer and allowed to stand undisturbed until thawed. As the sample liquified, some remaining suspended material precipitated to give an almost perfectly clear supernatant. The procedure was found easier and more effective than centrifuging and filtering.

88

Nitrate content of the petiole sap and fruit was measured with a quick-test method using nitrate-test strips (Reflectoquant Nitrate Test, Cat.No. 1.16995.0001, E.Merck, Darmstadt, Germany) and a reflectometer (Merck RQflex No.16970.0001). In the latter method nitrate ions are reduced to nitrite with Griess reagent. The nitrite reacts with an aromatic amine to form a diazonium salt that in turn reacts with N-(1-naphthyl)-ethylene-diamine to form a red-violet azo dye. The intensity of the dye is read by the reflectometer and is proportionate to the concentration of $NO_3^-$ present in the test solution (Schmidhalter 2005). The use of the reflectometer improves the objectivity of the strip method by standardising the time given for colour development and removing inaccuracies involved in visual comparison of strip colour against a colour chart (Schmidhalter 2005; Hartz et al. 1993; Prasad and Spiers 1984).

The nitrate-test strip was immersed in the thawed sample extract solution for two seconds simultaneously with the activation of the reflectometer's timer. The strip was introduced into the reading mechanism of the reflectometer and read 60 seconds after immersion in the sample. Accuracy of the values given by the reflectometer and $NO_3^-$ strips was checked against potassium nitrate standards. The difference between the standards and the reflectometer was less than +/- 0.5 ppm.

## 2.6 Fruit and leaf N%

Leaf samples were oven-dried and ground to less than 0.5 mm particle size in a UDY Cyclone Sample Mill (Thomas Scientific, USA). Fruit samples were lyophilized and ground in a coffee mill (Breville CG2B, Breville, USA). A standard Kjeldahl digestion including salicylic acid to ensure recovery of $NO_3^-$ similar to that described by (Temminghoff and Houba 2004). The method used 100 mg +/-10 mg of the dried powder (accurate to 1 mg) with 5 ml of the Kjeldahl solution added to 25ml graduated glass digest tubes. Duplicate extractions of each sample were made, including extractions from plant standards of known N content. The digest mixtures in the tubes were placed on a heating block and heated until dry. The residue was redissolved in 25 ml of distilled water, mixed thoroughly. The N and P content of the resulting extracted solutions was measured on an auto-analyser (Blakemore et al. 1987).

# Contents

## List of Tables

## List of Figures

# 3. Effects of nitrate fertiliser applied to the roots on fruit and vegetative characteristics of 'Hort16A' and 'Hayward' kiwifruit

## 3.1 Introduction

The replacement of nutrients removed in harvested products is often used as the basis for rational fertiliser use (Huett 1996). Judged against this standard, fertiliser inputs to kiwifruit orchards often exceed theoretical fertiliser requirements (Anon 2005; Sale 1997; Tagliavini et al. 1995). They are also high compared to fertiliser inputs for some other fruit crops such as apples and grapes (Wilson 2001, p29; Smart et al. 1986). High nitrogen (N) fertiliser rates could have an adverse effect on fruit quality and the environment (Weinbaum et al. 1992). The harmful environmental effects of leaching and runoff of N fertilisers are well documented (Tilman et al. 2002). Fruit quality attributes such as taste and flavour, nutritional value, and storage life could also be affected negatively by excessive N fertiliser inputs but these effects in kiwifruit are not well quantified (Prasad and Spiers 1992; Sher and Yates 1992; Sanchez et al. 1995). Excessive vegetative vigour can also be induced by N fertilisation and can be a significant cultural problem in kiwifruit production (Patterson and Currie 2011). Nevertheless, N fertiliser inputs are essential to maintain both fruit quality and crop yield (Marschner 2002). For example, in kiwifruit slow canopy development, poor bud break, and low photosynthetic rates have been used to justify high N inputs (Buwalda et al. 1990).

Nitrogen is the dominant element in plant nutrition and generally contributes about 80% of the total cation and anion uptake of plants (Marschner 2002). It is an important determinant of yield, is implicated in a wide range of fruit quality attributes and issues, and more than any other mineral nutrient changes the composition of plants (Marschner 2002). In most situations, the main form of N taken up by crops is nitrate ($NO_3^-$; Miller and Cramer 2004). Nitrate is chemically very reactive and its reduction and assimilation bears a high energy cost and may compete for energy resources in fruiting plants or in low light situations (Marschner 2002). Within the plant $NO_3^-$ is the primary signal molecule involved in N assimilation and can induce multiple gene responses in tissues within minutes of exposure (Crawford 1995; Wang et al. 2000). Nitrate uptake increases the synthesis of organic acids, decreases starch synthesis, changes plant hormone levels, and alters shoot:root allocation and root morphology

(Stitt 1999). Reduced starch synthesis with increasing levels of $NO_3^-$ was reported by Scheible (1997) and is consistent with the generally inverse relationship between carbohydrates and increasing levels of N nutrition (Marshner 2002). Since starch contributes up to 50% of total fruit dry matter (Beever and Hopkirk 1990), a reduction in starch concentration is likely to also reduce fruit dry matter concentration (DM%). Fruit DM% is an important measure of fruit quality since it is directly related to the taste and consumer preference for the fruit (Richardson et al. 1997b; Harker 2004). The capacity of $NO_3^-$ to increase plant water uptake has been noted in a wide range of species (McIntyre 1997; Cardenas-Navarro et al. 1999) and this could also reduce fruit dry matter concentration.

To investigate the effects of excessive N fertiliser and elevated $NO_3^-$ uptake on a range of kiwifruit vegetative characteristics and fruit quality attributes, high rates of $NO_3^-$ fertiliser were applied to four year old 'Hort16A' vines (*Actinidia chinensis*) growing in pots during the 2006-07 season, and to mature field-grown 'Hayward' vines (*A. deliciosa*) over four successive seasons (Appendix 2, Plates 1-3). By applying $NO_3^-$ fertiliser at regular intervals in the field trial or maintaining a constant fertigated supply in the pot trial, it was intended to maintain a high level of $NO_3^-$ uptake and content within the vine throughout the period of fruit development.

Specific experimental questions were:

1. Does maintaining a high supply of $NO_3^-$-N in the soil solution result in increased water uptake and reduced dry matter accumulation in the fruit?
2. Does $NO_3^-$ accumulate in the fruit?
3. What are the effects of an elevated $NO_3^-$ supply on vine growth?

## 3.2 Materials and methods

### 3.2.1 Pot-grown 'Hort16A'

During the 2006-07 season (Year 1), sixteen four-year-old pot-grown 'Hort16A' (*A. chinensis*) vines in a greenhouse at HortResearch, Palmerston North were randomly allocated to one of two different nitrogen (N) treatments:

(1) High nitrogen (HN): irrigated with 10mM N as calcium nitrate $Ca(NO_3)_2$;

(2) LN: irrigated with 0.5mM N as $Ca(NO_3)_2$ .

Pots had a surface area of 0.3 m$^2$ and volume of 160 L.The vines had been planted in the pots in 2004. Four split applications of solid fertiliser were applied to the surface of the pots to supply 3.24 g P, 9.24 g K, and 3.6 g Mg per pot. These rates were based on estimates of vine demand. LN vines also received gypsum ($CaSO_4$), to supply the same Ca (16.3 g/pot) as the HN vines received with the fertigated $Ca(NO_3)_2$. Trace elements Mn, Zn, and Fe were applied as foliar applications according to results of leaf analysis and pots were given two drenches with a seaweed extract solution. Pots were irrigated up to three times daily to maintain the media close to field capacity judged by the maintenance of low but regular drainage flow, which was collected. Regular monitoring of irrigation sprinkler output to each pot ensured equal delivery. The electrical conductivity (EC), pH and temperature of the drainage water of the pots was also monitored. Nitrogen was introduced into the irrigation water supply by means of two dispensers (Dosatron Inline Dispenser Model 2000, Bell-Booth Ltd, Palmerston North, NZ) connected to two separate irrigation lines, one for each of the N treatments. Media in the pots was a coarse bark/compost/pumice mix with a pH of between 6.0 and 6.4 measured using a saturated paste extract with 25 ml water to 10 g media (Warncke 1986). The unheated greenhouse was automatically ventilated at 24°C. Full bloom was 4$^{th}$ October 2006.

Leaves were sampled at 56, 85, and 110 days after full bloom (DAFB). On the second leaf sampling time (85 DAFB) leaf samples were taken morning (6.00 am), midday (12.00 pm), and late afternoon (4.00 pm) (times NZDST = solar real time + 1.5 hours), and whole shoots (long unterminated) were also collected. Fruit samples were collected at 85 and 184 DAFB.

### 3.2.2 Field-grown 'Hayward'

Mature 'Hayward' vines (*A. deliciosa*) growing on T-bar structures in the Massey University orchard, Palmerston North (140.7°S 174.7°E) received either high nitrogen (HN) or no nitrogen (LN) during the four seasons from 2006-07 to 2009-10. Full details of the vines and their management are given in Chapter 2. Twenty four female vines were selected from two rows referred to in the text as Row 1 and Row 2 separated by a single buffer row. Rows were 5 m apart. The vines were in pairs making a 'bay' delineated by posts of the T-bar structure. Each bay was separated by a bay containing two female vines on either side of a single male vine. Vine spacing within the treatment bays was 2.5 m. Nitrate fertiliser was broadcast onto the soil surface above the main root zone (approximately 25 $m^2$/bay) of six randomly selected bays at a rate equivalent to 50 kg N $ha^{-1}$ every three weeks from fruit set, or shortly before, until one month before harvest. The remaining six bays received no N fertiliser but, depending on the form of $NO_3^-$ fertiliser used, were supplied with equivalent amounts of potassium or calcium as the sulphate salt. Soil type is classified as a Manawatu fine sandy loam (Cowie and Rijkse 1977; Green and Clothier 1995). Soil samples were taken with a 3 cm diameter steel auger to 15 cm depth in late winter of 2006 and 2008. Eight cores were taken from each experimental bay and combined into two samples for HN and LN bays and submitted for standard soil analysis by the Fertilizer and Lime Research Centre at Massey University (Table 3.1).

Budbreak was in the first week of September and was considered complete by the third week of the same month. Full bloom in all seasons (2006-2009) was between 28 Nov – 5 Dec. Crop loads were relatively light in comparison to commercial vines and leaf:fruit ratios were high in each of the four seasons so that crop load and leaf:fruit ratios were not considered significant factors needing to be taken into account in interpreting the results. Vines were irrigated as necessary during dry periods in the summer but soil moisture was not routinely monitored.

Nitrogen was supplied as $Ca(NO_3)_2$ in each of the seasons, except in the 2007-08 season (Year 2), when $KNO_3$ was used. Applications equivalent to 50 kg N $ha^{-1}$ were made at approximately 20 day intervals from just before flowering until about 20 days before the final harvest. The final harvest occurred when fruit had reached °Brix levels between 6.2 (recommended maturity for exported fruit) and 8.0 which is close to the optimum maturity level in terms of fruit storage and eating quality of between 7 and 10 (Beever and Hopkirk 1990).

Table 3.1 Soil bulk density (BD), cation exchange capacity (CEC), plant available phosphorus (Olsen P), and exchangeable cations (Ca, Mg, K, and Na) in late August 2006 and 2008 of the 'Hayward' T-bar orchard used for the experiments with soil-applied N (0-15 cm depth soil cores taken from Rows 1 and 2).

| | pH | BD | CEC | Olsen P | Ca | Mg | K | Na |
|---|---|---|---|---|---|---|---|---|
| | | (g/mL) | (me/100g) | (ug/ml) | (me/100g) | (me/100g) | (me/100g) | (me/100g) |
| 29/08/06[1.] | | | | | | | | |
| HN | 6.1 | 0.9 | 16 | 38.6 | 10.3 | 1.5 | 1.3 | 0.1 |
| LN | 6.1 | 1.0 | 18 | 41.6 | 10.0 | 1.7 | 1.2 | 0.1 |
| 30/08/08 | | | | | | | | |
| HN | 6.3 | 0.9 | 15 | 44.6 | 10.6 | 1.6 | 1.1 | <0.05 |
| LN | 6.3 | 0.9 | 17 | 43.8 | 11.5 | 2.0 | 1.0 | <0.05 |

[1.] date soil cores collected.

### 3.2.2.1 Year 1 (2006-07 season)

Calcium nitrate was applied to the HN vines and $CaSO_4$ to the LN vines to supply equal amounts of calcium to the two treatments. Two fertiliser applications were made at 54 and 31 days before anthesis respectively. Further applications were made at full bloom and then after at three week intervals until two weeks before harvest. Leaf samples were collected early morning between 7 and 8am at 29, 45, and 105 days after full bloom (DAFB). Twenty youngest fully expanded leaves from a midway position of one side of the canopy were taken from each vine. Leaf petioles were removed from the leaves and frozen fresh to be used for $NO_3^-$ analysis, except for leaves collected 45 DAFB, when petioles were included with the laminar. Leaf material was weighed and oven dried at 65°C for calculation of moisture content. Full bloom was on 5/12/06. Fruit was sampled at 29, 76, and 176 DAFB.

### 3.2.2.2 Year 2 (2007-08 season)

In Year 2 the fertiliser treatments applied in Year 1 were repeated, except that $KNO_3$ was used instead of $Ca(NO_3)_2$. Equivalent potassium as sulphate was supplied to the LN vines. An additional 12 vines (Row 3) was included to give a total of 36 vines (18 × HN and 18 × LN). The first fertiliser application was made 15 days before full bloom and thereafter at three week intervals until two weeks before harvest. Full bloom was 3/12/07.

Fruit samples were taken at 45, 80, and 116 DAFB, and for the final harvest at 163 (Row 4), 167 (Row 1), and 174 (Row 2) DAFB.

### 3.2.2.3 Year 3 (2008-09 season)

In Year 3 the HN and LN treatments were again applied to the same vines as in Years 1 and 2. In this season $Ca(NO_3)_2$ was used as the N source. The first application of $Ca(NO_3)_2$ was made to the HN vines five days before anthesis. Subsequent applications were made at 12, 31, 40, 59, 93, 114, and 142 DAFB. In addition to the HN and LN treatments, an early season application of $KNO_3$ and $K_2SO_4$ to three HN and three LN bays respectively were made (Table 3.2). The additional spring fertiliser treatments were used to compare effects of fertiliser and vine N status on the rate of sap bleeding from the cut ends of canes in early spring and on early season canopy growth. Spring sap bleeding is an indication of physiological activity in the roots that generates positive pressures in the xylem and is a common phenomenon in vines (Ewers et al. 1991). The bays so treated were designated HN+SF and LN+SF. The experiment included a total of 36 vines in three rows. Full bloom was on 1/12/08 and fruit was harvested at 163 DAFB (12/05/09).

### 3.2.2.4 Year 4 (2009-10 season)

In this season the fertiliser treatments to the HN and LN bays were repeated. A trial with foliar N and the plant growth regulator CPPU was combined factorially with the soil-applied N, full details and results of which are given in Chapter 6. Only the results for fruit fresh weight (FW), dry weight (DW), and dry matter concentration (DM%) of HN and LN treatments are included in this chapter because they continue for a fourth year the experiments using the same high rates of soil-applied $NO_3^-$ to the same 'Hayward' vines and therefore are directly relevant to this section. Budbreak (50%) was on 1/09/09 and full bloom was on 4/12/09. Fruit was harvested 81 DAFB (23/02/10) and the final harvest was at 165 DAFB (18/05/10).

Table 3.2 Summary of high (HN) and low (LN) nitrogen fertiliser treatments in trials over four seasons between 2006 and 2010.

| Year | Season | Trial and N application method | Cv. | Main treat-ments | N form and rate | Chapter |
|---|---|---|---|---|---|---|
| 1 (Year 1) | 2006-07 | Greenhouse pot trial; fertigated | *A.chinensis* 'Hort16A' | HN | $Ca(NO_3)_2$ @ 10 mM $NO_3$-N <br> Plus P, K, Mg, S, and trace elements | 3 and 4 |
| | | | | LN | $Ca(NO_3)_2$ @ 0.5 mM $NO_3$-N <br> Plus P, K, Mg, S, and trace elements | |
| 1 (Year 1) | 2006-07 | Field trial; Soil-applied | *A.deliciosa* 'Hayward' | HN | $Ca(NO_3)_2$ 450 kgN/ha <br> Split applications from 54 days before anthesis | 3 and 4 |
| | | | | LN | Nil N, equivalent Ca as $CaSO_4$ | |
| 1 | | Te Puke long-term N fertiliser trial | *A.chinensis* 'Hort16A' | HN | 295 kg N/ha/yr as calcium ammonium nitrate | 4 |
| | | | | LN | Nil N | |
| 2 (Year 2) | 2007-08 | Field trial; Soil-applied | *A.deliciosa* 'Hayward' | HN | $KNO_3$ 450 kgN/ha <br> Split applications from anthesis | 3 |
| | | | | LN | Nil N, equivalent K $(K_2SO_4)$ | |
| 3 (Year 3) | 2008-09 | Field trial; Soil-applied | *A.deliciosa* 'Hayward' | HN | $Ca(NO_3)_2$ 400 kgN/ha from 5 days before anthesis | 3 |
| | | | | HN+SF[1.] | As for HN, plus 300 kg N as $KNO_3$ at budbreak | |
| | | | | LN | Nil N, equivalent Ca $(CaSO_4)$ as for HN | |
| | | | | LN+SF | As for LN, plus equivalent K as $(K_2SO_4)$ at budbreak | |
| 4 (Year 4) | 2009-10 | [2.]Field trial; Soil-applied | *A.deliciosa* 'Hayward' | HN | $Ca(NO_3)_2$ from anthesis | 3 and 6 |
| | | | | LN | Nil N, equivalent Ca as $CaSO_4$ | |

[1.]SF = spring fertiliser; [2.]Year 4 had foliar N and CPPU treatments as sub treatments and is fully reported in Chapter 6.

### 3.2.3 Measurement of total N and $NO_3^-$

*3.2.3.1 Leaf and fruit total N% and petiole sap $NO_3^-$.*

Leaf and fruit total N% was measured by Kjeldahl digestion followed by N analysis on an auto analyser (Blakemore et al. 1987). Nitrate content of the petiole sap and fruit was measured with a quick test method using nitrate-test strips (Reflectoquant Nitrate Test, Cat.No. 1.16995.0001, E.Merck, Darmstadt, Germany) and a reflectometer (Merck RQflex No.16970.0001). Full details of the methods for $NO_3^-$ extraction from the petioles and analysis of total N and $NO_3^-$ in fruit and leaf tissue are given in Chapter 2.

*3.2.3.1 Nitrate extraction from fruit*

Various methods to extract $NO_3^-$ from dried fruit samples were compared using dried and powdered fruit tissue, including hot and cold water, hot and cold acetic acid (2% v/v), and hot and cold methanol (Salomez and Hofman 2002). Cold water was found equal to any of other extracting solutions and was therefore used (Temminghoff and Houba 2004). Extracts were turbid probably due to soluble proteins (Dawes et al. 1991). Turbidity of the extract can interfere with the colorimetric assay (Massey 1991). Lyophilized and powdered tissue was found easier to clarify for colorimetric determination of $NO_3^-$ than oven dried ground tissue. Various clarifying agents were tried including Carrez reagent and activated charcoal (Pickston et al. 1980) and bentonite (Dawes et al. 1991). The easiest and most effective was bentonite and was therefore used. Bentonite is widely used in the fruit juice and wine industries to remove proteins (Dawes and Keene 1999). No allowance was made for interference by $NO_2^-$ since it is present only at very low levels in fruit generally (<1ppm) (Susin et al. 2006).

Two grams of lyophilized and powdered fruit tissue was weighed into centrifuge tubes and 30 ml deionized water plus 0.1g bentonite was added. The tubes were mixed on a rotary shaker for 1 hr followed by centrifuging at 10,000 rpm for 10 min. The supernatant was filtered (Whatman 41, Whatman® GE Healthcare Life Sciences, USA). The $NO_3^-$ content was read in the filtered supernatant with nitrate-test strips and reflectometer as described in Chapter 2, section 2.6.

### 3.2.4 Early season vine growth

*3.2.4.1 Sap collection, sap $NO_3^-$ and soluble solids content*

Pre- and post-bud-break sap flow rate and sap $NO_3^-$ content in vines of different N status and in vines receiving early spring fertiliser applications were determined in Year 3 (2008-09 season). Dates are referred to in relation to the date of 50% bud-break (15/09/08). Days after bud-break is abbreviated DPBB – days 'post' bud-break. Bud-break commenced shortly before the time of the first sap collection and was considered complete by 26/09/08 (11 DPBB).

Sap collection took place between 12.00pm and 3.00pm on three different dates by attaching a graduated 50 ml BD Falcon™ conical tube (BD Biosciences Co. USA) to the cut end of a cane following a similar procedure to that described by Ferguson (1980).

Collection 1: 5 days before bud-break (10/09/08).

The end of one selected cane on 24 vines receiving each of the above treatments was cut and the sap collected for 20 minutes. Sap volume, $NO_3^-$ content, Brix, and pH were recorded.

Collection 2: 1 DPBB (16/09/08)

The ends of three equal sized canes from each of 36 vines receiving one of each of the above treatments were cut and the sap collected for 20 minutes. Sap volume, $NO_3^-$ content, °Brix, and pH was recorded. The diameter of each of the canes used for sap collection was measured at the first wire and just above the cut end.

Collection 3: 21 DPBB (6/10/08)

The same canes used for the second sap collection were recut approximately 5 cm from the end of the cane (previous cut) and sap was collected for 20 minutes.

*3.2.4.2 Shoot growth*

On the same canes used for sap collection, the percentage of buds breaking, number of floral buds, shoot growth, and leaf area was recorded on the first four shoots from the end of each cane giving a total of 12 observations (4 shoots × 3 canes) per vine. Percentage bud break was recorded on 11 DPBB, shoot growth and number of floral buds was measured on 30 DPBB and 43 DPBB. Fruit numbers, shoot length, and leaf area was recorded on selected long unterminated shoots from the outer canopy zone on 88 DPBB (12 days after full bloom.

Leaves from each shoot were removed, weighed, and their area measured on a LI-COR Area Meter (Model Li 3100, Li-Cor Inc. Lincoln, Nebraska, USA).

### *3.2.4.3 Photosynthesis measurements*

Subtending leaves on similarly positioned (middle of the inclined part of the T-bar canopy) and sized fruiting shoots (4 per vine) were tagged for measurement of photosynthesis. Measurements on both sides of the T-bar canopy were taken between 1.5 and 0.5 hours before solar midday (midday NZDST) in sunny blue sky conditions with a portable photosynthesis system (LI-6400 PSC-591, Li-Cor, Lincoln, Nebraska, USA) on the same leaves on 57 DPBB (ambient air temp 22.6°C), 65 DPBB (20.2°C), and 75 DPBB (23.3°C). Mature leaves (youngest fully expanded, and fully exposed) on fruiting long shoots (>100 cm) from the same canopy zone were selected for the measurement made on 138 DPBB (62 DAFB) (31.3°C). Data was not collected from all of the LN+SF vines and so this treatment was not included.

## 3.2.5 Vegetative vigour

Summer pruning is a standard practice in commercial kiwifruit orchards to remove unwanted shoots and prevent tangling of shoots whose natural growth habit includes twining typical of many vines (Putz and Holbrook 1991, p77). The weight of prunings can be used as a measure of vegetative vigour (Burge et al. 1987). In Year 1, fresh weight of shoots removed in summer pruning was recorded 50 DAFB in the pot-trial with Hort16A, and 19 DAFB in Year 3 in the field trial with 'Hayward' vines. Pruning weights were also recorded in Year 4 but these results are reported in Chapter 6.

## 3.2.6 Weighing and drying fruit

Fruit samples were weighed to give fresh weight (FW) and then cut into four longitudinal slices. Two slices were placed in weighed paper bags and placed in a forced air oven at 60 °C and dried to constant weight to obtain dry weights (DW). The other two slices were placed in zip-lock plastic bags, weighed, and frozen for later analysis of oxalate, ascorbic acid, and calcium (results of these analyses are reported in Chapter 4).

Year 1. Fruit for the samples was taken from similar sized shoots towards the end of the canes close to the third wire of the T-bar support structure (outer canopy) and from terminated shoots between the leader and the first wire (inner canopy). In the pot-trial with

'Hort16A' fruit was sampled from a zone within 1.5 and 2.5 metres from the trunk of the vine. Fruit was weighed and then cut longitudinally into four pieces. Two pieces were placed in snap lock plastic bags, weighed, and frozen for chemical analysis. The remaining two pieces were placed in paper bags, weighed, and dried to a constant weight in a forced air oven at 60 to 70°C. When recording the dry weights, bags were removed in sets of four from the oven to minimise the effect of re-absorption of moisture in the interval between removal from the oven and recording of their weight.

Year 2. At the final harvest eight fruit from two canopy positions (inner and outer) and two shoot types (short and long) was taken for processing. The inner and outer canopy positions correspond to fruiting zones close to the main leader and zones at the distal ends of the canes respectively. Short shoots were terminated shoots <40 cm and long shoots were terminated or unterminated >100cm.

At each sampling fruit was weighed and then cut longitudinally into eight sections. Four slices were frozen and lyophilized for chemical analysis and the other four slices were oven dried for calculation of DM%.

Year 3 and 4. A slightly different procedure was used with fruit being cut equatorially rather than longitudinally. The full procedure is described in Chapter 2.

### 3.2.7 Seed counts
In Year 1 at the final harvest of the field trial with 'Hayward' vines, eight fruit from each vine and two canopy positions (inner and outer) were weighed (FW) and cut in half. All visible seeds were counted on the exposed surface of one half of a total of 384 fruit. Both halves were then bagged and dried in a forced air oven at 65°C to constant weight for dry weight.

### 3.2.8 Statistical analysis
The experiment was a randomised complete block with rows as blocks. In total there were 24 vines in two blocks with 12 HN and 12 LN vines within each block.

Data was averaged and standard errors of each mean are presented. Coefficient of variation (CV%) was used when treatments or variables were extremely different. Where relevant, data sets were analysed with single factor ANOVA using GLM procedure of SAS with treatment means compared using Fisher's Protected Least Significant Difference (SAS Institute Inc., 2004). When there were only two variables, means were compared with two-tailed T-tests in Excel. Normal or close to normal distributions were assumed. Significance of correlations was determined using a value of t derived from the formula $t = (r\sqrt{n} - 2)/(\sqrt{1} - r2)$ (Moore and McCabe 1993).

## 3.3 Results and discussion

In this section the effects of the fertiliser treatments on vine and fruit N and $NO_3^-$ content are presented first. Then the effects of the fertiliser treatments on early season growth and vegetative vigour are given. These effects are related to changes induced by $NO_3^-$ in vine water content. The final sections report the effects of elevated $NO_3^-$ uptake on some of the primary quality attributes of the fruit, including some unexpected variation between rows in the orchard used for the field trial, and differences between shoot types and canopy position.

### 3.3.1 Leaf N% and petiole $NO_3^-$

In the Year 1 pot-trial with 'Hort16A', leaf N% averaged 2.2% in the LN vines and 3.0% in the HN vines in October 2006. This level found in the LN vines was only slightly below the 'normal' or average range for 'Hort16A' at this time of the season (2.3 to 3.5% - Hills Laboratories 2010). However, these levels may have been buffered by vine reserves at this early point in the season. Mature perennial fruit species obtain a significant proportion of their annual N requirement from internally stored reserves, providing an important buffer against temporal fluctuations in the soil supply, especially during the early season period (Millard 1995). Even the relatively young (4 year old) vines in the pot-trial that had received a standard level of nutrition in the two seasons prior to the Year 1 trial seem likely to have contained up to 20% of their annual N demand in the form of internal reserves (Millard 1995). Nevertheless, by mid-season, leaf colour in the LN vines was a much lighter hue than the HN vines. This is consistent with LN vines having lower N status since leaf colour is highly correlated to chlorophyll content and thereby also to plant N status (Errecart et al. 2012).

In the Year 1 pot trial, the concentration of $NO_3^-$ in the soil solution of the pots for the high nitrate treatment (HN; 10 mM $NO_3$-N) was intended to create levels of $NO_3^-$ availability similar to those that can occur in the field following N fertilisation. For example, concentrations slightly above 10 mM were found in the soil solution of a kiwifruit orchard one week after an application of 70 kg N ha$^{-1}$ (Parfitt 1991); and Yanai et al. (1998) found levels above 10 mM were maintained in the soil solution for as long as 30 days in the presence of plants after an application of $Ca(NO_3)_2$ equivalent to 200 kg N ha$^{-1}$. The rate given to the pots for the low $NO_3^-$ treatment (LN; 0.5 mM $NO_3^-$-N) is less than the

concentration usually found in agricultural soils of 1-5 mM but can occur in kiwifruit orchards during winter (Marschner 2012; Parfitt 1991). In our experiment the low concentration of $NO_3^-$ was intended to minimise $NO_3^-$ uptake but not deliberately induce a N-deficiency. A low but constant supply of N will not necessarily lead to N deficiency since as each $NO_3^-$ ion is taken up it is immediately replaced by another, making the supply essentially inexhaustible (Larsson et al. 1992). Furthermore, the efficiency of uptake by roots increases at low soil solution concentrations due to the activation of high affinity $NO_3^-$ transport systems in roots (Miller and Cramer 2004). For example, 90% of optimum growth could be achieved in some plant species at $NO_3^-$ concentrations in the soil solution of only 14 µM $NO_3^-$-N (Clement et al. 1978).

In the 'Hayward' vines in the field trial, regular applications of $KNO_3$ fertiliser caused large increases in vine $NO_3^-$ content as measured in leaf petioles at all times during the season (Table 3.3). Leaf N% was also increased by the N fertiliser treatment and the level found in the LN vines was within the range considered normal for 'Hayward' vines at this time of the season (Table 3.3).

Petiole sap responds quickly to fertiliser application and therefore is considered a reliable indicator of current and seasonal $NO_3^-$ uptake (Errebhi et al. 1998). Prasad et al. (1986) suggested optimum leaf petiole levels in 'Hayward' vines were between 400 and 800 ppm. Buwalda et al. (1990) found leaf petiole $NO_3^-$ concentrations in N-fertilised and unfertilised kiwifruit ranged from 500 to 155 ppm respectively. Although levels found in LN vines were much less than this, there was no obvious evidence of N deficiency since leaf colour was dark green. Furthermore, leaf N% was well above the level of 1.5% considered indicative of N deficiency in 'Hayward' kiwifruit (Table 3.3; Barber et al. 1986). That there were no symptoms of nutrient deficiency shown by the vines at any time in any of the seasons indicates that the mineralisation of soil organic matter was able to maintain an adequate N supply to the LN vines. Mineralisation of soil organic matter in the absence of regular N fertilisation appears to have provided sufficient N to avoid deficiency in the long-term N fertiliser trial reported by Mills et al. 2008. Perham (1990) also made a reasoned argument for this possibility in kiwifruit orchards. The high levels found in HN vines confirmed that the fertiliser regime was effectively elevating vine $NO_3^-$ content, as was intended. The higher levels in HN vines than those reported by Buwalda et al. (1990) can probably be explained by the greater interval between the N fertiliser application and petiole sampling in their case. In

grapes $NO_3^-$ concentration in leaf petiole sap of up to 1820 ppm were found following N fertilisation (Roberts and Ahmedullah 1991) and in xylem sap of barley up to 2100 ppm (Miller and Cramer 2004). The effect of N fertiliser on kiwifruit leaf N% are similar to those reported elsewhere (Buwalda et al. 1990; Costa et al. 1997).

Table 3.3 Effect of high rates of N fertiliser (HN) or nil N fertiliser (LN) on 'Hayward' leaf N% and petiole sap $NO_3^-$ (ppm FW) in Year 2.

| Days after full bloom | Days after last fertiliser application (50 kg N/ha) | HN | LN |
|---|---|---|---|
| *Petiole sap $NO_3^-$ (ppm)* | | | |
| 37 | 10 | 1132 (48.0) | 54 (31.2) |
| 44 | 17 | 2894 (14.8) | 40 (49.8) |
| 178 | 15 | 2113 (52.7) | 31 (70.3) |
| | | | |
| *Leaf laminar total N%* | | | |
| 44 | 17 | 2.8 (0.1) | 2.2 (0.1)* |

Normal range for 'Hayward' leaf N% at this time of the season 2.2 to 3.0 (Hill Laboratories 2010). Coefficient of variation (%) in parenthesis for petiole $NO_3^-$ ; Standard error in parenthesis for leaf laminar N%; * $p<0.001$. DAFB: days after full bloom.

### 3.3.2 Fruit $NO_3^-$

Fruit $NO_3^-$ appeared to accumulate in the HN fruit over time with much higher levels being found at the final harvest (184 DAFB) than at the earlier sampling time (85 DAFB) (Table 3.4). There was considerable variability particularly in the HN vines (Table 3.4). The fruit from one vine averaged over 1000 ppm $NO_3^-$ but this vine was excluded from the data because it showed other signs of abnormality, such as very small leaves and lack of shoot growth.

The relatively low $NO_3^-$ content of the HN fruit at the earlier sampling time seems unusual since these vines had been growing with an elevated $NO_3^-$ supply for the previous four months. However, the much higher levels found by the time of the final harvest suggests vacuolar storage of excess $NO_3^-$ in *A. chinensis* fruit. Vacuolar storage of excess $NO_3^-$ is common in leaves of higher plants and prevents reduction since this occurs in the cytosol (Granstedt and Huffaker 1982). It is also a characteristic of so-called nitrophilous species (Bharucha and Dubash 1951).

Table 3.4 Effect of high (HN) and low (LN) levels of NO$_3^-$ availability on 'Hort16A' fruit NO$_3^-$ content (ppm DW) at two sampling times (Year 1).

| Days after full bloom | HN | LN | p-value |
|---|---|---|---|
| 85 | 36.0 (12.0) | 20.6 (4.1) | NS |
| 184 | 120.7 (45.7) | 17.4 (3.8) | <0.01 |

Standard error in parenthesis; p-value from two tailed T-test

Accumulation of NO$_3^-$ over time did not occur in fruit from 'Hayward' vines in the field trial in Year 1, although levels were always significantly higher in HN fruit (Table 3.5). The pattern of higher fruit NO$_3^-$ early in the season, as was found here in the HN fruit, are similar to that reported by Walton and De Jong (1990). They found 'Hayward' fruit NO$_3^-$ at 52 and 73 days after flowering of 140 and 110 ppm respectively and these then declined to undetectable levels by harvest. The earliest sampling time in Table 3.5 was 76 days after flowering and hence comparable to Walton and DeJong (1990). The higher levels found at harvest in HN fruit than was reported by Walton and DeJong (1990) presumably are caused by the continued application of NO$_3^-$ fertiliser. However, Pickston et al. (1980) reported NO$_3^-$ content of between 60-80 ppm in commercially grown mature 'Hayward' fruit, which confirms that there can be considerable variability in fruit NO$_3^-$ content. There were also higher levels of NO$_3^-$ in the unfertilised LN fruit (Tables 3.4 and 3.5) than were reported by Walton and DeJong (1990). However, these results may reflect the lack of sensitivity of the Merckoquant NO$_3^-$-strip method when NO$_3^-$ concentrations are below 3 ppm. With the sample weight and dilution factor used, low results of between 1 and 3 ppm in the prepared sample solution result in calculated NO$_3^-$ values of between 14 and 33 ppm as were found in LN fruit in both the 'Hort16A' pot trial and the 'Hayward' field trial. Even though the extracts were clarified before measurement of their NO$_3^-$ content, colorimetric methods of NO$_3^-$ determination are prone to inaccuracy due to inferences from coloured matter or turbidity (Carlson 1986). Although these results show very high levels of NO$_3^-$ can occur in leaf petiole sap following NO$_3^-$ fertilisation, there was little evidence of NO$_3^-$ accumulating to high levels in the fruit.

Table 3.5 Effect of high (HN) and low (LN) levels of NO$_3^-$ availability on 'Hayward' fruit NO$_3^-$ content (ppm DW) at three sampling times in Year 1.

| Days after full bloom | HN | LN | p-value |
|---|---|---|---|
| 76 | 131.8 (26.4) | 28.0 (3.6) | 0.002 |
| 105 | 52.6 (4.7) | 18.2 (3.6) | <.001 |
| 174 | 58.5 (12.6) | 25.0 (1.8) | <.001 |

Standard error in parenthesis; p-value from two tailed T-test.

### 3.3.3 Early season vine growth and vegetative vigour

*3.3.3.1 Sap flow*

The rate of exuding sap from cut canes was measured during the period of bud-break in Year 3 in 'Hayward' vines that had received high N fertiliser (HN) or no N fertiliser (LN) in the two previous seasons. At the two earliest sap collection times, 5 days before and 1 day after 50% budbreak respectively, LN vines had sap flow rates significantly higher than those of HN vines but by the time of the last collection 21 days after 50% budbreak (21 DPBB), the sap flow rate had declined in both sets of vines to similar levels (Figure 3.1).



Figure 3.7 Sap flow rate (ml/cane/20mins) from bleeding canes of 'Hayward' vines given high (HN) or nil (LN) N fertiliser rates during the previous two seasons (Year 1 and 2), or additional spring applied $KNO_3$ (HN+SF) or $K_2SO_4$ (LN+SF) fertiliser applied pre-budbreak of Year 3 as recorded at three collection times in early spring of Year 3. * $p<0.05$, **$p<0.01$, NS not significant, two tailed T-tests, n =18.

In kiwifruit, xylem sap bleeding from cut stems or canes in spring is probably due to the generation of a positive pressure in the roots rather than in the stem or other aerial parts (Peterlunger et al. 1990). The higher sap flow or root pressure of the LN vines could be due to earlier root activity in response to their lower N status and consequent higher N demand. Increased root growth is a common plant response to N-deficiency (Miller and Cramer 2004). However, core sampling of the same vines in July 2008 showed no differences (data not shown) between HN and LN vines in the weight of total or white roots (Mills and Morton 2009). Buwalda and Hutton (1988) suggested root pressure might not be solely dependent on

new root growth since water is also taken up by suberized root surfaces. Therefore, the difference between HN and LN vines might be due to increased hydrolysis of storage compounds creating lower solute potential leading to increased water uptake by the root system (Enns et al. 2000).

Spring-applied potassic fertiliser in Year 3 had no significant effect on sap flow rates in either the nitrate or sulphate form at the two earlier collection times (Table 3.6). However, the average flow rates of HN+SF vines became progressively higher than HN vines, until by the last collection time they were significantly different (p<0.01; Table 3.6).

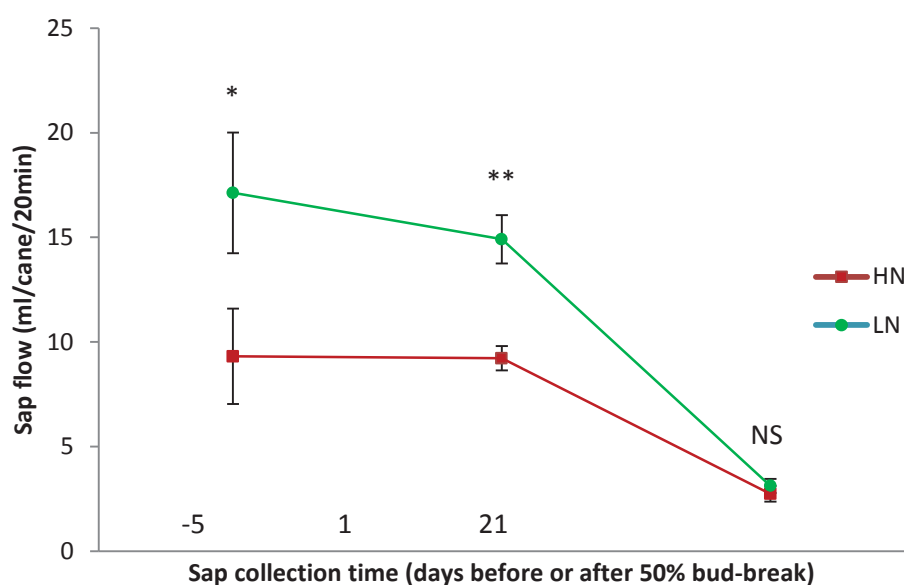Table 3.6 Sap flow rate (ml/cane/20mins) from bleeding canes of 'Hayward' vines given high (HN) or nil (LN) N fertiliser rates during the previous two seasons (Year 1 and 2), or additional spring applied $KNO_3$ (HN+SF) or $K_2SO_4$ (LN+SF) fertiliser applied pre-budbreak of Year 3 as recorded at three collection times in early spring of Year 3.

| Sap collection time (DPBB)[1] | HN | HN+SF | LN | LN+SF |
|---|---|---|---|---|
| -5 | 9.2 (3.0) | 9.6 (4.0) | 16.9 (2.6) | 17.5 (7.7) |
| 1 | 8.9 (0.7) | 10.3 (1.4) | 15.3 (1.4) | 13.4 (1.3) |
| 21 | 2.3 (0.3) | 4.4 (1.2) | 3.1 (0.4) | 3.4 (0.4) |

[1]DPBB: days after 50% bud-break. Standard error in parenthesis

Fertiliser might increase root pressure by osmotic effects either in the soil or, following uptake, in the roots (Enns et al. 2000). Clearwater et al. (2007) suggested that increases in kiwifruit sap exudation, following fertiliser applications, were due to osmotic effects especially in the case of nitrates. This is consistent with the increase in exudation in HN+SF by the last collection time (Table 3.6). Solute potential of the sap should be well correlated with readings of °Brix (Matthews et al. 1987). Significantly higher °Brix readings were found in sap from HN+SF vines at the earliest collection time but unfortunately °Brix was not determined at the last collection time (Table 3.7). The lack of a similar effect of increasing sap exudation in the case of $K_2SO_4$ applied to the LN vines might be due to the lower solubility and slower dissolution of $K_2SO_4$ compared to $KNO_3$ fertiliser forms (Elam et al. 1995).

Table 3.7 °Brix readings of sap from bleeding canes of 'Hayward' vines given high (HN) or nil (LN) N fertiliser rates during the previous two seasons (Year 1 and 2), or additional spring applied $KNO_3$ (HN+SF) or $K_2SO_4$ (LN+SF) fertiliser applied pre-budbreak of Year 3 as recorded at two collection times in early spring of Year 3.

| Sap collection time (DPBB)[1.] | HN | HN+SF | LN | LN+SF |
|---|---|---|---|---|
| -5 | 8.6 (1.1) | 11.0 (1.1) | 9.1 (1.5) | 10.8 (0.4) |
| 1 | 10.5 (0.3) | 10.5 (0.9) | 10.4 (0.6) | 9.8 (0.3) |

[1.]DPBB: days after 50% bud-break. Standard error in parenthesis

There was considerable variation in the rate of sap exudation between individual canes which almost matched the between vine variation (sap flow rate was not dependant on cane diameter - data not presented). The average coefficient of variation (cv%) between canes on individual vines was 33% compared to 41% between vines. If the function of spring root pressure in deciduous vines such as kiwifruit is to remove air and emboli from their relatively wide and extended xylem vessels, then the large variability in sap flow rate within vines might be related to the highly irregular distribution of such blockages in the canopy (Sperry et al. 1987).

At the first collection time, the average $NO_3^-$ content of sap from HN vines (HN and HN+SF), although higher, was not significantly different from the LN vines (LN and LN+SF) (Table 3.8). However, by the second collection time the $NO_3^-$ content of sap from HN and HN+SF vines was significantly higher than LN treatments, especially with spring applied N ($p < 0.01$; Table 3.8).

Table 3.8 Nitrate content (ppm) in sap from bleeding canes of 'Hayward' vines given high (HN) or nil (LN) N fertiliser rates during the previous two seasons (Year 1 and 2), or additional spring applied $KNO_3$ (HN+SF) or $K_2SO_4$ (LN+SF) fertiliser applied pre-budbreak of Year 3 as recorded at two collection times in early spring of Year 3.

| Sap collection time (DPBB)[1.] | HN | HN+SF | LN | LN+SF |
|---|---|---|---|---|
| -5 | 10.6 (7.9) | 12.5 (6.1) | 9.6 (3.0) | 6.2 (2.5) |
| 1 | 15.4 (5.1) | 36.0 (12.3) | 8.5 (2.4) | 6.5 (2.7) |

[1.]DPBB: days after 50% bud-break. Standard error in parenthesis

### 3.3.3.2 Shoot growth

Bud-break (%) on the same canes used for sap collection was recorded on 26/09/08 at which time bud-break was considered complete. Bud-break of HN vines was lower than LN vines (Table 3.9) but fertiliser application increased bud-break in both LN and HN vines (Table 3.9). However, the large variability especially in the LN vines prevented the differences between the treatments being statistically significant probably because data for HN+SF and LN+SF was limited to six observations (Table 3.9).

It has been claimed elsewhere that spring fertiliser application, by generating higher root pressures, promotes bud-break in kiwifruit (Smith and Miller 1991). This is consistent with the pattern shown in Table 3.9, although vine N status appears important also, as it was in the generation of spring xylem sap pressure (Figure 3.1, Table 3.6).

Table 3.9 Bud-break (%) for vines in early spring of Year 3 for 'Hayward' vines given high (HN) or nil (LN) N fertiliser rates during the previous two seasons (Year 1 and 2), or additional spring applied $KNO_3$ (HN+SF) or $K_2SO_4$ (LN+SF) fertiliser applied pre-budbreak of Year 3 as recorded at 100% budbreak (26/09/08).

|                | HN   | HN+SF | LN   | LN+SF |
| -------------- | ---- | ----- | ---- | ----- |
| Bud break (%)  | 25.0 | 30.2  | 30.8 | 39.1  |
| SEM            | 2.3  | 3.3   | 5.1  | 7.9   |
| n              | 30   | 6     | 30   | 6     |

Standard error in parenthesis, n = number of canes

By 30 DPBB (approximately one month after 50% bud-break) HN vines appeared to have longer shoots, greater number of leaves per shoot, and greater number of floral buds per shoot than LN vines (Table 3.10). However, the differences were not statistically significant and were mainly due to more of the shoots on LN canes being stunted, with buds which had burst but whose shoots had failed to develop. Fertiliser applied just before budbreak had little effect on any of these parameters with the values in HN+SF and LN+SF being respectively similar to HN and LN (Table 3.10).

Table 3.10 Average shoot length (cm), number of leaves/shoot, and number of floral buds/shoot recorded 30 days after 50% budbreak (DPBB) and shoot length and number of shoots <20cm recorded on 43 DPBB in spring of Year 3 for 'Hayward' vines given high (HN) or nil (LN) N fertiliser rates during the previous two seasons (Year 1 and 2), or additional spring applied $KNO_3$ (HN+SF) or $K_2SO_4$ (LN+SF) fertiliser applied pre-budbreak in Year 3.

| | HN | HN+SF | LN | LN+SF |
|---|---|---|---|---|
| 30 DPBB[1] | | | | |
| Shoot length (cm) | 13.7 (2.5) | 13.5 (4.2) | 12.1 (2.2) | 13.3 (4.7) |
| Leaves/shoot | 8.7 (0.8) | 8.3 (1.2) | 8.1 (0.7) | 7.9 (1.3) |
| Floral buds/shoot | 3.6 (0.5) | 4.1 (0.8) | 3.3 (0.5) | 3.5 (0.9) |
| 43 DPBB | | | | |
| Shoot length (cm) | 34.4 (4.9) | 37.5 (8.3) | 32.3 (4.9) | 31.9 (9.7) |
| Shoots <20cm | 3.1 (0.7) | 1.3 (0.5) | 3.9 (0.5) | 4.3 (0.9) |

[1] DPBB: days after 50% bud-break. Standard error in parenthesis

The number of fruit per shoot, average leaf size, and leaf area per shoot varied insignificantly between the treatments (Table 3.11). In contrast to these results, increases in leaf size in response to N fertilisation of kiwifruit was reported by Costa et al. (1997). Average leaf density, measured in terms of leaf weight per unit of leaf area, although higher for leaves from LN vines (LN and LN+SF) than leaves from HN vines (HN and HN+SF), did not vary significantly between the fertiliser treatments (P>0.05; Table 3.11). Increased leaf thickness in trees of low N status was reported in citrus (Bondada et al. 2001) and apples (Buwalda and Lenz 1992).

Table 3.11 Fruit/shoot, leaf area (cm2/cm shoot), leaf density (mg/cm2) recorded 12 days after full bloom in Year 3 for 'Hayward' vines given high (HN) or nil (LN) N fertiliser rates during the previous two seasons (Year 1 and 2), or additional spring applied $KNO_3$ (HN+SF) or $K_2SO_4$ (LN+SF) fertiliser applied pre-budbreak in Year 3.

| | HN | HN+SF | LN | LN+SF |
|---|---|---|---|---|
| Fruit/shoot | 4.5 (0.3) | 4.5 (0.8) | 3.8 (0.3) | 4.5 (0.6) |
| Leaf area ($cm^2$/cm shoot) | 27.0 (1.2) | 29.1 (3.9) | 28.0 (1.5) | 26.0 (0.8) |
| Leaf area ($m^2$/shoot) | 1.94 (0.13) | 2.01 (0.28) | 1.78 (0.10) | 2.09 (0.10) |
| Leaf density ($mg/cm^2$) | 34.4 (0.8) | 34.3 (1.2) | 35.5 (1.1) | 37.8 (1.4) |

Standard error in parenthesis.

The 18% increase in fruit number/shoot between HN and LN could represent a significant increase in yield. Increases in kiwifruit yield associated with high rates of N fertilisation were attributed to increased fruit number by Buwalda et al. (1990) and Smith and Miller (1991).

The length of the shoots was measured two weeks after the first measurement time by which time shoots from HN+SF vines had grown faster than the other treatments with an average growth increment of 24.2 cm over the two weeks between the two measurement times (Table 3.10). The average growth rate of shoots from HN vines (20.8 cm) over the same period was similar to that of the LN shoots (20.3 cm), while the slowest growth rates were shown by LN+SF shoots (18.6 cm). With shoot growth rate it was N fertiliser in particular that was effective while vine N status and other fertiliser forms were not influential. This contrasts with the effects on budbreak where fertiliser in general and the N status of the vines both appeared to be related to the percentage of buds growing (Figure 3.1, Table 3.6). Nevertheless, there was considerable variability, especially in the spring-fertilised treatments. The number of terminated shoots noted (at 43 DPBB) among the selected shoots was greatest for LN+SF (average 4.5 terminated shoots out of 12) and this might account for some of the variability for this treatment (Table 3.10). However, HN+SF, which also showed comparatively large variability in shoot length, had the least number of terminated shoots (3.3 out of 12). Most noticeable was the number of shoots less than 20 cm long, which shows the invigorating effect of early spring-applied N fertiliser (Table 3.10). Increased shoot length with N fertilisation was also found by Costa et al. (1997) although in their case the difference in shoot length between the highest rate of N (450 kg N ha-1) and the nil N control was only 6% compared to 16% between HNF and LN in Table 3.10.

The effect of N fertiliser on vegetative vigour is even more clearly seen in the 72% increased weight of leaf and shoots removed from HN compared to LN vines during summer pruning (Figure 3.2). By this time HN vines had also received two N fertiliser applications with 500 g $CaNO_3$ (approximately equal to 50 kg N ha$^{-1}$) given to each HN and HN+SF vine, the first application being made five days before full bloom and the second 12 DAFB. A similar increase in vegetative vigour (73%) was found in the pot-trial with 'Hort16A' vines in Year 1 (Figure 3.3).

118
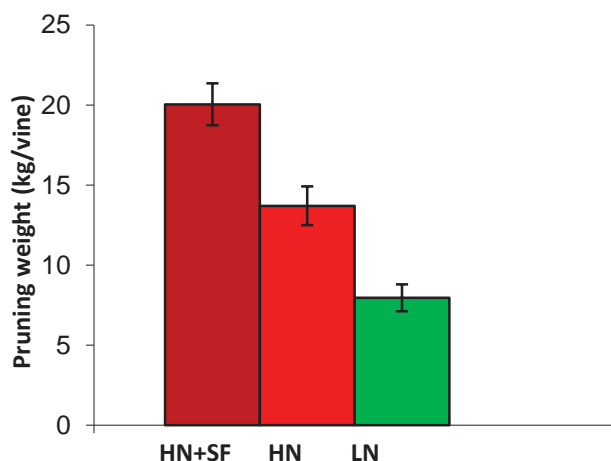
Figure 3.8 Weight (kg/vine) of shoots removed in summer pruning 19 days after full bloom (95 days after 50% budbreak) in Year 3 for 'Hayward' vines given high (HN) or nil (LN) N fertiliser rates during the previous two seasons (Year 1 and 2), or additional spring applied $KNO_3$ (HN+SF) applied pre-budbreak in Year 3. Error bars are standard error, n=3.
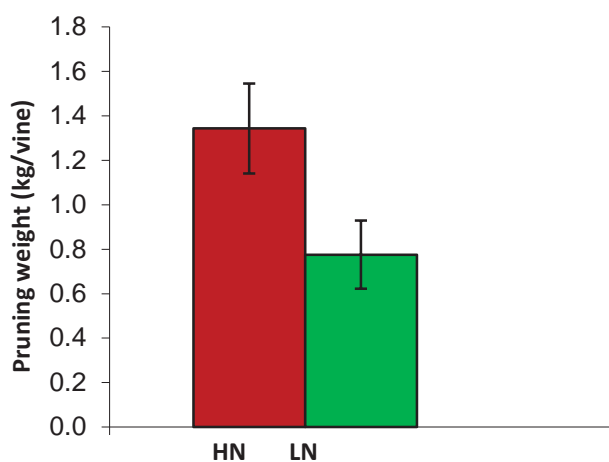


Figure 3.9 Weight of shoots removed during summer pruning 50 days after full bloom from potted 'Hort16A' vines fertigated with high (HN) or low (LN) rates of $NO_3^-$-N in Year 1. Error bars are standard error, n= 10 (LN), n = 6 (HN).

A 72 – 73% increase in shoot vigour in terms of the weight of summer prunings was the most outstanding effect of the elevated $NO_3^-$ supply given to the HN 'Hayward' and 'Hort16A' vines compared to their respective LN counterparts. Additional early season $NO_3^-$ (HN+SF) created an even bigger vegetative response in the 'Hayward' vines with a 150% increase in the weight of prunings compared to LN vines (Figure 3.2).

### 3.3.4 Vine water content

In the 'Hort16A' pot trial, leaves and shoots from HN vines had lower DM% (higher water content) than LN vines at all sampling times (Table 3.12). Lower leaf and shoot DM% reflects the greater succulence of the HN vegetative tissues; increased succulence is commonly associated with an increased N supply and can be used to predict leaf N content (Hodgson et al. 2011). The difference between the treatments reduced over time, possibly reflecting a phenological reduction in vegetative vigour with associated lower succulence as the season progressed (Table 3.12).

Table 3.12 Effect of high (HN) or low (LN) fertigated nitrate supply on pot-grown 'Hort16A' leaf and shoot DM% in Year 1.

| Days after full bloom | HN | LN | p-value |
|---|---|---|---|
| *Leaf (DM%)* | | | |
| 56 | 23.9 (0.8) | 28.6 (1.7) | ≤0.02 |
| 85 | 34.2 (0.7) | 35.8 (0.5) | <0.03 |
| 110 | 28.7 (2.4) | 29.5 (1.6) | NS |
| *Whole shoot (DM%)* | | | |
| 85 | 24.1 (0.4) | 27.7 (1.1) | <0.01 |

Standard error in parenthesis, p-values from one-tailed T-test, n=4.

In the pot trial with 'Hort16A', leaf DM% increased diurnally with a more rapid increase in the morning in both HN and LN vines (Figure 3.4). The diurnal pattern suggests LN vines continued to lose water during the afternoon, which could indicate more open stomata than HN vines. In beans, N supplied plants had greater water uptake during the night, while N deficient plants appeared to be less able to regulate stomatal aperture under conditions of water stress during the day (Shimshi 1970). This would be consistent with the data in Figure 3.4, where HN vines have reduced DM% (higher water content) early in the morning (6.00 am) and LN vines apparently continued losing water during the afternoon when some water stress in the greenhouse conditions are likely to have developed. The small volume of the pots in relation to the area of the canopy, plus elevated temperatures, both in the greenhouse

atmosphere and within the pots, on hot afternoons meant that water stress often developed despite adjustments being made to the irrigation volume.
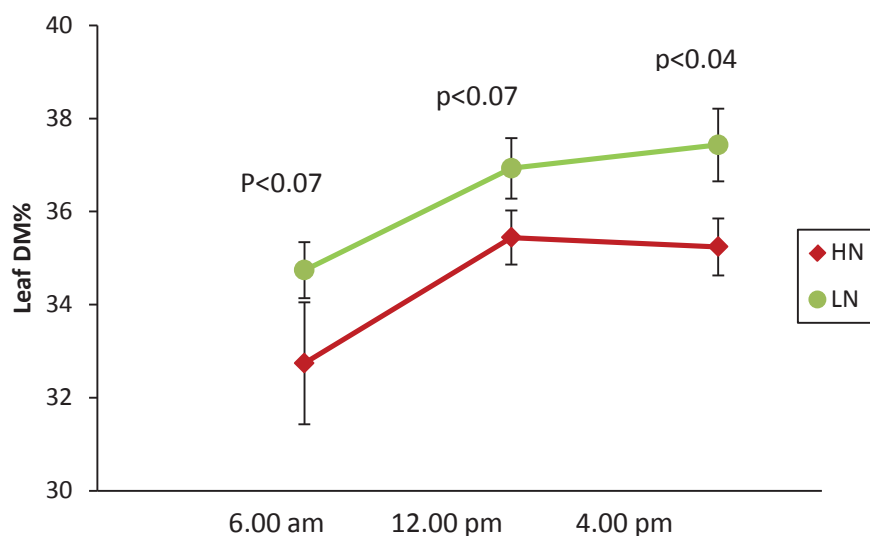


Figure 3.10 Diurnal changes in leaf DM% in 'Hort16A' vines grown with high (10 mM) or low (0.5 mM) nitrate supply. Error bars are standard error, n=6, p-values from one tailed T-test.

In the field trial with soil-grown 'Hayward' vines, higher levels of succulence (i.e., lower DM%) were also found in HN leaves, shoots, and leaf petiole (Table 3.13). For example, the FW of leaf petioles of the HN vines was 30% greater than those from LN vines, whereas petiole DW varied by only 14% between the two treatments. When comparing the succulence of the shoots, the largest difference was found in the long shoots (>50cm), which is consistent with the greater physiological activity of this class of shoots as evident by their increased vigour.

Nitrate has been associated with increased water uptake in a wide range of species with osmotic effects or activation of aquaporins being two suggested mechanisms involved (McIntyre 1997; Cardenas-Narvarro et al. 1999; Guo et al. 2007; Zhang et al 2012). Increased water uptake with increasing levels of $NO_3^-$ might be explained by the contribution $NO_3^-$ makes to the osmotic potential of cells, a role for which it is well suited because of its relatively secure confinement in cell vacuoles (Smirnoff and Stewart 1985). Some of the immediate products of $NO_3^-$ reduction, such as proline, glycinebetaine and various soluble amino acids and proteins are also osmolytes whose levels increase in response to increasing rates of N fertiliser, even in well watered plants (Zhang et al. 2012). Benign osmotica such as

proline and glycinebetaine are likely to accumulate to higher levels in the cytoplasm (Leigh et al. 1981). In kiwifruit, increasing the rate of N fertilisation from nil to 250 kg N ha$^{-1}$ increased the total free amino acid pool of the fruit by up to about 360% at harvest (Clark et al. 1992). Apart from $NO_3^-$ and the soluble products of its reduction, a third class of osmotically active compounds could also be present in higher concentrations in the N fertilised vines, namely non-nitrogenous organic acids (Stitt 1999). In kiwifruit, organic acids such as quinic acid are believed to have a particularly important role as osmotica (Nardozza et al. 2010). The effect of the N treatments on fruit acidity is reported in Chapter 4 (section 4.3.1).

Apart from osmotic effects, $NO_3^-$ might increase vine water content by increasing aquaporin activity (Guo et al. 2007b). Aquaporins contribute significantly to symplastic water fluxes and hydraulic conductivity (Guo et al. 2007b). Therefore the osmotic effects of $NO_3^-$ and its associated soluble metabolites, as well as its affects on aquaporin expression, could account for the increased water content of HN vines.

In contrast to $NO_3^-$, the effect of ammonium uptake is associated with suppression of aquaporins and reduced water uptake (Guo et al. 2007a). Nitrogen and water are often the main limiting factors to growth in plants and therefore can be seen as the main determinants of vegetative vigour (McIntyre 1987, 2001; Benson et al. 1992; Jose et al. 2003; Zhang et al. 2012).

Table 3.13 Effect of N supply on DM% of 'Hayward' leaves, petioles, and whole shoots sampled 29, 45, and 105 DAFB in Year 1 and leaves sampled 174 DAFB in Year 2. Samples collected between 7am and 8am (NZDST).

| Days after full bloom | | HN | LN | p-value |
|---|---|---|---|---|
| 29 | Leaf laminar | 28.0 (0.7) | 28.7 (0.6) | NS |
| | Leaf petiole | 12.5 (0.3) | 14.1 (0.2) | <0.001 |
| 45 | Long Shoots | 23.0 (0.3) | 24.5 (0.6) | <0.04 |
| | Short Shoots | 24.8 (0.4) | 25.1 (0.4) | NS |
| 105 | Leaf | 26.7 (0.3) | 28.7 (0.2) | <0.001 |
| 176 | Leaf | 30.7 (0.8) | 29.0 (0.6) | <0.06 |

Standard error in parenthesis, p-values from two-tailed T-test. Year 1: 2006-07 season; Year 2: 2007-08 season.

### 3.3.5 Photosynthesis

The photosynthetic rate (Pn) of HN and LN vines were similar at each of the measurement times (Figure 3.5). However, when additional fertiliser was applied to HN vines just prior to bud-break in August 2007 these vines (HN+SF) had higher Pn than either HN or LN vines at each of the measurement times (Figure 3.5).

The rates of Pn for all treatments were however similar to those reported by Buwalda et al. (1991b) and by Greer et al. (2002) on vines in the same orchard used for our trials. Increases in Pn with N fertilisation of kiwifruit vines was also reported by Costa et al. (1997) and by Buwalda and Meekings (1993). Rates of Pn for all treatments in November were much less than recorded on 31 January. This might have been because different leaves were used for the January measurement. For the November measurements subtending leaves on terminated shoots were used whereas for the January measurements leaves on non-terminated shoots in positions distal from the fruit were selected. It is unclear why the measurement made 75 DPBB was lower especially when compared to the previous two measurements (57 and 65 DPBB). Ambient air temperature and incident photosynthetically active radiation was similar for the first three measurement times.
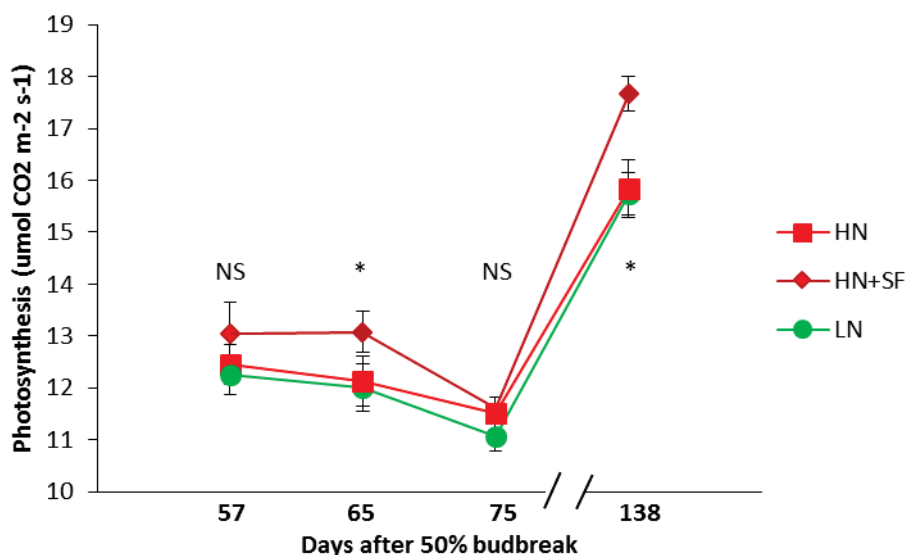


Figure 3.11 Midday (NZDST) photosynthetic rates in Year 3 for 'Hayward' vines given high (HN) or nil (LN) N fertiliser rates during the previous two seasons (Year 1 and 2), or additional spring applied $KNO_3$ (HN+SF) applied pre-budbreak in Year 3. Note: measurements for the first three times were made on the same mature leaf subtending fruit on terminated shoots, but the last measurement (138 days after budbreak) was made on youngest fully expanded leaves from unterminated shoots. Error bars are standard error; * $p < 0.05$, NS not significant, p-values from ANOVA.

The recorded levels of Pn at 138 DPBB are similar to the maximum rates reported by Buwalda et al. (1991b) in similarly aged leaves. Kiwifruit leaves are slow to reach maximum Pn compared to many other crop plants and hence leaf age and time of emergence has a large influence on the rate of Pn (Buwalda et al. 1991b).

There was no significant difference in photosynthetic rates between the two sides of the canopy, although photosynthesis on the eastern side was always higher (data not shown). The eastern side also had lower average stomatal conductance, internal $CO_2$ concentration, and higher leaf temperature (data not shown). The difference between eastern and western sides probably reflects the time of day when the measurements were made, with the eastern side having been more exposed to direct sunlight than the western side.

### 3.3.6 Fruit fresh and dry weight relationships

Fresh weight of mature 'Hort16A' fruit from the pot-trial in Year 1 was not significantly different between the high and low $NO_3^-$ treatments (Table 3.14). However, fruit DM% from the HN vines was significantly lower than that of fruit from the LN vines (P<0.01; Table 3.14). This difference was due to LN fruit having accumulated about 10% more dry matter than the HN fruit (p<0.05; Table 3.14). The reduced dry matter accumulation may have been due to increased competition for assimilates from more vigorous shoots (Figure 3.3; Snelgar et al. 2012).

Table 3.14 Effect of high (HN) or low (LN) $NO_3^-$ supply on fresh weight (FW), dry weight (DW), and dry matter concentration (DM%) of pot-grown 'Hort16A' fruit harvested 184 days after full bloom in Year 1.

| | FW (g) | | DW (g) | | DM% | |
|---|---|---|---|---|---|---|
| | HN | LN | HN | LN | HN | LN |
| | 78.0a | 80.3a | 15.3b | 17.0c | 19.6d | 21.2e |
| Standard error | 2.1 | 2.6 | 0.6 | 0.6 | 0.6 | 0.2 |

Different letters denote significant difference within parameter pairs, DW: p<0.05, DM%: p<0.01, two-tailed T-tests, n=6.

In Year 1 of the field trial with 'Hayward' vines, an elevated N supply significantly increased fruit FW only at the earliest sampling time approximately 30 days after full bloom (DAFB) (Table 3.15). Although by this early stage of fruit development HN fruit had accumulated

18% more dry matter, fruit water uptake had been increased by 27% leading to a significant reduction in DM% (Table 3.15). By the time of the final harvest 176 DAFB there was little difference in FW between HN and LN vines, although DM% of HN fruit was still significantly reduced compared to LN fruit (Table 3.15). Again this effect can be attributed to increased water uptake by HN fruit without a proportionate increase in dry matter accumulation.

Table 3.15 Fresh weight (FW), dry weight (DW), and dry matter concentration (DM%) of fruit from 'Hayward' vines given high (HN) or nil (LN) rates of N fertiliser as recorded at different times in Year 1.

| | FW (g) | | DW (g) | | DM% | |
|---|---|---|---|---|---|---|
| DAFB | HN | LN | HN | LN | HN | LN |
| 29 | 10.1a | 8.0b | 0.8c | 0.7d | 8.3e | 8.9f |
| 76 | 56.9a | 57.6a | 6.5b | 6.4b | 11.4c | 11.1c |
| 176 | 90.0a | 87.1a | 14.8b | 14.9b | 16.4c | 17.1d |
| Standard error | | | | | | |
| 29 | 0.5 | 0.4 | 0.04 | 0.03 | 0.7 | 0.9 |
| 76 | 4.4 | 4.3 | 0.6 | 0.5 | 0.4 | 0.3 |
| 176 | 1.2 | 1.5 | 0.3 | 0.2 | 0.3 | 0.1 |

Different letters beside values within rows for each parameter denote significant differences: 29 days after full bloom (DAFB) $p<0.01$; 176 DAFB $p<0.05$; two-tailed T-tests; n=6.

Late season increases in DM% are a common feature of fruit development (Coombe 1976; Han and Kawabata 2002; Currie and Richardson 2007), which is one reason that tree ripened fruit tends be of better quality in terms of flavour and taste than that of fruit harvested at an earlier stage of maturity in order to facilitate marketing demands (Beever and Hopkirk 1990).

Therefore a separate set of vines was harvested twice with a one-week interval to observe differences between HN and LN vines in late season fruit growth. At the first harvest there was little difference between the treatments in any of the measured fruit attributes (Table 3.16). At the second sampling time fruit from both treatments had increased FW equally by an average of about 10 g (12.5%) but fruit from LN vines had accumulated 22% more dry matter since the first sampling time compared to 15% more by HN fruit in the same period. The HN fruit had also accumulated 12% more water compared to 10.5% in LN fruit.

Consequent to these changes, the LN fruit now had higher DM% than HN fruit (p<0.05; Table 3.16).

Table 3.16 Effect of delaying the harvest for one week on 'Hayward' fruit fresh weight (FW), dry weight (DW), and dry matter concentration (DM%) from vines given high (HN) or nil (LN) N fertiliser in Year 1..

| | FW (g) | | DW (g) | | DM% | |
|---|---|---|---|---|---|---|
| DAFB | HN | LN | HN | LN | HN | LN |
| 178 | 80.1 | 83.0 | 14.2 | 14.3 | 17.7 | 17.3 |
| 185 | 90.2 | 93.4 | 16.3 | 17.5 | 18.1 | 18.9 |
| Difference (%) | 12.6 | 12.5 | 14.8 | 22.4 | 2.3 | 9.2 |
| Standard error | | | | | | |
| 178 | 1.8 | 3.5 | 0.4 | 0.6 | 0.4 | 1.0 |
| 185 | 2.8 | 3.9 | 0.5 | 0.6 | 0.3 | 0.3 |

DAFB: days after full bloom. n = 6. No significant differences between HN and LN treatments.

In Year 2 there were also significant increases in FW of HN fruit at an early stage of fruit development (Table 3.17). In addition there was the same convergence during the middle stages of fruit development as was found in Year 1 (Table 3.14), so that HN and LN fruit became very similar in terms of FW and DW, only to become different again as the fruit matured (Table 3.17).

As suggested above in relation to the Year 1 results, this is consistent with $NO_3^-$ having a role in water relations of the vine, since during early and late stages of development fruit becomes especially responsive to water influx due to osmotically generated hydraulic gradients (Han and Kawabata 2002; Rose and Bennett 1999; Nardozza et al. 2010).

In Year 3 fruit was only sampled at maturity and the differences between HN and LN were minor and not statistically significant (Table 3.18).

Table 3.17 Fresh weight (FW), dry weight (DW), and dry matter concentration (DM%) of fruit from 'Hayward' vines given high (HN) or nil (LN) rates of N fertiliser as recorded at different times in Year 2.

| DAFB | FW (g) | | DW (g) | | DM% | |
|---|---|---|---|---|---|---|
| | HN | LN | HN | LN | HN | LN |
| 45 | 51.9a | 47.1b | 4.0c | 3.7c | 7.7c | 7.7c |
| 80 | 81.3a | 81.5a | 9.6b | 9.5b | 11.7c | 11.7c |
| 116 | 107.3a | 100.3b | 15.9c | 14.9d | 14.8e | 14.8e |
| 167 | 121.5a | 114.9b | 17.7c | 17.2c | 14.6d | 15.0e |
| Standard error | | | | | | |
| 45 | 0.8 | 0.9 | 0.1 | 0.1 | 0.04 | 0.03 |
| 80 | 1.2 | 1.4 | 0.2 | 0.2 | 0.1 | 0.1 |
| 116 | 1.6 | 1.5 | 0.3 | 0.3 | 0.1 | 0.1 |
| 167 | 1.4 | 1.6 | 0.2 | 0.3 | 0.2 | 0.1 |

Different letters beside values in rows denote significant difference within a parameter pair, two tailed T-tests; 45 days after full bloom (DAFB) FW: $p < 0.05$, 107 DAFB FW and DW: $p < 0.01$; 167 DAFB FW: $p < 0.01$, DM%: $p < 0.05$; n=12.

Table 3.18 Fresh weight (FW), dry weight (DW), and dry matter concentration (DM%) of fruit from 'Hayward' vines given high (HN) or nil (LN) rates of N fertiliser as recorded 163 days after full bloom in Year 3 (no significant differences).

| | FW (g) | | DW (g) | | DM% | |
|---|---|---|---|---|---|---|
| | HN | LN | HN | LN | HN | LN |
| | 96.4 | 95.3 | 15.0 | 15.1 | 15.5 | 15.8 |
| Standard error | 2.8 | 4.7 | 0.6 | 1.0 | 0.3 | 0.4 |

In Year 4 fruit from HN vines was significantly heavier than LN fruit in February (81 DAFB) and also in May at the final harvest (165 DAFB) (Table 3.19). By 81 DAFB, HN fruit had accumulated 11.6% more dry matter than LN fruit, and because water influx had only increased by 5.6%, the DM% of HN fruit also significantly increased (Table 3.19). However, by 165 DAFB, dry matter accumulation in LN fruit was almost the same as that of HN fruit and because water influx to HN fruit was 6.7% more than to LN fruit, DM% was now significantly higher in LN fruit (Table 3.19).

Table 3.19 Fresh weight (FW), dry weight (DW), and dry matter concentration (DM%) of fruit from 'Hayward' vines given high (HN) or nil (LN) rates of N fertiliser as recorded at two different times in Year 4.

| DAFB | FW (g) | | DW (g) | | DM% | |
|---|---|---|---|---|---|---|
| | HN | LN | HN | LN | HN | LN |
| 81 | 75.3a | 70.9b | 8.6c | 7.7d | 11.4e | 10.8f |
| 165 | 100.3a | 93.6b | 15.1c | 15.0c | 15.0d | 16.0e |
| Standard error | | | | | | |
| 81 | 1.9 | 1.3 | 0.2 | 0.3 | 0.3 | 0.2 |
| 165 | 2.0 | 4.9 | 0.5 | 1.0 | 0.3 | 0.9 |

Different letters denote significant difference within parameter pairs; 81 days after full bloom (DAFB) FWand DW: $p<0.01$; DM%: $p<0.05$; 165 DAFB FW: $p<0.01$, DM%: $p<0.05$; n=12. In each of the four seasons of our field trial with Hayward vines, fruit DM% was reduced in

HN vines exposed to high levels of soil-applied $NO_3^-$ fertiliser (Tables 3.15 to 3.19). The reduction in DM% was mainly due to apparent increased water uptake by the developing fruit (Table 3.20), which supports the conclusion that one of the main effects of high $NO_3^-$ supply is to alter vine water relations. It has been suggested that fruit growth in kiwifruit remains highly sensitive to vine water status throughout fruit development and that increases in vine water content lead to increases in fruit size (Judd et al. 1989; Miller et al. 1998). However, the contrast in the effects of high N supply at different stages of fruit development as noted in the results presented for each of the three seasons in which it was recorded (Year 1, Year 2, and Year 4) suggests that the sensitivity of fruit to alteration in vine water status varies during the course of fruit development. Increases in vine water content will not necessarily lead to increased fruit water uptake without a concurrent increase in the capacity for cell wall expansion (Boyer 2001). There are similarities in the enzymatic processes leading to increased elasticity of cell walls during early stages of fruit development and their softening during later stages as fruit matures (Rose and Bennett 1999). Increased water uptake of HN fruit was limited to early and late stages of fruit development consistent with cell walls becoming more elastic at these two stages (Tables 3.15, 3.16, 3.17, and 3.19).

Increases in fruit size of *A. deliciosa* following N fertiliser application have been reported by others (e.g. Prasad et al. 1986b; Costa et al. 1977; Vizzotto et al. 1999; Tagliavini et al. 1995) and in 'Hort16A' by Mills et al. (2008). For example, 'Hayward' vines fertilised at rates up to 450 kg N ha (similar to the rate applied to our HN vines) over four seasons showed an

average FW increase of 8.7% compared to fruit from unfertilised vines (Vizzotto et al. 1999). This is higher than the four season average of 5.0% increased FW for HN compared to LN fruit in Table 3.20. However, the effect of N on FW differed considerably between seasons in both studies, ranging from 3.0% in 2007 to 6.8% in 2010 (Table 3.20), and from 3.0 to 12.8% in the Italian study (Vizzotto et al.1999). This variability probably explains why some have found no effect of N fertilisation on kiwifruit FW (e.g., Buwalda et al. 1990; Testoni et al. 1990) and is probably due to the numerous environmental and cultural variables that influence fruit development.

In the field trial with Hayward vines, FW was significantly increased in HN vines in two out of four seasons but DW hardly differed between HN and LN fruit in all seasons (Tables 3.13, 3.15, 3.16, and 3.17). The potential for DW and FW to change independently of each other underlines their separated regulation (Han and Kawabata 2002). In many fruit species there is a linear increase in dry matter production with increasing N rates and at times this has been associated with increases in fruit size (Huett 1996). However, although total dry matter production of the canopy can be increased with N fertilisation, the associated stimulation of shoot growth makes the vegetative component a stronger competitive sink that tends to monopolise the additional supply of assimilates. Thus in apples, the proportion of total dry matter partitioned to fruit decreased as N fertiliser rate and total dry matter production increased (Xia et al. 2009). Of course this would only apply once the optimum leaf area has been exceeded. The net effect of shoot competition on fruit growth also depends on how prolonged the vigour or shoot flushing is and its temporal coincidence with fruit growth and demand for assimilates. This is consistent with studies on kiwifruit showing fruit growth and dry matter accumulation reduced when shoot vigour increased (Minchin et al. 2010; Snelgar et al. 2012). Fruit size can still be increased concurrently with increased vegetative vigour, since increases in fruit FW are due to increased water uptake driving cell expansion rather than being mainly due to increased dry matter accumulation. In kiwifruit, shoots can compete with fruit for dry matter more strongly than for water, so that with increasing shoot growth, fruit DW is reduced more than FW (Snelgar et al. 2012). Furthermore, the hydraulic gradients regulating fruit water uptake might even be enhanced with N fertilisation due to the effect of $NO_3^-$ on increasing plant water uptake (Section 3.3.4; McIntyre 1997).

Table 3.20 Average fresh weight (FW), dry weight (DW), water content (W), and dry matter concentration (DM%) expressed as percentage differences between fruit from 'Hayward' vines given high (HN) or nil (LN) rates of nitrogen fertiliser for each season (Year 1 to Year 4) and overall for all four seasons.

| | Difference (%) between HN and LN fruit | | | |
|---|---|---|---|---|
| | FW | DW | W | DM% |
| Year 1 | 3.0 | 1.5 | 3.3 | -0.3 |
| Year 2 | 6.0 | 3.6 | 6.5 | -2.2 |
| Year 3 | 1.2 | -1.0 | 1.6 | -2.1 |
| Year 4 | 6.8 | 0.4 | 7.3 | -6.0 |
| overall | 5.0 | 1.8 | 5.4 | -2.8 |

### 3.3.7 Correlations between fruit properties, yield, and seed number

In the combined data for all four seasons of the field trial with 'Hayward' vines, fruit FW was negatively correlated to DM% ($r^2 = 0.47$, p<0.001) but correlations between DM% and DW were either weak or absent altogether (Table 3.21). This is consistent with increases in FW being due to increased water uptake without proportionate increases in dry matter accumulation, an effect that was associated with $NO_3^-$ fertiliser in the previous section (section 3.3.6). However, the relationship was not limited to the HN vines but appeared to be a general condition and therefore not caused by $NO_3^-$ fertiliser but by other factors operating within the orchard. In each of the seasons, correlations between FW and DM% appeared stronger for HN vines but because these were variously positive or negative (e.g., compare Year 1 with Year 4, Table 3.22), the overall correlation shown in Table 3.21 is not stronger for HN ($r^2 = 0.43$) than for LN ($r^2 = 0.48$) fruit for which the same relationship was always negative.

Table 3.21 Correlations between fresh weight (FW) and dry matter concentration (DM%), and between dry weight (DW) and DM% in mature 'Hayward' fruit from vines given high (HN) or nil (LN) rates of nitrogen fertiliser and in two different rows (Row 1 and Row 2).

| | n | FW-DM% $r^2$ | Equation | DW-DM% $r^2$ | Equation |
|---|---|---|---|---|---|
| Row 1 | 42 | 0.37*** | y = -0.0481x + 20.253 | 0.02 | y = -0.0928x + 16.851 |
| Row 2 | 40 | 0.64*** | y = -0.0674x + 22.96 | 0.25*** | y = -0.3884x + 22.509 |
| Combined | 82 | 0.47*** | y = -0.0595x + 21.785 | 0.05* | y = -0.1769x + 18.628 |
| HN | 40 | 0.43*** | y = -0.0576x + 21.52 | 0.03 | y = -0.1392x + 17.829 |
| LN | 42 | 0.48*** | y = -0.0598x + 21.881 | 0.07 | y = -0.1941x + 19.083 |
| Row 1 HN | 21 | 0.28* | y=-0.0411x+19.393 | 0.00 | y=-0.0166x+15.392 |
| Row 1 LN | 21 | 0.42** | y=-0.0514x+20.712 | 0.15 | y=-0.2561x+19.535 |
| Row 2 HN | 19 | 0.65*** | y=-0.0679x+22.997 | 0.25* | y=-0.402x+22.627 |
| Row 2 LN | 21 | 0.62*** | y=-0.0665x+22.889 | 0.23* | y=-03625x+22.189 |

*** $p<0.001$, **$p<0.01$, *$p<0.05$. N treatments: HN: 400-450 kg N ha-1 yr-1, LN: nil N. Combined data for four seasons.

The form of the correlations also varied within each of the two rows. In Row 2 there was a stronger correlation between FW and DM% ($r^2 = 0.64$) than in Row 1 ($r^2 = 0.37$) (Table 3.21). Average fruit FW of Row 2 was only slightly less than that of Row 1 (1.2 g difference) but DM% was significantly higher ($p<0.002$, 2-sided T-test) than Row 1. The stronger negative correlation between both FW and DM% and between DW and DM% in this row was present in both HN and LN fruit.

The variability of these correlations is also shown in Table 3.22 when the relationships in Year 1 and Year 4 are compared. In Year 1 the correlations between FW and DM% were negative, although of varying strength in the two rows and N treatments, and there were no significant correlations between DW and DM%. However, in the Year 4, although there was no overall correlation (combined data for both rows), there was a negative correlation between FW and DM% in Row 2 (HN+LN); and HN fruit showed a positive correlation while LN had a negative correlation. The positive correlations in HN fruit in Year 4 can be traced to HN fruit from Row 1 ($r^2=0.83$) with only a small contribution from HN fruit in Row 2. In contrast, the concurrent negative correlation in LN fruit was present in both rows, although it was stronger in Row 2 ($r^2=0.44$) compared to $r^2=26$ in Row 1. The differences

131

between the two rows may have arisen from differences in pollination and/or soil properties, as is discussed in more detail below.

Table 3.22 Correlations between fresh weight (FW) and dry matter concentration (DM%), and between dry weight (DW) and DM% in mature 'Hayward' fruit from vines given high (HN) or nil (LN) rates of nitrogen fertiliser and in two different rows (Row 1 and Row 2) in Year 1 and Year 4.

| | FW-DM% | | | DW-DM% | |
|---|---|---|---|---|---|
| | n | $r^2$ | | $r^2$ | |
| Year 1 | | | | | |
| Row 1 | 12 | 0.65** | y = -0.0914x + 25.004 | 0.12 | |
| Row 2 | 12 | 0.27 | y = -0.0925x + 25.27 | 0.1 | |
| Combined[1.] | 24 | 0.41*** | y = -0.0993x + 25.768 | 0.01 | |
| HN | 12 | 0.49* | y = -0.1039x + 26.263 | 0.01 | |
| LN | 12 | 0.35* | y = -0.0999x + 25.738 | 0.09 | |
| Year 4 | | | | | |
| Row 1 | 12 | 0.04 | y = 0.0288x + 12.296 | 0.55** | y = 0.4848x + 7.961 |
| Row 2 | 10 | 0.40* | y = -0.0703x + 22.836 | 0.01 | y = -0.0674x + 17.151 |
| Combined | 22 | 0.04 | y = -0.0297x + 18.437 | 0.03 | y = 0.4308x + 9.0644 |
| HN | 10 | 0.47* | y = 0.0914x + 5.8636 | 0.81*** | y = 0.4736x + 7.8683 |
| LN | 12 | 0.21 | y = -0.0818x + 23.658 | 0.19 | y = 0.3866x + 10.188 |

[1.]Combined data for both N treatments and rows.

The average crop load in Year 2 were 17.57 kg/vine in Row 1 and 21.63 kg/vine in Row 2 but the difference was not statistically significant (p>0.05). The planting density of the orchard was 2.5 m × 5 m giving a theoretical vine canopy area of 12.5 $m^2$, which would make these yields about a third of what can be achieved in high producing 'Hayward' orchards (Patterson and Currie 2011). However, the actual canopy area was lower due to design of the T-bar support structures so that the canopy area was probably closer to 7.5 $m^2$. Based on this area the vine yields are calculated as being equivalent to 22 and 28 tonnes per hectare for Row 1 and Row 2 respectively, which are within the normal range for 'Hayward' vines (Sale and Lyford 1990).

Table 3.23 Summary of differences between rows and nitrogen treatments in fruit fresh weight (FW), dry matter (DM%), and crop load, and correlations between these three factors in Year 2.

| | Row 1 | | Row 2 | |
|---|---|---|---|---|
| | HN | LN | HN | LN |
| FW (g) | 121.5 | 114.9 | 120.2 | 113.1 |
| DM% | 14.6 | 15.0 | 15.1 | 15.2 |
| Crop load | | | | |
| (kg/vine) | 12.4 | 22.7 | 24.4 | 18.8 |
| Correlations ($r^2$) | | | | |
| FW:crop load | 0.01 | -0.33 | +0.12 | +0.09 |
| DM%:crop load | -0.39 | +0.09 | -0.62 | +0.64 |
| FW:DM% | -0.21 | 0.01 | -0.35 | 0.01 |

Nitrogen treatments: HN: 400-450 kg N ha$^{-1}$ yr$^{-1}$, LN: nil N. n=6.

Average vine crop load varied considerably between the treatments but because of the large inter-vine variation the differences were not statistically significant (ANOVA $p<0.05$; Table 3.23). There were no significant correlations between yield (i.e. crop load) and fruit FW or DM% (Table 3.23). However, a negative correlation between 'Hayward' FW and crop load was found by Burge et al. (1987). The absence of a consistent relationship between fruit FW and crop load suggests other factors such as vine water status were more influential in determining FW. Neither does crop load appear able to explain the contrasting correlations reported in Table 3.21 and 3.22.

Differences between the two rows were also found in correlations between seed number and FW and DW in Year 1. In this case there were highly significant correlations between both FW and DW in Row 1 but not in Row 2 (Table 3.24). The correlations were positive which is consistent with the known importance of seed number in determining the sink strength of the fruit (Lai et al. 1989a, 1989b; Ho 1992).

Table 3.24 Correlations between seed count and fresh weight (FW), dry weight (DW), and dry matter concentration (DM%) of fruit from 'Hayward' vines given high (HN) or nil (LN) nitrogen fertiliser rates in different rows in Year 1.

| | LN | | HN | | HN+LN | |
|---|---|---|---|---|---|---|
| | $r^2$ | n | $r^2$ | n | $r^2$ | n |
| Row 1and2 | | | | | | |
| FW | 0.06 | 26 | +0.27** | 26 | 0.02 | 48 |
| DM% | +0.19* | 26 | 0.01 | 26 | 0.00 | 48 |
| DW | 0.06 | 26 | +0.25* | 26 | 0.03 | 48 |
| Row 1 | | | | | | |
| FW | +0.66** | 14 | +0.53** | 14 | +0.41*** | 26 |
| DM% | 0.00 | 14 | 0.01 | 14 | 0.01 | 26 |
| DW | +0.60** | 14 | +0.41* | 14 | +0.41*** | 26 |
| Row 2 | | | | | | |
| FW | -0.14 | 14 | 0.03 | 14 | 0.04 | 26 |
| DM% | 0.00 | 14 | +0.24 | 14 | 0.01 | 26 |
| DW | 0.08 | 14 | +0.30 | 14 | 0.02 | 26 |

*p<0.05, **p<0.01, ***p<0.001. n=6.

Overall, including both rows and canopy positions, HN fruit averaged 26 seeds per fruit compared to 29 in LN fruit (an 11% difference) (p=0.01, two-tailed T-test, n=24). The difference in seed number between HN and LN fruit was more pronounced in Row 2 than Row 1 (Table 3.25). Since the N fertiliser applications in this season began 54 days before anthesis, the lower seed number in HN fruit might have been due to a more leafy canopy limiting access to the flowers by bees and the artificially-applied pollen. However, increased competition for assimilates from N stimulated shoot growth could also have limited floral development pre-anthesis, a possibility that deserves further investigation. Average seed counts in Row 1 were about 8% higher than in fruit from Row 2 (p<0.06, n=24, 2-tailed T-test). However, the similarity in average fruit FW, DW, and DM% between the two rows (Table 3.23) suggests that variation in seed number was not a factor able to explain the differences between these rows found in the various correlations reported in Tables 3.21 – 3.24).

Table 3.25 Seed counts of equatorial sections of fruit from inner and outer canopy positions in 'Hayward' vines given high (HN) or nil (LN) rates of nitrogen fertiliser (Year 1).

|  | HN inner | HN outer | LN inner | LN outer | p-value | n |
|---|---|---|---|---|---|---|
| Row 1 | 29.88 | 26.31 | 30.75 | 27.88 | 0.062 | 6 |
| se | 0.75 | 0.95 | 1.05 | 1.14 |  | 6 |
| Row 2 | 23.58 | 24.49 | 27.94 | 30.42 | 0.094 | 6 |
| se | 0.96 | 0.79 | 2.05 | 1.70 |  | 6 |
| Row 1 and2 | 26.73 | 25.40 | 29.34 | 29.15 | 0.086 | 12 |
| se | 0.89 | 0.63 | 1.17 | 1.04 |  | 12 |

se: standard error; p-value from ANOVA. Canopy positions: Inner, fruiting zones close to the main leader; outer, fruiting zones at the distal ends of canes.

The contrasting correlations between the two rows suggests that the coordination of dry matter and water accumulation by the fruit was different, with Row 2 tending to accumulate a larger proportion of water relative to dry matter than Row 1. The source of this variation might be due to soil spatial heterogeneity. Although the centers of the two rows were only 10 metres apart being separated by a single buffer row, there were noticeably more stones present throughout the profile in Row 2 than in Row 1. Factors such as soil heterogeneity could alter xylem water potential and affect the different relationships between FW and DM% observed in the two rows (Clearwater et al. 2004). Inconsistent relationships between FW and DM% were also found in a large-scale study of 'Hayward' fruit from 36 orchards over four seasons (Woodward and Clearwater 2008). The correlations could be either positive or negative, changing between orchards and seasons. These results support the idea that there is no clear relationship between water and dry matter accumulation by the fruit because both are influenced differently and, sometimes, independently of each other by environmental, cultural, and seasonal factors (Han and Kawabata 2002; Woodward and Clearwater 2008). The possibility of having either positive or negative correlations between FW and DM% would influence the relative quality of different fruit size categories, since a negative correlation means smaller fruit would tend to have higher quality (i.e., higher DM%), whereas a positive correlation would favour larger fruit size categories.

Even in seasons where there was no significant increase in average FW in HN vines, such as in Year 3, the proportion of fruit in larger size categories was increased (Figure 3.6). This has also been reported previously with kiwifruit where no significant difference in FW with N

fertiliser was found until the fruit was sorted into size categories; then increases in the preferred size ranges with increased N rates were found (Prasad et al. 1986; Tagliavini et al. 1995). This was also been the case in other fruits, such as apples (Wargo et al. 2003). Larger kiwifruit are generally more valuable and even small increases in FW can be commercially important (Snelgar et al. 2012).
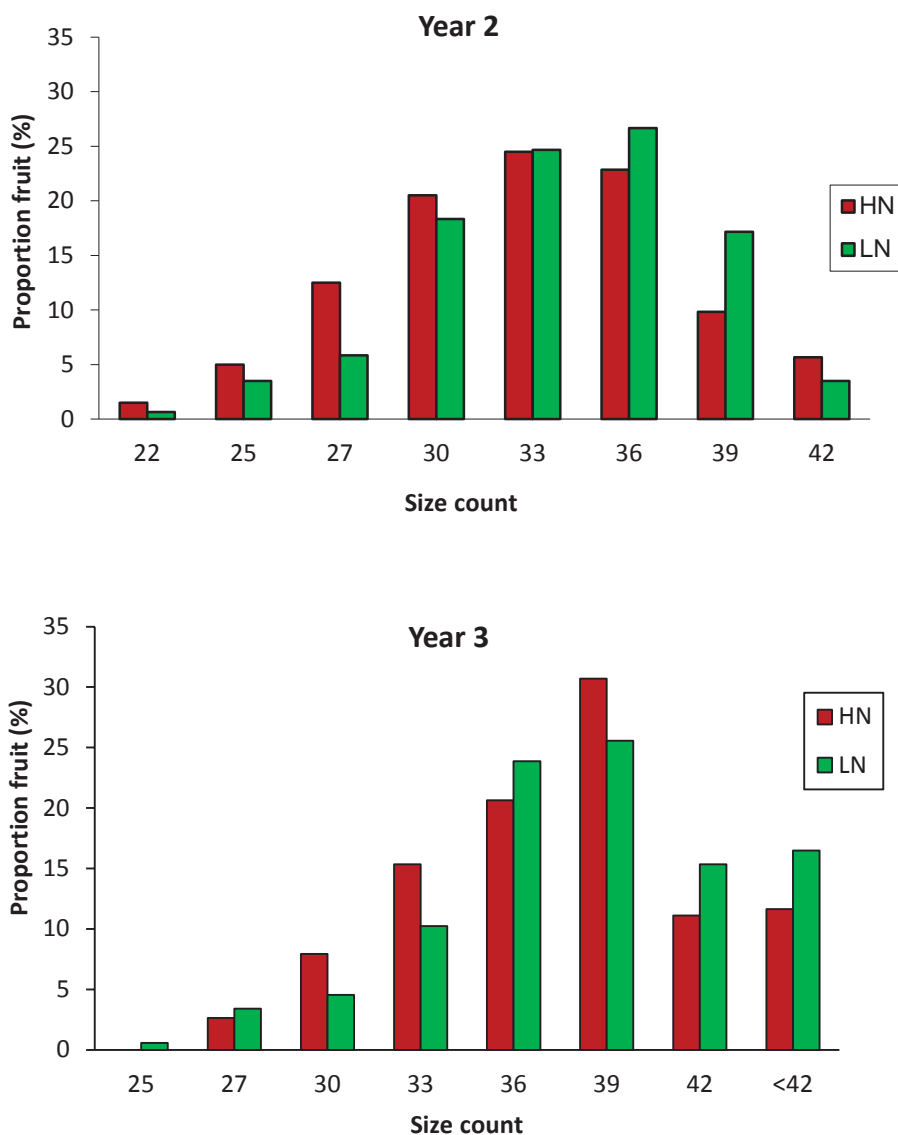


Figure 3.12 The size distribution of mature 'Hayward' fruit in Year 2 and Year 3 from vines given high (HN) or nil (LN) rates of N fertiliser. Size count categories refer to the number of fruit in a 3.6 kg standard tray.

Nevertheless, the relative quality of fruit in terms of DM% within different size categories will depend on whether the correlation between FW and DM% is negative or positive. For example, in Year 4 there was a strong positive correlation in Row 1 for HN fruit so that fruit from this group weighing between 105 and 133 g (Count 33 to 27) had an average DM% of 15.5 compared to an average of 14% for fruit sized between 80 to 90 g (Count 39). In contrast there was a negative correlation in Row 2 for LN fruit and fruit from this line weighing between 105 and 133 g had an average DM% of 15 compared to 16.7% for smaller fruit between 80 and 90 g.

### 3.3.8 Canopy position

In Year 1 fruit from HN and LN vines were sampled from inner and outer canopy positions. The inner and outer canopy positions correspond to fruiting zones close to the main leader and zones at the distal ends of the canes respectively. There were no significant effects of canopy position or N treatment in fruit FW, DW, or DM% (Figure 3.7). However fruit from the inner canopy and HN vines tended to be heavier than fruit from the outer canopy or LN vines respectively (Figure 3.7). Generally canopy position had little effect on the average number of seeds per equatorial slice (Table 3.25).

In Year 2, fruit was sampled from the inner and outer canopy positions as in Year 1, but also from two different shoot types in the outer canopy. Fruit from different canopy positions and shoot types differed significantly. Overall in the outer canopy, the average FW of fruit from long shoots (shoots >100cm) was 123 g compared to 112 g for fruit from short shoots (shoots <40cm) (p<0.01, ANOVA). Fruit from long shoots in the inner canopy were midway between these with an average weight of 118 g. The average accumulation of dry matter by fruit on long shoots differed little between canopy positions (18.0 g fruit$^{-1}$) but was significantly less in fruit on short shoots in the outer canopy (16.9 g; p<0.001) (Table 3.8C). The largest average DM% was found in fruit from long shoots in the inner canopy (15.2%) compared to 14.6% for both shoot types in the outer canopy (p<0.05, ANOVA). The higher DM% of fruit from inner canopy long shoots was apparently due to lower water accumulation in these fruit whereas in the outer canopy the difference between long and shoot shoots was due to a fairly proportional decrease in dry matter and water accumulation by fruit on short shoots. The apparently weaker sink strength of fruit on short shoots may have been due to weaker floral development leading to reduced seed numbers as suggested by Lai et al. (1990).
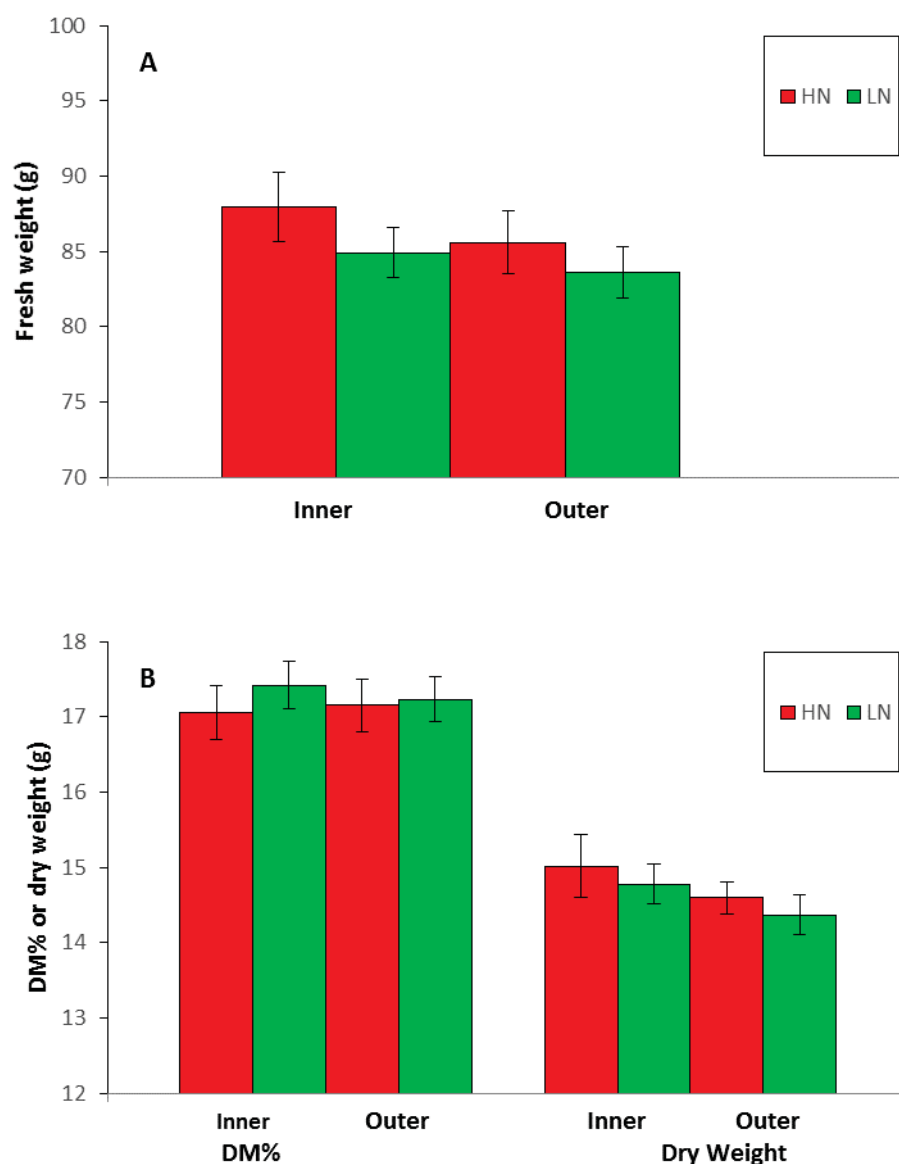
Figure 3.13 A: Fresh weight and B: dry matter concentration (DM%) and dry weight of fruit from inner and outer canopy positions on 'Hayward' vines given high (HN) or nil (LN) rates of N fertiliser in Year 1.

On all three shoot types HN fruit was heavier than LN fruit (Figure 3.8A). The heaviest fruit was HN fruit from long shoots in the outer canopy, which was 6% heavier than LN fruit from equivalent shoots (p<0.01, two-tailed T-test; Figure 3.8A). However, this fruit had significantly lower DM% compared to the LN fruit from equivalent shoots (p<0.01, two-tailed T-test; Figure 3.8B).
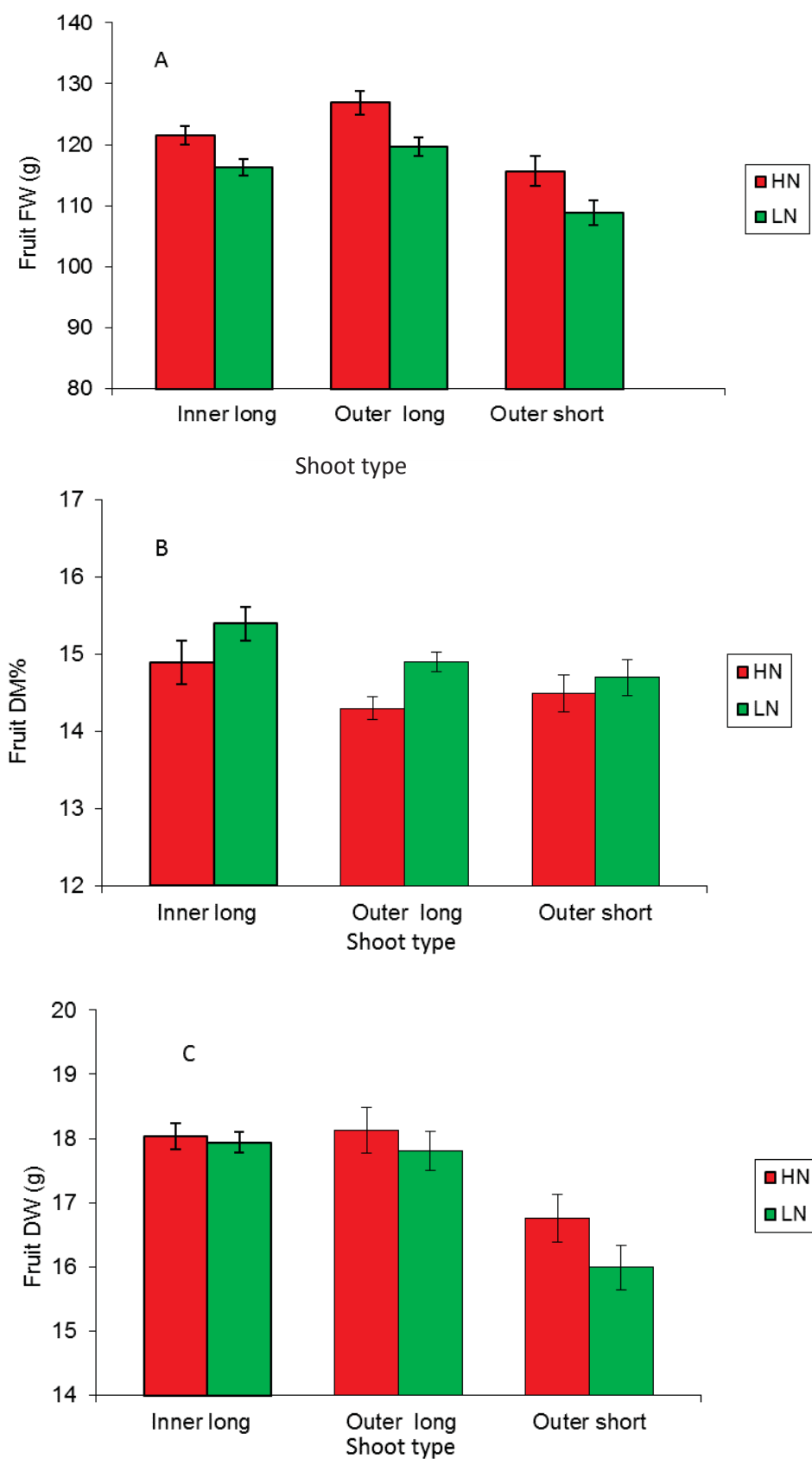
Figure 3.14 A: Fresh weight (FW); B: dry matter concentration (DM%); and C: dry weight (DW) of fruit from different shoot types on 'Hayward' vines given high (HN) or nil (LN) rates of N fertiliser in Year 2. n = 12. Error bars are standard error.

The accumulation of dry matter by fruit on long shoots differed little between treatments and canopy positions (Table 3.8C). However, water influx was increased by $NO_3^-$ in all shoot types by 6.8% and 6.5% in long and short shoots respectively from the outer canopy, and by 5.2% in long shoots from the inner canopy.

Because $NO_3^-$ stimulates shoot vigour there is increased competition for dry matter from the vegetative sinks, so that even though the photosynthetic rate of the shoots in the HN vines is increased, the fruit gains little advantage due to it being the weaker of the two sinks (Patterson and Currie 2011; Minchin et al. 2010). However, the relative weakness of the kiwifruit as a sink for assimilates applies less to its potential to take up water. In this respect the fruit is much more competitive in the sense of being extremely sensitive to changes in vine water content (Judd et al. 1989; Miller et al. 1998). The greater increase in water influx in HN compared to LN fruit in the outer canopy than in the inner canopy and the higher DM% of fruit from inner canopy positions is also consistent with $NO_3^-$ affecting water gradients within the vine (see Sections 3.3.4 and 3.3.6). This is because water potential gradients in trees are basically linear with increasing distance from the ground (Hellkvist et al. 1974); it therefore follows that because $NO_3^-$ increases vine water content (Section 3.3.4) the effect would be greater in more distal locations within the canopy. Other studies have also found a tendency for fruit from outer canopy positions to have lower DM% than fruit closer to the cordon (Boyd et al. 2004; King et al. 2006; Wakefield and Max 2007).

Only in the short shoots did HN fruit appear to attract more dry matter (p=0.10 two-sided T-test; Figure 3.6C). This may have been due to the greater availability of N increasing the sink strength of the fruit on short shoots without an associated increase in competition of the shoot since these were terminated shoots. This would be consistent with the demonstrations provided by Minchin et al (2010) and Snelgar et al. (2012) of the effect of the vegetative vigour of a shoot on the growth of the fruit on that shoot. Fruit from short shoots may also have been less competitive for N due to their generally weaker sink strength as suggested by the findings of Mills et al. (2008) and therefore more responsive to the increased supply of N in HN vines. This provides further evidence that it is the N-stimulated shoot growth that prevents large increases in fruit dry matter accumulation through the increased competitiveness of the vegetative component for assimilates, even although the photosynthetic capacity of the canopy might be increased by higher leaf areas and rates of photosynthesis (Wang et al. 2006).

## 3.4 Summary and conclusions

Regular fertigation (Year 1 pot-trial with 'Hort16A') or soil applications of nitrogen fertiliser (Year 1 to 4 field trial with 'Hayward') designed to maintain constant high levels of nitrate availability (amounting to total annual applications equivalent to 450 kg N ha$^{-1}$ in Years 1, 2, and 4, and 650 kg N ha$^{-1}$ in Year 3 in the case of the field trial with soil-applied nitrogen) resulted in elevated nitrate uptake but levels in the fruit were generally less affected. The high levels of nitrate uptake were associated with increased vine and fruit water uptake and a large stimulation of shoot growth. With the increased competitive strength of the vegetative sink for assimilates, fruit from vines given a supra-optimal nitrate supply were unable to balance the increased water influx with proportional increases in dry matter accumulation. Therefore fruit from the vines given a supra-optimal nitrate supply, although often larger, had reduced dry matter concentration compared to fruit from vines given either a low nitrate supply (fertigated pot-trial in Year 1) or nil nitrogen fertiliser (field trial in Years 1 to 4). However, dry matter accumulation in fruit from vines given supra-optimal nitrogen supply was not generally reduced by high nitrogen uptake, which argues against an inhibition of starch synthesis by nitrogen as was hypothesised. Instead the main effect was to alter vine water relations causing an increase in vine and fruit water uptake, and supporting an increase in vegetative vigour. There was evidence of adaptive responses in vines not fertilised with nitrogen to compensate for the lower nitrogen supply. One such response was suggested by the higher xylem sap pressure developed in the unfertilised vines in spring, possibly due to earlier initiation of root growth or increased hydrolysation of stored nutrient reserves.

# Contents

# List of Tables

## List of Figures

# 4. Effects of nitrate fertiliser applied to the roots on some physical and biochemical quality attributes of 'Hayward' and 'Hort16A' fruit

## 4.1 Introduction

Nitrate ($NO_3^-$) is the main soluble form of N in fertilised agricultural soils taken up by crops (von Wiren et al. 1997; Miller and Cramer 2004). Nitrate is chemically very reactive and its reduction and assimilation bears a high energy cost. Therefore it may compete for limited energy resources with reduction of $CO_2$ in fruiting plants or in low light situations, leading to reduced carbohydrate synthesis (Marschner 2002). Within the plant $NO_3^-$ is considered to be the primary signal molecule involved in N assimilation and can induce multiple gene responses in tissues within minutes of exposure (Crawford 1995). For example, in *Arabidopsis* shoots 183 genes were identified as responding within 20 minutes of exposure to $NO_3^-$, while in tomato roots over 1200 genes responded to $NO_3^-$ exposure within 96 hours (Wang et al. 2001). Nitrate uptake increases the synthesis of organic acids, decreases starch synthesis, changes plant hormone levels, and alters shoot:root allocation and root morphology (Stitt 1999). Nitrate also leads to wide ranging and rapid up- and down- regulation of enzyme transcription levels involved in N and C metabolism (Stitt 1999).

Firmness, soluble solids content, and titratable acidity are important attributes of fruit quality and are used as measures of quality in kiwifruit (Beever and Hopkirk 1990; McMath et al. 1992). All three can be affected by N fertilisation and potentially therefore, by nitrate. Increasing N fertiliser rate has been related to decreased fruit firmness at harvest (Buwalda et al. 1990; Vizzotto et al., 1999; Testoni et al., 1990) or during storage (Vizzotto et al., 1999; Testoni et al. 1990; Prasad and Spiers 1992; Johnson et al. 1997; Smith et al. 1994). The direct effect of N fertilisation on soluble solids content in kiwifruit is less clear but there are numerous reports of reduced carbohydrate synthesis being attributed to increased N uptake (e.g., Marschner 2002; Stitt, 1999; Scheible et al., 1997; Aloni et al., 1991; Table 1.5). The composition of organic acids, their concentration and relative amounts, in kiwifruit may be affected by N fertilisation, as is the case in peaches (Jia et al. 1999), grapes (Renquist and Reid 2001), sour cherry (Hansen 1997), and citrus (Albertini et al. 2006; Smith 1966).

Given the reactive properties of $NO_3^-$ it seemed likely that providing high rates of $NO_3^-$ fertiliser could alter the levels of secondary metabolites and minerals important for fruit quality. Oxalate, ascorbic acid, and calcium are important nutritional and physiological components of the fruit and are interrelated since ascorbic acid is a likely precursor for oxalate synthesis and oxalate is involved in cytosolic $Ca^{+2}$ regulation. Kiwifruit contain relatively high levels of oxalate (Beever and Hopkirk 1990). Oxalate crystals in kiwifruit are considered an 'anti-nutrient' (Rassam and Laing 2005) because of their potential to cause irritation in the mouths and throats of some people eating the fresh fruit (Perera et al. 1990).

In species where $NO_3^-$ assimilation occurs in the shoots, oxalic acid can be the charge balancing acid and has the advantage that it can be precipitated as calcium oxalate in the vacuoles thereby maintaining osmotic potentials (Raven and Smith 1976). Rinallo and Modi (2002) reported oxalate levels in kiwifruit varied with different N sources and was increased by $NO_3^-$ compared to $NH_4^+$, which is consistent with other studies. For example, oxalate content in tea increased with increasing leaf $NO_3^-$ content and concentration in the nutrient solution (Morita et al. 1999). Increased oxalate content in association with $NO_3^-$ fertilisation was also found in sugar beet (Joy 1964), tomato, and spinach (Libert and Franceschi 1987).

Kiwifruit contain relatively large amounts of ascorbic acid (vitamin C), an attribute used to promote the fruit as being particularly healthy (Ferguson and Ferguson 2003). Vitamin C content is consistently reduced in fruit as N fertiliser rates increase (Benbrook 2005).

Calcium is of particular importance for postharvest quality of kiwifruit (Hopkirk et al. 1990). There are some reports of reduced Ca uptake being associated with increased N fertilisation rates in kiwifruit (Mills et al. 2008) and other crops (Spiers 1992; Spiers and Braswell 1993; Torre et al. 2004).

Evidence exists that protein and phenolic synthesis are competitive metabolic pathways, and increasing N fertilization causes increased protein synthesis at the expense of phenolics or secondary metabolites. Although kiwifruit have low levels of phenolics compared to some other fruits, these compounds are generally important for the flavour and taste of fruit (Dawes and Keene 1999). The evidence presented above suggests there are numerous ways high or 'supra-optimal' levels of $NO_3^-$ supply and uptake could have adverse effects on fruit quality.

In this chapter, the effects of $NO_3^-$ supply on fruit firmness, soluble solids, calcium, titratable acid levels and phenolics are reported from fruit harvested during two seasons of the N fertiliser trial described in the previous chapter.

Specific experimental questions were:

1. What are the effects of $NO_3^-$ supply on fruit quality parameters, like fruit firmness, soluble sugars and titratable acid levels?
2. Is the oxalate content of the fruit increased by high levels of $NO_3^-$ consistent with its increased synthesis in response to higher rates of $NO_3^-$ reduction?
3. Given the possible competitive relationship between phenolic and protein synthesis, is the phenolic content of the fruit reduced by high levels of $NO_3^-$ consistent with increased protein synthesis?

## 4.2 Materials and Methods

The attributes of quality reported here are data obtained from the Year 1 and Year 2 (2006-07 and 2007-08 seasons) with 'Hayward' and 'Hort16A' vines in the experiments described in Chapter 3 and with treatments summarised in Table 3.2. Fruit was also harvested in the same season as the Year 1 experiments, from an existing long-term N fertiliser trial underway in Te Puke; a district in the main commercial kiwifruit growing region in New Zealand (Mills et al. 2007). This fruit was used for ascorbate and oxalate analysis to complement the data for these metabolites obtained from the Year 1 field trial with 'Hayward' and pot-trial with 'Hort16A'.

### 4.2.1 Firmness, soluble solids content, and titratable acidity

In Year 1 'Hayward' fruit was harvested from inner and outer canopy positions from HN and LN vines at 178 days after full bloom (DAFB) and used for the measurement of firmness with a penetrometer and soluble solids content (SSC) measured in °Brix with a refractometer.

In Year 2 fruit from HN and LN vines was harvested 174 DAFB and used for the measurement of fresh weight (FW), dry matter concentration (DM%), SSC and firmness. The remainder of the fruit from this harvest were then placed into low temperature storage (1°C)

for 20 weeks after which period it was reassessed for DM%, SSC, and firmness. A portion of each fruit used for both measurements was frozen and put aside for acidity measurement.

Although SSC is a generally accepted measure of fruit maturity and ripeness, the proportion of dry matter that has been solubilised (soluble solids of the whole fruit as a percentage of fruit dry matter (SSFDM%) might be a better indicator that would allow the maturity of different fruit lines to be more accurately compared (Feng et al. 2003). Data for DM% and SSC at harvest in Year 2 was used to calculate this value. Procedures used for measurement of fruit firmness and soluble solids content are described in Chapter 2 (sections 2.5.2 and 2.5.3).

For measurement of titratable acidity (TA) and pH a puree was prepared from 2.6 g of lyophilised fruit tissue mixed with 30 ml of deionised water and shaken for 1 hour on a rotary shaker and then titrated using an automatic titrator (Mettler-Toledo DL21 Mettler-Toledo International Inc.) with 0.1N NaOH to an endpoint of 8.2 with the results expressed as per cent citric acid equivalents (Marsh et al. 2004). The instrument simultaneously read the pH of the sample solution. The endpoint was intended to ensure the complete dissociation of the main acids (citric, quinic, and malic acids), while avoiding over-titration causing dissociation of weak bases that do not make a significant contribution to fruit acidity (Lobit et al. 2002). The end point conformed to that used by others for kiwifruit (Fisk 2006; Marsh et al. 2004; Matsumoto et al. 1983).

### 4.2.2 Oxalate and ascorbic acid

Oxalate was measured in frozen samples following the method of (Rassam and Laing 2005). Ascorbic acid was measured simultaneously with this procedure. The sample material included fresh frozen fruit samples from the final harvest of both the 'Hort16A' pot trial, the 'Hayward' field trial in Year 1 and samples of 'Hort16A' that had been collected at different times during the same season from vines in a long term N fertiliser trial in Te Puke (Mills et al. 2008). In this trial, vines had been given rates of N fertiliser equivalent to 295 kg N ha$^{-1}$ yr$^{-1}$ (HN) or nil N fertiliser (LN). Samples from the 'Hayward' field trial were segregated into fruit from outer and inner canopy positions.

Fruit samples were cyro-powdered and duplicated extractions made with 150 – 200 mg of sample in 5 volumes of 0.5 N HCl, 4 mM TCEP (Tris[2-carboxyethyl] phosphine; Thermo

Fisher Scientific Inc., Rockford, IL, USA), vortexed for 20 seconds, incubated in a heating block at 40°C for 2 hours, and centrifuged under refrigeration at 13,000 g for 10 min. Samples were kept on ice for immediate processing, or stored at -80°C for later analysis. Each duplicated sample extract was duplicated again for separation of ascorbic from oxalate by HPLC on an Aminex HPX-87H HPLC column (BioRad). The column was eluted with 2.8mM $H_2SO_4$ at a flow rate of 0.6 ml/min. The ascorbate peak was quantified by measuring the absorbance at 245 nm [retention time (RT) 9.6 min] using a freshly prepared ascorbic acid standard (Sigma, St.Louis). The oxalate peak fraction (RT 6.2 min) was collected between 6 and 7.5 min and stored -80 °C until analysis. Oxalic was assayed enzymatically in 96-well microtiter plates (Greiner Bio-One GmbH) in a colorimetric assay of $H_2O_2$ using oxalate oxidase/horseradish peroxidase. Standard curves were calculated for each incubation from working standards prepared with 40 µg oxalic acid ($H_2C_2O_4.2H_2O$; Sigma) per ml of 2.8mM $H_2SO_4$. Samples or oxalic standard (100 µl) were added to 100 µl of colour reagent (modified Sigma Oxalate Reagent A, see details in Rassam and Laing 2005), then quantified by measuring the absorbance at 600 nm to give a control value, before 4 µl of oxalate/horseradish peroxidase (Oxalate reagent B, Sigma #591-2) was added to each well, the plate incubated at 32°C for 25 minutes, and the absorbance at 600nm was measured again. If results varied by more than 5% between duplicated injections for ascorbic or between duplicates in the oxalate assay the sample was re-analysed. A total of 59, 42, and 26 samples were analysed for the field trial ('Hayward' fruit), pot trial ('Hort16A'), and Te Puke field trial ('Hort16A') respectively (see Table 3.2). Resulting data for ascorbic acid and oxalate was averaged for the duplicated extractions and incubations, and presented as mg/100g FW. Residual extraction solutions were stored at -80°C for later analysis of Ca content.

### 4.2.3 Calcium

Calcium concentration was measured by (flame) atomic absorption spectrometry in the same sample material used for the oxalate analysis (see 4.2.2). Preparation of the samples was by digestion of the dried fruit or leaf tissue with hot concentrated $HNO_3$ followed by evaporation and resolublisation in 2M HCl with strontium and caesium added for ionic suppression.

### 4.2.4 Phenols

Phenolic contents were quantified by the Folin-Ciocalateau colorimetric method (FC; Waterhouse 2002; Singleton et al. 1974; Imeh and Khokhar 2002). The lyophilized unbroken longitudinally sliced samples of fruit collected 174 DAFB from the 'Hayward' vines were separated into pulp and skin using a sharp scalpel. Samples were pooled from two fruit from each of 12 vines (6 × HN, 6 × LN) to give four final samples of six fruit in each (1× HN pulp, 1)×HN skin, 1× LN pulp, and 1× LN skin). Free phenols were extracted from about 0.5 g of sample mixed with 25 ml of 50% methanol and heated for 2 hours at 90°C in a capped centrifuge tube with intermittent shaking. The procedure was repeated for total phenols but this time with the weighed sample mixed with 25 ml of 1.2M HCl in 50% methanol. After cooling the extracts were centrifuged for 5 minutes at 5000 rpm and filtered (Whatman 41, Whatman® GE Healthcare Life Sciences, USA). A working standard was prepared by dissolving 0.5 g gallic acid in 10 ml methanol and brought up to 100 ml with deionized water. From this a series of standards of 50, 100, 250, and 500 mg/l were prepared. A 500 µl aliquot of sample extract or standard was added to a 15ml centrifuge tube, followed by 7.5 ml deionized water and 500 ul of Folin-Ciocalateau phenol reagent (Sigma-Aldrich, Buchs, Switzerland). The contents of the tube were mixed thoroughly by inverting and incubated for 6 minutes at room temperature. At the end of this incubation period, 3 ml of sodium carbonate solution (200 g anhydrous $NaCO_3$ $l^{-1}$) was added to each tube and mixed, and the tube was incubated for exactly 2 hours. Contents of each tube were then transferred to a 2ml crystal cuvette and read at 765 nm on a spectrometer (Philips PU 8625 UV/VIS, BioLab Scientific Ltd). Duplicate readings were made of each sample. Results were expressed as mg gallic acid equivalents (GAE)/100g DW and values obtained for free phenolic content were subtracted from total phenolic content to estimate conjugated phenolics. The value for bound reducing compounds (total phenols minus free phenols) might be a more reliable measure of phenolic substances in our analysis since interferences from invert sugars and ascorbic acid may have distorted the results for either extraction considered on its own (Singleton et al. 1974; Waterhouse 2000). Taking account of these issues and because our analysis was in the nature of a preliminary investigation, it was decided to instead report the results in terms of FC-sensitive substances or 'reducing capacity'. This is supported by the view of Singleton et al. (1974) that "...the assay should be considered a measure of oxidisable substrates not just phenols..." which was also the approach adopted by Okuse and Rygo (1981). By this means we could avoid the need to quantify the ascorbate and invert sugar content of the samples.

# 4.3 Results and discussion

In this section the effects of the N fertiliser treatments on: (1) fruit SSC ($^o$Brix), titratable acidity, and firmness (kgf); (2) fruit oxalate, ascorbic acid, and calcium contents; and (3) the results of phenolic analysis are presented in turn. Statistical correlations between these attributes of quality are examined and how the effects were modified by the position of the fruit in the canopy.

## 4.3.1 Soluble solids, acidity, and firmness

The average firmness of HN 'Hayward' fruit harvested 178 DAFB in Year 1 from Row 2 was lower than LN fruit, but the difference was not statistically significant ($p>0.05$, two-tailed T-test). The soluble solids content (SSC) of HN fruit compared to LN fruit varied by only 0.3 °Brix units (Figure 4.1). A similar difference in firmness between HN and LN fruit was found in fruit from Row 1 and the difference was maintained during storage (Figure 4.2). In Year 2, fruit was harvested from two canopy positions (inner and outer) and two shoot types (short and long). The inner and outer canopy positions correspond to fruiting zones close to the main leader and zones at the distal ends of the canes respectively. Short shoots were terminated shoots <50 cm and long shoots were terminated or unterminated >90cm. Soluble solids contents at harvest were higher in LN fruit than in HN fruit in both rows but only in Row 1 was the difference statistically significant ($p<0.05$; Figure 4.3). Canopy position and shoot type had no consistent effect on SSC (Figure 4.3); and the only significant difference was found between fruit from long shoots from the outer canopy of HN vines in Row 1 ($p<0.05$ ANOVA; Figure 4.3B).

Figure 4.15 Effect of high rates of nitrogen fertiliser (HN) or nil nitrogen fertiliser (LN) on firmness (kgf) and SSC (°Brix) of 'Hayward' fruit 178 days after full bloom in Year 1. n=6, dual measurements on 72 fruit; error bars are standard error.
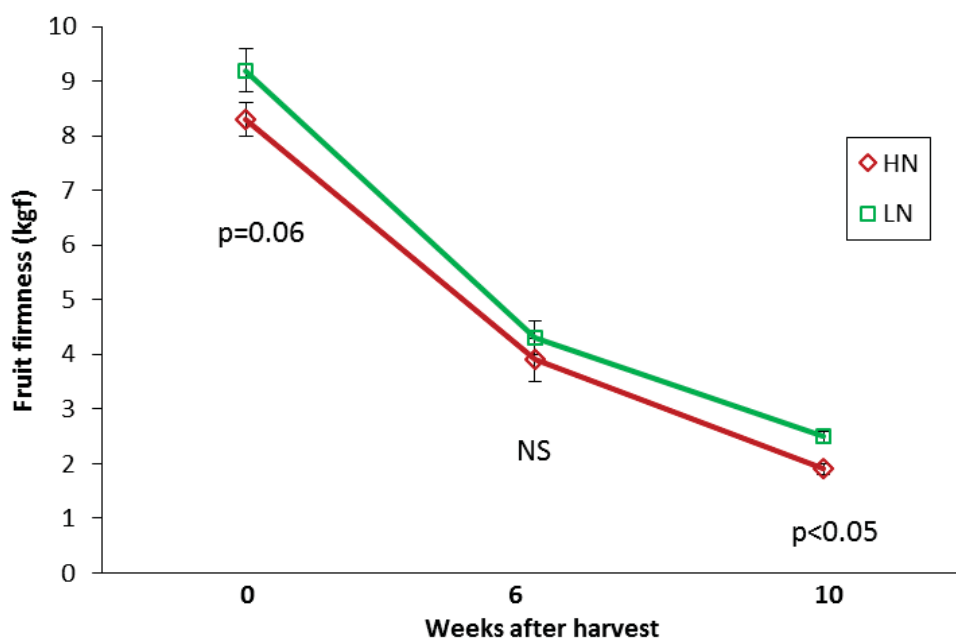


Figure 4.16 Effect of high rates of nitrogen fertiliser (HN) or nil nitrogen fertiliser (LN) on 'Hayward' fruit firmness harvested 176 DAFB in Year 1, and after 6 and 10 weeks storage at 1°C. p-values from two-tailed T-test, n=12, error bars are standard error.
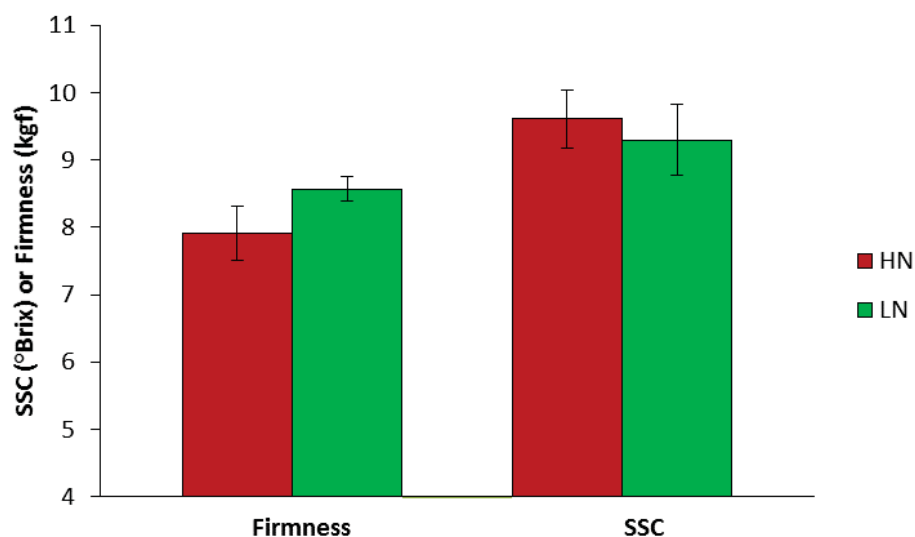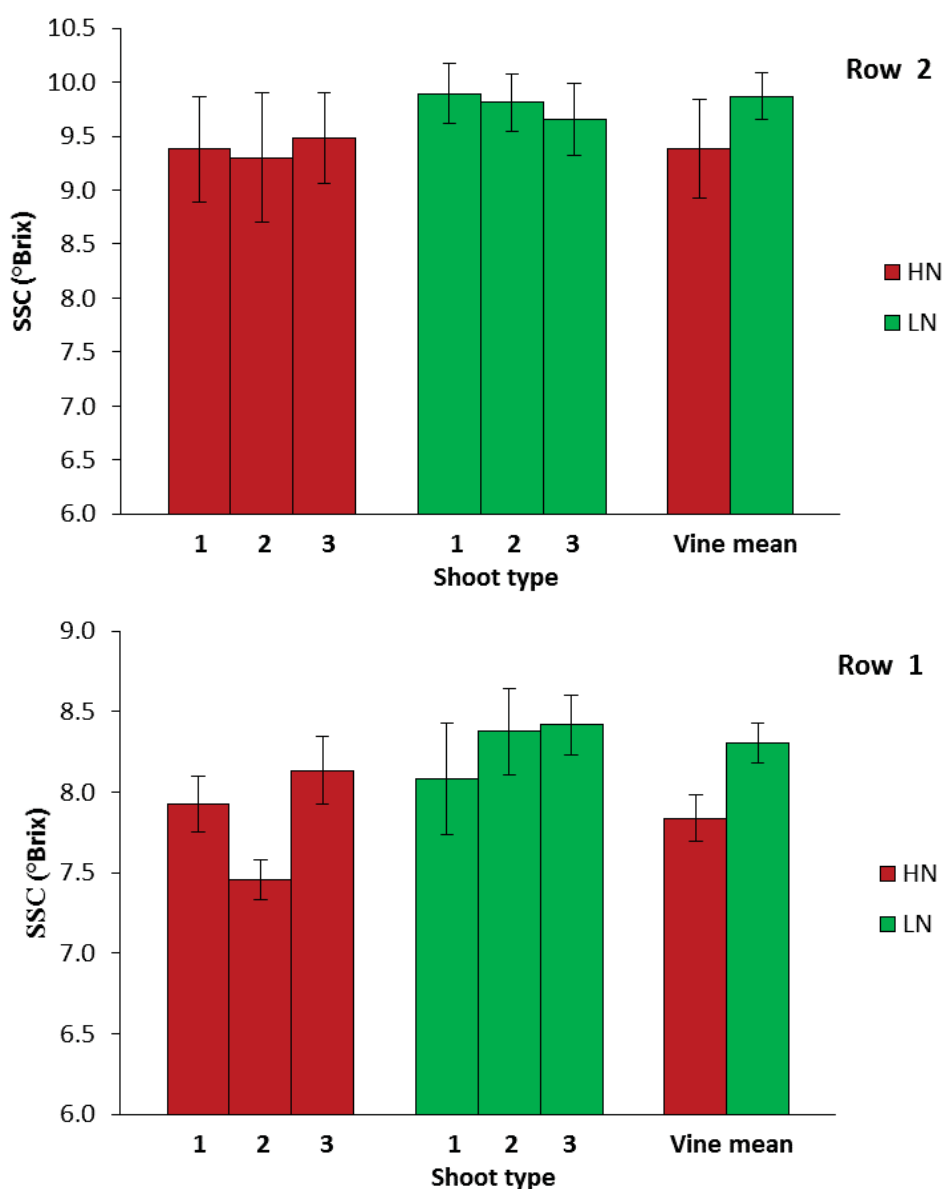
Figure 4.17 Effect of high rates of nitrogen fertiliser (HN) or nil nitrogen fertiliser (LN) on soluble solids content (SSC) of 'Hayward' fruit from different shoot types and combined to give a vine mean in Year 2 for two different rows of vines within the orchard.
Row 2 harvested 174 DAFB, Row 1 harvested 167 DAFB. Shoot types: 1, long shoots inner canopy; 2, long shoots outer canopy; 3, short shoots outer canopy. n=6, dual readings from a 48-fruit sample in each row; error bars are standard error.

Fruit from the Year 2 harvest was used to monitor changes after 20 weeks in cool storage at 1°C. Before storage there was no significant difference between HN and LN fruit in FW or DM% (Table 4.1). After storage, data for FW was not recorded, but DM% was still not significantly different between HN and LN vines (Table 4.1). Higher DM% values were obtained in fruit stored for 20 weeks compared to fruit assessed at harvest, and differences

were probably due to different drying methods used to obtain DWs for the two measurement times. The pre-storage DW was obtained with oven drying to a constant weight and was fully dehydrated, whereas the post-storage DW was obtained by lyophilisation and may have contained some residual moisture (full methodology and problems associated with incomplete drying are described in Chapter 2, section 2.5.1). There was also no significant difference between HN and LN fruit pre-storage in fruit firmness or SSC (Table 4.1). After low temperature storage for 20 weeks HN fruit had higher SSC (p<0.01), two-tailed T-test), TA% (p<0.03), and SSC:TA ratio (p<0.04) than LN fruit (Table 4.1).

Table 4.6 Effect of high rates of N fertiliser (HN) or nil N fertiliser (LN) onfruit fresh weight (FW), dry matter concentration (DM%), firmness, soluble solids content (SSC), titratable acidity (TA), Brix:TA ratio, and soluble solids of the whole fruit as a percentage of fruit dry matter (SSFDM%) at harvest 174 days after full bloom and after 20 weeks storage at 1°C in Year 2.

|  | HN | Standard error | LN | Standard error |
|---|---|---|---|---|
| *At harvest* |  |  |  |  |
| FW (g) | 126.3a | 2.5 | 121.1a | 1.7 |
| DM% | 15.7a | 0.2 | 15.7a | 0.1 |
| Firmness (kgf) | 9.0a | 0.4 | 8.8a | 0.3 |
| SSC (°Brix) | 9.7a | 0.3 | 9.9a | 0.2 |
| SSFDM (%) | 44.0a | 1.8 | 46.8a | 0.8 |
| *After 20 weeks storage* |  |  |  |  |
| DM% | 16.4a | 0.4 | 16.1a | 0.1 |
| SSC (°Brix) | 13.4a | 0.1 | 13.0b | 0.1 |
| TA% | 1.50a | 0.11 | 1.16b | 0.03 |
| Brix:TA ratio | 9.1a | 0.6 | 11.2b | 0.2 |

All values based on n=6 (60 fruit per sample) except post-storage SSC and TA where individual fruit samples were pooled (n = 4); different letters beside values within rows denote p<0.05 two-tailed T-test.

The values obtained for fruit firmness are within the range reported for mature 'Hayward' at harvest (Beever and Hopkirk 1990). Softer fruit at harvest and during storage has been previously associated with high rates of N fertilisation of 'Hayward' vines (Prasad and Spiers 1991; Vizzotto et al. 1999; Johnson et al. 1997). There was a negative correlation between fruit firmness and SSC ($r^2$=0.36, n=40) but only in HN fruit ($r^2$=0.56) (Figure 4.4A). Feng et al. (2003) reported a similar negative correlation between firmness and SSC ($r^2$=0.47) at

harvest in 'Hayward'. Generally this correlation could be attributed to the relationship between both fruit attributes to fruit maturation and ripening (Beever and Hopkirk 1990). The lack of correlation in the LN fruit suggests that the two variables are not directly related. Fruit softening during maturation is due to a combination of cell wall disassembly and water loss (Rose and Bennett 1999; Ghiani et al. 2011; Saladie et al. 2007). The main barrier to fruit transpiration is the cuticle (Maquire et al. 2001). Cuticle permeability can be increased by N fertilisation (Prior et al. 1997; Bondada et al. 2001). Increased water loss due to increased permeability of the cuticle might therefore be a factor in the reduction of firmness with N fertilisation in kiwifruit.

Feng et al. (2003) found that the proportion of dry matter that has been solubilised (SSFDM%) gave a stronger correlation to firmness ($r^2$=0.75) than did SSC. Correlating firmness with SSFDM% only showed a stronger correlation in in HN fruit ($r^2$=0.67), being almost non-existent in LN fruit (Figure 4.4B). The similarity in the correlations for HN fruit (Fig 4.4B) and the results reported by Feng et al (2003) might reflect the prevalence of N fertilisation in commercial orchards and suggests the usefulness of SSFDM% as a maturity indicator might depend on the N status of the vines. Benge et al. (2000) found conventionally grown 'Hayward' did not differ significantly in firmness at harvest to organically grown fruit (presumably grown with smaller N fertiliser inputs), although SSC was generally higher in conventionally grown fruit. In Table 4.1 the average value for SSC and SSFDM% was higher for LN than HN fruit suggesting that LN fruit was more mature, although the differences were not statistically significant. Nevertheless, it is consistent with the higher SSC levels for LN fruit reported in Figure 4.3.
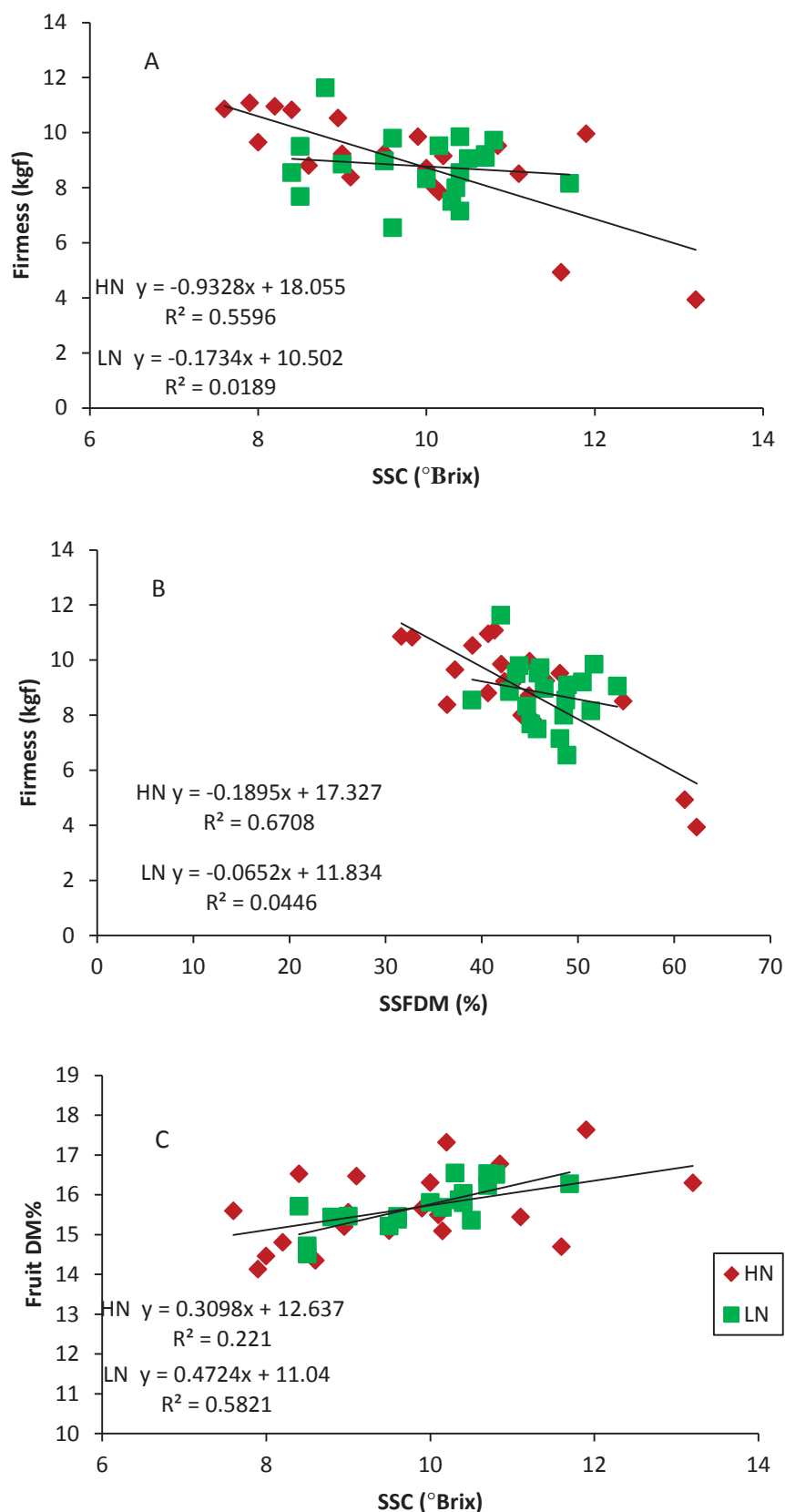
Figure 4.18 Correlations between A, fruit firmness and soluble solids content (SSC); B, firmness and proportion of dry matter solubilised (SSFDW%); and C, dry matter concentraton (DM%) and SSC of mature fruit from 'Hayward' vines given high rates of N fertiliser (HN) or nil N fertiliser (LN) and harvested 174 DAFB in Year 2. n=20.

Fruit DM% was positively correlated to SSC at harvest with a stronger relationship in LN than HN fruit (Figure 4.4C). Fruit from LN vines might be accumulating dry matter during the final stages of fruit maturation at a faster rate than HN vines as suggested by the data in Chapter 3 (Table 3.16) thus leading to a stronger positive correlation between DM% and SSC.

After 20 weeks low temperature storage TA% was significantly higher in HN than LN fruit (Table 4.1). Similarly Vizzotto et al. (1999) found that the acidity of 'Hayward' fruit from N fertilised vines was increased during storage relative to unfertilised vines. The ratio of SSC:TA was also significantly lower for HN fruit, which is indicative of HN fruit being poorer tasting (Woodward and Clearwater 2007).

Changes in the composition and concentration of organic acids in response to N fertilisation has been reported for peaches (Jia et al. 1999), grapes (Renquist and Reid, 2001), sour cherry (Hansen 1997), and citrus (Albertini et al. 2006; Smith 1966). Nitrate fertilisation stimulates the synthesis of organic acids needed for amino acid synthesis or storage as counter-anions (Scheible et al. 1997; Stitt 1999). The main acids in kiwifruit are citric, malic, and quinic (Okuse and Ryugo 1981); however amino acids also contribute to the total acidity of fruit (Lobit et al. 2002). Increasing the rate of N fertilisation increased the total free amino acid content of kiwifruit by up to about 360% at harvest (Clark et al. 1992).

Clark et al. (1992) measured the levels of individual amino acids in 'Hayward' fruit and found that at the time of harvest the dominant species, arginine, had increased from 4 $\mu$mol g$^{-1}$ in the nil-N fruit to 25 $\mu$mol g$^{-1}$ (dry weight basis) in fruit from vines receiving 250 kg N ha$^{-1}$. Jia et al. (2000) found that N fertilisation at 250 kg N ha$^{-1}$ also increased the amino acid content of peaches. Futhermore, sensory panels determined increased contents of asparagine and arginine in high N fruit were responsible for the unsavoury and poor flavour of the peaches (Jia et al. 2000). Kiwifruit from unfertilised vines had higher proportions of amino acids such as glutamate (Clark et al. 1992), which in peaches were associated with improved flavour (Jia et al. 2000). The strong correlation between fruit arginine and N content (Clark et al. 1992) and the increase in fruit N with increasing N fertilisation rates (Chapter 5, Table 5.5; Buwalda et al.1990; Costa et al. 1997) indicates not only a decrease in fruit flavour but also an increase in titratable acids (Lobit et al. 2002) are possible outcomes of high levels of N fertilisation.

Titratable acidity was positively correlated to fruit DM% at harvest (Figure 4.5), which may be typical for 'Hayward' kiwifruit (Woodward and Clearwater 2007). However, the correlation was only present in LN fruit (Figure 4.5). It is unclear whether this difference between HN and LN fruit is the result of the higher water content of HN fruit leading to a dilution of acidity.
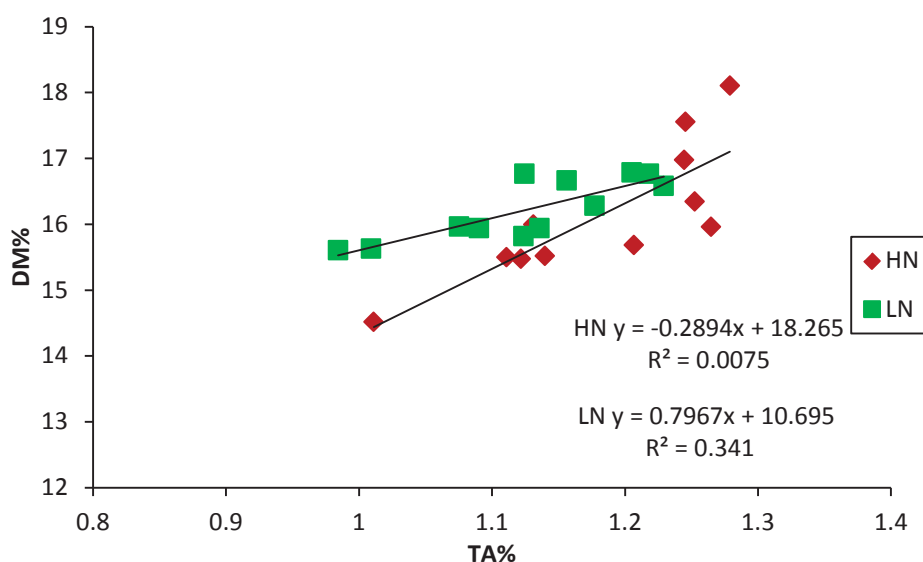


Figure 4.19 Effect of high (HN) or low (LN) rates of $NO_3^-$ fertiliser on correlations between TA% and DM% in 'Hayward' fruit at harvest 174 DAFB in Year 2; (fruit from short shoots in the outer canopy).

## 4.3.2 Oxalate, ascorbic acid, and calcium

### 4.3.2.1 Oxalate

The oxalate content of fruit ranged from 11 to 29 mg/100g FW in the pot-trial with 'Hort16A' and from 8 to 36 mg/110g FW in the field trial with 'Hayward' (Figure 4.6). These levels are consistent with the observations of Honow and Hesse (2002), who reported oxalate levels in *A. deliciosa* from 0.8 to 47.3 mg/100g FW, and Rassam and Laing (2005), who reported levels between 18 and 45 mg/100g FW in a selection of *A. chinensis* genotypes. In the pot trial, 'Hort16A' fruit from HN vines had lower average oxalate content than fruit from LN vines but the difference was below the 95% level of probability (p=0.075 two sided T-test; Figure 4.6). However, in the field trial HN 'Hayward' fruit also had lower oxalate content than LN fruit, and the difference was statistically significant (p<0.01, two sided T-test; Figure 4.6).

160

The results are not consistent with oxalate being the principle charge balancing acid for $NO_3^-$ assimilation in kiwifruit as it is believed to be in some other oxalate accumulating plants (Raven and Smith 1976). However, Rinallo and Modi (2002) found higher $NO_3^-$ levels were associated with higher oxalate levels in kiwifruit leaves where most oxalate accumulation occurs (Rassam et al. 2007). The transport of oxalate from leaves to fruit was considered unlikely by Rassam et al. (2007) due to its bivalency limiting movement across membranes. However, Libert and Franceschi (1987) suggested oxalate probably is phloem mobile because of the limited presence of $Ca^{2+}$ within the phloem. Nevertheless, although most $NO_3^-$ assimilation probably occurs in leaves, it also takes place in other tissues especially those containing chloroplasts such as the fruit (Beevers and Hageman 1969). Since oxalate crystals in kiwifruit are considered an 'anti-nutrient', the results suggest there might be beneficial effects on fruit nutritional quality of $NO_3^-$ fertilisation in terms of reduced oxalate content.



Figure 4.20 Effect of high (HN) or low (LN) rates of $NO_3^-$ fertiliser on the oxalate content (mg/100g FW) of mature fruit from pot-grown 'Hort16A' and field-grown 'Hayward' vines. Error bars are indicating standard error.

### 4.3.2.2 Ascorbic acid

Ascorbic acid levels ranged from 95 to 178 mg/100g FW in 'Hort16A' and from 53 to 127 mg/100g FW in fruit from 'Hayward' vines (Table 4.7). Rassam and Laing (2005) found average fruit ascorbic acid concentrations ranged from 98 to 163 mg/100g FW in a selection of *A. chinensis*, while Beever and Hopkirk (1990) reported a range of between 80 and 120 mg/100g FW for 'Hayward'. Nevertheless, there can be considerable variation between

seedlings of the same species, between vines within an orchard, and between fruit on the same vine (Ferguson and MacRae 1991). Muggleston et al (1998) reported that 'Hort16A' contained up to 50% more ascorbic acid than 'Hayward' whereas the difference is over 80% in Figure 4.7 due to 'Hort16A' being at the upper part of the range reported for this cultivar and 'Hayward' being at the lower range reported. Levels of ascorbic acid might be increased by oxidative stresses. These might be created by water deficits, hormonal interactions due to root restriction, or elevated temperatures in the root zone: three factors which may have been more prevalent for the 'Hort16A' in the greenhouse pot-trial (Kaack et al. 2001; Bar-Tal et al. 1995).

In the pot trial with 'Hort16A' vines HN fruit had higher levels of ascorbic acid than LN fruit ($p<0.01$) but in the field trial with 'Hayward' vines, HN fruit showed lower levels than LN fruit, although this difference was not statistically significant (two-tailed T-test) (Figure 4.7). Apart from being different species, the growing conditions for the 'Hort16A' and 'Hayward' vines were different, particularly in that the LN vines in the pot-trial had a much lower level of N nutrition than did the vines in the field where mineralisation of organic matter in the soil maintained an adequate level of nitrogen.

Reduction in ascorbic acid (or vitamin C) with increases in N fertilisation have been widely reported but increases at high or supra-optimal levels of N have also been found (Mozafar 1993). Decreases in ascorbic acid concentration similar to those shown in 'Hayward' fruit in Figure 4.7 of about 10% were found with increasing levels of soil $NO_3^-$ in carrot (Kaack et al. 2001).
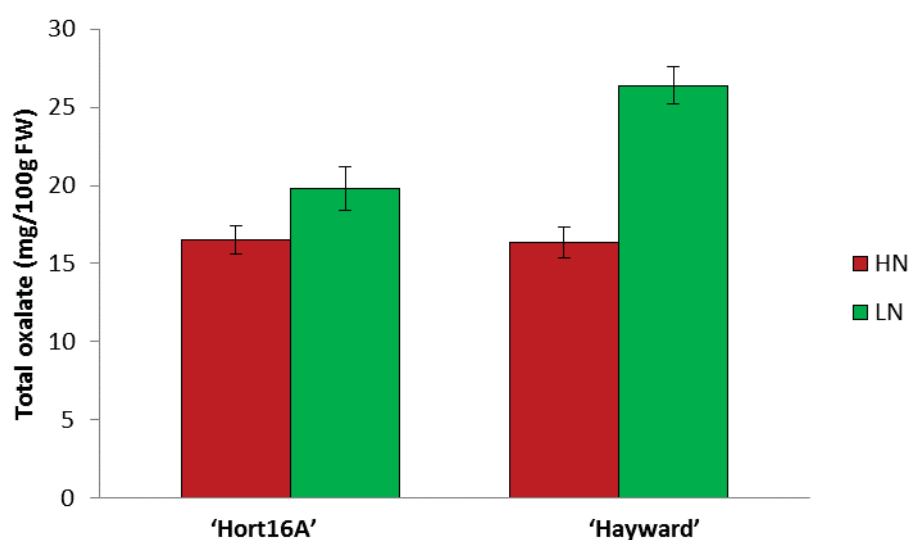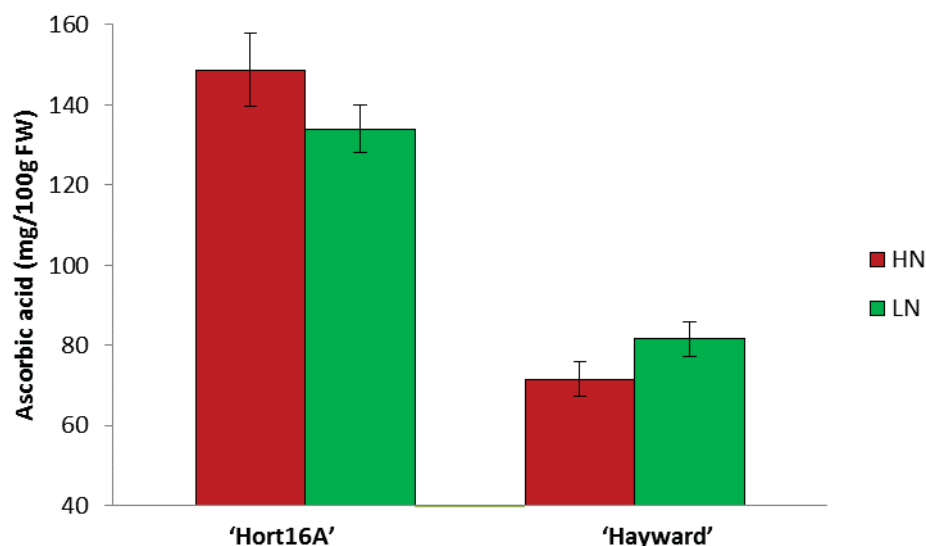
Figure 4.21 Effect of high (HN) or low (LN) rates of $NO_3^-$ fertiliser on the ascorbic acid content (mg/100g FW) of mature fruit from pot-grown 'Hort16A' and field-grown 'Hayward' vines.

Ascorbic acid is considered a likely precursor for oxalate so a relationship between the two might be expected (Rassam and Laing 2005; Franceschi and Nakata 2005). There was a positive correlation between these metabolites in the 'Hayward' vines with the relationship being strongest in the HN vines (Figure 4.8) although no correlation was found in the 'Hort16A' fruit from the pot trial ($r^2$=0.02; data not presented). 'Hort16A' fruit was also collected during the same season from a separate N fertilisation trial on a commercial orchard in Te Puke and was included in our oxalate and ascorbic acid analysis. These results also showed a positive correlation between the two metabolites and, furthermore, it was stronger in HN fruit than in LN fruit (Figure 4.9).



Figure 4.22 Relationship between ascorbic acid and oxalate in mature fruit from 'Hayward' vines given high (HN) or low (LN) rates of $NO_3^-$ fertiliser.

Figure 4.23 Relationship between ascorbic acid and oxalate in field grown 'Hort16A' fruit from a long-term nitrogen fertilisation trial in Te Puke sampled at different times during the season. HN: 295 kg N ha$^{-1}$ yr$^{-1}$; LN: nil N fertiliser.

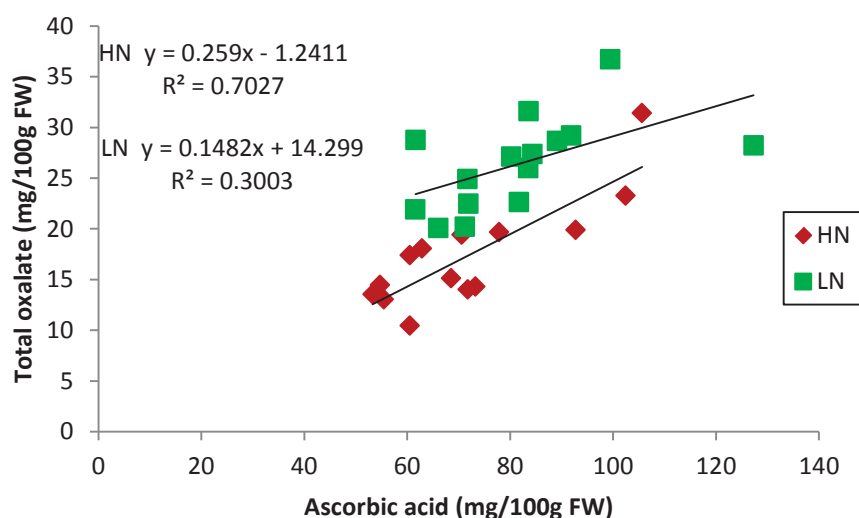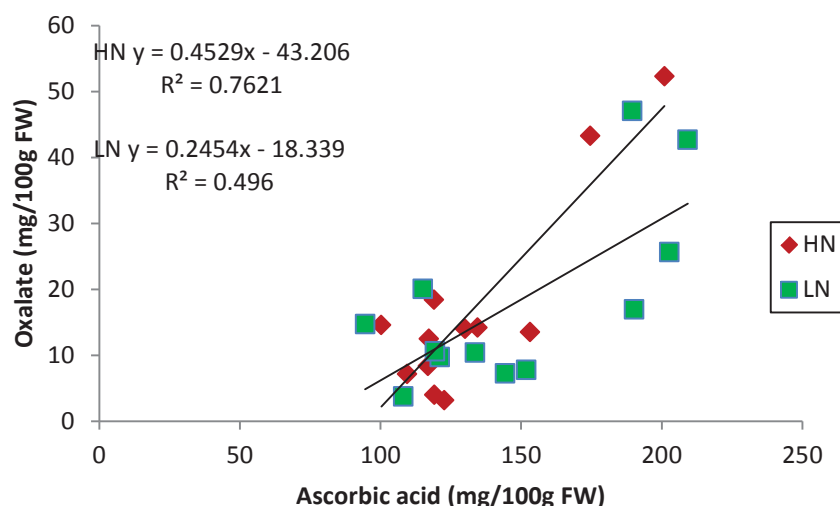Our results differ from those reported by Rassam and Laing (2005) who found no correlation between ascorbic acid and oxalate ($r^2$ =0.04) in whole fruit of six *A. chinensis* genotypes. Nevertheless, when comparing the ratios of ascorbate and oxalate in different tissue zones to those in the whole fruit, a clear clustering of values was found by Rassam and Laing (2005), suggesting that conversion of ascorbate to oxalate and oxalate turnover was being strongly regulated. A correlation within whole fruit may have been hidden in Rassam and Laing's (2005) study by the large variation between the genotypes in contents of ascorbate and oxalate (more than four-fold variation in contents) and in ascorbic:oxalate ratios. In Figures 4.8 and 4.9 the correlations were done separately for each cultivar perhaps explaining the different results.

In 'Hayward' fruit, the ascorbic acid to oxalate ratio in HN fruit was 4.4 compared to 3.1 in the LN fruit, due mainly to the large difference in oxalate content between the two N treatments (Figure 4.6 cf. Figure 4.7). Also in the Te Puke field trial, it was the oxalate that varied most between HN and LN fruit. Rassam and Laing (2005) suggested that there is no direct biosynthetic dependence between ascorbic and oxalate but that both are being regulated by some common factor such as the availability of a shared substrate. For example, the common substrate might be a sugar for which there is an increased competitive demand from the canopy of HN vines, which is reflected in the closer correlation between oxalate and ascorbate in HN fruit. Nevertheless, the correlation between oxalate and ascorbic acid in the Te Puke field trial might in this case reflect the declining concentration of both metabolites during fruit development (Figure 4.10A, B). A similar pattern in the seasonal changes in

164

concentration of the two metabolites in kiwifruit was found by Rassam et al. (2005), Watanabe and Takahashi (1998), and Ferguson and MacRae (1991).
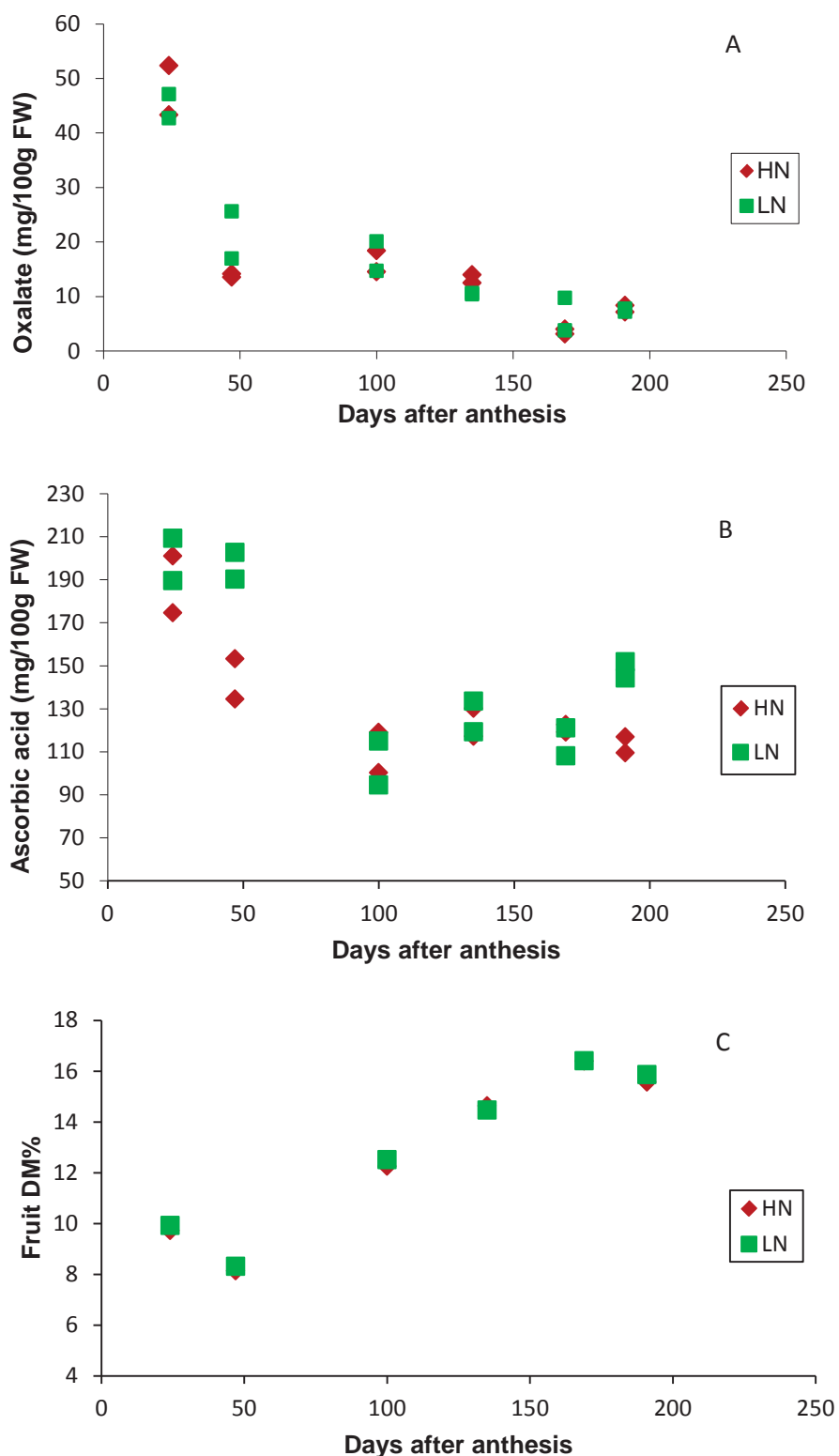


Figure 4.24 Effect of high (HN) or low (LN) rates of nitrogen fertiliser on the concentrations of: A, oxalate; B, ascorbic acid; and C, dry matter (DM%) of 'Hort16A' fruit from a long-term nitrogen fertilisation trial in Te Puke, sampled at different times during the season. HN: 295 kg N ha$^{-1}$ yr$^{-1}$; LN: nil N fertiliser.

Although the FW of the fruit from the Te Puke trial used for oxalate and ascorbate analysis was not recorded, the shape of the curve plotting changes in DM% of these fruit for each of the sampling times (Figure 4.10C) follows a normal pattern for kiwifruit (Figure 1.1B; Richardson and Currie 2007). Since the DM% curve was so typical, reasonable approximations of FW were calculated from the graph of 'Hort16A' fruit FW development given by Richardson and Currie (2007) and used to graph total oxalate and ascorbic acid contents per fruit during the season. This shows that while the ascorbic acid content of the fruit increased steadily right through to harvest, oxalate followed a more variable pattern with an apparent decline (most noticeable in LN fruit) from about 100 days after anthesis before it began to rise again late in the season (Figure 4.11). Using data for seasonal changes in FW and oxalate concentration provided by Wanatabe and Takahashi (1998) a similar pattern for oxalate accumulation was found for another *A. chinensis* cultivar ('Yellow Queen') although not for 'Hayward', which showed a curvilinear response more similar to that of ascorbic acid.
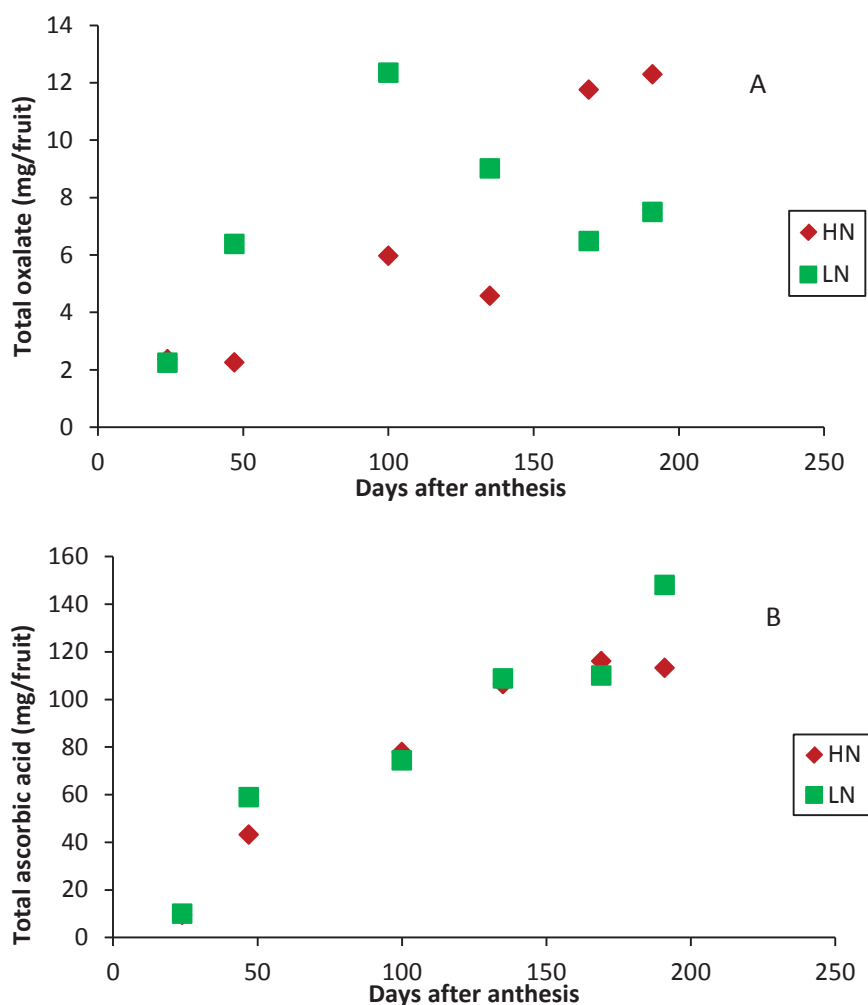


Figure 4.25 Effect of high (HN) or low (LN) rates of nitrogen fertiliser on the total contents of: A, oxalate; and B, ascorbic acid of 'Hort16A' fruit from a long-term nitrogen fertilisation trial in Te Puke sampled at different times during the season. HN: 295 kg N ha$^{-1}$ yr$^{-1}$; LN: nil N fertiliser.

In many plants, including kiwifruit, oxalate precipitates as insoluble calcium oxalate crystals, called raphides, and this process is believed to be involved in the regulation of $Ca^{2+}$ levels in the cell (Webb 1999). Cytosolic $Ca^{2+}$ at levels much above $10^{-7}$ M interfere with a variety of important cellular processes such as the precipitation of inorganic phosphorus, ionic competition with $Mg^{2+}$ for binding sites, and its function as a second messenger (Webb 1999; Marschner 2012). It is unclear to what extent Ca can be remobilised from these calcium - oxalate complexes, but it seems likely that perturbations in the biochemistry of this process could impact on the many important cellular functions Ca has; especially in postharvest fruit quality such as its role in fruit firmness (Clark et al. 1987; Hopkirk et al. 1990). The pattern of seasonal changes in fruit oxalate content shown in Figure 4.11A is consistent with the catabolism of calcium oxalate as the fruit reaches commercial maturity on the vine (about 150-160 days after anthesis).
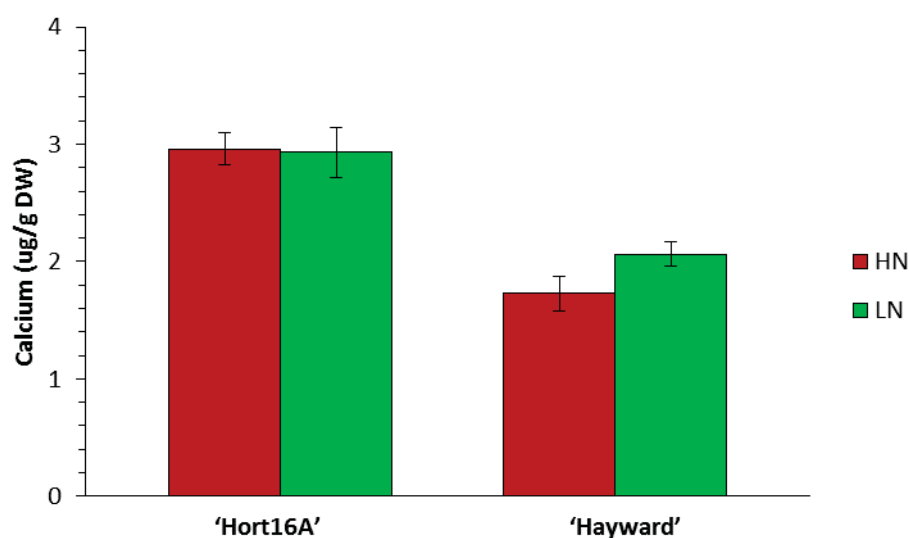


Figure 4.26 Effect of high (HN) or low (LN) rates of $NO_3^-$ fertiliser on the calcium content (mg/g dry weight) of mature fruit from pot-grown 'Hort16A' and field-grown 'Hayward' vines.

### 4.3.2.3 Calcium

Calcium content (Fig 4.14) of the 'Hayward' fruit was within the range reported for mature 'Hayward' fruit by Hopkirk et al. (1990). However, the levels found in the pot trial with 'Hort16A' were about two-fold higher than those reported by Mills et al. (2008) and Clark et al. (2004). Clark et al. (2004) suggested that seasonal changes in soil conditions as well as changes in fruit transpiration and xylem functionality (Montanaro et al. 2006) may influence

Ca accumulation by the fruit. In the pot-trial, Ca availability and uptake may have been better maintained by the daily fertigation keeping the media moist and well supplied with calcium ions.

The Ca content of 'Hort16A' fruit from the pot trial hardly varied between the two N treatments (Figure 4.12). However, the Ca concentration of the HN 'Hayward' fruit was reduced by about 16% compared to that of the LN fruit although the difference was not statistically significant ($p>0.05$ two sided T-test; Figure 4.12). Although leaf Ca was not measured in Year 1 in which the current data for Ca were obtained, it was measured in Year 2 when the treatments were essentially repeated and the Ca content of leaves from the HN vines (2.5 to 2.6%) was reduced by about 13% compared to the LN vines (2.8 to 3.1%), comparable to the 16% reduction found in HN fruit.

Mills et al. (2008) found only small reductions in 'Hort16A' leaf and fruit Ca with a N fertilisation rate of 295 kg N ha$^{-1}$. However, no reduction in leaf or fruit Ca levels were found with N rates up to 200 kg N ha$^{-1}$ in 'Hayward' by Testoni et al. (1990) or by Tagliavini et al. (1995). Inconsistent effects of N fertilisation on Ca levels have been reported in other crops, with levels being reduced in raspberry (Spiers 1992), grapes (Spiers and Braswell 1993) and mango (Torre et al. 2004); increased in blueberry (Bryla et al. 2012) and capsicum (Kowalska and Wlodzimierz 2012); or unaffected in grape (Peuke 2009) and raspberry (Kowalenko 2006). Nitrate, while not directly antagonistic to $Ca^{2+}$ uptake, to some extent regulates the soil solution concentration of cations such as $K^+$ and $Mg^{+2}$, which are directly antagonistic to $Ca^{2+}$ uptake (Okajima 1977). Calcium accumulation in aerial organs depends primarily on the transpiration-driven xylem flow (Saure 2005). Therefore, reductions in fruit Ca following N fertilisation could be caused by reductions in fruit transpiration due to restricted air flow in a denser canopy (Saure 2005) (see also the discussion about the effects of N on fruit transpiration in Chapter 9, section 9.6).

Calcium is not evenly distributed through the fruit but occurs in localised spots (Mills et al. unpublished). Calcium concentrations in the skin are more than double those in the flesh in 'Hayward' (Clark and Smith 1988). Similarly, fruit oxalate concentrations vary widely between different tissue zones with concentrations in skin being more than double those in the seedless flesh (Rassam and Laing 2005). Insoluble and physiologically inactive calcium oxalate can contain 50% of the total Ca in the fruit (Watanabe and Takahashi 1998). Since

calcium oxalate raphides are mainly seen between locules in the inner pericarp and not in the epidermal region it is unclear whether the oxalate is dominated by the insoluble form in the skin (Watanabe and Takahashi 1998; Perera et al. 1990). This could be important in understanding the relationships between Ca, N, and various storage disorders where these two nutrients are implicated (Boyd et al. 2004; Prasad and Spiers 1992). There was weak positive correlation between oxalate and calcium in both HN and LN 'Hayward' fruit (HN: $R^2$= 0.18, y = 0.0357x + 1.1684; LN: $R^2$ = 0.22, y = 0.0429x + 1.0067). The calcium:oxalate ratio was significantly higher in HN (0.12) than in LN fruit (0.09) (p=0.04, two tailed T-test) from the 'Hayward' field trial. The ratio was also higher in HN fruit (0.23) than LN fruit (0.15) from the 'Hort16A' pot trial (p=0.06). Analysis of oxalate and Ca in the different tissue zones of the fruit is needed to better understand the effects of N on the local distribution of these related components.

### 4.3.2.4 Canopy position

Concentrations of ascorbic acid, oxalate, and Ca were generally higher in fruit from the inner canopy than fruit from the outer canopy (Table 4.2). The inner and outer canopy positions correspond to fruiting zones close to the main leader and zones at the distal ends of the canes respectively. The differences between inner and outer canopy were significant (p<0.05) for ascorbic acid and for calcium. Lower levels of all three constituents were found in HN compared to LN fruit from both canopy positions (Table 4.2).

Light is involved in the regulation of ascorbate levels (Massot et al. 2012), and both light and transpiration are involved in fruit Ca accumulation (Biasi and Altamura 1996). The higher levels of both ascorbate and Ca found in fruit from inner canopy positions in the Year 1 'Hayward' vines might therefore reflect a lower leaf area index (LAI) in this part of the canopy. A lower LAI would result in more exposed fruit resulting in higher light exposure, which in turn promotes both transpiration and enhanced vascular development (Montanaro et al. 2006; Biasi and Altamura 1996). Higher Ca concentration in fruit from inner canopy positions was also reported by Thorp et al. (2003) and Remorini et al. (2007) found higher ascorbic acid levels in exposed kiwifruit. Interestingly, in tomato a reduction of light irradiation by 70% for seven days reduced fruit ascorbate concentration by 10% (Massot et al. 2012) the same as the reduction in ascorbate in fruit from outer compared to inner and in HN compared to LN fruit in the 'Hayward' field trial.

Table 4.7 Effect of high (HN) or low (LN) rates of $NO_3^-$ fertiliser on the ascorbic acid (mg/100g fresh weight), oxalate (mg/100g fresh weight), calcium (µg/g dry weight) content of 'Hayward' kiwifruit from different canopy positions.

| | Inner canopy | | Outer canopy | |
| | HN | LN | HN | LN |
| --- | --- | --- | --- | --- |
| Ascorbic acid | 75.8 (6.5)a | 87.4 (6.4)a | 68.5 (6.6)a | 73.1 (2.3)a |
| Oxalate | 17.2 (1.7)a | 28.5 (1.5)b | 17.7 (2.4)ac | 23.2 (1.0)c |
| Ca | 1.94 (0.13)ab | 2.23 (0.12)b | 1.44 (0.25)ac | 1.84 (0.15)ad |

Inner and outer canopy positions correspond to fruiting zones close to the main leader and zones at the distal ends of the canes respectively. Different letters within rows designate significant differences at $p<0.05$ Fisher's Protected LSD, n=6.

### 4.3.3 Phenolics

Although the phenolic content of kiwifruit is low compared to many other fruits (Dawes and Keene 1999), reported levels vary widely due to different extracting conditions, maturity levels, and methods of quantification. The FC method is sensitive to any oxidisable substrates present in the sample which, in the case of fruit and especially in the case of kiwifruit, includes invert sugars (both glucose and fructose are the main sugars present in mature kiwifruit) and ascorbic acid (Waterhouse 2000). Applying a correction factor for ascorbate, as advised by Waterhouse (2000), was considered unrealistic in this work, since the degradation of ascorbate during the heating step could not be accounted for (Lee and Kader 2000). Furthermore, results from the previous season showed ascorbate content was likely to differ between HN and LN samples, even if they did not vary significantly between the pulp and skin (Rassam and Laing 2005). Similarly, in the case of the invert sugars, although correction factors are available (Singleton et al. 1974; Waterhouse 2000), sugar levels were also likely to differ significantly between HN and LN fruit. Taking account of these issues and because the analysis was in the nature of a preliminary investigation, it was decided to instead report the results in terms of FC-sensitive substances or 'reducing capacity'. This is supported by the view of Singleton et al. (1974) that "...the assay should be considered a measure of oxidisable substrates not just phenols…"; which was also the approach adopted by Okuse and Rygo (1981). By this means the need to quantify the ascorbate and invert sugar content of the samples was avoided.

The phenolic content based on the reducing capacity found in mature 'Hayward' fruit from Year 2 ranged from 1437 to 2134 mg gallic acid equivalents (GAE)/100g dry weight (DW) in the pulp and between 3123 to 4230 mg GAE/100 g DW in the skin (Figure 4.13). These concentrations are similar to those reported by Imeh and Khokhar (2002) for ripe 'Hayward' fruit. These researchers used similar extraction and quantification methods. Lower levels of about 220 mg GAE /100g DW were reported by Tavarini et al. (2008); levels around 500 mg GAE/100g DW were found by Latocha et al. (2010) and by Okuse and Ryugo (1981). Each of these studies had differences in the extraction and/or quantification procedures used, which probably accounts for some of the variation in levels of phenolics found.

In each of the published studies cited in the previous paragraph, the objective for phenolic analysis appears to have been in relation to the nutritive value of the fruit. In this context hot acidic methanolic extractions seem less appropriate since they will remove tightly bound compounds such as polymeric lignins from cell walls that have little nutritional relevance (Antolovich et al. 2000) and will also degrade phenols of importance in the nutritional context (Merken et al. 2001). However, the method was appropriate in this case since the purpose was to conduct an exploratory study of the effects of N on general phenolic metabolism without regard to any specific phenolic function.
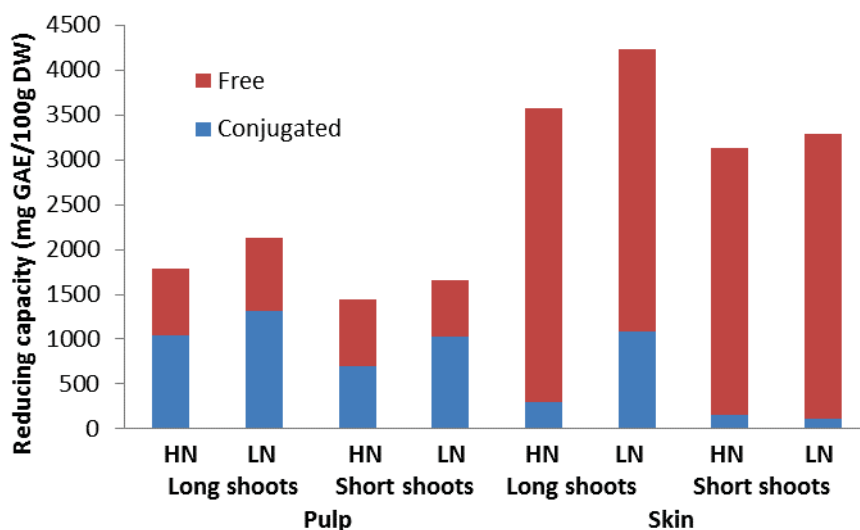


Figure 4.27 Effect of high (HN) or low (LN) rates of $NO_3^-$ fertiliser on the total, free, and bound compounds contributing to the reducing capacity (mg GAE/100g DW) of pulp and skin tissues of mature 'Hayward' fruit from short and long shoots from the outer canopy (average values of triplicate extractions of pooled samples).

The reducing capacity of compounds extracted from the skin was higher than those of the pulp (Figure 4.13). This is a typical pattern in most fruit, especially in respect to soluble or free phenolics (Antolovich et al. 2000; Robards et al. 1999). Fruit from long shoots had higher amounts of phenolics than short shoots, in both skin and pulp tissues. This may reflect the generally higher level of physiological activity of fruit on these stronger growing shoots, as shown by their increased FW and DW in comparison to fruit from short shoots (Figure 3.6 A, C). On both shoot types, higher levels of total reducing compounds (bound + free or 'total phenolics') were found in the pulp and skin of LN fruit than in HN fruit (Figure 4.13). This could have implications for fruit storage since phenolic compounds are involved in repairing oxidative damage and thus are important for the maintenance of postharvest fruit quality (Zhu et al. 2008; Robards et al. 1999).

Levels of bound reducing compounds in the LN fruit also appeared greater in the skin of fruit from long shoots and in the pulp of both shoot types (Figure 4.13). Accumulation of cell wall bound phenolic compounds is associated with increased disease resistance in plants, particularly to fungal pathogens, and with reduced water loss from fruit during storage (El Modafar et al. 1999; Tesfay et al. 2011). A relationship between bound phenolics in the epidermis and reduced water loss would be consistent with the observed link between N fertilisation and the loss of fruit firmness during storage (Figure 4.2, Table 4.1).

Evidence exists that protein and phenolic synthesis are competitive metabolic pathways, and increasing N fertilization causes increased protein synthesis at the expense of phenolics or secondary metabolites (Jones and Hartley 1999). Plant demand for protein increases with increasing growth rates and hence the correlation between phenolic content and plant vigour. Increasing N rates can lower the activity of enzymes important in phenolic synthesis and can also alter some of the active properties of phenolic compounds (Kiraly 1964). There are no published reports on the effect of N fertiliser on the phenolic content of kiwifruit, although higher phenolic content in organic than conventional 'Hayward' fruit was reported by Amodio et al. (2007).

## 4.4 Summary and conclusions

The various effects on fruit chemical and physical properties of a supra-optimal nitrate supply given to kiwifruit vines described in this chapter are summarised in Figure 4.16.

Increased availability and uptake of nitrate in vines given a supra-optimal nitrate supply was associated with softer fruit with generally lower soluble solids content at harvest than fruit from unfertilised vines. After storage fruit from vines given a supra-optimal nitrate supply had higher levels of titratable acidity and a lower ratio of soluble solids to titratable acidity. These differences suggest that fruit from vines given a supra-optimal nitrate supply may have lower postharvest quality in terms of storability and flavour.
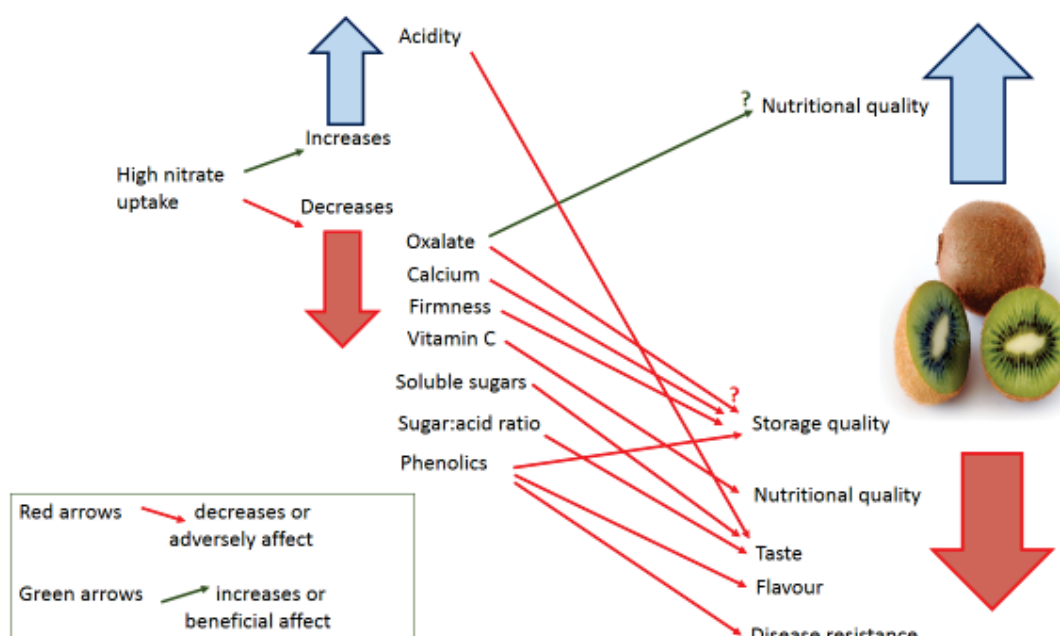


Figure 4.28 Diagrammatic representation of the effects of elevated nitrate uptake by kiwifruit vines on selected characteristics of mature fruit.

Fruit oxalate content was reduced in fruit from vines given a supra-optimal nitrate supply rather than increased as was hypothesised. Oxalate crystals in kiwifruit are considered an 'anti-nutrient' because of their potential to cause irritation in the mouths and throats of some people eating the fresh fruit (Rassam and Laing 2005). Therefore the lower levels of oxalate in vines given a supra-optimal nitrate supply could represent an improvement in fruit nutritional quality in respect to oxalate. Effects of an elevated nitrate supply on ascorbic acid

were less consistent. Higher levels of ascorbic acid were found in fruit from potted 'Hort16A' vines fertigated with high compared to low rates of nitrate; but lower levels were found in fruit from 'Hayward' vines given a supra-optimal nitrate supply than in fruit from unfertilised vines in the field trial. It is unclear from the present study if the different effects of nitrogen fertiliser on ascorbic acid in the two different kiwifruit cultivars was due to different experimental conditions (potted vines in a greenhouse environment compared to field grown vines) or is an actual cultivar difference.

Phenolic contents were generally lower in fruit from 'Hayward' vines given a supra-optimal nitrate supply than in fruit from unfertilised vines, and especially in the skin of fruit from long shoots. This effect of nitrogen might have implications for disease resistance and water loss during storage affecting firmness.

Reduced fruit firmness, soluble solids, calcium, and phenolic contents in the skin could have implications for the storage quality of kiwifruit. Further work is needed to investigate possible connections between calcium, oxalate, and phenolic content on properties of the skin affecting water loss and firmness. Further research is needed to determine whether oxalate levels could be reduced by lower levels of nitrate fertilisation so that other adverse effects of elevated nitrate uptake described in this chapter and in Chapter 3 could be simultaneously minimised.

# Contents

# List of Tables

## List of Figures

# 5. The response of 'Hort16A' fruit to foliar applications of nitrogen and a proprietary biostimulant

## 5.1 Introduction

The New Zealand-bred kiwifruit cultivar 'Hort16A' (*Actinidia chinensis*), is a vigorous deciduous vine with yellow-fleshed fruit. Nitrogen (N) fertilisation of kiwifruit orchards is recommended to maintain vine health and regularly produce heavy crops (Sale 1997). Careful management of N fertiliser inputs is necessary to avoid possible adverse effects associated with excess nitrogen availability (Mills et al. 2008a). Two such adverse effects are the induction of excessive vegetative vigour and nitrate ($NO_3^-$) leaching (Mills et al. 2008a). Excessive vegetative vigour is associated with poor fruit quality and increased labour costs for canopy management (Patterson and Currie 2011). Nitrate leaching contaminates ground water and, increasingly, growers must conform to production standards and local environmental regulations (Mills et al. 2008). Foliar applications of nutrients can supplement the soil supply and thereby reduce the quantity of fertiliser needing to be applied to the soil (El-Otmani et al. 2004). Foliar-applied N sprays can reduce both the risk of $NO_3^-$ leaching and of excessive vegetative vigour, compared to soil-applied N (Dong et al. 2005). It may also allow supplementary N to be supplied at specific times during the season when there is an increased demand for N such as during periods of rapid fruit growth (Klein 2002). Therefore, foliar-applied N might be useful for the N management of kiwifruit orchards.

Biostimulants are defined as "… materials, apart from fertilisers that promote plant growth when applied in small quantities…" (Khan et al. 2009). The proprietary biostimulant Benefit Kiwi®is widely applied to 'Hort16A' grown in New Zealand to increase fruit size (Currie et al. 2005). However, when fruit size is increased by biostimulants, the dry matter concentration (DM%) of the fruit is often diluted due to water uptake being increased more than dry matter (Patterson and Currie 2011; Currie et al. 2005). The DM% of the fruit is an important attribute for kiwifruit because it correlates positively with the taste and consumer preference for the ripe fruit (Patterson and Currie 2011). Furthermore, growers receive an incentive payment for fruit with high DM%.

Fruit size can also be increased by foliar-applied N, particularly when applied during early fruit development. Foliar-applied N applied as urea during the early season increased the size of apples (Dong et al. 2005), citrus (Lovatt 1999), and guava (Kundu et al. 2007). However, the effects of foliar-applied N during early fruit development in kiwifruit and its effects on fruit size and DM% has not been previously reported. It is also not known if foliar-applied N would interact with a biostimulant. However, it seems reasonable to expect fast growing tissues treated with biostimulants to be more likely to suffer temporary N deficiency and therefore to be responsive to timely applications of foliar-applied N.

Uptake of foliar-applied nutrients can be stomatal, trichomal, or by direct cuticular absorption (Haynes and Goh 1977). Kiwifruit appear to be well suited to foliar-applied N nutrition as their leaves have numerous anomocytic stomata on the underside of leaves and many trichomes (Ferguson 1990). Such a dense indumentum probably also assists spray retention. When kiwifruit are grown on the pergola training system, the underside of leaves and the fruit hanging below the canopy are well exposed to foliar sprays applied from the ground. Furthermore, kiwifruit have a thin epidermal layer and high rates of surface conductance, compared to some other fruit such as apple, making efficient absorption of foliar-applied N likely, especially during early fruit development (Smith et al. 1995).

This chapter reports the effects on the fresh weight (FW) and DM% of 'Hort16A' fruit of aqueous solutions of either urea or potassium nitrate ($KNO_3$) applied to leaves and fruit at different times and frequencies during early fruit development. The specific experimental questions asked were:

1. What are the effects of N applied as a foliar spray during early fruit development on 'Hort16A' fruit FW and DM% and how does the time of application influence these effects?
2. Are these effects similar when fruit is treated with a proprietary biostimulant?

## 5.2 Materials and methods

The experiment was conducted in a commercial orchard near Hastings in Hawke's Bay (39º 39′S 176 º 52′ E) over two non-consecutive seasons. The vines were planted in 1980 as cv. 'Hayward' (*A. deliciosa*) but top-worked to 'Hort16A' (*A. chinensis*) in 1997 (Appendix 2, Plate 6). Vine spacing was 5m × 4.8m. Soil type is a Hastings silt loam (Molloy 1998) and soil samples were collected from the area used for the experiment in the winter prior to each season of the trial (Table 5.1). Fertiliser inputs over both seasons included 20 kg P, 100 kg K, 50 kg Mg, 30 kg Ca, and 60 kg S per hectare. In the 2007-08 season, 100 kg N and in the 2009-10 season 50 kg N per hectare was applied as calcium ammonium nitrate. The relatively low N inputs used in this orchard are part of a management strategy of the orchardist aiming to reduce the vegetative vigour of the vines.

Table 5.8 Trial site pre-budbreak soil pH, bulk density (BD), cation exchange capacity (CEC), plant available phosphorus (Olsen P), exchangeable cations (Ca, Mg, K, and Na) (0-15cm depth) for both seasons.

| Season | pH | BD (g/mL) | CEC (me/100g) | Olsen P (µg/ml) | Ca (me/100g) | Mg (me/100g) | K (me/100g) | Na (me/100g) |
|---|---|---|---|---|---|---|---|---|
| 2007-08 | 6.7 | 0.7 | 27 | 55 | 20.1 | 3.1 | 0.95 | 0.13 |
| 2009-10 | 6.6 | 0.8 | 22 | 44 | 16.7 | 2.5 | 0.70 | 0.12 |

During two seasons 1% (w/v) foliar sprays of $KNO_3$ (Yara Krista-K Plus, Yara International Ltd, Norway) or low-biuret urea (Yara Urea Tech, Yara Fertilizers NZ Ltd; biuret content 0.65-0.80%) were applied to the fruit and adjacent leaves of selected canes of vines within a single row containing uniform vines with complete canopies. Urea and $KNO_3$ have a similar salt index at this concentration, which is also considered the maximum safe level for foliar application of both of these N fertilisers (Weinbaum 1978; Lea-Cox and Syvertsen 1995). Three sprays at 10 day intervals were applied in three staggered series from 20 days after full bloom (DAFB) in 2007-08 and from 15 DAFB in 2009-10, with the final spray of the third series being applied at 80 DAFB and 75 DAFB in the two respective seasons (Table 5.2). A fourth series of seven sprays covered the whole period (Table 5.2).

Each treatment was repeated on canes also treated with the proprietary biostimulant Benefit Kiwi®(Valagro Farm, Italy; henceforth abbreviated to 'BK'), which was applied (0.3% v/v)

three times coinciding with the first set of foliar sprays (N1, U1). The product's label claims that BK contains 5.0% (w/v) organic N as nucleotides, specific amino acids, and vitamin co-factors and promotes cell division.

In the 2007-08 season the treatments were all allocated to canes on a single vine, and repeated six times (6 vines). In the 2009-10 seasons 12 vines were used and the $NO_3^-$ and urea treatments were separately applied to different vines, also giving six replications. In both seasons, a water-only control and a BK-only control were used to compare the effects of the foliar-applied N sprays. The experimental design was a complete block with vines as blocks and with treatments allocated randomly to selected canes (2 per vine per treatment) on each vine.

### Treatments

Control- water

Benefit Kiwi® (BK) 30 ml/10 Litres

Urea (U1, U2, U3, U4) 1% (w/v)

Urea + BK (U1+BK, U2+BK, U3+BK, U4+BK)

$KNO_3$ (N1, N2, N3, N4) 1% (w/v)

$KNO_3$ + BK (N1+BK, N2+BK, N3+BK, N4+BK)

The pH of solutions was between 6.7 and 7.4 and was not adjusted. A non-ionic organo-silicon surfactant (Breakthru®, Evonik industries AG, Germany) was added to the foliar solutions at the rate of 0.02% v/v. Treatments were applied with 1 litre spray bottles in the 2007-08 season and with a manually operated 'back pack' spray unit in the 2009-10 season.

Table 5.9 Timing of application of foliar treatment series for the two seasons (2007-08 and 2009-10).

| Foliar treatments | | Number | Spray timing (days after full bloom)[a] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| No BK | Plus BK | of sprays | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 |
| N1/U1 | N1+BK/U1+BK | 3 | | 2007-08 / 2009-10 | | | | | | | |
| N2/U2 | N2+BK/U2+BK | 3 | | | | | 2007-08 / 2009-10 | | | | |
| N3/U3 | N3+BK/U3+BK | 3 | | | | | | | 2007-08 / 2009-10 | | |
| N4/U4 | N4+BK/U4+BK | 7 | | 2007-08 / 2009-10 | | | | | | | |

[a]Full bloom: 2007-08 season 11 October, 2009-10 season 16 October. BK: Benefit Kiwi®

## 5.2.1 Sampling and chemical analysis

### *5.2.1.1 Sampling*

In the 2007-08 season fruit from all treatments was harvested on 2/04/08 (162 DAFB) while in the 2009-10 season fruit from all treatments was taken on 3/12/09 (55 DAFB), 8/01/10 (84 DAFB) 8 days after last foliar-applied N application, and for the final harvest fruit and leaves from all treatments were taken on 22/03/10 (157 DAFB). Sixteen to 20 fruit per treatment were weighed for FW and two equatorial slices from each fruit were taken. One slice was weighed and dried to constant weight (60ºC) in a forced air oven to obtain dry weight (DW) and allow calculation of DM% (DW/FW ×100), while the remaining sample was frozen and freeze dried for subsequent N and carbohydrate analysis. Twenty youngest fully-expanded leaves from non-fruiting shoots on each vine were pooled to give one sample per treatment.

### *5.2.1.2 Nitrogen analysis*

Leaf, fruit, and seed N% was measured by Kjeldahl digestion followed by N analysis on an auto-analyser (Blakemore et al. 1987). Duplicate extractions of each sample were made. Seeds were removed from a set of lyophilized fruit equatorial slices (mature fruit samples), counted and weighed before being ground in a mortar for N analysis. A set of slices from the early harvest was processed with or without skins to check that superficial foliar spray residues were not influencing the N% results.

### *5.2.1.3 Soluble sugar analysis*

Fruit soluble sugar contents were analysed by an adaptation of the phenol-sulphuric colorimetric method described by Dubois et al. (1956). Lyophilized fruit samples were ground in a small coffee grinder until able to pass through a 40 mesh filter (particle size <0.5 mm). The ground material was re-dried overnight to ensure equal moisture content between the samples and small subsamples were then weighed into 15ml incubation tubes. Unused sample material was stored in screw capped 35ml plastic containers (P35) in a bench top desiccator. Ten ml of 80% aqueous ethanol was added to the tubes and the tubes were placed on a rotary shaker for 48 hours. The tubes were centrifuged at 1000 g for 10 minutes and the supernatant poured off into another set of P35 containers. Each sample was washed twice more with 5 ml of 50% aqueous ethanol, centrifuged and the supernatant added to the first extracted solution. The residual sample was frozen and put aside for starch analysis. The extracts were then made up to exactly 25 ml. Aliquots of the extracts were taken for processing as described by Dubois et al. (1956) and absorbance read at 490 nm on a

spectrophotometer (Philips PU 8625 UV/VIS, BioLab Scientific Ltd.). The precision of the method was verified by triplicate extractions of the same material and the cold ethanol extraction was compared to water-bath incubation with hot ethanol and a range of extraction times. Moisture content of the stored lyophilized samples was monitored by re-weighing before use. The same sample material was used for the N analysis.

### 5.2.1.4 Starch

A procedure for starch analysis was developed based on the glucose oxidase method described by Kilburn and Taylor (1969) with modifications according to information given by Rasmussen and Henry (1990), Seager and Haslemore (1993), and Sigma-Aldrich (n.d.). A heat stable α-amylase (Sigma, 15,000 to 30,000 U/ml) from *Bacillius lichenformis* in saline sucrose solution was used. Care was taken to ensure sufficient enzyme was supplied during the procedure in accordance with the quantities used in the different methods consulted. Multiple assays were completed with different quantities of enzyme and incubation time. For the α-amylase 750 U/sample with incubation for 1 hour at 95 -100 °C was found optimal. For the amyloglucosidase step, 120 Units of enzyme was used and incubation was for 2 hours at 60 °C. For the glucose oxidase step the samples were incubated for 30 minutes at 37° C. Absorbance was read at 540 nm on a spectrophotometer (Philips PU 8625 UV/VIS, BioLab Scientific Ltd.).

## 5.2.2 Statistical analysis

The experiments were complete randomised blocks with vines as blocks, and treatments applied to individual canes or pairs of canes on each vine. In the 2007-08 season all treatments were applied to each of six vines. In the 2009-10 season six individual vines were randomly allocated to receive either potassium nitrate or urea foliar treatments, with a total of twelve vines.

Statistical analysis of the effects of the foliar treatments on the fruit was by ANOVA using SAS statistical software (SAS Institute Inc. 2004) with treatment means compared using Dunnett's test for significance. This test compares all treatment means against a selected reference mean (Chew 1976).

## 5.3 Results

Effects of the foliar spray treatments on the N content of the leaves and fruit in the second season are reported first in this section. Following are the effects of the treatments on the early season growth of the fruit, also in the second season. Next are the effects of the foliar treatments on fruit FW and DW at final harvest (commercial maturity) in each of two seasons. After a brief report comparing treatment effects on seed weight, the effects on fruit carbohydrates in the second season are presented. A full discussion of the results appears in section 5.4.

### 5.3.1 Leaf N%

Leaf N% in most of the treatments at the end of the 2009-10 season were below the industry established 'normal' range (1.5 to 2.0%) for 'Hort16A' for this time of the growing season (Hill Laboratories 2010) (Table 5.3). Only treatments receiving seven foliar applications of urea had leaf N% within the normal range (Table 5.3). Urea appeared to increase leaf N% more than potassium nitrate ($KNO_3$). There was also some increase in leaf N% in treatments receiving BK sprays, although this was not apparent where seven N sprays were also applied.

Table 5.10 Nitrogen concentration (N%) at 157 days after full bloom in the 2009-10 season of 'Hort16A' leaves treated with Benefit Kiwi®(+BK), no BK (-BK), and foliar-applied urea or $KNO_3$ (1% w/v).

| Treatment | Number of N sprays | | |
| --- | --- | --- | --- |
| | 0 | 3 | 7 |
| -BK (control) | 1.16 | 1.23 | 1.70 |
| +BK | 1.21 | 1.39 | 1.51 |
| Urea | 1.17 | 1.41 | 1.88 |
| $KNO_3$ | 1.20 | 1.25 | 1.34 |

Data from duplicate extractions of pooled samples, n=1. Treatments included Control, BK, N1, U1, NB1, UB1, N4, U4, NB4, UB4.

### 5.3.2 Fruit N%

For the early sampling time fruit N% ranged from 1.0% to 1.1% (Table 5.4) compared to levels at an equivalent time of the season of 1.3% for fruit from unfertilised vines and 1.4% for fruit from vines given 145 kg N ha$^{-1}$ yr$^{-1}$ in the long-term N fertiliser trial reported by Mills et al. (2008). Similarly, for the second sampling time, fruit N% ranged between 0.7% and 0.8% (Table 5.5), compared to a range at the same seasonal time of between 0.9% and 1.3% for fruit from fertilised and unfertilised vines respectively reported by Mills et al. (2008).

Fruit N% was also increased by N sprays in a pattern consistent with the amount of N applied with the two different N forms and number of applications (Tables 5.4 and 5.5).

Table 5.11 Nitrogen concentration (N%) at 84 days after full bloom (8/01/2010; 8 days after last foliar-applied N application) of 'Hort16A' fruit treated with Benefit Kiwi® (+BK), no BK (-BK), and foliar-applied N as either urea or $KNO_3$ (1% w/v).

| Foliar treatment | Number of N sprays | | | ANOVA |
| | 0 | 3 | 7 | p-value |
| --- | --- | --- | --- | --- |
| -BK (control) | 1.06 (0.05)a | 1.36 (0.15)b | 1.65 (0.22)b | 0.051 |
| +BK | 1.04 (0.08)a | 1.23 (0.10)ab | 1.55 (0.17)b | 0.043 |
| | NS | NS | NS | |
| Urea | 1.14 (0.07)a | 1.54 (0.09)b | 1.98 (0.10)c | >.001 |
| $KNO_3$ | 0.98 (0.05)a | 1.07 (0.05)ab | 1.20 (0.07)b | 0.037 |
| | NS | * | * | |

Standard error in parenthesis, n = 6. Reported range for 'Hort16A' at same time of season in Mills et al. 2008: 1.3 in unfertilised vines to 1.4% in vines given 145 kg N ha$^{-1}$ yr$^{-1}$ Treatments included for analysis Control, BK, N1, U1, NB1, UB1, N4, U4, NB4, UB4. Different letters within rows denote significant difference at p<0.05; * denotes significant difference within column for treatment pair at p<0.05 Fisher's protected LSD; NS: not significant.

Table 5.12 Nitrogen concentration (N%) at 157 days after full bloom of 'Hort16A' fruit treated with Benefit Kiwi®(+BK), no BK (-BK), and foliar-applied N as either urea or $KNO_3$ (1% w/v).

| Foliar treatment | Number of N sprays | | | ANOVA |
| | 0 | 3 | 7 | p-value |
| --- | --- | --- | --- | --- |
| -BK (control) | 0.82 (0.04)a | 0.89 (0.05)ab | 1.01 (0.06)b | 0.033 |
| +BK | 0.74 (0.03)a | 0.80 (0.04)b | 0.95 (0.05)c | 0.002 |
| | NS | NS | NS | |
| Urea | 0.78 (0.03)a | 0.92 (0.04)b | 1.07 (0.04)c | >.001 |
| $KNO_3$ | 0.78 (0.04) | 0.78 (0.05) | 0.89 (0.05) | NS |
| | NS | * | * | |

Standard error in parenthesis, n = 12. Reported range for 'Hort16A' at same time of season in Mills et al. 2008: 0.9% in unfertilised vines to 1.3% in vines given 145 kg N ha$^{-1}$ yr$^{-1}$. Treatments included Control, BK, N1, U1, NB1, UB1, N4, U4, NB4, UB4. Different letters within rows denote significant differences Fisher's protected LSD; * denotes significant difference within column for treatment pair at p<0.05; NS: not significant.

### 5.3.3 Seed nitrogen and phosphorus content

The foliar-applied N treatments had no significant effects on the concentration in the seeds of N or P measured on a DW basis (Table 5.6).

Table 5.13 Seed N% and P% of fruit given 0, 3, or 7 applications of foliar-applied N and harvested 157 days after full bloom.

| % of dry weight | Number of N sprays | | | |
| --- | --- | --- | --- | --- |
|  | 0 | 3 | 7 | ANOVA |
| N% | 2.39 (0.02) | 2.39 (0.01) | 2.40 (0.02) | NS |
| P% | 0.72 (0.01) | 0.70 (0.01) | 0.72 (0.01) | NS |

Standard error in parenthesis, n = 12, NS: not significant p>0.05. Pooled samples from Control, Benefit Kiwi® and both foliar nitrogen forms from the early series (N1, U1, NB1, UB1, N4, U4, NB4, and UB4).

### 5.3.4 Fruit growth

#### *5.3.4.1 Early season fruit growth 2009-10 season*

A trend for fruit FW and DW to be increased by BK and foliar-applied N was already apparent 55 DAFB (20 days after the third application of the foliar treatments) (Table 5.7). At this stage the FW of fruit treated only with foliar-applied N was 9% heavier than the control, while fruit treated only with BK® was 10% heavier, and fruit treated with both foliar-applied N and BK was 15% heavier than the control respectively. The trends for increased FW were more strongly established by the time of the second sampling 84 DAFB, particularly for fruit treated with seven foliar-N applications and with BK (Table 5.7). Fresh weight increases were highly significant compared to the control in the case of seven foliar-applied N applications (11% increase, p<0.003) and BK only (16.6% increase, p<0.001). Seven foliar-applied N applications in combination with BK also gave highly significant FW increases compared to BK only (7.4% increase, p<0.015). Similar trends were present for DW, although these were only significant when BK-treated fruit was compared to the control (p<0.001). Fruit DM% was reduced by both BK and foliar-applied N (Table 5.7). At 84 DAFB in non-BK treated fruit, the reduction was highly significant with seven applications of foliar-applied N compared to the control (p<0.001). Treatment with BK also reduced DM% compared to the control (p<0.001) but although DM% appeared slightly lower with foliar-applied N plus BK compared to BK alone, these differences were not statistically significant (Table 5.7).

Table 5.14 Effect of 0, 3, or 7 foliar-applied N applications plus or minus Benefit Kiwi® (BK) on fresh weight (FW), dry weight (DW), and dry matter concentration (DM%) of 'Hort16A' fruit at 55 and 84 days after full bloom (DAFB) in the 2009-10 season.

| +/-BK | Number of N sprays | | | p-value | Number of N sprays | | | p-value |
|---|---|---|---|---|---|---|---|---|
| | 0 | 3 | 7 | | 0 | 3 | 7 | |
| | -BK (control) | | | | +BK | | | |
| **55 DAFB** | | | | | | | | |
| FW (g) | 25.6 | 27.9 | | 0.060 | 28.0 | 29.4 | | >0.1 |
| se | 0.85 | 0.63 | | | 0.94 | 0.91 | | |
| DW (g) | 4.05 | 4.26 | | >0.1 | 4.22 | 4.38 | | >0.1 |
| se | 0.11 | 0.09 | | | 0.14 | 0.13 | | |
| DM% | 7.94 | 7.65 | | 0.057 | 7.54 | 7.46 | | >0.1 |
| se | 0.12 | 0.06 | | | 0.07 | 0.06 | | |
| **84 DAFB** | | | | | | | | |
| FW (g) | 57.5 | 61.1 | 64.7 | 0.003 | 67.0 | 68.0 | 72.4 | 0.015 |
| se | 1.54 | 1.09 | 1.37 | | 1.33 | 1.16 | 1.42 | |
| DW (g) | 6.24 | 6.38 | 6.54 | >0.1 | 7.07 | 7.13 | 7.48 | >0.1 |
| se | 0.13 | 0.18 | 0.17 | | 0.14 | 0.15 | 0.18 | |
| DM% | 10.90 | 10.42 | 10.10 | 0.001 | 10.56 | 10.48 | 10.32 | >0.1 |
| se | 0.20 | 0.17 | 0.14 | | 0.15 | 0.10 | 0.11 | |

Foliar treatments: data from combined urea and $KNO_3$ foliar treatments. se: standard error. n = 12, p-values from ANOVA.

### 5.3.4.2 Final harvest 2007-08 season

In the first season, fruit treated with Benefit Kiwi®alone (BK), with an average weight of 114 g, was 15 g heavier than fruit from the untreated control (p<0.05; Figure 5.1A). Dry matter per cent (DM%) was reduced in BK (16.2%) compared to the control (16.9%) (p<0.05). Nitrate sprays had no significant effect on fruit FW in the absence of BK, although N4 (seven $NO_3^-$ sprays between 20 and 80 DAFB) was 7 g heavier than the control. Within the series of treatments that included BK, fruit from N2+BK (three $NO_3^-$ sprays between 35 to 55 DAFB plus BK) was 15 g heavier than BK (p<0.05). A similar increase was found for N4+BK (seven sprays between 20 and 80 DAFB plus BK). Dry matter per cent was maintained within the treatment series not including BK relative to the control, and also within the series including BK relative to BK even when FW had been increased by the foliar-N treatments as in the case of N4, N2+BK, and N4+BK (values within individual bars are DM% of those fruit

whose average FW the bar displays; Figure 5.1A).The DM% of N3+BK (16.7%) was significantly increased relative to BK (p<0.05) (Figure 5.1A).
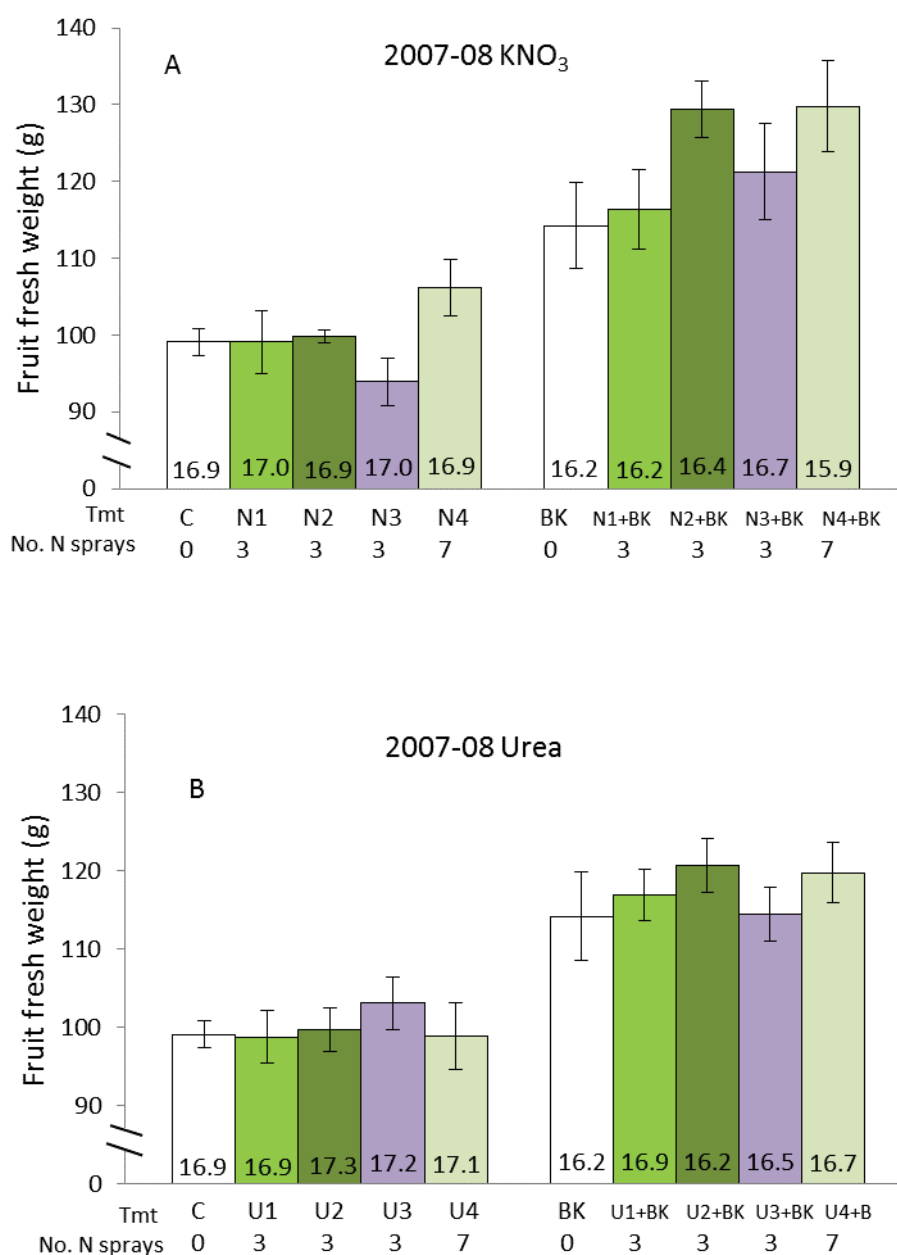


Figure 5.29 Effect of different number and timing of foliar sprays of $KNO_3$ (N1, N2, N3, N4; top figure, A) or urea (U1, U2, U3, U4; bottom figure, B), with Benefit Kiwi®(+BK) or without BK (five bars on left of each figure) on 'Hort16A' fruit fresh weight (FW) and dry matter concentration (DM%: values within each bar) at 162 days after full bloom (2007-08 season). Standard error bars, n = 6. C: control. See Table 5.2 for complete treatment definitions and colour code.

Foliar urea sprays without BK had no significant effect on fruit FW (Figure 5.1B). Foliar urea treatments combined with BK increased FW compared to B by up to 6.5 g (U2+BK), but these increases were not significant (Figure 5.1B). The DM% of U1+BK (16.9%) and U4+BK (16.7%) was significantly increased relative to BK ($p<0.05$) and was equal (U1+BK) or not significantly different (U4+BK) to the control (Figure 5.1B).

### 5.3.4.3 Final harvest 2009-10 season

In the vines used for the foliar-applied $KNO_3$ treatments, fruit from BK with an average weight of 100.1 g was 15 g heavier than the control (85.2 g) ($p<0.05$) (Figure 5.2A). Dry matter concentration was reduced from 16.1% to 15.5%, although this was not statistically significant ($p>0.05$). Within the treatments not including BK, N4 showed the largest response, being 10 g heavier than the control ($p<0.05$) and these fruit were not significantly smaller than BK (Figure 5.2A). Within the treatments not including BK but including three applications of $KNO_3$, N1 appeared the most effective giving an average 7 g increase in FW (Figure 5.2A). Fruit weight was also increased by $KNO_3$ sprays in BK-treated fruit, the largest response being for N4+BK ($p<0.05$), followed by N1+BK, N2+BK, and lastly by N3+BK (Figure 5.2A). Within the treatment series not including BK, the two largest FW responses, N1 and N4, had reduced DM% compared to the control, although this difference was not statistically significant ($p>0.05$) (Figure 5.2A). Within the BK treated series increases in FW were associated with smaller variations in DM%.
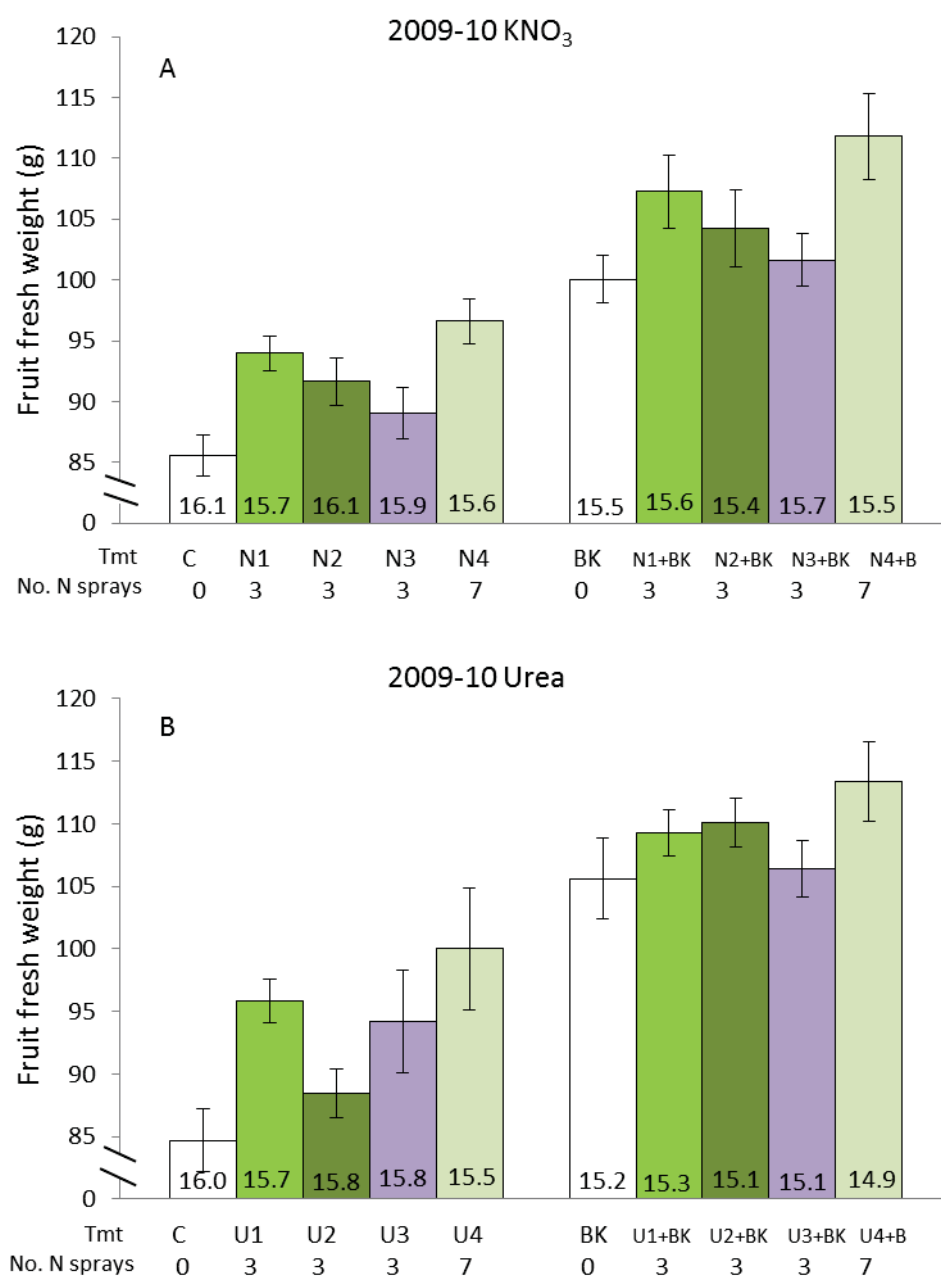
Figure 5.30 Effect of different numbers and timing of foliar sprays of KNO$_3$ (N1, N2, N3, N4; top figure, A) or urea (U1, U2, U3, U4; bottom figure, B), with Benefit Kiwi®(+BK) or without BK (five bars on left of each figure) on 'Hort16A' fruit fresh weight (FW) and dry matter concentration (DM%: values within each bar) at 157 days after full bloom (2009-10 season). Standard error bars, n = 6. C: control. See Table 5.2 for complete treatment definitions and colour code.

In the vines used for the urea treatments, fruit from BK, with an average weight of 105.6 g, was 21 g heavier than fruit from the control (84.7 g) (p<0.05) (Figure 5.2B). Dry matter concentration was reduced from 16.0% in the control to 15.2% in BK (p<0.05). There was generally a larger FW response to urea in the absence of BK and compared to the control, than when it was included and compared to BK. Within the treatments not including BK, the largest response was for U4 which was 15 g heavier than the control (p<0.05) (Figure 5.2B). This was followed by U1 (11 g heavier than the control: p<0.05) and U3 (9.5 g heavier than the control; p<0.05). In the foliar urea plus BK treatments were significantly heavier than BK. The largest response was for U4+BK, which was 7.7 g heavier than BK (Figure 5.2B). Although DM% was significantly reduced in the BK series compared the non-BK series, foliar-applied urea had little effect on dry matter concentration.

### 5.3.5 Seed weight

A trend was apparent for foliar-applied N and BK sprays to increase seed weight, although these differences lacked statistical significance (Table 5.8).

Table 5.15 Average weight (mg) of seeds from mature 'Hort16A' fruit treated with Benefit Kiwi®(+BK), no BK (-BK), and 0, 3, or 7 applications of foliar-applied N as either urea or $KNO_3$ (1% w/v) (fruit harvested 157 DAFB, 2009-10 season).

| Foliar treatment | Number of N sprays | | | |
| | 0 | 3 | 7 | ANOVA |
| --- | --- | --- | --- | --- |
| -BK (control) | 1.13 (0.02) | 1.17 (0.03) | 1.18 (0.01) | NS |
| +BK | 1.16 (0.02) | 1.19 (0.03) | 1.20 (0.04) | NS |
| Urea | 1.15 (0.01) | 1.17 (0.03) | 1.20 (0.03) | NS |
| $KNO_3$ | 1.15 (0.02) | 1.18 (0.03) | 1.18 (0.02) | NS |

Standard error in parenthesis, n=12, NS: not significant p>0.05. Pooled samples from Control, BK and both foliar N forms from the early series (N1, U1, NB1, UB1, N4, U4, NB4, and UB4).

## 5.3.6 Fruit carbohydrates

There was a significant reduction (p<0.05) in the concentration of soluble sugars as the number of foliar N applications was increased, in fruit sampled eight days after the last foliar N application (Figure 5.3). The same trend remained in mature fruit harvested in March although the differences were now much less and not statistically significant (Figure 5.3). Both forms of foliar-applied N with or without BK reduced soluble sugar content similarly (Table 5.9).
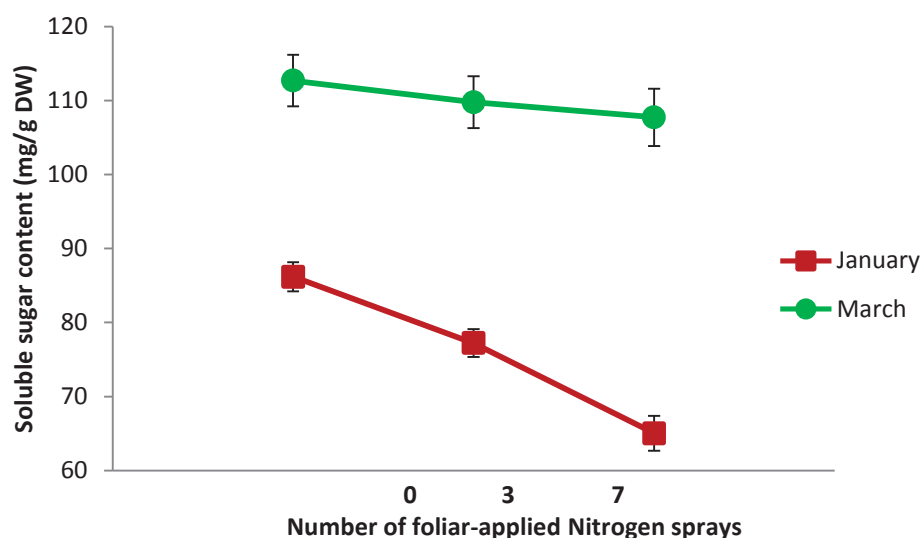


Figure 5.31 Soluble sugar content (mg/g dry weight) 84 days after full bloom (January) and 157 days after full bloom (March) in 2009/10 season of 'Hort16A' fruit treated with 0, 3, or 7 applications of foliar-applied N as either urea or $KNO_3$ (1% w/v). Standard error bars.

Table 5.16 Soluble sugar content (mg/g dry weight) of 'Hort16A' fruit treated with Benefit Kiwi® (+BK), no BK (-BK), and 0, 3, or 7 applications of foliar-applied N as either urea or KNO3 (1% w/v) 84 days after full bloom (2009-10 season).

| Foliar treatment | Number of N sprays | | | |
|---|---|---|---|---|
| | 0 | 3 | 7 | ANOVA |
| -BK (control) | 83.1 (2.2)a | 73.6 (2.0)b | 66.2 (3.6)b | <.001 |
| +BK | 89.6 (3.1)a | 80.8 (2.8)b | 63.9 (3.0)c | <.001 |
| Urea | 85.0 (3.1)a | 74.4 (2.5)b | 63.3 (4.1)c | <.001 |
| $KNO_3$ | 87.3 (3.1)a | 80.1 (3.2)a | 66.8 (3.5)b | <.001 |

Standard error in parenthesis, n=12. Different letters within rows denote significant differences, Fisher's protected LSD, p<0.05.

Table 5.17 Total soluble sugar contents (SSC), dry weight (DW), fresh weight (FW), and dry matter concentration (DM%) of 'Hort16A' fruit treated with 0, 3, or 7 applications of foliar-applied N (fruit sampled 84 days after full bloom) with relative differences (%) between 3 and 7 foliar-applied N applications and the unsprayed control (nil N sprays) (2009-10 season).

| No. sprays | SSC (mg) | DW (g) | FW (g) | DM% |
|---|---|---|---|---|
| 0 | 576.1 | 6.7 | 62.5 | 10.7 |
| 3 | 523.1 | 6.8 | 64.6 | 10.5 |
| 7 | 455.5 | 7.0 | 68.5 | 10.2 |
| p-value | <.001 | NS | 0.004 | 0.005 |
| Relative difference | | | | |
| 0-3 | -9.2% | 1.0% | 3.3% | -2.6% |
| 0-7 | -20.9% | 4.8% | 9.6% | -4.8% |

ANOVA p-value, n = 24.

The starch concentration of the fruit did not appear to be affected by the foliar-applied N treatments or by BK at either 84 DAFB (Table 5.11) or 157 DAFB (Table 5.12).

Table 5.18 Starch content (mg/g dry weight) of 'Hort16A' fruit 84 days after full bloom and treated with Benefit Kiwi®(+BK), no BK (-BK), and 0, 3, or 7 applications of foliar-applied N as either urea or $KNO_3$ (1% w/v) (2009-10 season).

| | Number of N sprays | | |
|---|---|---|---|
| Foliar treatment | 0 | 3 | 7 |
| -BK (control) | 194.8 (10.0) | 194.0 (6.5) | 192.6 (9.9) |
| +BK | 204.5 (11.2) | 198.4 (12.6) | 209.0 (6.2) |
| Urea | 198.4 (12.7) | 205.2 (12.3) | 198.5 (9.2) |
| $KNO_3$ | 201.0 (8.4) | 187.2 (4.4) | 203.2 (8.7) |

Standard error in parenthesis, n=6

Table 5.19 Starch content (mg/g DW) of 'Hort16A' fruit 157 days after full bloom and treated with Benefit Kiwi®(+BK), no BK (-BK), and 0, 3, or 7 applications of foliar-applied N as either urea or $KNO_3$ (1% w/v) (2009-10 season).

| | Number of N sprays | | |
|---|---|---|---|
| Foliar treatment | 0 | 3 | 7 |
| -BK (control) | 405.8 (2.0) | 411.3 (4.0) | 412.9 (3.9) |
| +BK | 412.4 (8.3) | 417.0 (4.9) | 416.1 (6.8) |
| Urea | 411.8 (8.3) | 412.4 (3.6) | 419.4 (5.3) |
| $KNO_3$ | 406.4 (2.5) | 415.8 (5.5) | 409.6 (5.0) |

Standard error in parenthesis, n=6.

## 5.4 Discussion

The low leaf and fruit N% of the vines is consistent with reduced use of N fertiliser on the orchard used for the trial. It also suggests that the stimulation of fruit growth obtained with foliar-applied N sprays was at least partly due to the alleviation of N deficiency. However, the leaf samples were taken towards the end of the season when significant re-translocation of N from leaves to the vine occurs, especially when the N supply is less than optimum (Cheng et al. 2002). Therefore, the level of N limitation during the period of fruit growth may have been less than the data at first suggests. Furthermore, historical production performance of the orchard used for the experiments had been good and above average for the region. For example in the 2011 season, the orchard produced 13,775 tray equivalents (te) per hectare and an average count size of 33.52, compared to regional averages of 10,935 te and 33.11 count size (data provided by the orchard manager). It is widely considered that responses to foliar-applied nutrients are limited to situations where a nutrient deficiency exists (Weinbaum et al. 2002; Mengel 2002). However, there are also reports of fruit growth being stimulated by foliar-applied N even in well-fertilised fruit trees (El-Otmani et al. 2002; Lovatt 1999). The maintenance of yield and fruit size in 'Hort 16A' when N fertiliser was reduced or withheld and leaf N% fell below the accepted industry 'normal' range was reported by Mills et al. (2009). Such ranges are based on the average or 'normal' range established from cumulative historical data. They are not based on empirical yield-N rate trials and therefore are not necessarily well correlated to yield or fruit size.

When the leaf and fruit N% values are used to calculate total N content, the estimated increase in N with the foliar treatments was proportional to the amount of N contained in the respective N form (urea or $KNO_3$) and number of applications (data not presented). This suggests efficient uptake of both N forms as has been reported elsewhere (Furuya and Umemiya 2002; Bowman and Paul 1992). During analysis of the fruit for N content, removing the skin made no difference to the results (data not presented). Therefore, the increases in N content of the foliar treated fruit were not caused by superficial residues from the foliar sprays. The relative importance of direct uptake through the fruit epidermis as opposed to uptake through leaves and subsequent translocation to the fruit remains to be determined. However, direct uptake rather than translocation might be indicated by the results of seed N content since seeds are directly connected to the vascular system of the fruit

(Beever and Hopkirk 1990) and might therefore be expected to show an elevated N content if foliar-applied N was being translocated to the fruit from the leaves. Fruit N% appeared lower in BK-treated fruit, which is consistent with a so-called 'growth dilution effect' (Mills et al. 2008b; Smith et al. 1987), since the absolute N content (g/fruit) remained the same.

Mills et al. (2008) found that although N levels of both fruit and leaves were lower in unfertilised 'Hort 16A' vines compared to fertilised ones, the leaves appeared to be less affected. From this it appears that in situations of reduced N supply shoots are prioritised or compete more strongly for the smaller pool of available N than fruit. Competition for N between shoots and fruit is likely to be particularly intense during early fruit development when both are growing rapidly. This evidence and reasoning provides a causative explanation for the significant increases in FW with foliar-applied N applied during the early stages of fruit development reported here.

Relatively large FW increases coincident with significant reductions of DM% were found in in all treatments that included BK. These effects are similar to those reported elsewhere for this biostimulant (Currie et al. 2005). Benefit Kiwi® is believed to contain compounds that act to increase or prolong cell division and expansion within the fruit and thereby increase the sink strength of the fruit (Woolley and Currie 2006). Sink strength generally refers only to the capacity of fruit to attract assimilates and not its capacity for water uptake (Ho 1988) even though water uptake is generally more important in terms of its contribution to increases in fruit size (Coombe 1976). Although BK increased the sink strength of the fruit, as indicated by an increase in average fruit DW compared to the control of between 8.7% in the 2007-08 season and 18.4% in the 2009-10 season, its main effect was on water uptake as indicated by the larger increase in FW of between 16% and 25% in the 2007-08 and 2009-10 seasons respectively. This suggests that BK increases cell expansion to a greater extent than it does cell division.

Although increases in FW obtained with foliar-applied N were smaller than those obtained with BK, DM% was generally maintained with foliar-applied N treatments. Given the important role of N in most aspects of plant growth it seems likely that foliar-applied N can stimulate a wider range of physiological processes both in the leaves and the fruit, and thus has the potential to strengthen source and sinks simultaneously. Foliar-applied N has been shown to increase leaf chlorophyll and photosynthesis in citrus and apple (Romero-Aranda

and Syvertsen 1996; Fisher et al. 1948). The hyperbolic relationship between leaf N% and rates of photosynthesis implies a greater effect at lower levels of leaf N (Fernandes and Rossiello 1995), such as existed in the vines in the present study (Table 5.3). Foliar-applied N applied during early fruit development might also increase or prolong hormonally regulated processes such as cell division and expansion in the fruit. Lovatt (1999) reported that foliar urea increased the rate and duration of cell division by stimulating polyamine biosynthesis, which is known to have a role in cell division. Nitrate can also act in a regulatory role, influencing membrane permeability and water uptake, as well as stimulating ABA and cytokinin biosynthesis (Guo et al. 2007; Sakakibara et al. 2006; Peuke and Dieter 1998). Thus both N forms have the potential to act as metabolic regulators as well as being nutrient substrates.

Both foliar $KNO_3$ and foliar urea applied during early fruit development were shown to have similar potential for increasing fruit size despite the different amounts of N they contain. This suggests the stimulation of fruit growth by $KNO_3$ was not only due to an alleviation of N deficiency, since about one third as much N was supplied and taken up from the $KNO_3$ treatments than from the equivalent treatments using urea. This might be due to the additional effects of potassium. Potassium nitrate is used to supply potassium to many crops but it is generally difficult to separate the effects of either $K^+$ or $NO_3^-$ (Howard et al. 2000).

In both seasons, the response to foliar-applied N appeared to vary according to the developmental stage of the fruit. In the 2007-08 season, fruit was most responsive to both forms of N applied between 40 and 60 DAFB, whereas in the 2009-10 season the greatest response was to N applications made between 15 and 35 DAFB. This variability between the two seasons might be explained by interrupted and prolonged flowering due to a sudden cooling, in 2009-10 season, which complicated an accurate estimation of full bloom. Therefore, the most effective time for foliar-applied N application is probably between 20 and 60 DAFB. There were several instances when the response to three foliar-applied N sprays during this period was similar to seven sprays over the whole treatment period. This might be because temporary deficits of N affecting fruit growth are most intense at this stage. This might be related to the simultaneously occurring change in dependence on endogenous N reserves to dependence on exogenous sources of N, and when competition from rapidly growing shoots and fruit is most intense (Weinbaum et al. 2002). Alternatively, it could be because the permeability of the fruit epidermis is substantially reduced after about 60 DAFB,

which is likely to also reduce the uptake of foliar sprays (Xiloyannis et al. 2001; Smith et al. 1995; Haynes and Goh 1977). Either way, it supports the theory that the timing of supplemental N applications can be more important than the total amount of N applied or plant N status (Lovatt 1999).

The reduction in the concentration and total amount of soluble sugars in the fruit (Figure 5.3; Table 5.10) may reflect the diversion of carbon from non-structural carbohydrates to protein and amino compounds. A similar reduction in soluble sugars and non-structural carbohydrates following foliar urea application was found in grapevines (Xia and Cheng 2004). Approximately 60% of the carbon reduction in non-structural carbohydrates was found in proteins and amino acids reflecting the increased demand for carbohydrates for carbon skeletons and energy associated with N assimilation (Xia and Cheng 2004). The reduction in soluble sugars does not appear to be due to a reduction in assimilate supply since fruit DW was increased by foliar-applied N (Table 5.10). Although phytotoxic effects due to foliar-applied N formulations can reduce photosynthesis, such effects at the conservative concentrations used in the present study are likely to be transitory, persisting for less than 24 hours after application (Orbovic et al. 2001). Reduced soluble sugars could also have been due to an increased rate of starch synthesis, although the results of starch analysis did not support this. However, it remains a possibility since the low accuracy of the starch analysis did not allow any definitive conclusion to be made. The low accuracy was due to difficulties involved in the multi-step three enzyme process (Rose et al. 1991). An increase in FW with increasing number of foliar-applied N sprays (nearly 10% for seven N spray applications in the combined data (Table 5.10, Figures 5.1 and 5.2) despite the reduction in soluble sugars is consistent with organic acids being more important than soluble sugars in kiwifruit for maintaining osmotic pressures necessary for rapid fruit growth especially during the early stages (Nardozza et al. 2010). Another possible adverse effect of foliar-applied nitrogen on 'Hort16A' might involve the masking of the appealing yellow pigmentation of the fruit by delayed chlorophyll degradation. However, this seems unlikely since increases in fruit N% affected by foliar-applied N were relatively small. Mills et al. (2009) found the yellow colouration of 'Hort 16A' was only slightly affected when fruit N% at harvest was above about 1.4%.

## 5.5 Summary and conclusions

The results support the use of foliar-applied nitrogen during early fruit development to simultaneously increase fresh and dry weight of 'Hort 16A' fruit, allowing significant fruit size increases with the maintenance of dry matter concentration. Despite some inconsistency in effects between seasons, both the urea and potassium nitrate forms of foliar-applied nitrogen show similar potential for increasing fruit size either in conjunction with the proprietary biostimulant Benefit Kiwi® or on their own. However, more research is needed to better define the optimum timing and number of applications. It seems unlikely that foliar-applied nitrogen would adversely affect the appealing yellow pigmentation of the fruit by delaying chlorophyll degradation since increases in fruit N% affected by foliar-applied N were relatively small. More work is needed to understand the mechanisms of the effects of foliar-applied N on fruit growth, especially the effects on fruit soluble sugars and starch, and the effects of foliar-applied N in vines of differing N status. Apart from being able to increase fruit size, foliar-applied N is also a more efficient method for the management of N inputs. Improved efficiency of fertiliser use can help improve the environmental sustainability of kiwifruit orchards.

# Contents

## List of Tables

# List of Figures

# 6. Response of 'Hayward' kiwifruit to foliar applications of nitrogen, CPPU, and seaweed

## 6.1 Introduction

Kiwifruit (*Actinidia deliciosa*) is a vigorous deciduous vine and the green-fleshed fruit of the 'Hayward' cultivar is a major New Zealand export product. Industry profitability relies on high levels of productivity and the maintenance of fruit quality. High yields represent a significant annual removal of nutrients from orchard soils; and the nutrient demand for annual renewal of the kiwifruit canopy is also high (Buwalda and Smith 1987). Thus relatively high fertiliser inputs to supply these demands have been common in New Zealand kiwifruit orchards (Anon 2005). For example, nitrogen inputs of over 200 kg N ha$^{-1}$ have historically been recommended for kiwifruit orchards (Buwalda et al. 1990; Sale 1997). However, high N inputs can induce excessive vegetative vigour, which is associated with reduced fruit quality and increased labour costs for canopy management (Snelgar et al. 2012; Mills et al. 2008). This along with increasing concern about the adverse environmental effects of N leaching means that the need to optimise N inputs and improve the nutrient use efficiency (NUE) of kiwifruit orchards is now widely accepted.

Foliar nutrient application has significant advantages in terms of NUE with higher proportions of the applied nutrient being recovered by the crop compared to soil-applied nutrients (Weinbaum et al. 2002). Foliar-applied N sprays can reduce the risk of $NO_3^-$ leaching and control vegetative vigour compared to soil-applied N (Dong et al. 2005). Late season foliar application of N in deciduous fruit crops has been shown to benefit fruit growth in the following spring by increasing tree reserves used to support early season growth (Johnson et al. 2001). Foliar nutrition allows timely applications to meet temporary deficits at particular stages during crop development; early season foliar application of N has been reported to increase fruit size of apples (Dong et al. 2005), citrus (Lovatt 1999), and guava (Kundu et al. 2007). Such a temporary N deficit might occur in kiwifruit during early fruit development stages when fruit is rapidly growing and must compete with shoots for nitrogen. This temporary deficit might be magnified when N inputs are reduced to manage vigour or to meet sustainability goals.

Larger sized fruit are generally more valuable and fruit size is a major component of yield. Biostimulants and plant growth regulators can increase the size of fruit especially when applied during early fruit growth stages when processes such as cell division and expansion are most active (Famiani et al. 1997a,b; Shargal et al. 2006). Apart from size, fruit dry matter concentration (DM%) is a key fruit quality parameter because it correlates positively with the taste and consumer liking for the ripe kiwifruit (Patterson and Currie 2011). Growers receive an incentive payment for fruit with high DM% (Patterson and Currie 2011). Significant increases in fresh weight (FW) will almost certainly be mainly due to increased water uptake by the fruit since water typically accounts for between 80 and 85% of the FW of a kiwifruit. However, unless dry matter accumulation by the fruit is simultaneously increased by a similar proportion, DM% of the fruit will be decreased. This effect has emerged as a problem associated with the use of biostimulants and plant growth regulators aiming to increase fruit size in kiwifruit (Patterson and Currie 2011).

There has been limited research regarding the use of foliar N in kiwifruit. Two trials on 'Hayward' have been published, the first with a commercial foliar fertiliser product containing N applied ten times during mid and late season and the second using 4% urea foliar-applied twice postharvest. Both of these trials failed to find any beneficial effects resulting from the N treatments (Mulligan 2007; Boyd et al. 2007). The present work was undertaken to ascertain whether four consecutively applied early season foliar N applications could stimulate fruit growth of 'Hayward' kiwifruit and if any FW increases would be accompanied by proportional increases in DW thus maintaining fruit DM%. Two common forms of N used for foliar application, 1% $KNO_3$ and 1% urea were applied over two seasons. Although different amounts of N were supplied by the two N forms, a 1% aqueous solution is commonly reported as being the maximum safe rate for pre-harvest foliar use of both $KNO_3$ and urea (Weinbaum, 1978; Lea-Cox and Syvertsen 1995). Furthermore $KNO_3$ and urea have a comparable salt index (73.6 vs 75.4 units, respectively) so in using a 1% solution for both N forms all leaves and fruitlets were treated with solutions of comparable osmotic concentration (Lea-Cox and Syvertsen 1995).

A similar trial on the gold-fleshed cultivar 'Hort16A' kiwifruit (A. *chinensis*) found both urea and $KNO_3$ provided significant increases in FW without reducing DM% (Chapter 5). In the

experiments with 'Hort16A' the proprietary biostimulant Benefit Kiwi® (Valagro SpA, Atessa, Italy) was added as a factor to gauge the effect of foliar N under conditions where fruit growth has already been stimulated. In both seasons an additive effect was apparent with the combination of foliar-applied N and biostimulant although the effects were at times inconsistent (Chapter 5, Figures 5.1 and 5.2). In 'Hayward' it has been found that CPPU, a synthetic-cytokinin active compound, has a greater effect in stimulating fruit growth than Benefit Kiwi® (Woolley and Currie 2006). Therefore, this was used as a factor to stimulate fruit growth in one season of the work with 'Hayward' described here. In this season the foliar treatments were combined with high or low (nil) rates of soil-applied $NO_3^-$ so that nutritional effects could be differentiated from other physiological effects of foliar N application on fruit growth.

Foliar urea was also applied alone or combined with a commercial seaweed extract (SM6 Seaweed Extract© 2010 Chase Organics (GB) Limited) during the same early period of fruit growth. Seaweed contains active cytokinins and can have biostimulatory properties when used as a foliar treatment (Jameson 1993; Chouliaras et al. 1997; Norrie and Keathley 2006). The purpose of this experiment was to determine whether possible stimulatory properties of the seaweed extract on 'Hayward' would be enhanced by the simultaneous application of supplemental N in the form of urea.

Specific experimental questions were:

1. Can foliar N applied during early fruit development increase fruit FW of 'Hayward'?
2. Are the effects of urea and $KNO_3$ applied at the same concentration similar?
3. Are the responses to foliar-applied N in vines given high rates of soil-applied N fertiliser different than in vines given no soil applications of nitrogen?
4. How do these effects change in fruit also treated with CPPU and, in the case of urea, also treated with a seaweed extract?

## 6.2 Materials and methods

### 6.2.1 Experiment 1 (2009-10 season)

Twelve pairs of mature 'Hayward' vines in the Massey University orchard growing on a T-bar training system were selected from two non-adjacent rows (Row 1 and 2). Each pair of treatment vines (a bay) was separated by a bay containing two female vines on either side of a single male vine (Chapter 2, Figure 2.1). Vine spacing was 2.5 m × 5 m. Calcium nitrate was broadcast onto the soil surface above the main root zone (approximately 25 m$^2$/bay) of six randomly selected bays at a rate equivalent to 50 kg N ha$^{-1}$ every three weeks from fruit set until one month before harvest. The remaining six bays received no N fertiliser but calcium sulphate was applied to supply equivalent quantities of Ca. No soil-applied fertiliser was applied during the previous three seasons. The vines receiving N fertiliser were designated 'HN' while those receiving no N fertiliser were designated 'LN'. Soil type is a Manawatu fine sandy loam (Cowie and Rijkse 1977; Green and Clothier 1995; Table 6.1). Full bloom was estimated as being 4th December 2009.

Table 6.1 Soil pH, mineral N, plant available phosphorus (Olsen P), exchangeable cations (Ca, Mg, K, and Na), cation exchange capacity (CEC), and bulk density (BD) pre-bud break for the Massey orchard (0-10cm depth) (Experiment 1) and the Hawkes Bay orchard (0-7.5cm depth) (Experiment 2).

| Season | | pH | Mineral - N (µg/g) | Olsen P (µg/g) | K (me/100g) | Ca (me/100g) | Mg (me/100g) | CEC (me/100g) | BD (g/ml) |
|---|---|---|---|---|---|---|---|---|---|
| 2009-10 (expt 1) | HN | 6.3 | 15.3 | 45 | 1.11 | 10.6 | 1.64 | 15.3 | 0.91 |
| | LN | 6.3 | 9.7 | 44 | 0.98 | 11.5 | 1.95 | 17.3 | 0.91 |
| 2010-11 (expt 2) | | 6.6 | | 57 | 0.82 | 16.2 | 2.93 | 23.0 | 0.82 |

A randomly selected group of six adjacent canes on each vine was treated with four consecutive foliar sprays (1% w/v) of KNO$_3$ (Yara Krista-K Plus, Yara International Ltd, Norway) or low-biuret urea (Yara Urea Tech, Yara Fertilizers NZ Ltd; biuret content 0.65-0.80%) at seven day intervals from 20 days after full bloom (DAFB) (Table 6.2; Appendix 2, Plate 5). This was the first series designated N1. The same foliar N treatments were repeated in a later sequence commencing 41 DAFB and was designated N2. The remaining canes on each vine were used as a control. One side of the canopy of each bay was randomly allocated to receive a single foliar application of N-(2-chloro-4-pyridyl)-N'-phenylurea (CPPU; 20 mg

$L^{-1}$) at 20 DAFB. Spray solutions were applied with 15L back-pack spray units (Croplands Swissmex) with a separate spray unit being used for the CPPU treatment.

Table 6.21 Periodicity of foliar N applications and CPPU application for Experiment 1.

| Date | Application days after full bloom | | |
| --- | --- | --- | --- |
| | CPPU | N1 | N2 |
| 24/12/09 | 20 | 20 | |
| 5/01/10 | | 32 | |
| 14/01/10 | | 41 | 41 |
| 25/01/10 | | 52 | 52 |
| 2/02/10 | | | 60 |
| 9/02/10 | | | 66 |

Because the use of surfactants introduces another variable with potential for direct physiological effects independent of the main spray ingredient (Orbovic et al. 2001) no surfactants were added to the foliar treatments in the 2009-10 season. This decision was also supported by the consideration that urea has some surfactant properties by itself (Yamada et al., 1965) and also that surfactants can increase runoff and therefore can actually reduce the amount of urea retained on the leaf (Leece and Dirou 1979).

Twelve fruit from each treatment on each vine were harvested from the fruiting zone of the canopy (as described in Thorp et al. 2003) on the 23/02/10, 81 days after full bloom (DAFB) and 21 days after the last foliar spray and again on 18/05/10 (165 DAFB) when fruit was physiologically mature. At the early harvest time fruit was sampled from long shoots but at the final harvest fruit from short shoots was taken. Fruit was sorted into four equal numbered sets of ascending size and weighed as a set.

Leaf samples were taken on 10/02/11, 21 days after an application of $Ca(NO_3)_2$ fertiliser to the HN vines. The samples consisted of 20 youngest fully-expanded leaves (lamina plus petiole) from non-fruiting shoots and were taken from each vine. Leaves were thoroughly rinsed with distilled water before being dried. Leaf N% was measured in duplicate extractions by Kjeldahl digestion followed by N analysis on an auto analyser (Blakemore et al. 1987). For petiole $NO_3^-$, 12 -14 leaves from each vine in Row 1 were collected at midday on 23/02/10 (five days after an application of $Ca(NO_3)_2$ fertiliser to the HN vines) and the leaves

from each pair of vines were pooled to give one sample per bay. The petioles were detached from the laminar and immediately frozen. Sap was then squeezed from the thawed petioles and diluted with clean water as necessary to enable measurement with Reflectoquant® nitrate test strips read with a meter (full method given in Chapter 2, section 2.6). Fruit N% was obtained from lyophilized whole fruit tissue collected on 23/02/10 and analysed as for the leaves.

All urea foliar treatments were made to Row 2 and nitrate to Row 1. This prevented a direct comparison of the effects of foliar urea and nitrate. One HN bay at the end of Row 2 was omitted from the data analysis because both vines showed symptoms of decline due to a suspected root disorder. This omission reduced the replicates for the HN treatments from 12 to 10.

### 6.2.2 Pilot study (2009-10 season)

During the same season and in the same orchard a further set of six vines was selected and three treatments were applied to a randomly selected group of six adjacent canes on each vine. These treatments were: $KNO_3$ (1% w/v), $K_2SO_4$ (1% w/v), and a water-only control. The treatments were applied at the same time and intervals as described above for the N1 series. The purpose of this experiment was to compare the effects of $K^+$ and $NO_3^-$ since both ions might be physiologically active and play a part in any observed effects of the $KNO_3$ foliar treatments.

### 6.2.3 Experiment 2 (2010-11 season)

In the 2010-11 season the experiment outlined above was repeated with some modifications to a set of six mature 'Hayward' vines growing on a pergola system in a commercial kiwifruit orchard in Hawkes Bay. Vine spacing was $6 \times 4$ m and the soil type was a Hastings silt loam (Table 6.1). The vine canopies were divided into four quadrants and four treatments were allocated randomly to each quadrant. Full bloom was estimated as being 25/11/10.

An acidifying-surfactant agent (Spray-aide®; active ingredient: 780 g/L alkylaryl polyoxyethylene glycol phosphate ester; Miller Chemical and Fertilizer Corporation Hanover, Pennsylvania, 17331, USA.) was added to the foliar N solutions at the rate of 88 mg/L. Distilled water was used for the control and the N solutions. The decision to add an

acidifying surfactant (Sprayaide™) to both foliar solutions in the 2010-11 season was due to the discovery that the pH of the standard $KNO_3$ spray solution as used in the previous season was very high (pH 9.41) due to impurities in the hydroponic grade $KNO_3$ used. Other research has also found increased uptake of N from acidic foliar N spray solutions (El-Otmani et al. 2002; Achilea et al. 2002).

The foliar N treatments were:

Urea 1% (Yara Urea Tech, Yara Fertilizers NZ Ltd; biuret content 0.65-0.80%; pH initial 7.56, buffered 3.98)

$KNO_3$ 0.5% (Yara Krista-K Plus, Yara International Ltd, Norway; pH initial 8.20, buffered 5.1-5.3)

$KNO_3$ 1% (pH initial 9.41, buffered 6.25-6.28)

Water only control (pH 6.87-6.91)

Table 6.22 Periodicity of application of foliar N treatments to vines during the 2010-11 season (Experiment 2).

| Treatment application number | Date | DAFB |
|---|---|---|
| 1 | 6/12/2010 | 11 |
| 2 | 16/12/2010 | 21 |
| 3 | 27/12/2010 | 33 |
| 4 | 3/01/2010 | 40 |

Four sprays were applied commencing at 10 DAFB, with subsequent applications at 21, 33, and 40 DAFB. Due to some leaf damage following the third application of urea (later identified as being due to very low pH of the spray solution), the concentration of the fourth application of urea was reduced to 0.5%. Fruit and leaf samples were taken at 55 DAFB (19/01/11), 15 days after the last foliar application and again when fruit reached commercial maturity at 154 DAFB (1/05/11). Leaves were sampled from each vine and foliar treatment and processed for N content as described in section 6.2.1. Sixteen fruit from each of three shoot types (long shoots >90 cm; short shoots <50 cm; and pruned shoots, i.e., zero leafed) from each treatment on each vine were collected from the inner fruiting zone of the canopy (about 100 cm from the main leader).

Apart from the application of the foliar treatments the vines were managed inclusively with the rest of the commercial orchard by the orchardist; the experimental vines received the standard spray, fertiliser, and canopy management programs in place in this orchard. Fertiliser inputs for the season of the trial included 108 kg N ha$^{-1}$ applied in spring as calcium ammonium nitrate. The orchard management included zero-leafing in February. Zero-leafing is a pruning technique where originally long shoots are shortened back to the most distal fruit ('zero leafing'), designed to reduce competition from the canopy for assimilates to the benefit of the fruit (Patterson et al. 2009).

### 6.2.4 Experiment 3 (2009-10 season)

Eight mature 'Hayward' kiwifruit vines in the Massey University experimental orchard were used for a series of foliar seaweed extract and 1% urea sprays during the 2009-10 season. The vines were in pairs in bays. One vine in each bay was sprayed with seaweed and a group of canes on each vine was also sprayed with a 1% solution of low biuret urea. Four applications were made starting from 14 DAFB (full bloom was estimated as being on 4 December 2009) (Table 6.4). The vines had had no recent history of fertiliser inputs and none during the previous three seasons (further details of the orchard and its management history are given in Chapter 2).

Table 6.23 Application program for treatments used in Experiment 3.

| Application number | Date | DAFB | Spray solution composition |
| --- | --- | --- | --- |
| 1 | 19/12/09 | 14 | Seaweed SM6 0.5%, urea 1% |
| 2 | 28/12/09 | 24 | Seaweed SM6 1.0%, urea 1% |
| 3 | 5/01/10 | 32 | Seaweed SM6 1.0%, urea 1% |
| 4 | 14/01/10 | 41 | Seaweed SM6 1.0%, urea 1% |
| 5 | 22/01/10 | 49 | Seaweed SM6 1.0%, urea 1% |

### 6.2.5 Calculation of the amount of N delivered directly to the surface of each fruit in the foliar treatments

Sixteen fruit ranging in size from 30 to 72 g to cover the size range representative of fruit during the time the foliar treatments were applied, were carefully weighed, dipped in water, drained, and then re-weighed. The difference in weight was used to estimate the amount of N

spray likely to be retained on the fruit surface and potentially available to be absorbed into the fruit.

## 6.2.6 Fruit quality parameters

At each harvest fruit was sorted into sets of four similar sized fruit and weighed together. One fruit from each group was selected for measurement of soluble solids content and firmness. Firmness was measured, after removing a thin layer of skin with a sharp craft knife on two opposite sides of the fruit, with a Effegi Fruit Pressure Tester model FT 327 fitted with a 7.9 mm diameter tip. An Atago Hand Refractometer model N-20 was used to measure soluble sugar content in °Brix of juice manually expressed from caps cut from each end of the fruit with the craft knife. Two equatorial slices from each fruit were then taken. One slice was immediately put in a small plastic ziplock bag, weighed, and frozen at -20°C for later chemical analysis. The other slice was placed in a paper bag, weighed, and forced draft oven-dried to a constant weight at between 75 and 80°C for DW and DM% calculation.

## 6.2.7 Statistical analysis

The experiments were treated as a completely randomised block design with vines as blocks and internal replication with the exception of the two foliar N forms in the 2009-10 season, which being applied to separate rows could not be compared statistically. However, the two rows were combined for comparing the main effects of foliar N and soil-applied N. Statistical analysis used the GLM procedure of SAS (SAS Institute Inc., 2004) with treatment means compared using Fisher's Protected Least Significant Difference. Where appropriate means were compared with two-tailed T-tests in Excel.

## 6.3 Results and discussion

In this section, the results of three experiments are presented and discussed separately in subsections dedicated to each experiment. In the first subsection are the results of Experiment 1 in which foliar-applied N was combined factorially with CPPU and high rates of soil-applied N. This experiment was conducted during the 2009-10 season using vines in the Massey University experimental orchard and is partly a continuation of the fertiliser experiment reported in Chapter 3 (Year 4). Some minimal repetition of the results reported in Chapter 3 for Year 4 was therefore deemed unavoidable. A small pilot study to compare the effects of $KNO_3$ and $K_2SO_4$, is included at the end of the first subsection. The second subsection deals with Experiment 2, in which foliar N in two forms and at different rates was applied to vines as a series of foliar sprays during early fruit development in a commercial orchard in Hawkes Bay during the 2010-11 season. The third subsection returns to the 2009-10 season and the Massey University experimental orchard to give the results of another factorial experiment with foliar-applied urea and a commercial seaweed extract applied during early fruit development. Effects of the treatments on fruit growth are presented along with some biochemical and physical measurements.

### 6.3.1 Experiment 1

#### *6.3.1.1 Leaf and fruit nitrogen*

Leaf N% in the HN vines was within the reported optimum range for kiwifruit in January of between 2.2 and 3.0% (Hill 2010) but was below this range in the LN vines (Table 6.5). However, it is unlikely that the LN vines were significantly N-deficient since their leaves retained a healthy green colour throughout the season and deficiency symptoms do not generally appear in *A. deliciosa* until leaf N% falls below 1.5% (Smith et al. 1985). Petiole $NO_3^-$ ranged between 2575 to 5540 ug $g^{-1}$ in the HN vines and 15 to 41 ug $g^{-1}$ in the LN vines (Table 6.5). Petiole N is a reliable indicator of current N uptake (Prasad and Ravenwood 1986) and the extremely large range between LN and HN vines confirms that the experimental treatments were effective in establishing a contrasting level of $NO_3^-$ uptake.

Table 6.24 Mid-February (81 days after full bloom) leaf and fruit N% and petiole $NO_3^-$ ($\mu g\ g^{-1}$) of 'Hayward' vines given four sprays of urea or $KNO_3$ (1% w/v) at approximately 10 day intervals between 20 and 52 days after full bloom and receiving high (HN) or low (LN) rates of soil-applied N fertiliser (Experiment 1).

| | HN | LN | Difference (%) | Significance | n |
|---|---|---|---|---|---|
| Leaf N% | 2.56 (0.07) | 1.89 (0.05) | 35.4 | p<0.01 | 12 |
| Petiole $NO_3^-$ ($\mu g\ g^{-1}$) | 3615 (46*) | 30 (45*) | | p<0.001 | 12 |
| Fruit N% | | | | | |
| Row 1 | | | | | |
| Control | 1.41 (0.04) | 1.08 (0.05) | 30.4 | p<0.006 | 3 |
| Foliar $KNO_3$ | 1.49 (0.06) | 1.06 (0.04) | 40.6 | p<0.004 | 3 |
| Row 2 | | | | | |
| Control | 1.49 (0.10) | 1.03 (0.03) | 44.8 | p<0.05 | 3 |
| Foliar Urea | 1.65 (0.05) | 1.28 (0.10) | 28.9 | p<0.05 | 3 |

Standard error in parenthesis, * coefficient of variation; p-values from two-tailed T-tests.

Average N% of control fruit from HN vines in mid-February was between 30% and 45% higher than that of LN vines in each of the two rows respectively (Table 6.5). Nevertheless, fruit from both treatments were within the range reported for 'Hayward' at the same time of the season (Smith et al. 1991; Clark et al. 2004). Neither forms of foliar-applied N significantly increased fruit N% as measured one month after the last foliar application (Table 6.5). However, foliar urea appeared to have a larger effect than foliar $KNO_3$ in both HN and LN vines, consistent with the approximate three times greater amount of N supplied with urea than $KNO_3$ (Table 6.5). The measured increase in total N content of fruit treated with foliar N averaged 15.7 mg and 7.7 mg N per fruit for urea and $KNO_3$ respectively, which is well within the potential N increase due to direct fruit absorption, as shown in Table 6.6 and represents approximately 15.5 and 7.5% of total fruit N at the time of sampling for urea and $KNO_3$ respectively.

Table 6.25 Estimated volume of spray (ml) and weight of N (mg) reaching fruit in single or four foliar applications of urea or $KNO_3$ (1% w/v).

| Number of foliar applications | Vol. spray reaching fruit (ml) | Urea (mg N) | $KNO_3$ (mg N) |
|---|---|---|---|
| 1 | 1.78 | 8.21 | 2.50 |
| Standard deviation | 0.36 | 0.50 | 1.63 |
| 4 | 7.12 | 32.83 | 9.99 |

n = 16

### 6.3.1.2 Effect of soil-applied N on vegetative growth

The weight of prunings removed during summer pruning was used as a measure of vegetative vigour (Burge et al. 1987). The weight of prunings removed from HN vines in Row 1 at 13 DAFB was more than double the weight removed from LN vines. One month later when Row 2 was similarly pruned the difference between HN and LN vines in the weight of prunings was even greater (Figure 6.1). This large increase in vegetative vigour was also found in other experiments with soil-applied N (Chapter 3, Figure 3.2; Chapter 7, Section 7.2). The increase in vigour may be related to increased water and $NO_3^-$ uptake in N fertilised vines, these two being key drivers of cell expansion and shoot growth (McIntyre 1997).
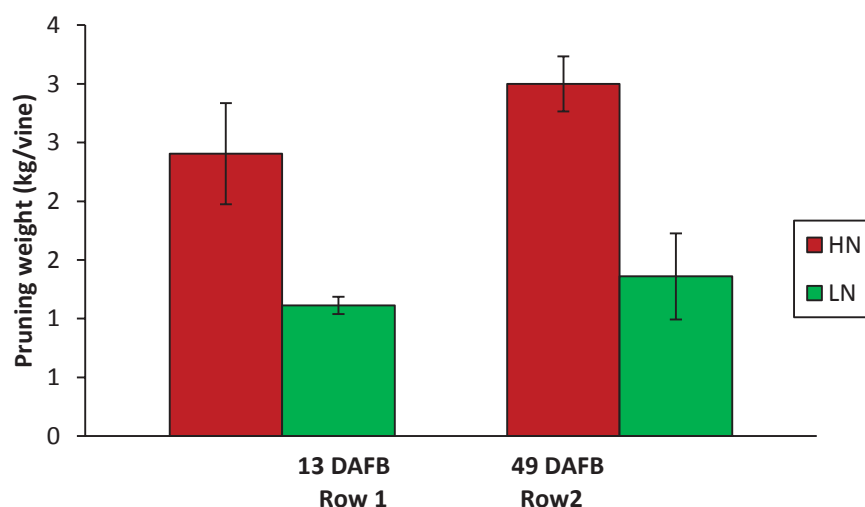


Figure 6.32 Effect of high (HN) or nil (LN) rates of soil-applied N fertiliser on the weight of shoots removed during summer pruning of two different rows of 'Hayward' vines at two times. n= 8 (Row 1), n =12 (Row 2). DAFB = days after full bloom.

### 6.3.1.3 Effect of soil-applied N and CPPU on fruit growth

At the early harvest 81 DAFB, FW of HN fruit was about 6 g (7.6%) heavier than LN fruit (p=0.02; Table 6.7). Dry matter (i.e., the dry weight, DW) and water uptake (the difference between FW and DW) increased in HN fruit in similar proportions (9.7 and 7.6%

216

respectively). This suggests that the sink strength of the fruit has been increased by the high $NO_3^-$ supply allowing fruit to attract more assimilate from the shoots, even although the vigour and competitive capacity of the shoots is particularly strong at this time of the season (Figure 6.1). CPPU increased fruit FW at early harvest by between 40 and 50% in HN and LN vines respectively (Table 6.7). Similar increases in DW were found with CPPU treatment, and as was found for FW, the largest responses were by LN fruit. The larger response by LN fruit to CPPU meant that there were no longer significant differences in FW or DW between HN and LN (Table 6.7).

In CPPU treated fruit both HN and LN fruit were stimulated with apparently no additive effect from the high $NO_3^-$ supply. This suggests that the response to soil-applied $NO_3^-$ was not because N was nutritionally limiting. If N was limiting fruit growth there might have been a bigger response to CPPU in HN fruit. The results are consistent with the effects of $NO_3^-$ on fruit during early fruit development being physiologically similar in some respects to those of CPPU. 'Hayward' fruit growth responds to cytokinins, which explains the strong response to CPPU, a cytokinin-like compound (Cruz-Castillo et al. 2002). Nitrate has strong interactions with cytokinins and induces an increase in cytokinin synthesis and transport within the plant (Sakakibara et al. 2006). The difference in the size of the response to CPPU and $NO_3^-$ might be because $NO_3^-$ simultaneously increases the strength of a major competing sink (i.e., the shoots), whereas CPPU does not (Famiani et al. 1997b).

Table 6.26 Effects of high (HN) or nil (LN) rates of soil-applied N fertiliser and treatment with CPPU or no CPPU (Control) on 'Hayward' fruit fresh weight (FW), dry weight (DW), water (W), and dry matter concentration (DM%) at early harvest (EH, 81 days after full bloom) and final harvest (FH, 165 days after full bloom).

|  | FW (g) | | DW (g) | | W (g) | | DM% | |
|---|---|---|---|---|---|---|---|---|
|  | HN | LN | HN | LN | HN | LN | HN | LN |
| EH |  |  |  |  |  |  |  |  |
| Control | 76.8a | 70.9b | 8.5a | 7.7b | 68.4a | 63.2b | 11.0a | 10.8a |
| CPPU | 107.6c | 106.6c | 11.1c | 11.1c | 96.5c | 95.6c | 10.3b | 10.4b |
| %diff | 40.2 | 50.4 | 31.1 | 44.3 | 41.0 | 51.1 | -6.2 | -3.9 |
| FH |  |  |  |  |  |  |  |  |
| Control | 100.3a | 93.6b | 15.1a | 15.0a | 85.2a | 79.1b | 15.0a | 16.0b |
| CPPU | 139.8c | 134.8c | 18.7b | 19.6b | 121.0c | 115.3c | 13.4c | 14.5d |
| %diff | 39.3 | 44.0 | 23.8 | 30.0 | 42.1 | 45.8 | -10.8 | -9.5 |

Different letters beside values within rows for each parameter denote significant differences at p<0.05 and within columns for each parameter at p<0.01, ANOVA LSD, n = 12. Water (W) is the calculated difference between FW and DW, DM% is the calculated ratio of DW to FW expressed as a percentage.

By the time of the final harvest 165 DAFB, the average weight of HN fruit was nearly 7 g (9%) heavier than LN fruit (p=0.01; Table 6.7). However, the increase in FW was due to increased water influx without a proportionate increase in DW, which was now similar to that of LN fruit (Table 6.7). Consequently DM% of the mature fruit from the HN vines was significantly reduced (p<0.01; Table 6.7). The results suggest that the advantage in sink strength apparent in HN fruit at early harvest was not maintained, and during the time between early and final harvests, LN fruit had managed to attract dry matter at an increased rate compared to HN fruit over the same period, allowing these fruit to compensate for their initial slower growth.

Between early and final harvest, LN fruit gained 7.4 g of dry matter compared to an increase of 6.6 g in HN fruit over the same period (Table 6.7). This is shown even more clearly in Figure 6.2 where the proportion of total fruit dry matter accumulation after early harvest relative to before, is higher in LN fruit compared to HN in both non-CPPU and CPPU treated fruit. Figure 6.2 also shows that the effect of CPPU was to increase the proportion of dry matter accumulated before early harvest. As was noted at early harvest the effect of CPPU at final harvest as a percentage difference from control fruit was larger for LN than HN fruit for FW and its DW and water components (Table 6.7). The increased water influx in HN fruit might be due to the effect of $NO_3^-$ of increasing vine water content and thereby steepening the hydraulic gradient driving water flux to the fruit (Table 6.7; Chapter 3, sections 3.3.4, 3.3.6; Chapter 9, sections 9.2, 9.3, Figures 9.1, 9.2; McIntyre 1997).
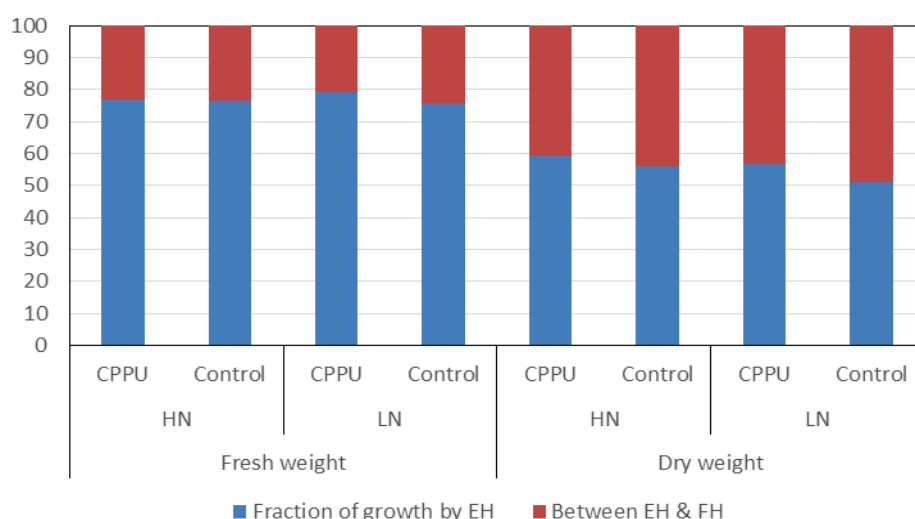


Figure 6.33 Effect of high (HN) or nil (LN) rates of soil-applied N fertiliser on 'Hayward' fresh weight (FW) and dry weight (DW) in CPPU treated and control (no CPPU) fruit as a percentage of the total accumulated by early harvest (EH, 81 days after full bloom) and between EH and final harvest (FH, 165 days after full bloom).

218

The large differences in FW, DW, and water content of CPPU treated fruit present at early harvest were still present at final harvest (Table 6.7). However, although the percentage differences between CPPU treated and control fruit for FW (and water content) remained similar to those found at early harvest, the difference for DW was smaller. This provides some further evidence that fruit grow towards an inherent and fixed potential, which can however be reached at varying growth rates and following different growth curves (Snelgar et al. 2012).

The DW and water differences between HN and LN in the CPPU treated fruit at final harvest were not statistically significant. Nevertheless, changes to both DW and water were apparently influential in causing the significant difference in DM% between HN and LN at final harvest ($p<0.05$, Table 6.7). This is in contrast to control fruit where a similar difference between HN and LN in DM% at final harvest was found to be due to a difference in water influx only. The lower dry matter accumulation of HN compared to LN fruit treated with CPPU might be related to a decreased availability of assimilate due to competition for assimilates from the shoots in the HN vines.

The patterns apparent in Figure 6.2 are consistent with findings from tissue culture experiments, where treatment with exogenous cytokinins caused cell division and expansion to proceed at faster rates initially but because the potential for expansion of untreated cells was similar, in time, these cells grew to an equal size (Stoynova-Bakalova et.al. 2011). Thus there might be capacity for compensatory growth where the smaller number of cells in untreated tissues are able to expand more to partially compensate for the increased growth of treated tissues (Stoynova-Bakalova et al. 2011). Similarly with N, although an adequate supply of N increased the rate of cell elongation in barley leaves, this was partially compensated for in N-limited plants by doubling of the period of cell elongation so that final size of leaf cells in N-limited plants was not markedly lower than those of N sufficient plants (Fricke et al. 1997).

Treatment of *A. deliciosa* with CPPU increases cell division and expansion in the fruit (Woolley et al. 1991; Patterson et al. 1993; Lewis et al. 1996), which are typical responses to cytokinins in plant tissues (Ron'zhina et al. 2003; Stoynova-Bakalova et al. 2011). There is also a large and rapid increase in hexose sugars in the CPPU treated fruit (Antognozzi et al. 1996), perhaps due to another known effect of cytokinins - that of strong enhancement of

plasmodesmata that connect the fruit parenchyma cells with the phloem-sieve tube complex (Maule 2003). However, since the differentiation and development of amyloplasts lags behind cell division (Lopez-Juez and Pyke 2005), it might be that the accumulation of hexose sugars cannot be efficiently utilised for starch synthesis. Together with the effect of cytokinins on cell wall yielding this might mean there is now a much increased hydraulic gradient to the fruit (Boyer 1988), explaining the rapid size response of the fruit to CPPU. The increase in cell expansion is most pronounced in the small cells of the fruit parenchyma tissue, the very cells within which most of the starch-storing amyloplasts are sited (Antognozzi et al. 1997; Patterson et al. 1993). But although the size of the small parenchyma cells was increased by 31% by CPPU treatment in their experiments, the starch content of the fruit was only increased by 10% by the time of peak starch content (approximately 120 DAFB). Cytokinins including CPPU are reported to increase the formation of amyloplasts and the synthesis of starch (Miyazawa et al. 2002) and CPPU also promotes chloroplast development (Antognozzi et al. 1996). Since both organelles occupy the same limited space in the cytoplasm, sandwiched between the vacuole and the cell wall, there may be some competition for space between them (Possingham 1980). Furthermore, the increased size of the vacuoles in the small cells of the CPPU treated fruit resulting from the rapid influx of water following the increased hydraulic gradient, may have reduced the relative size of the cytoplasm available for the development of either plastid. Thus alterations to the hydraulic gradient regulating water flux to the fruit are involved in the size responses found with CPPU and with soil-applied $NO_3^-$. However, both CPPU and soil-applied $NO_3^-$ failed to increase fruit sink strength to the same extent as they stimulated fruit water uptake, resulting in significant reductions in DM%.

### 6.3.1.4 Foliar-applied N

At early harvest, foliar-applied N (1% sprays of $KNO_3$ or urea) showed small increases in average fruit FW, with both DW and water being increased, but only for the earlier application time (FN1) and only in LN vines (Table 6.8). Although the differences with foliar-applied N were not statistically significant within the HN or LN vines, neither were the differences between HN and LN fruit treated with foliar-applied N at the earlier time (FN1), suggesting that the foliar treatments had increased the growth of LN fruit.

Within LN vines treated with CPPU, the results show a decrease in FW, DW, and water with foliar-applied N at the earlier application time (FN1), which was close to being significant

(p<0.06 for FW and DW and p<0.07 for water; Table 6.8). The second and later foliar series (FN2) had little effect on any of the parameters in either control or CPPU treated fruit. Within the control fruit the increase in growth with foliar N in LN but not in HN fruit suggests a nutritional deficiency for N. However, if this was the case a similar response would be expected in LN fruit treated with CPPU, but this was not apparent. A situation of N deficiency is also not consistent with data presented in Table 6.7 or by the fruit N% data (Table 6.5) which showed levels within the normal range.

Table 6.27 Effect at 81 days after full bloom of foliar N applied at two different times (FN1 and FN2) on fresh weight (FW), dry weight (DW), and water content (W) of 'Hayward' fruit from vines given high (HN) or nil (LN) rates of soil-applied N fertiliser and with CPPU or without (Control).

| | FW (g) | | DW (g) | | W (g) | |
|---|---|---|---|---|---|---|
| | HN | LN | HN | LN | HN | LN |
| Control | 76.9a | 70.9a* | 8.5a | 7.6a* | 68.4a | 63.3a* |
| Control+FN1 | 77.5a | 75.7a | 8.2a | 8.2a | 69.3a | 67.4a |
| Control+FN2 | 74.8a | 72.0a | 8.1a | 7.9a | 66.7a | 64.1a |
| CPPU | 107.6b | 106.6b | 11.1b | 11.1b | 96.5b | 95.6b |
| CPPU+FN1 | 111.8b | 100.0b* | 11.7b | 10.4b* | 100.1b | 89.6b* |
| CPPU+FN2 | 107.2b | 102.3b | 11.1b | 10.7b | 96.1b | 91.6b |

* denotes significant difference at $p<0.05$ within row for parameter pair; different letters within columns denote significant differences at $p<0.01$; FN1: four foliar N applications at approximately 10 day intervals between 20 and 52 days after full bloom; FN2: four foliar N applications at between 6 and 10 day intervals between 41 and 66 days after full bloom (1% urea or 1% $KNO_3$ w/v).

At final harvest in control fruit from the LN treatment (no CPPU), a similar increase in FW was found for the earliest foliar-applied N series (FN1) as was obtained from the soil application of $NO_3^-$ fertiliser; but because of a proportionately equal increase in DW and water, fruit DM% was maintained at similar level (Table 6.9). Foliar-applied N also increased the average FW and DW of HN fruit, and although these differences were not statistically significant, the increase in water uptake with the early foliar series was statistically significant ($p<0.05$; Table 6.9). The second foliar series (FN2) appeared to have a smaller effect on FW than FN1 as was also the case at early harvest (Table 6.8). Dry matter accumulation may have been less effected by timing of the foliar-applied N series in both HN and LN fruit. Although the mechanism of the effect is unclear the results do suggest that

foliar sprays of N applied during early fruit development can increase fruit growth and dry matter accumulation, particularly in vines of lower N status.

In the CPPU-treated fruit, foliar-applied N had little effect on FW within the respective HN and LN vines (Table 6.9). However, the results show the same increase in FW for HN compared to LN fruit as was noted for EH (Table 6.8). This increase was statistically significant ($p < 0.05$) in the case of the first foliar N series (Table 6.9). The results with CPPU are not consistent with N becoming a limiting factor when fruit growth (and theoretically fruit N demand) is increased by a biostimulant.

Table 6.28 Effect at final harvest (165 days after full bloom) of foliar N applied at two different times (FN1 and FN2) on fresh weight (FW), dry weight (DW), water content (W), and dry matter concentration (DM%) of 'Hayward' fruit from vines given high (HN) or nil (LN) rates of soil-applied N fertiliser and with or without CPPU (CPPU, C).

| | FW (g) | | DW (g) | | W (g) | | DM% | |
|---|---|---|---|---|---|---|---|---|
| | HN | LN | HN | LN | HN | LN | HN | LN |
| Control[1.] | 100.3a | 93.6a* | 15.1a | 15.0a | 85.2a | 79.1a* | 15.0a | 16.0a* |
| Control+FN1 | 106.7a | 101.1b | 15.6a | 16.0a | 91.1b | 85.1b* | 14.6a | 15.8a* |
| Control+FN2 | 103.8a | 98.3ab | 15.6a | 15.8a | 88.2ab | 82.5ab | 15.0a | 16.1a* |
| CPPU[1.] | 139.8b | 134.8b | 18.7b | 19.6b | 121.0c | 115.3c | 13.4b | 14.5b* |
| CPPU+FN1 | 143.2b | 131.5b* | 19.5b | 19.1b | 123.8c | 112.3c* | 13.5b | 14.5b* |
| CPPU+FN2 | 140.0b | 130.5b | 18.9b | 19.2b | 121.1c | 111.3c* | 13.5b | 14.6b* |

* denotes pair of values within rows are significantly different $p < 0.05$; different letters within columns denote significant difference $p < 0.05$. ANOVA LSD; n = 10 (HN), n = 12 (LN).

[1.] Note that data for C and CPPU has also been given in Table 6.7. FN1: four foliar N applications at approximately 10 day intervals between 20 and 52 days after full bloom; FN2: four foliar N applications at between 6 and 10 day intervals between 41 and 66 days after full bloom (1% urea or 1% $KNO_3$ w/v).

The generally smaller response to the second later foliar series (FN2) than the first series (FN1) supports the idea that fruit is responsive to foliar N for a limited and transitory period as was found for 'Hort16A' (Chapter 5). The generally smaller response to foliar-applied N in CPPU treated fruit is consistent with CPPU and foliar-applied N sharing a common mechanism, since the application of foliar N could not stimulate growth where the growth potential had already been realised by CPPU. The significant difference between HN and LN fruit DM% was consistently maintained with foliar-applied N and CPPU, which is consistent with the effect of soil-applied N on fruit being through the simultaneous increase in vine

water content and shoot sink strength. The difference between HN and LN fruit water uptake was also very similar in C and CPPU-treated fruit with a 5 and 8% increase respectively (Table 6.9). This is consistent with an increased hydraulic gradient being developed in the HN vines, which remains a constant effect regardless of physiological differences in the fruit induced by CPPU treatment.

The effects of potassium nitrate ($KNO_3$) and urea for the earliest foliar N series were similar (Table 6.10). When data for HN and LN were combined there was a 7.9 and 6.8% increase for FW in $KNO_3$ (p<0.06) and urea (p<0.01) respectively (Table 6.10). Dry weight and water were increased in relatively equal proportions but only the increase in water was significant (p≤0.05; Table 6.10).

Table 6.29 Effect at final harvest (165 days after full bloom) of foliar-applied urea and $KNO_3$ (1% w/v) on fresh weight (FW), dry weight (DW), water content (W), and dry matter concentration (DM%) of 'Hayward' fruit.

|  | FW (g) | DW (g) | W (g) | DM% | n |
|---|---|---|---|---|---|
| Control | 97.5 (1.8) | 14.7 (0.4) | 82.7 (1.5) | 15.1 (0.3) | 12 |
| $KNO_3$ 1% | 105.1 (3.3) | 15.7 (0.6) | 89.4 (2.7) | 14.9 (0.2) | 12 |
| %diff | 7.9 | 6.4 | 8.1 | -1.2 | |
| p-value | 0.055 | 0.172 | 0.048 | 0.604 | |
| | | | | | |
| Control | 96.7 (2.0) | 15.3 (0.3) | 81.8 (1.9) | 15.8 (0.2) | 14 |
| Urea 1% | 103.3 (1.7) | 16.0 (0.2) | 87.3 (1.6) | 15.5 (0.2) | 14 |
| %diff | 6.8 | 4.3 | 6.7 | -1.9 | |
| p-value | 0.013 | 0.064 | 0.028 | 0.323 | |

[1.] Standard error in parenthesis, p-values from two-tailed T-test. Four foliar N applications at approximately 10 day intervals between 20 and 52 days after full bloom.

The difference in FW with foliar N was greatest in LN fruit treated with $KNO_3$ (Table 6.11). The 9% increase in FW of LN fruit treated with $KNO_3$ coincided with a proportionately equal increase in DW so that DM% was maintained in these larger fruit (Table 6.11). The smaller increase in DW of HN fruit treated with $KNO_3$ resulted in these fruit having significantly lower DM% than the equivalent LN fruit (p<0.01; Table 6.11). Similar trends were found with urea, with DW and water being increased relatively equally in LN fruit so

that DM% was maintained, but not so equally in HN fruit which had lower average DM% than HN control fruit (Table 6.11).

Table 6.30 Effect at final harvest (165 days after full bloom) of foliar-applied urea and KNO$_3$ (1% w/v) on fresh weight (FW), dry weight (DW), and dry matter concentration (DM%) of 'Hayward' fruit from vines given high (HN) or nil (LN) rates of soil-applied N fertiliser.

| | FW (g) | | DW (g) | | DM% | |
|---|---|---|---|---|---|---|
| | HN | LN | HN | LN | HN | LN |
| Control | 100.8 (3.0) | 94.1 (0.9) | 14.9 (0.8) | 14.6 (0.3) | 14.7 (0.4) | 15.5 (0.3) |
| KNO$_3$ (FN1) | 107.6 (3.7) | 102.6 (5.6) | 15.5 (0.6) | 15.9 (0.9) | 14.4 (0.2) | 15.5 (0.2)** |
| %diff | 6.8 | 9.0 | 3.9 | 9.0 | -2.3 | -0.1 |
| P-value | 0.186 | 0.196 | 0.582 | 0.215 | 0.463 | 0.970 |
| Control | 99.5 (2.4) | 93.1 (2.8) | 15.4 (0.5) | 15.5 (0.4) | 15.5 (0.2) | 16.5 (0.2)** |
| Urea (FN1) | 105.4 (4.0) | 99.2 (1.7) | 15.8 (0.7) | 16.2 (0.3) | 14.9 (0.3) | 16.3 (0.1)** |
| %diff | 5.9 | 6.5 | 2.2 | 4.2 | -3.4 | -1.4 |
| P-value | 0.261 | 0.067 | 0.681 | 0.245 | 0.156 | 0.246 |

*p≤0.01; n=6, except for HN Urea when n=4. FN1: four foliar N applications at approximately 10 day intervals between 20 and 52 days after full bloom.

Both foliar N forms appeared to be more effective for increasing the growth of LN fruit, which suggests fruit growth was N limited. There may be temporary N deficits during early fruit development stages due to the increased demand of rapidly growing tissues within the fruit and competition for N from on-going canopy shoot growth. For example, in *A. chinensis* Mills et al. (2008) found a bigger reduction in N content in the fruit than in the leaves when fertiliser N was withheld suggesting that the leaves were given priority over fruit for the available N. The measured increase in N content of fruit treated with the four foliar N applications averaged 15.7 mg and 7.7 mg N per fruit for urea and KNO$_3$ respectively, which is well within the potential N increase due to direct fruit absorption (see Chapter 5, Table 5.6). Nevertheless efficient translocation of foliar-applied N from leaves to reproductive sinks has been demonstrated (Klein and Weinbaum 1984; Cimato et al. 1990). For example, Klein and Weinbaum (1984) found 57% of ^{15}N-enriched urea applied to the leaves of olive ended up in the fruit. Furthermore, the translocation from leaves to fruit can be rapid with 20% of the ^{15}N-enriched urea being found in fruit 3 days after its application to the foliage. Such an effect is consistent with the generally accepted efficiency of foliar-applied N for supplying supplementary nutrition to sites where temporary deficits exist (Klein 2002; Mengel 2002).

During early fruit development the young fruitlets need relatively high N concentrations for protein and enzyme synthesis to support the active metabolism associated with rapid cell division (Cheng et al. 2007).

The response to foliar-applied N appears not to depend entirely on the N status of the vines since a similar response was found in the HN vines as in the LN vines (Table 6.9). A response to foliar-applied N in well-fertilised crops was also reported for citrus (El-Otmani et al. 2004; Ali and Lovatt 1994; Lovatt 1999). In HN vines the increased vigour (or sink strength) of the shoots might not only reduce the fruit's capacity to attract carbohydrates but also the products of N assimilation (Porro et al. 2010). This might explain the similar response to foliar-applied N in the HN vines. An increased assimilate supply resulting from increased N content of the leaves cannot be discounted as a factor in the responses to foliar-applied N although this seems more likely to apply to LN than HN vines (Cheng et al. 2007). Although the effects of foliar-applied N on leaf N% were minimal (Table 6.14) this may be because by the time of sampling most of the foliar-applied N had been translocated out of the leaves, so the possibility that photosynthesis was enhanced by the foliar-applied N during early fruit development remains.

### 6.3.1.5 Fruit firmness and soluble solids contents

Nitrogen supply did not significantly affect fruit firmness or soluble solids content (SSC), although the average value of both variables was lower in HN than LN fruit. CPPU treatment did not affect SSC of the fruit but did make the fruit much softer ($p < 0.001$; Figure 6.3). The effect of soil-applied-N on fruit firmness and SSC confirms results reported in Chapter 4. The effect of CPPU of reducing firmness in 'Hayward' fruit at harvest has been reported by others (Antognozzi et al. 1997; Faminai et al. 1997), but the difference disappeared rapidly during storage (Faminai et al. 1997). The very small difference in SSC in CPPU treated fruit in our experiment might be because the fruit was more mature at the time of harvest (°Brix at harvest between 8.0 and 8.5 in our study compared to 6.5 to 7.0 in Faminai et al. 1997).
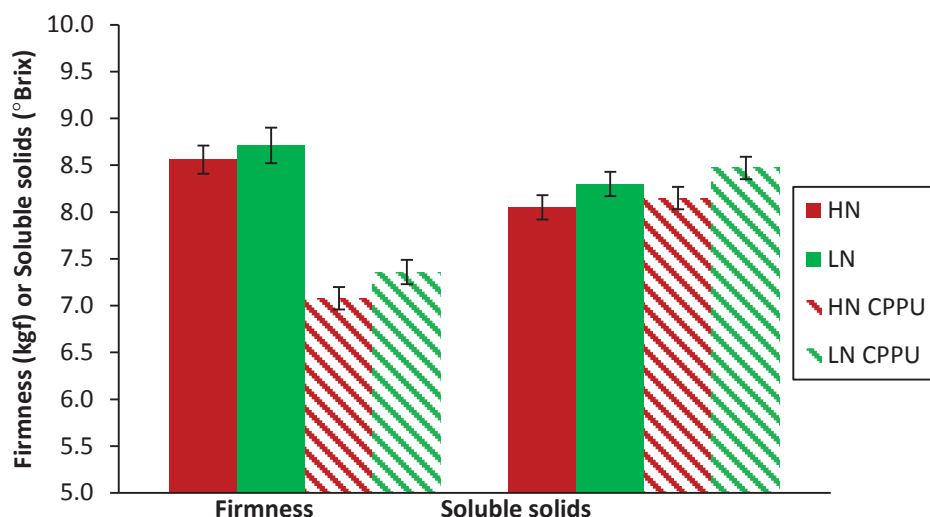
Figure 6.34 Effect of high (HN) or nil (LN) rates of soil-applied N fertiliser and CPPU on fruit firmness (kgf) and soluble solids content (°Brix) of mature kiwifruit at final harvest (165 days after full bloom). Error bars are indicating standard error.
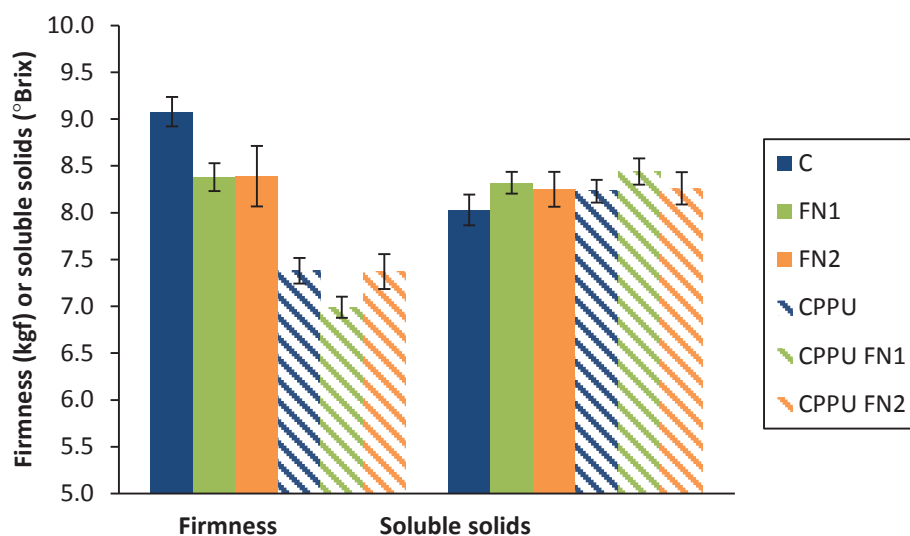


Figure 6.35 Effect of foliar-applied N and CPPU on fruit firmness (kgf) and soluble solids content (°Brix) of mature kiwifruit at final harvest (165 days after full bloom). Error bars indicate standard error. FN1: four foliar N applications at approximately 10 day intervals between 20 and 52 days after full bloom; FN2: four foliar N applications at between 6 and 10 day intervals between 41 and 66 days after full bloom (1% urea or 1% $KNO_3$ w/v).

Fruit was significantly softer in both foliar-applied N series (FN1 and FN2) than in the control (p=0.017, ANOVA; Figure 6.4). Both forms of foliar-applied N (urea and $KNO_3$) reduced fruit firmness and there were no consistent differences in the degree of softening between the two N forms (data not shown). With CPPU treatment, the average firmness of

FN1 fruit was less than that of the CPPU-only control but the difference was not significant overall and was not apparent in the later foliar application series (FN2) (overall p=0.078 ANOVA; Figure 6.4). There were no significant differences in SSC with foliar N or CPPU, although average SSC levels for these treatments were slightly higher than control (Figure 6.4). The reduced firmness and higher SSC of CPPU treated fruit has been used as evidence of earlier maturation (Antognozzi et al. 1997; Famiani et al. 1997). However, it might be more accurate to attribute the reduced firmness to higher water content (i.e. more succulent). Support for this relationship was found in the significant positive correlation between DM% and firmness within the non-CPPU treated fruit (Table 6.12). However, the relationship was not present in the CPPU treated fruit, where there were no correlation between DM% and firmness, except for a weak negative correlation in HN fruit only (Table 6.12).

Table 6.31 Effect of high (HN) or nil (LN) rates of soil-applied N fertiliser and CPPU on correlations between fruit dry matter concentration (y) and firmness (x) at final harvest (165 days after full bloom).

| N supply | CPPU | df | $r^2$ | P-value | Slope | Regression equation |
|---|---|---|---|---|---|---|
| HN+LN | - | 54 | 0.20 | <0.001 | / | y = 0.391x + 2.362 |
| HN | - | 21 | 0.24 | <0.02 | / | y = 0.4932x + 1.2507 |
| LN | - | 29 | 0.22 | <0.01 | / | y = 0.5244x + 0.3892 |
| HN | + | 26 | 0.14 | <0.10 | \ | y = -0.4273x + 12.805 |

### 6.3.1.6 Potassium nitrate compared with potassium sulphate

In a separate pilot study undertaken concurrently with the main experiment described above, the effect of $KNO_3$ was compared to $K_2SO_4$ by applying 1% solutions of both salts to groups of canes on selected vines. Although the average difference in FW of fruit from the $KNO_3$ treatment compared to the control (5.8% increase) was similar to that found in the main trial (Experiment 1), an ANOVA found no significant differences between the treatments in FW, DW, or DM% (Table 6.13). Although these results provide some tentative evidence that $KNO_3$ is more effective than $K_2SO_4$ under the conditions of this trial, the results cannot determine whether the effect of $KNO_3$ was due to its N or K component. Uptake of K from $K_2SO_4$ may have been reduced due to the lower solubility of this salt compared to $KNO_3$ (Elam et al. 1995) especially in the un-buffered solutions (Howard 1998) used in the experiment reported in Table 6.13 (Howard 1998). Nevertheless, both $KNO_3$ and $K_2SO_4$ are widely used for foliar K nutrition (Howard 1998).

Table 6.32 Effect of foliar sprays of $KNO_3$ and $K_2SO_4$ (1% w/v) on fruit fresh weight (FW), dry weight (DW), and dry matter concentration (DM%) at final harvest (165 days after full bloom).

|  | Control | $KNO_3$ | $K_2SO_4$ | ANOVA |
|---|---|---|---|---|
| FW (g) | 109.2 (2.1) | 115.6 (3.3) | 110.7 (2.7) | NS |
| DW (g) | 17.1 (0.3) | 17.8 (0.7) | 16.9 (0.7) | NS |
| DM% | 15.7 (0.2) | 15.3 (0.3) | 15.3 (0.5) | NS |

n=6. NS: not significant p>0.05

## 6.3.2 Experiment 2

### 6.3.2.1 Leaf N%

Leaf N% of the experimental vines in the 2010-11 season were slightly below the reported optimum range for 'Hayward' kiwifruit in December-February of between 2.2 and 3.0% (Hill 2010) and did not vary significantly between any of the treatments (Table 6.14). Average yield (5151 tray equivalents/ha) for the orchard in the season used for this experiment were slightly below the regional average of 5938 te/ha, but fruit size (count 36.75) was above the regional average of count 34.10. However, the relatively low yield was most likely due to poor canopy management since leaf colour was dark green (being indicative of N sufficiency) throughout the season (personal observations; Appendix 2, Plates 4 and 6).

Table 6.33 Mid-January (55 days after full bloom) leaf N% from different foliar-applied N treatments in 'Hayward' vines (Experiment 2, 2010-11 season).

|  | Control | 1% KNO | 0.5% KN | 1% Urea |
|---|---|---|---|---|
| Leaf N% | 2.11 | 2.17 | 2.11 | 2.16 |
| Standard error | 0.03 | 0.03 | 0.03 | 0.03 |

n = 6.

### 6.3.2.2 Fruit growth

At 55 DAFB there were no significant differences between the foliar-applied N treatments in FW, DW, water, or DM% (Table 6.15). However, the average FW and DW of the 0.5% $KNO_3$ treatment was higher than any of the other treatments. Also the urea treated fruit had lower values for all four parameters (Table 6.15).

Table 6.34 Effect of different forms and rates of foliar-applied nitrogen on 'Hayward' fruit fresh weight (FW), dry weight (DW), water content, and dry matter concentration (DM%) 55 days after full bloom.

|  | Control | 0.5% $KNO_3$ | 1% $KNO_3$ | 1% Urea | ANOVA |
|---|---|---|---|---|---|
| FW (g) | 66.8 (1.7) | 69.0 (0.7) | 66.1 (1.7) | 63.2 (1.6) | NS |
| DW (g) | 4.7 (0.1) | 4.8 (0.1) | 4.5 (0.2) | 4.3 (0.2) | NS |
| Water (g) | 62.1 (1.6) | 64.2 (0.7) | 61.6 (1.5) | 58.9 (1.5) | NS |
| DM% | 7.0 (0.1) | 7.0 (0.1) | 6.9 (0.1) | 6.8 (0.1) | NS |

n = 6. NS: not significant $p > 0.05$

At 154 DAFB 1% (w/v) foliar sprays of $KNO_3$ or urea had no significant effect on FW (Table 6.16). However, foliar sprays of 0.5% $KNO_3$ increased the fresh weight (FW) of mature fruit by about 8.6% ($p < 0.01$; Table 6.16). Dry matter accumulation and water influx were increased relatively equally by the 0.5% $KNO_3$ treatment to maintain similar DM% of the fruit (Table 6.16).

Table 6.35 Effect of different forms and rates of foliar-applied nitrogen on 'Hayward' fruit fresh weight (FW), dry weight (DW), water content, and dry matter concentration (DM%) 154 days after full bloom.

|  | | | | | ANOVA |
|---|---|---|---|---|---|
|  | Control | 0.5% $KNO_3$ | 1% $KNO_3$ | 1% Urea | p-value |
| FW (g) | 100.3a | 108.9b | 103.1a | 102.7a | 0.009 |
| DW (g) | 17.6a | 19.2b | 18.1ab | 18.0ab | 0.073 |
| Water (g) | 81.9a | 90.2b | 86.0a | 85.8a | 0.002 |
| DM% | 17.6a | 17.6a | 17.5a | 17.5a | 0.992 |

n = 6. Different letters within rows designate significant differences Fisher's unprotected LSD.

In both seasons (Experiment 1 and 2), $KNO_3$ gave larger responses than urea although much less N was supplied by $KNO_3$ than urea. For example, the 0.5% $KNO_3$ treatment, which was the most effective treatment in the second season, supplied only 4.5 g N per vine compared to 26 g N for the 1% urea treatment. This provides further evidence that increases in fruit growth with foliar-applied N are not purely due to the nutritional effect of the additional nitrogen (see also discussion in Chapter 5, section 5.4).

It is possible that the response to $KNO_3$ was a K effect although the results comparing $KNO_3$ with $K_2SO_4$ were inconclusive (Table 6.13). There might also be a synergistic effect of $NO_3^-$ and $K^+$ when they are applied together with $KNO_3$. Potassium nitrate ($KNO_3$) is widely used in crops to improve fruit quality and yield and can be an effective source of both K (Sing and McNeil 1992; Coker et al. 2009; Achilea et al. 2002) and N (Weinbaum 1978). It has been widely used for foliar K nutrition where K uptake is limited by soil conditions (Calvert 1969; Howard et al. 1998; Nelson et al. 2005). In many studies there is an assumption of responses to $KNO_3$ being due to either K or N. In respect to the greater effectiveness of the 0.5% compared to the 1% $KNO_3$ treatment, the stimulation of assimilate export from leaves was reported to be stimulated by very low concentrations of foliar KCl but not by higher concentrations (Doman and Geiger 1979). Uptake of K can also be greater at lower foliar application rates and this might also affect the uptake rate of the accompanying anion, explaining possible synergetic effects (Fernandez and Eichert 2009).

### 6.3.2.3 Shoot type

Fruit from long shoots had significantly higher FW and DW than fruit from short or pruned shoots (Table 6.17). The pruned shoots were originally long shoots that had been shortened back to the most distal fruit ('zero leafing'), a horticultural practice designed to reduce competition from the canopy for assimilates to the benefit of the fruit (Patterson et al. 2009). Patterson et al. (2009) reported zero leafing gave a 10 g increase in FW compared to fruit from un-pruned shoots on the same cane, and this was accompanied by a proportionate increase in DW so that DM% was maintained. The reduction in FW and dry matter accumulation in fruit on pruned shoots in our study (Table 6.17) may have occurred because the zero-leafing had in some cases not been done correctly with the cut not being made close enough to the last fruit allowing some regrowth to occur. A large reduction in fruit growth and particularly dry matter accumulation can occur when shoots are made to re-grow in this manner (Minchin et al. 2010; Snelgar et al. 2012) and improperly pruned shoots were probably sufficiently common within our experimental vines to influence the results in this way. Reduced FW and DW found in fruit from short shoots compared to long shoots is consistent with other experiments (Chapter 3, section 3.3.7; Volz et al. 1991; Kulczewski 2003).

Table 6.36 Effect of shoot type on 'Hayward' fruit fresh weight (FW), dry weight (DW), water content, and dry matter concentration (DM%) 154 days after full bloom.

| | Shoot type | | | ANOVA |
| --- | --- | --- | --- | --- |
| | Short | Long | Pruned | p-value |
| FW (g) | 99.0a | 110.1b | 101.7a | <0.001 |
| DW (g) | 17.2a | 19.6b | 17.7a | <0.001 |
| Water (g) | 81.8a | 90.5b | 83.9a | <0.001 |
| DM% | 17.4a | 17.8a | 17.4a | 0.09 |

n=24. Shoot type: short <50 cm, long > 90cm, pruned a long shoot cut back to the most distal fruit. Different letters within rows denote significant differences, Fisher's protected LSD, $p<0.05$.

Although pruning appeared to make the shoots behave like naturally short shoots, the factors involved may be different. Smaller fruit on short shoots may be due to fewer seeds and later anthesis, which respectively weaken the sink strength of the fruit and reduce the time available for growth (Lai et al. 1990). However, on pruned long shoots smaller fruit is probably due to a combination of increased competition from re-growths and a reduced capacity to attract assimilate due to the removal of a proximal source (Tombesi et al. 1993). The greater effect of the foliar N on the pruned shoots compared to long and short shoots supports the idea that the main effect of foliar N is to increase sink rather than source strength since pruned shoots had a smaller leaf area available for absorption of the foliar N than the other two shoot types. This conclusion is also supported by the greater response to foliar N by fruit from pruned shoots than by fruit from the naturally short shoots since the potential for growth had already been established in the fruit on pruned-shoots before the shoots were shortened.

Fruit from the 0.5% $KNO_3$ foliar treatment had the highest FW on all three shoot types (Table 6.18). The effect of this foliar treatment appeared greatest on pruned shoots with a 9.9% increase and least on long shoots with a 7.3% increase compared to their respective unsprayed control ($p< 0.01$; Table 6.18). The 0.5% $KNO_3$ treatment also significantly increased fruit DW in long shoots and showed a similar pattern in short and pruned shoots (Table 6.18). The lack of statistical significance, particularly in the pruned shoots, is mainly due to high variability in the 1% $KNO_3$ treatment. When the 0.5% $KNO_3$ treatment alone is compared to the unsprayed control, the significance level of the FW and DW differences in all shoot types is $p<0.05$ with the biggest increase in pruned shoots (10.9% higher DW than

control). The foliar urea treatment showed only small differences in FW compared to the control, although average DW in the pruned shoots with urea was similar to the $KNO_3$ treatments (Table 6.18). In the previous season, foliar urea gave a 6% increase in FW of 'Hayward' fruit (Table 6.10) but only small responses were found with urea in Experiment 3 (see section 6.3.3). Similarly variable results were obtained with foliar urea in two seasons in 'Hort16A' (Chapter 5). Foliar treatments appeared overall to increase DW more in pruned shoots than in short or long shoots (Table 6.18).

Table 6.37 Effect of different forms and rates of foliar-applied nitrogen on fresh weight (FW), dry weight (DW), and dry matter concentration (DM%) of 'Hayward' fruit from different shoot types 154 days after full bloom.

|  |  | Control | 0.5% $KNO_3$ | 1% $KNO_3$ | 1% Urea | p-value |
|---|---|---|---|---|---|---|
| FW (g) | Short | 95.4a | **103.2b** | 99.9ab | 97.5ab | 0.080 |
|  | Long | 108.9a | **116.9b** | 104.6a | 109.9a | 0.002 |
|  | Pruned | 96.5 | **106.1** | 104.8 | 100.7 | 0.134 |
| DW (g) | Short | 16.5 | **17.9** | 17.5 | 16.9 | 0.263 |
|  | Long | 19.5a | **20.9b** | 18.5a | 19.5a | 0.005 |
|  | Pruned | 16.8 | **18.6** | 18.2 | 18.2 | 0.225 |
| DM% | Short | 17.3 | **17.4** | 17.5 | 17.3 | 0.992 |
|  | Long | 18.0 | **17.9** | 17.7 | 17.8 | 0.918 |
|  | Pruned | 17.4 | **17.6** | 17.4 | 17.4 | >1.0 |

Values with different letters within rows are significantly different at p<0.05, Fisher's protected LSD, n=6. Shoot type: short <50 cm, long > 90cm, pruned a long shoot cut back to the most distal fruit.

Pruning of long shoots significantly reduced fruit FW by 12.8% and DW by 16.2% compared to un-pruned long shoots (p<0.001). The 0.5% $KNO_3$ treatment appeared to buffer the negative effects of pruning, so that fruit sprayed with 0.5% KNO3 on pruned shoots were slightly smaller (2.6%) than unsprayed control fruit on long shoots (relationships within upper blue triangle in Table 6.18). Although fruit DW on pruned shoots treated with 0.5% $KNO_3$ was still 4.8% reduced compared to control fruit on long shoots, the difference was not statistically significant (lower blue triangle in Table 6.18). The effect of 1% $KNO_3$ on restoring growth potential of fruit on the pruned shoots was similar to the 0.5% $KNO_3$ treatment (Table 6.18).

### 6.3.2.4 Firmness and soluble solids contents

Fruit from long shoots was firmer than fruit from short shoots and firmness of fruit from pruned shoots was intermediate to the other two shoot types (Figure 6.5). Soluble solids content (SSC) was not significantly different between the shoot types although the average values were higher in fruit from short shoots (Figure 6.5). The lower firmness and SSC in short shoots suggests that this fruit was maturing earlier than fruit from the other two shoot types. There were no overall significant differences in firmness or SSC (ANOVA $p>0.05$) between the foliar treatments, although fruit from long shoots treated with 0.5% $KNO_3$ was significantly firmer than the unsprayed control ($p=0.03$, two tailed T-test). The foliar urea treatment was least firm and had the highest SSC levels of the foliar treatments, which might indicate this fruit was more mature (Figure 6.5). The urea treated fruit from short shoots was the least firm and had the highest SSC, which could indicate that this fruit was the most mature overall the foliar-applied N treatments and shoot types. Lower firmness and higher SSC on short shoots compared to long shoots was also reported by Pyke et al. (1996), but only for fruit from the T-bar training system with the relationship being reversed for pergola trained vines.
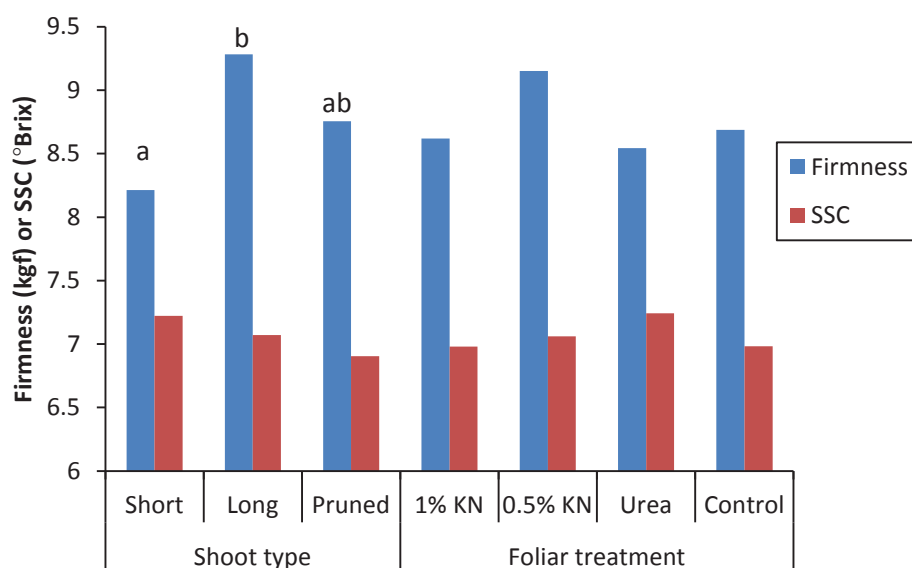


Figure 6.36 Effect of shoot type and the form and rate of foliar-applied nitrogen on 'Hayward' fruit firmness and soluble solids content (SSC) 154 days after full bloom. Shoot type: short <50 cm, long > 90cm, pruned a long shoot cut back to the most distal fruit.

### 6.3.2.5 Spray solution pH

The results from the 2010-11 season showed a significant FW increase for the 0.5% $KNO_3$ treatment but no significant increase for either the 1% $KNO_3$ or the 1% urea treatments

(Table 6.16). However, in the 2009-10 season 1% KNO$_3$ and 1% urea gave similar sized FW responses to each other and to the 0.5% KNO$_3$ treatment of the second season. In the 2010-11 season there was some marginal leaf necrosis in the urea treatment following the third application (for which reason the final urea application was reduced to 0.5%). The acidifying surfactant added to the foliar N spray solutions in this experiment lowered the pH of the resulting 0.5% KNO$_3$ to 5.0 and the 1% KNO$_3$ solution to 6.26. The lower pH of the 0.5% KNO$_3$ treatment might explain why it was more effective than the 1% KNO$_3$ treatment, but does not explain the effectiveness of the un-buffered 1% KNO$_3$ solution of the previous season (Section 6.3.1, Experiment 1). The effect of the acidifying surfactant was different in the case of urea where the pH was made too low (pH 3.98) due the same rate of acidifying surfactant being used despite the lower initial pH of the urea solution (pH 7.56) compared to the un-buffered KNO$_3$ solution (pH 9.41). The very low pH of the urea solution probably explains both the leaf damage and poorer response found with this treatment in the 2010-11 season compared to the results reported in Experiment 1 and 3, and in other experiments with urea reported in Chapters 5 and 7. Nevertheless the initial pH of the un-buffered urea solution may have been less than optimal and some acidification of the spray solution would probably increase uptake. For example, El-Omani et al. (2004) reported significantly increased citrus leaf uptake of foliar urea when pH of the spraying solution was lowered from 7.6 in the un-buffered solution to between 5.5 and 6.0. Similarly, Faust and Swietlik (1984) reported studies that found highest rates of urea uptake by apple leaves was between pH 5.4 and 6.6, lowest at pH 7.3 and intermediate at pH 8.0.

Care has to be exercised with foliar N applications, as both N forms are potentially phytotoxic, especially at higher concentrations. Agricultural grade urea contains variable amounts of biuret, a potentially phytotoxic compound formed during urea manufacture. Low biuret urea is therefore recommended for foliar application. However, crops vary in their tolerance to biuret and the biuret tolerance of kiwifruit has not been established. The 0.65 to 0.80% biuret content of the urea used in these experiments is above that considered safe for biuret sensitive crops such as citrus (Albrigo 2002). Slight to moderate leaf damage with 1% KNO$_3$ applied to citrus was reported by Calvert (1969) and 'Partial blights of the leaf margins' with 3% KNO$_3$ on grapes was reported by Altindisli et al. (1999). Clearly the tolerance of kiwifruit to biuret and to the various forms of foliar N needs to be more clearly established.

## 6.3.3 Experiment 3

In this experiment, five consecutive foliar applications of 1% urea (w/v) were applied to a group of randomly selected canes on each of eight vines from 15 DAFB. A 1% solution (v/v) of a commercial seaweed extract was applied at the same time to the whole canopy of four of the eight vines. Fruit was differentiated during harvest into fruit from short (< 50cm) and long shoots (> 90 cm) as well as from the four foliar treatments.

### 6.3.3.1 Effect of shoot type on fruit

Average FW of mature fruit harvested 165 DAFB from long shoots was 4.2% heavier than fruit from short shoots but the difference was not statistically significant (Table 6.19). Dry matter concentration differed only slightly between the two shoot types, but fruit from long shoots had accumulated 5.7% more dry matter (DW) than fruit from short shoots ($p=0.06$; Table 6.19). There were no significant differences between the two shoot types in fruit firmness or SSC (Table 6.19). In this experiment the differences in FW and DW between long and short shoots were quite small (about 5% reduction in each variable in short compared to long shoots) and not statistically significant. Nevertheless, the pattern was consistent with that found in Experiment 2 (section 6.3.2 above) and during Year 2 (Chapter 3, Figure 3.6). In both of these other experiments fruit FW and DW was about 10% greater on long compared to short shoots and the differences were statistically significant.

Table 6.38 Effect of shoot type on fresh weight (FW), dry weight (DW), dry matter concentration (DM%), firmness, and soluble solids content (SSC) of 'Hayward' fruit 165 days after full bloom.

|  | Shoot length | | | |
|---|---|---|---|---|
|  | < 50 cm | > 90 cm | %diff | p-value |
| FW (g) | 101.2 (1.7) | 105.5 (2.0) | 4.2 | NS |
| DW (g) | 14.9 (0.3) | 15.8 (0.3) | 5.7 | 0.06 |
| DM% | 14.8 (0.2) | 15.0 (0.3) | 1.6 | NS |
| Firmness (kgf) | 8.4 (0.2) | 8.5 (0.2) | 1.1 | NS |
| SSC (°Brix) | 8.9 (0.2) | 9.1 (0.2) | 3.2 | NS |

Standard error in parenthesis, p-value from two-tailed T-test, n =16. Shoot type: short <50 cm, long > 90cm.

### 6.3.3.2 Effect of foliar spray treatments

The foliar treatments had similar effects on both shoot types. Overall foliar urea application increased FW and DW of the fruit by between 4 and 5% for each variable but the effects were not statistically significant. The effect of urea on control vines (no seaweed) was also small

and not statistically significant with an increase of about 4% in both variables (Table 6.20). Vines treated with seaweed extract only were not different from control vines and when also treated with urea the fruit showed a similar response as did urea on the control vines, with FW and DW increased by 5.4 and 4.0% respectively (Table 6.20). The average firmness of fruit treated with seaweed extract alone was about 11% greater than fruit treated with urea plus seaweed, urea alone, or the unsprayed control but the difference was not statistically significant ($p > 0.05$ ANOVA). Average SSC of fruit from vines not sprayed with seaweed extract was nearly 12% higher than that of fruit from vines treated with seaweed ($p < 0.01$, ANOVA) but urea appeared to have little effect on SSC (Table 6.20).

Table 6.39 Effect of foliar sprays of urea (1% w/v) and a seaweed extract (1% v/v) on 'Hayward' fruit fresh weight (FW), dry weight (DW), dry matter concentration (DM%), firmness and SSC (°Brix) 165 days after full bloom.

| | Control | Urea | Seaweed | Seaweed+Urea | ANOVA p-value |
|---|---|---|---|---|---|
| FW (g) | 99.0 (3.8) | 103.4 (2.7) | 102.8 (2.5) | 108.3 (2.2) | NS |
| DW (g) | 14.9 (0.6) | 15.5 (0.4) | 15.2 (0.4) | 15.8 (0.4) | NS |
| DM% | 15.1 (0.3) | 15.1 (0.3) | 14.8 (0.3) | 14.6 (0.2) | NS |
| Firmness (kgf) | 8.1 (0.3) | 8.3 (0.2) | 9.0 (0.1) | 8.3 (0.2) | NS |
| SSC (°Brix) | 9.4 (0.2)a | 9.6 (0.3)a | 8.5 (0.3)b | 8.5 (0.2)b | <0.01 |

Standard error in parenthesis, n = 8. Different letters within rows denote significant differences, Fisher's protected LSD, $p < 0.05$.

There are few other reports of the effects of foliar seaweed applications to kiwifruit. In a Greek study, one or two applications of a 1-2% seaweed extract applied to selected canes increased average fruit FW by up to 23% and advanced maturity of the fruit by about 10 days (Chouliaras et al. 1997). However, a similar trial in New Zealand with three different commercial seaweed extracts found no significant effects on kiwifruit FW (Snelgar et al. 2006). Lovatt and Ferguson (2006) found positive interactions between seaweed and urea on pistachio nuts.

## 6.4 Summary and conclusions

In the first experiment (Experiment 1, section 6.3.1), soil-applied nitrogen fertiliser increased fruit fresh weight but not fruit dry weight and this resulted in fruit dry matter concentration being significantly reduced (Table 6.7). Therefore, the main effect of soil-applied nitrogen was to increase fruit water uptake. Because of the role of water in driving cell expansion, an increase in vegetative vigour is commonly associated with an increase in water uptake (McIntyre 1987). In Experiment 1 there was a two-fold increase in canopy vigour in the vines given a supra-optimal level of nitrogen fertilisation, as measured by the weight of material removed during summer pruning in mid-January.

The failure of soil-applied nitrogen to increase fruit dry matter accumulation might be due to increased competition for assimilates from the more vigorous canopy (Patterson and Currie 2011; Snelgar et al. 2012). Fruit are seen to be more competitive for water than dry matter (Snelgar et al. 2012). It is also possible that carbohydrate synthesis in the leaves was reduced by the excessive nitrogen supply in the vines given a supra-optimal level of nitrogen fertilisation due to the increased energy and carbon demands of nitrogen assimilation (Marschner 2002).

Foliar-applied nitrogen also increased fruit fresh weight, but in these instances increased water influx was accompanied by a proportionately similar increase in dry matter accumulation, so that dry weight was also increased and dry matter concentration maintained (Table 6.9). In the second season (Experiment 2 section 6.3.2) the same pattern appeared, especially with the 0.5% potassium nitrate treatment (Table 6.16). There may be temporary nitrogen deficits during early fruit development stages due to the increased demand of rapidly growing tissues within the fruit and competition for nitrogen from on-going canopy shoot growth. However, the absence of a similar effect of foliar-applied nitrogen in CPPU-treated fruit argues against nitrogen being limiting for fruit growth.

An alternative explanation might be that foliar-applied nitrogen during early fruit development stimulates fruit growth in a manner typical of plant growth regulators such as CPPU, i.e., through interaction with hormone or genetic regulatory systems within the fruit. However, foliar-applied nitrogen might be less disruptive of fruit physiology than CPPU,

allowing a smaller but more balanced stimulation of fruit growth; and does not alter vine water relations or the strength of competing vegetative sinks in the same way that soil-applied nitrogen does.

Results from Experiment 3 suggest a positive interaction between foliar-applied nitrogen and a seaweed extract might occur. This deserves further attention and different preparations of seaweed and forms of foliar-applied nitrogen, e.g., potassium nitrate should be explored.

The results support the use of foliar-applied nitrogen during early fruit development either as urea or potassium nitrate. However, further work is needed to define optimum rates and timing. Further work is also needed to understand the effects of foliar-applied potassium nitrate on kiwifruit, including the respective roles (and possible synergies) of the nitrate and potassium ions. From the results reported in this chapter it can be concluded that advantages of foliar-applied nitrogen in the nutritional management of kiwifruit are not limited to reduced environmental impacts associated with a more efficient way to supply nitrogen. The results suggest that foliar-applied nitrogen can also be used to increase the value of the crop due to larger fruit with good levels of dry matter concentration.

# Contents

## List of Tables

## List of Figures

# 7. Effects of foliar-applied urea on fruit and vegetative growth in 'Hayward' kiwifruit vines differing in N status

## 7.1 Introduction

Careful management of the nitrogen (N) fertilisation of kiwifruit orchards is necessary to maintain vine health and produce regular heavy crops without inducing excessive vegetative vigour or nitrate ($NO_3^-$) leaching (Mills et al. 2008). Excessive vegetative vigour is associated with poor fruit quality and increased labour costs for canopy management (Patterson and Currie 2011; Elfing 1988). Nitrate leaching contaminates ground water and, increasingly, must be accounted for by growers who must conform to production standards and local environmental regulations (Mills et al. 2008). Excessive fertiliser use also adds unnecessarily to orchard operating expenses and is therefore economically inefficient.

In Chapters 3 and 6 high rates of soil-applied $NO_3^-$ fertiliser were found to be associated with large increases in vegetative vigour (Figure 3.2; Figure 6.1) but had only relatively minor effects on fruit FW and DM%. In Chapters 5 and 6, beneficial effects on fruit growth were found with small amounts of N applied as foliar sprays during early fruit development for both 'Hort16A' and 'Hayward' cultivars. In these previous chapters there was no equivalence between the rates of N applied to the soil and the foliar applications. That is high rates of N (about 450 kg N ha$^{-1}$ yr$^{-1}$) were applied with the soil treatments and only small amounts were applied with the foliar treatments (ranging from an equivalent per hectare rate of about 4 kg N in three foliar applications of $KNO_3$ up to about 32 kg N with seven foliar applications of urea). There are no published studies on the comparative effects of foliar and soil-applied N on vegetative vigour of kiwifruit.

The experiment reported in this chapter compares the effects on 'Hayward' fruit quality and vine vegetative vigour of a moderate rate of soil-applied N fertiliser with those of an equal amount of N applied in a series of foliar-applied urea sprays. Specific experimental questions addressed were:

1. In terms of vine vigour how do the effects of foliar-applied N compare to soil-applied N at equivalent application rates?

2. What is the effect on fruit FW and DM% of a moderate rate of soil-applied N compared to the same amount foliar-applied?

## 7.2 Materials and Methods

The experiment was carried out on mature T-bar 'Hayward' kiwifruit vines in the Massey University orchard in Palmerston North (140.7°S 174.7°E) during the 2009-2010 season. Details of the orchard and its prior management history are given in Chapter 2. Briefly, the previous management had been according to organic 'Biogro' organic systems since the year 2000. Since that time fertiliser inputs had been minimal and no soluble inorganic nitrogen had been applied. Twelve uniform vines were selected. Vines were in pairs (bays) 2.5 m apart within the row, and one of four treatments was randomly allocated to each vine or bay. The treatments were:

LN - no nitrogen fertiliser;

LNF - no nitrogen fertiliser applied to the soil, plus foliar sprays of 1% urea (w/v);

MN - a moderate rate of nitrogen fertiliser applied to the soil;

MNF - a moderate rate of nitrogen fertiliser applied to the soil, plus foliar sprays of 1% urea.

For MN and MNF treatments, calcium nitrate fertiliser (Calcinit® Yara International Ltd, Norway; 15.5% N) was applied to the root area (25 m$^2$) of selected bays on 25/11/2009 at a rate equivalent to 80 kg N ha$^{-1}$. Foliar-applied urea sprays (Yara Urea Tech, Yara Fertilizers NZ Ltd; 46% N, biuret content: 0.65-0.80%) commenced 10 November and were repeated at 10 day intervals with a total of 11 sprays supplying N at a cumulative rate equivalent to 80 kg N ha$^{-1}$. The final urea spray was on 2/03/2010.

Soil within the bays was sampled and analysed for mineral N on 6/11/09 prior to the start of the experiment. Soil samples were extracted in 2M KCl (Blakemore et al. 1987). Leaf samples (20 youngest mature leaves on non-fruiting shoots from the

leader zone of each vine) were collected on 6/1109, 13/01/10 (8 days after the 7th foliar-applied urea application), and 1/04/10 (29 days after 11th foliar-applied urea application). Dried leaf was extracted by Kjeldahl digestion followed by N analysis by auto analyser (Blakemore et al. 1987). Petiole sap $NO_3^-$ was measured on the samples collected 6/11/09 using a nitrate-test strips (Reflectoquant® E.Merck, Germany) and a reflectometer (Reflectometer RQflex, E.Merck, Germany). Details of this method are given in Chapter 2. Vines were summer pruned 13/01/10 and 5/03/10 and the fresh weight of the prunings recorded.

Fruit was harvested on 18/05/10, approximately 165 days after anthesis (full bloom 4/12/09). Fruit was sampled separately from short (<50 cm) and long (>100 cm) shoots because differences in fruit size and sink strength have been associated with shoot type (Lai et al. 1990). Fresh weight was recorded and equatorial slices were taken from each fruit (24 fruit/vine) and oven dried at 65ºC to obtain fruit dry weight (DW) and allow calculation of dry matter concentration (DM%; DM% = [DW/FW]×100) and fruit water content. Brix and firmness readings were also taken on six fruit per vine following the method given in Chapter 2 (sections 2.5.2, 2.5.3). Runoff from the foliar sprays was estimated after collection in shallow trays placed under selected vines.

Statistical analysis was by ANOVA with means separation by Fisher's Protected Least Significant Difference and using SAS statistical software (SAS Institute Inc. 2004). Students T-tests were used when comparing two variables using Excel (Moore and McCabe 1993).

## 7.3 Results and discussion

This section begins by establishing vine N status before the start of the experiment and then reports leaf N content after treatments have been applied. Following are the effects of the treatments on vegetative vigour and on fruit growth. Differences in the response of fruit growth depending on shoot type are described and the section ends by reporting the effects of the treatments and shoot type on firmness and soluble solids content.

### 7.3.1 Vine nitrogen status

At the start of the experiment before N treatments had been applied there were no significant differences in petiole sap $NO_3^-$ concentration between the sets of vines allocated to each of the four treatments. At this time (6/11/09) average petiole sap $NO_3^-$ in each of the bays ranged from 299 to 711 ppm with both of these extreme values being found in bays used for the LN and LNF treatments (Table 7.1). Soil mineral N and soil $NO_3^-$ also did not differ significantly between the bays, although the average content, especially for soil $NO_3^-$ was noticeably lower in bays allocated for the LN treatments (i.e. LN and LNF) than in MN bays (vines allocated for the MN and MNF treatments) (Table 7.1). Because the soil analysis data was not available until after the first of the N applications had been made due to a delay in processing the samples, the allocation of bays could not be re-arranged to achieve a more equal distribution of the soil mineral N content between the LN and MN treatments. The implication of this unintentional bias is that we cannot attribute the difference in N status of the MN and LN bays solely to the $Ca(NO_3)_2$ applied to the soil. Nevertheless, the correlation between soil $NO_3^-$ and petiole sap $NO_3^-$ was weak ($r^2 = 0.11$), as were those between petiole sap $NO_3^-$ before the start of the experiment and January pruning weight ($r^2 = 0.07$) and leaf N% ($r^2 = 0.04$). Since petiole sap $NO_3^-$ is generally accepted to be an accurate indicator of the current supply of N to the plant (Prasad and Ravenwood 1986), it seems reasonable to conclude that the differences between LN and MN bays was at least partly due to the soil application of nitrogen.

Table 7.40 Soil mineral N and soil $NO_3^-$ (0 – 20 cm depth), and vine petiole sap $NO_3^-$ (ug g$^{-1}$) on 6/11/09 for each of the six bays before the start of the experiment. LN: no soil applications of N fertiliser, MN: soil application equivalent to 80 kg N ha$^{-1}$.

| | Soil mineral N (ug g$^{-1}$) | | Soil $NO_3^-$ (ug g$^{-1}$) | | Petiole $NO_3^-$ (ug g$^{-1}$) | |
|---|---|---|---|---|---|---|
| | LN | MN | LN | MN | LN | MN |
| | 13.45 | 12.03 | 6.28 | 6.07 | 352.55 | 381.12 |
| | 7.35 | 14.63 | 2.50 | 8.32 | 299.30 | 693.28 |
| | 9.87 | 12.44 | 5.55 | 7.54 | 711.55 | 372.92 |
| Mean | 10.23 | 13.04 | 4.78 | 7.31 | 454.47 | 482.44 |

Soil mineral N based on one sample per bay (pooled from 10 cores), petiole $NO_3^-$ one sample per bay (20 petioles/sample).

## 7.3.2 Runoff from foliar sprays

Run-off of foliar-applied urea to the soil beneath the vines was just 2% of the volume applied and was estimated as being equivalent to a combined total of 1.8 kg N ha$^{-1}$ and was therefore considered not significant as an extra source of N to the vines.

## 7.3.3 Leaf nitrogen content

Eight days after the seventh foliar-applied urea application (13/01/10) leaf N% of LN vines was slightly below the reported optimum range for 'Hayward' vines at this time of the season (2.2 – 3.0%; Hill 2010) but leaf N% of MN vines was within the optimum range and was significantly higher than LN vines (Figure 7.1). Nevertheless, the LN vines were not severely N-limited since there were no visual foliar symptoms of N deficiency. Such symptoms do not appear in kiwifruit until leaf N% falls to about 1.5% (Smith et al. 1985). Foliar-applied urea had little effect on leaf N% of LNF vines but significantly increased it in MNF vines compared to MN ($p<0.05$; Figure 7.1). The leaves of LN vines might have had reduced permeability to urea caused by thicker cuticles and epicuticular wax layers, as was reported for citrus trees of low N status (Bondada et al. 2001). However, by this time the foliar-applied urea treatments had supplied the equivalent of about 34 kg N ha$^{-1}$, so it seems likely that at least some of the foliar-applied N had been absorbed by the LN vines. An alternative explanation is that there had been faster translocation of urea in response to increased N demand of sinks elsewhere in the vine (Gonzalez et al. 2010). Translocation rates of foliar-applied urea are influenced by the N demand and strength of competing sinks such as fruit (Cimato et al. 1990; Klein and Weinbaum 1984).

Figure 7.37 Effect of no additional nitrogen (LN), foliar sprays of urea (LNF), soil-applied nitrogen (MN) and soil plus foliar-applied nitrogen (MNF) on leaf N% (± standard error) at mid and late season.

By 1/04/10 (29 days after the last foliar-applied urea application) leaf N% in MN and MNF bays had fallen to levels similar to the LN bays, which had remained almost constant (Figure 7.1). The only significant difference between the treatments in leaf N% remaining by April was that between LN and MNF ($p < 0.05$; Figure 7.1). Only the LNF had leaf N% below the optimum level for 'Hayward' kiwifruit in April (2.0 – 2.7; Hill 2010). This supports the suggestion based on the January levels that the translocation of N from leaves of LN vines to developing fruits was promoted by foliar-applied urea.

### 7.3.4 Vegetative growth

Soil-applied N [80 kg N ha$^{-1}$ as Ca(NO$_3$)$_2$] doubled the weight of shoots removed during summer pruning ($p < 0.05$; Figure 7.2). The weight of prunings can be used as a measure of vegetative vigour (Burge et al. 1987). Stimulation of vine shoot growth by relatively low rates of N fertiliser has also been reported for grape vines, where an application of only 50 kg N ha$^{-1}$ at bud break increased shoot biomass by 70% (Conradie 2001). The large increase in shoot biomass shown in Figure 7.2 and reported by Conradie (2001) is consistent with the characteristic capacity of woody vines for rapid growth in response to increases in resource availability and particularly in response to soil N (Dillenburg et al. 1993). Rapid vegetative responses to increases

in $NO_3^-$ supply is also a characteristic of so-called nitrophilous species (Fichtner and Schulze 1992; Bharucha and Dubash 1951).



Figure 7.38 Effect of no additional nitrogen (LN), foliar sprays of urea (LNF), soil-applied nitrogen (MN) and soil plus foliar-applied nitrogen (MNF) on the weight of shoots removed during summer pruning. Combined data from two pruning times (January and March). Error bars indicate standard error.

Foliar-applied urea did not increase vegetative vigour in either LNF or MNF despite the significant quantity of N supplied with the 11 urea sprays (Figure 7.2). Other authors have also reported foliar-applied N to be less stimulatory to vegetative growth than soil-applied N (Klein 2002; Dong et al. 2005). It might be that foliar-applied urea does not stimulate vegetative growth because it is assimilated more directly, bypasses the root system, and avoids a large increase in soil and plant $NO_3^-$ concentration as would occur with a soil N application (Chapter 1, section 1.3.5). The result is therefore consistent with $NO_3^-$ being fundamentally involved in the vegetative growth response to N fertiliser.

### 7.3.5 Fruit growth

Soil-applied N had no significant effects on fruit FW, DW, or DM% although average FW was increased by about 5% and DM% was reduced by a similar amount (Table 7.2). This pattern is consistent with the results of experiments reported in Chapters 3 and 6, where the main effect of soil-applied $NO_3^-$-N was to increase water influx to

the developing fruit without a proportionate increase in dry matter accumulation. This might be because the more vigorous growth of the shoots in the MN vines has increased their sink strength relative to the fruit, thereby monopolizing any increased supply of assimilates that might have resulted from the increased leaf area or photosynthetic capacity of these vines.

However, foliar-applied urea significantly increased fruit FW (P<0.05) in LNF compared to LN vines and average FW of MNF was also greater than MN although in these vines the difference was not statistically significant (Table 7.2). Foliar-applied N may have also increased fruit DW in both LNF and MNF compared to LN and MN respectively, although these differences were not statistically significant (Table 7.2). The increases in average DW obtained with foliar-applied N were not sufficient to prevent a decrease in average DM% with both foliar and soil-applied N (Table 7.2). However, the effect of N on DM% was only significant in the case of MNF compared to LN. Thus fruit size increases obtained with fertiliser N may have been accompanied by reductions in fruit quality in terms of DM% regardless of how the N was supplied.
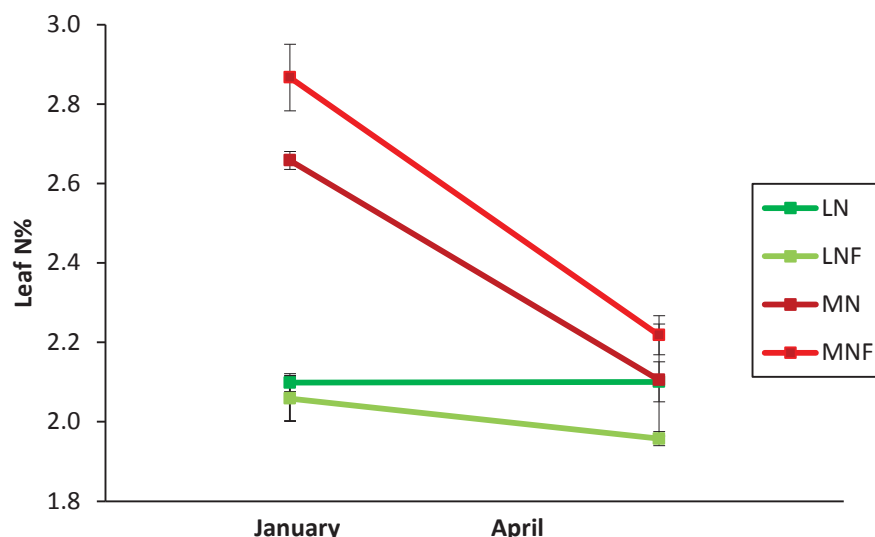
Table 7.41 Effect of no additional nitrogen (LN), foliar sprays of urea (LNF), soil-applied nitrogen (MN) and soil plus foliar-applied Nitrogen (MNF) on mature 'Hayward' fruit fresh weight (FW), dry weight (DW), and dry matter concentration (DM%) at harvest 165 days after full bloom (18 May 2010).

|        | LN            | LNF           | MN             | MNF           |
|--------|---------------|---------------|----------------|---------------|
| FW (g) | 97.5 (2.7)a   | 105.5 (1.9)b  | 102.6 (2.6)ab  | 106.4 (2.1)b  |
| DW (g) | 15.9 (0.5)a   | 16.6 (0.4)a   | 16.0 (0.3)a    | 16.5 (0.6)a   |
| DM%    | 16.3 (0.2)a   | 15.7 (0.3)ab  | 15.6 (0.2)ab   | 15.5 (0.3)b   |

Standard errors in parenthesis, values with different letters within the same row are significantly different at p≤0.05 Fisher's Protected LSD, n=6.

The 5% increase in average FW obtained with a moderate rate of N (80 kg N ha$^{-1}$) applied to the soil just before anthesis (MN cf. LN) was similar to those obtained with a high rate (450 kg N ha$^{-1}$) spread with a series of split applications over the period of fruit development (Chapter 3, Table 3.20). Reduction in DM% in MN compared to LN shown in Table 6.2 was also within the range found over four seasons with 450 kg N ha$^{-1}$ (Chapter 3, Table 3.20). Thus regardless of the amount of $NO_3^-$-N fertiliser

248

applied to the soil the effect was to increase fruit water uptake rather than dry matter accumulation. As already discussed in Chapters 3 and 6 the reason might be found in the relationships between $NO_3^-$ and the regulation of shoot vigour and vine water content.

The 8% increase in average FW obtained with 80 kg N ha$^{-1}$ supplied by foliar application of urea in the absence of soil-applied N (LNF) was also similar to the size of the FW increases obtained with only four applications of foliar-applied urea in the same season and orchard (Chapter 5, Table 5.11). The 4% increase in average DW in LNF compared to LN was also the same as that obtained with four foliar-applied N applications, as reported in Chapter 6, Table 6.10. Based on the results shown in Table 7.2 and Figure 7.2, eleven foliar applications of 1% urea is an effective way to supply N where soil N fertiliser is being withheld as part of a vigour management strategy.

### 7.3.6 Effect of shoot type

Fruit was sampled separately from short (<50 cm) and long (>90 cm) shoots because differences in fruit size and sink strength have been associated with shoot type (Chapter 3, section 3.3.8; Chapter 6, section 6.3.2.3; Lai et al. 1990). Soil-applied N appeared to have less effect on FW and DW of fruit from long shoots than it did on fruit from short shoots (Table 7.3). Foliar-applied N appeared to have similar effects on both short and long shoots in the absence of soil-applied N (LNF cf. LN; Table 7.3) but appeared less effective in short shoots when combined with soil application (MNF cf. MN; Table 7.3). The reduction in DM% in all N treatments compared to LN was statistically significant in the case of long shoots but only in the case of MNF in short shoots (Table 7.3).
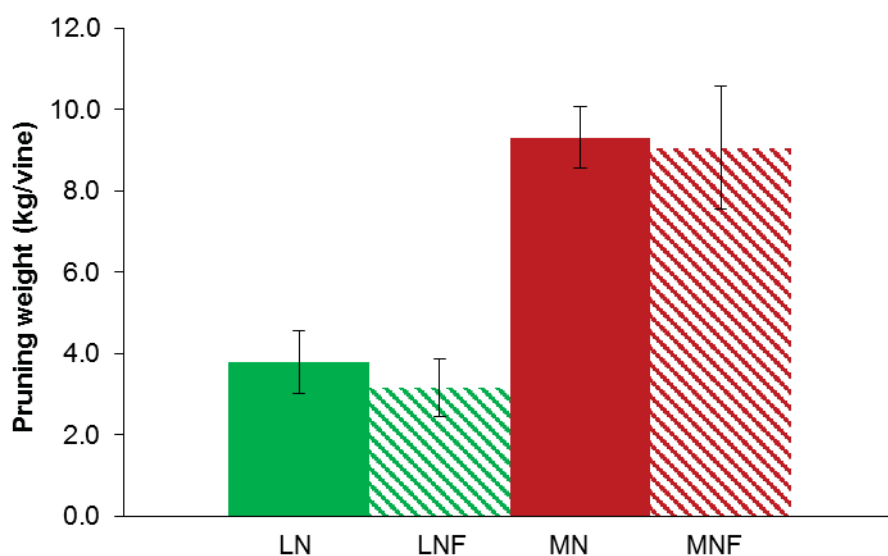
Table 7.42 Effect of no additional nitrogen (LN), foliar sprays of urea (LNF), soil-applied nitrogen (MN) and soil plus foliar-applied nitrogen (MNF) on fresh weight (FW), dry weight (DW) and dry matter concentration (DM%) of 'Hayward' fruit from long (>90 cm) and short shoots (<50 cm) harvested 165 days after full bloom.

| | Shoot type | LN | LNF | MN | MNF |
|---|---|---|---|---|---|
| FW (g) | Long | 99.2 (3.2) | 106.8 (3.6) | 101.7 (4.0) | 110.5 (1.6) |
| | Short | 95.7 (4.8) | 104.3 (2.0) | 103.6 (4.1) | 102.2 (1.2) |
| DW (g) | Long | 16.5 (0.4) | 17.1 (0.4) | 16.1 (0.4) | 17.7 (0.4) |
| | Short | 15.2 (0.7) | 16.0 (0.6) | 15.9 (0.6) | 15.2 (0.4) |
| DM% | Long | 16.7 (0.3)a | 16.1 (0.3)b | 15.8 (0.2)b | 16.0 (0.3)b |
| | Short | 15.9 (0.1)a | 15.3 (0.4)ab | 15.4 (0.3)ab | 14.9 (0.3)b |

Standard error in parenthesis, values with different letters within the same row are significantly different at p≤0.05 Fisher's Protected LSD (applies to DM% only – there were no significant differences for FW or DW), n = 3.

Overall, using the combined data for all treatments, fruit from short shoots was of similar size (FW) to fruit from long shoots, but had 8% less dry matter (DW; $p \leq 0.002$) and consequently DM% was also significantly reduced (Table 7.4). This difference between the two shoot types was noted previously in the same T-bar orchard (Chapter 3, Figure 3.8) and in a commercial pergola orchard in a different district (Chapter 6, Table 6.18). The effect of shoot type was also more pronounced in the absence of soil-applied N (LN) in both the 2007-08 season (Chapter 3, Figure 3.8) and in the 2009-10 season (Table 7.3) especially in respect to dry matter accumulation. It is of interest that the largest increases in fruit growth with foliar-applied N were in found in fruit from the short shoots in the absence of soil-applied N (LNF cf. LN) (Table 7.3). Foliar-applied N was also more effective in increasing fruit growth in short shoots than in long shoots in the 2010-11 season reported in Chapter 6 and Table 6.18, although in this case using 0.5% $KNO_3$ rather than 1% urea.

Mills et al. (2008) found that although N% levels of both fruit and leaves were lower in unfertilised 'Hort 16A' vines compared to fertilised ones, the leaves appeared to be less affected. From this it appears that in low N situations shoots are prioritised or compete more strongly for the smaller supply of N than fruit. Competition for N between shoots and fruit is likely to be particularly intense during early fruit development, when both are growing rapidly. It seems reasonable to believe that the relative sink strength or 'priority' of fruit on short shoots would be less than that of

fruit from long shoots, in terms of its ability to attract N as well as in terms of carbohydrates. This might explain the results reported here (Table 7.3) that show significant increases in FW with foliar-applied N applied during the early stages of fruit development and especially in short shoots from LNF.

Table 7.43 Fresh weight (FW), dry weight (DW), dry matter concentration (DM%), and water (W) of mature 'Hayward' fruit from short (<50 cm) and long (>90 cm) shoots at harvest 165 days after full bloom.

|        | Short | Long  | %diff | p-value |
|--------|-------|-------|-------|---------|
| FW (g) | 101.5 | 104.5 | 3.0   | NS      |
| DW (g) | 15.6  | 16.9  | 8.3   | 0.002   |
| DM%    | 15.4  | 16.1  | 5.1   | 0.002   |
| W (g)  | 85.9  | 87.7  | 2.1   | NS      |

p-value from two-tailed T-tests

## 7.3.7 Firmness and soluble solids

The range of values for soluble solids content (SSC) and firmness are within the 'normal' range encountered in 'Hayward' fruit harvested commercially when the fruit is physiologically mature but not yet fully ripe (Beever and Hopkirk 1990). Both foliar and soil-applied N tended to reduce fruit SSC at harvest, but the differences were not statistically significant (Figure 7.2). Fruit firmness was also reduced by the N treatments but the effect was significant only in the case of MNF ($p < 0.04$, ANOVA), which was softer than any of the other treatments (Figure 7.2). Because SSC was not increased in conjunction with reduced firmness there is no indication that any of the N treatments advanced the maturity of the fruit (Beever and Hopkirk 1990).
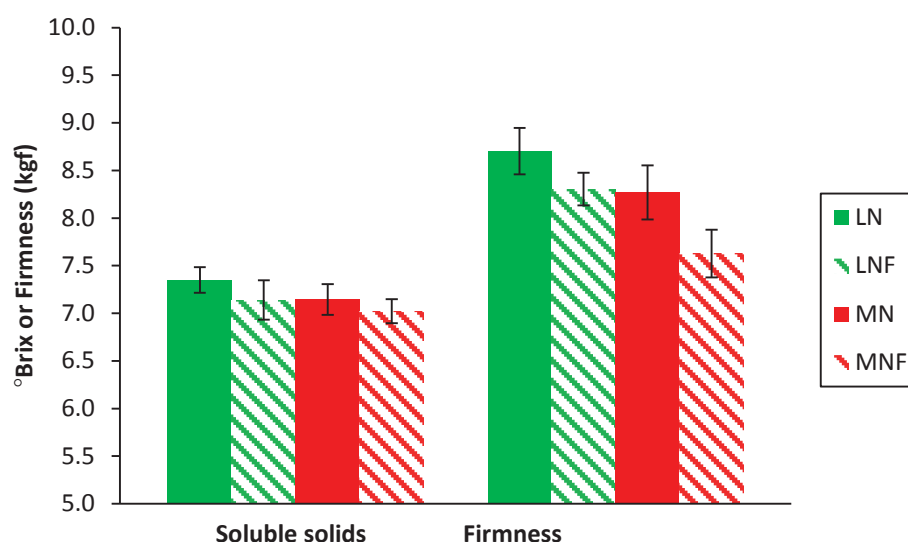
Figure 7.39 Effect of no additional nitrogen (LN), foliar sprays of urea (LNF), soil-applied nitrogen (MN), and soil plus foliar-applied nitrogen (MNF) on fruit soluble solids content (°Brix) and firmness (kgf) at harvest 165 days after full bloom. Error bars indicate standard error.

There were no significant differences in SSC or firmness between short and long shoots from the same canopy position (mid outer canopy) when the data for the N treatments was combined. There were also no significant differences between long and short shoots within the individual N treatments (Table 7.5). Although the average firmness of MNF fruit was lowest in both shoot types, the difference was largest in short shoots where MNF fruit was significantly softer than LN and LNF fruit (Table 7.5). Inconsistent patterns of difference between short and long shoots in °Brix and firmness at harvest were found in other experiments (Chapter 4, Figure 4.2; Chapter 6, Figure 6.5 and 6.19). Pyke et al. (1996) found fruit from short shoots on T-bar vines had slightly higher Brix and were softer than fruit from long shoots. However, inconsistent and complex patterns of spatial variation in brix and firmness within kiwifruit vines and shoots and between individual fruits are generally found (Hopkirk et al. 1986; Pyke et al. 1996). Factors such as the distribution of fruit and light within the canopy, pruning style, and vine vigour probably influence the effect of shoot type on fruit characteristics.

Table 7.44 Effect of no additional nitrogen (LN), foliar sprays of urea (LNF), soil-applied nitrogen (MN), and soil plus foliar-applied nitrogen (MNF) and shoot type on fruit soluble solids content (°Brix) and firmness (kgf) at harvest 165 days after full bloom.

| | Shoot type | LN | LNF | MN | MNF |
|---|---|---|---|---|---|
| °Brix | Long | 7.4 (0.1) | 7.3 (0.3) | 7.2 (0.2) | 6.9 (0.1) |
| | Short | 7.3 (0.3) | 7.0 (0.3) | 7.1 (0.3) | 7.2 (0.2) |
| Firmness | | | | | |
| (kgf) | Long | 8.5 (0.3) | 8.1 (0.3) | 8.4 (0.5) | 7.9 (0.2) |
| | Short | 8.9 (0.4) | 8.5 (0.1) | 8.1 (0.4) | 7.4 (0.5) |

Standard error in parenthesis

## 7.4 Summary and conclusions

Although moderate rates of soil-applied nitrogen fertiliser had little effect on the fresh weight or dry matter concentration of the fruit, vegetative vigour was more than doubled. However, the same amount of nitrogen applied to the canopy in a series of urea sprays was able to increase fruit fresh weight without an effect on vegetative vigour. Foliar-applied urea appeared able to stimulate fruit growth even in vines also fertilised with soil applications of nitrogen fertiliser. This supports the idea that foliar applications of urea can be an effective alternative and/or supplement to soil applications of nitrogen fertiliser for kiwifruit vine management. Any increase in costs associated with the urea foliar sprays would be more than offset by the savings in pruning labour costs. Furthermore, foliar application of nitrogen is associated with reduced nitrate leaching compared to soil-applied nitrogen fertiliser, which could potentially reduce the environmental impact and improve the sustainability of kiwifruit production.

# Contents

## List of Tables

## List of Figures

# 8. Manipulation of fruit water and dry matter content by treatments applied during early and late stages of fruit development in 'Hayward' kiwifruit

## 8.1 Introduction

Dry matter percentage (DM%) is an important fruit property strongly linked to fruit quality (Burdon et al. 2004). When fruit size is increased by techniques such as the use of growth stimulants, it can lead to reduced DM% due to increased rates of water uptake without a commensurate increase in DM accumulation (see Chapters 5 and 6). The development of a kiwifruit berry (KF) can be divided (arbitrarily) into three main stages (Chapter 1, Figure 1.1): Stage 1 (anthesis to about 58 days later) in which fruit size increases rapidly; Stage 2 (from about 58 to about 76 days after flowering) – a period of slow growth; and Stage 3 (from about 76 to about 160 days after flowering) when fruit grows more rapidly again until maturity and harvest (Hopping 1976). It was hypothesised that during late Stage 1 of fruit development and again during late Stage 3, fruit cells increase their capacity for expansion and water uptake (see Chapter 1, sections 1.2.2 and 1.3.3; Rose and Bennett 1999). If the water potential gradient between the vine and the fruit is steepened, either by increasing vine water status (i.e., vine water uptake) or increasing the concentration of osmotica in the fruit at either of these times (late Stage 1 or late Stage 3), then the ratio between water and dry matter accumulation by the fruit should shift to favour water uptake, thus reducing DM%.

This chapter reports an experiment intended to explore the effects of manipulating water potential gradients at certain stages of fruit development. Some vines were exposed to excessive water availability, while control vines received no additional water. However, the un-watered control vines were not exposed to a severe water deficit since all the vines had deep root systems able to access plentiful water from below 50 cm depth (Green and Clothier 1995). Therefore this is not a deficit irrigation experiment in the normal sense, where the objective is to expose some plants to a controlled water deficit (Behboudian and Mills 1997). Rather, here the aim was to increase water uptake above normal levels in some of the vines, while maintaining the

257

control vines at close to normal water statuses. Other treatments were designed to interact with the water treatments by modifying cell expandability with N-(2-chloro-4-pyridyl)-N'-phenylurea (CPPU), increasing the supply of assimilates (girdling and CPPU), preventing transpiration by wrapping the fruit, and stimulating fruit growth with foliar-applied urea. The treatments were arranged to give maximum effect during the critical two stages of fruit development identified above. The experimental questions were:

1. How does increasing the availability of water to the vine during early and late stages of fruit development affect fruit water uptake?
2. How are the effects of surplus water modified by girdling, treatment with CPPU, wrapping to prevent transpiration, and/or with nitrogen applied as foliar sprays of urea during early fruit development?

## 8.2 Materials and methods

The experiment used 14 mature 'Hayward' kiwifruit vines growing on T-bar structures (Sale and Lyford 1990) in the Massey University research orchard (details of the orchard and its management are given in Chapter 2). The soil is a Manawatu fine sandy loam and the vines have deep root systems so that botanically significant water deficits are unlikely in most seasons (Cowie and Rijkse 1977; Green and Clothier 1995). The vines were spaced three metres apart in pairs (bays) with each bay separated by a buffer bay (containing two female and one male vine) (Figure 8.1). Full bloom was 4/12/09.

Polythene tents were constructed to cover the soil to the drip line and 90 cm deep trenches were dug on either side of the bays to confine the surface roots within the covered area (Appendix 2, Plate 5). The inner side of the trench was lined with polythene and then the trench was refilled. Separate irrigation lines were installed for two water regimes – un-watered (the control; UW) and 'surplus water'(SW). Micro-sprinklers regulated by automatic timer valves were arranged to wet the whole ground area under the covers. The output of the sprinklers was regularly monitored to ensure water application was equal between the bays and uniform within each bay. Initial

applications were set to deliver 50 mm of water to bring the soil of the surplus-water bays to field capacity (FC), and thereafter two daily irrigations to replace estimated evapotranspiration loss. There were three replicates (i.e., three pairs of vines) for each of two water regimes.

| Row 6 | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| /// | /// | /// | /// | **5** | 6 | 7 | **8** | /// | /// | /// | /// |
| 1 | **2** | **3** | 4 | /// | /// | /// | /// | 9 | **10** | 11 | **12** |
| Bay 1 water | | Bay 2 dry | | Bay 3 water | | Bay 4 dry | | Bay 5 water | | Bay 6 dry | |

| Row 7 | |
|---|---|
| **13** | 14 |
| /// | /// |
| Bay 7 | water |

Figure 8.40 Aerial view of layout for experiment using 2 rows within the orchard (Row 6 and 7); 14 vines in 7 bays (2 vines/bay) with each bay separated by a buffer bay (not shown). Girdled vine number in bold and underlined. Side of canopy treated with CPPU shown by diagonal cross hatch.

| +/- surplus water | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| C | | U2 | | C | | U2 | | |
| CP | | CP+U2 | | CP | | CP+U2 | | |
| Vine 1 – Trunk Girdle | | | | Vine 2 – no Trunk Girdle | | | | |

Figure 8.41 Aerial view of treatment allocation. Detail of one bay containing two vines. Vertical lines represent individual canes and their allocated treatments. Oval shape (⬭) represents the vine trunk.

### 8.2.1 Treatments

A spray of N-(2-chloro-4-pyridyl)-N'-phenylurea at 10 ppm (CPPU) with 0.02% v/v non-ionic organo-silicone surfactant (Breakthru®, Evonik Industries, Germany) was applied at 40 days after full bloom (DAFB; 13/01/10) to one half of the canopy of each vine. This date was chosen because later CPPU application appears to increase the capacity for fruit cell expansion more than division compared to earlier application times (Famini et al. 1997). Four 1% sprays of urea (Yara Urea Tech, Yara Fertilizers NZ Ltd; biuret content 0.65-0.80%) (unadjusted pH 7.56) were applied at approximately 10 day intervals between 34 DAFB (07/01/10) and 60 DAFB (2/02/10) to selected groups of canes on both sides of the canopy of each vine with a manual

backpack sprayer (Croplands Swissmex). No adjuvants (e.g. surfactants) were added to the foliar urea solutions. A trunk girdle was applied to one vine in each bay at 44 DAFB (17/01/10) and repeated at 134 DAFB (17/04/10). The timing of the girdling treatments was chosen to coincide with the two water cycles (see below for details), which also coincided with the two stages during fruit growth when the capacity for cell expansion is highest (Richardson and Currie 2007; Currie and Richardson 2007). Details of the treatment allocation to the vines are further depicted in Figure 8.2.

From 47 to 67 DAFB (20 January to 9 February) water was applied twice every 24 hours to pairs of vines receiving the surplus water treatment to maintain the upper layers of soil close to field capacity. This is the first water cycle (early fruit development). At 74 DAFB (16/02/10, 27 days after the start of the first water cycle) fruit was sampled (early harvest) and the un-watered vines were then irrigated to field capacity. On the day before the start of the first water cycle, 24 fruit on each vine (12 on each side of the canopy) were measured in three dimensions before being carefully wrapped in tinfoil and plastic film. These fruit were also harvested on 16/02/10 (early harvest).

No further water was given to any of the vines until the second water cycle. For the second water cycle (late fruit development), water was again applied as for the first water cycle, this time for the period between 135 and 158 DAFB. Fruit was then harvested at 163 DAFB (16/05/10; 28 days after the start of the second wet cycle; final harvest).

Soil water was measured gravimetrically on soil samples collected 1.5 hours after an irrigation cycle. Gravimetric values were converted to volumetric soil water content by multiplying the gravimetric values by a soil density factor (0.87) derived from soil test data for this orchard (McLaren and Cameron 1996). Leaf water potential $\Psi_{leaf}$ was measured with a Scholander pressure chamber (Boyer 1969; Mpelasoka et al. 1997). Measurement of $\Psi_{leaf}$ was done in the field with each leaf being excised from the shoot and mounted immediately in the pressure chamber to minimise water loss from the uncovered excised leaf (Nuzzo et al. 1990). Measurements were taken at midday at the end of the first water cycle (09/02/10).

For the early harvest, six fruit, and for the final harvest, 18 fruit from each treatment and vine were taken and fresh weight (FW) recorded. Fruit was sliced and an equatorial slice from each fruit was weighed and then placed in a forced air oven and dried (65° C) to constant weight. From the DW data fruit DM% was calculated (DM% = [DW/FW] × 100).

## 8.2.2 Statistical analysis

Data was analysed with single factor ANOVA using GLM procedure of SAS with treatment means compared using Fisher's Protected Least Significant Difference (SAS Institute Inc., 2004). When there were only two variables, means were compared with two-tailed Students T-tests in Excel. Normal or close to normal distributions were assumed.

## 8.3 Results and discussion

This section begins by presenting measurements of the effects of the surplus water treatment (SW) on soil water content and vine water potential. The effects of the first water cycle on fruit, as observed at the early harvest, are presented first, including treatment effects on fruit wrapped to prevent transpiration. The results from the final harvest, which followed soon after the end of the second water cycle, follow and the chapter concludes with the effects of the foliar-applied urea treatments.

### 8.3.1 Soil water content

The SW treatment significantly increased the soil water content in the 0-20 cm depth during both water cycles ($p<0.002$; Table 8.1). The high water content of SW at the end of the first cycle indicates the soil of these bays was saturated. Although the soil of the un-watered bays (UW) was drier than SW, it was still quite moist with a water content well above the wilting point of about 7% v/v for this soil type (Clothier and Green 1995; McLaren and Cameron 1996; Table 8.1). During the period between the two wetting cycles, the soil in both sets of bays had similar water contents (Table 8.1). At the end of the second water cycle the water content of the soil in UW bays had fallen slightly but was still well above wilting point, while the soil in SW bays was below saturation but significantly wetter than UW bays (Table 8.1). Because the vines in this orchard have a relatively deep root system, reported in earlier research as extending to about 0.9 m beneath the surface, it is probable that the UW vines were able to obtain sufficient water from deeper in the soil profile to maintain transpiration rates (Clothier and Green 1995).

Table 8.45 Volumetric soil water content (% v/v) during and between the first and second water cycles from surplus water and un-watered (control) bays (0-20 cm depth).

|  | Un-watered | Surplus water | p-value |
| --- | --- | --- | --- |
| First cycle | 19.16 (0.97) | 40.68 (2.02) | <0.001 |
| Between cycles | 16.71 (1.53) | 17.67 (1.67) | >0.2 |
| Second cycle | 16.02 (1.02) | 25.81 (0.69) | <0.002 |

Standard error in parenthesis, p-value from two-tailed T-test, n=6.

## 8.3.2 Leaf water potential

That UW vines were able to obtain sufficient water was confirmed by pressure chamber measurements that showed UW and SW vines had similar midday leaf water potentials ($\Psi_{leaf}$) at the end of the second wetting cycle (Table 8.2). The recorded values for $\Psi_{leaf}$ were also similar to previously reported midday $\Psi_{leaf}$ for irrigated kiwifruit vines and well above the levels associated with leaf wilting of $\leq$ -0.9 Mpa (Judd et al. 1989). However, the average $\Psi_{leaf}$ of girdled vines was almost 40% lower than un-girdled vines (p<0.02, 2-tailed T-test) with the effect being similar in both UW and SW vines (Table 8.2). In *A. chinensis* girdling had no significant effect on midday $\Psi_{leaf}$, although that of girdled vines was about 16% lower than un-girdled vines (Black et al. 2012). The smaller effect reported by Black et al. (2012) might be due to their experimental vines being grown in pots within a greenhouse, which may have lowered leaf-air vapour pressure deficits. In girdled grapes, studies have reported more positive midday and pre-dawn $\Psi_{leaf}$ (Williams et al. 2000; Yamane et al. 2009), while in persimmon, girdling resulted in lower daytime $\Psi_{leaf}$ and reduced shoot growth (Fumuro 1998).

Table 8.46 Midday leaf water potential (KPa×100) from surplus water and un-watered (control) vines at the end of the first water cycle 67 days after full bloom (09/02/10).

|  | Un-watered (KPa×100) | Surplus water (KPa×100) |
|---|---|---|
| No Girdle | − 3.00 (0.60)a | − 3.04 (0.64)a |
| Girdle | − 4.24 (0.28)b | − 4.16 (0.32)b |

Standard error in parenthesis. Trunk girdle applied at the start of the water cycle (44 days after full bloom). Different letters denote significant differences, p<0.05, two-tailed T-test.

## 8.3.3 First water cycle

At the start of the first water cycle, average fruit weight was approximately 56 g and 70 g for the non-CPPU and CPPU treated fruit respectively, which are typical weights for 'Hayward' fruit at this developmental stage (47 DAFB; Richardson and Currie 2007) and time after CPPU application (7 days; Antognozzi et al. 1996). The first water cycle supplied surplus water to the SW vines for 21 days (between 47 and 67 DAFB), and one week later, fruit was sampled (early harvest, 74 DAFB). By this time, average fruit weight had increased to 69 g in UW and 75 g in SW reflecting a growth rate of 3.0 and 4.4 g/fruit/week in UW and SW respectively. Growth rates

between 3.5 and 7 g/week for 'Hayward' fruit at similar developmental stages were reported by Green et al. (1990). Compared to this, the growth rates over the period of the 1st water cycle were slow.

At early harvest fruit from un-girdled SW vines had accumulated 9.5% more water and was 8.7% heavier than fruit from un-girdled UW vines (p≤0.02; Table 8.3). Because there was little difference in dry matter accumulation in fruit from SW, DM% was reduced by 6.5% compared to UW (p≤0.02; Table 8.3). In the girdled vines there was a smaller response to surplus water, with fruit not being significantly affected in their water or dry matter contents, although average FW and DW were increased by 4.7 and 3.7% respectively (Table 8.3). However, girdling significantly increased fruit FW by 9.4% in the UW vines (p<0.04) and affected a smaller increase (5.4%) in SW vines (p=0.14). In UW vines dry matter accumulation might have been increased by about 4% with girdling but because of the larger increase in water influx in the fruit from girdled vines (10%, p<0.04), fruit DM% was reduced by 5.3% (p<0.06). In the SW vines fruit dry matter and water contents appear to have been increased proportionately by girdling (average DW increased by 5.7% and W by 5.3%) so that DM% remained unchanged.

The increased water influx in the fruit of un-girdled vines is consistent with the capacity for cell expansion during Stage 1 of fruit growth being more or less realised according to factors that influence the hydraulic gradient to the fruit. Although $\Psi_{leaf}$ did not provide evidence of higher vine water content in SW vines (Table 8.2) this might be because at the time of measurement the soil was temporarily saturated (Table 8.1), which can depress $\Psi_{leaf}$ (Else et al. 1995). The water logging was probably only a transient condition because care had been taken to avoid anoxic conditions in the root zone by limiting the quantity of water applied during the water cycle to that needed to maintain the soil at field capacity (see section 8.2). Furthermore, there was no leaf desiccation on any of the vines, a symptom that is reported to appear quickly if kiwifruit roots are waterlogged for longer than two days (Smith et al. 1990).

Table 8.47 The effect of surplus soil water during early fruit development (47 – 67 days after full bloom) on fresh weight (FW), dry weight (DW), water content (W), and dry matter concentration (DM%) of 'Hayward' fruit from un-watered control (UW) and surplus water (SW) vines, +/- girdle[1], and +/- CPPU, as measured at early harvest (74 DAFB).

|  |  | - CPPU | | +CPPU | |
|---|---|---|---|---|---|
|  |  | - Girdle | +Girdle | - Girdle | +Girdle |
| FW (g) | UW | 69.0 (1.6)a | 75.5 (2.3)b | 90.5 (3.3)c | 96.2 (3.9)c |
|  | SW | 75.0 (1.9)a | 79.0 (1.9)a | 95.5 (2.6)b | 96.5 (2.5)b |
| Difference (%) |  | 8.7 | 4.7 | 5.5 | 0.3 |
|  | p-value | 0.02 | >0.1 | >0.1 | >0.1 |
|  |  |  |  |  |  |
| DW (g) | UW | 7.1 (0.1)a | 7.4 (0.3)a | 8.6 (0.4)b | 8.7 (0.5)b |
|  | SW | 7.3 (0.2)a | 7.7 (0.2)a | 8.5 (0.3)b | 8.9 (0.3)b |
| Difference (%) |  | 2.2 | 3.7 | -1.8 | 1.8 |
|  | p-value | >0.1 | >0.1 | >0.1 | >0.1 |
|  |  |  |  |  |  |
| W (g) | UW | 61.9 (1.6) | 68.1 (2.1) | 81.9 (3.0) | 87.4 (3.4) |
|  | SW | 67.7 (1.7) | 71.3 (1.7) | 87.0 (2.3) | 87.6 (2.3) |
| Difference (%) |  | 9.5 | 4.8 | 6.2 | 0.2 |
|  | p-value | 0.02 | >0.1 | >0.1 | >0.1 |
|  |  |  |  |  |  |
| DM% | UW | 10.4 (0.2)a | 9.8 (0.1)b | 9.5 (0.2)bc | 9.1 (0.2)c |
|  | SW | 9.7 (0.1)a | 9.7 (0.1)a | 8.9 (0.1)b | 9.2 (0.1)b |
| Difference (%) |  | -6.5 | -1.1 | -7.0 | 1.7 |
|  | p-value | 0.02 | >0.1 | 0.02 | >0.1 |

[1]Trunk girdle applied at the start of the water cycle (44 days after full bloom). Standard error in parenthesis; different letters within a row denote significant difference p<0.05, two-tailed T-test; p-value, two-tailed T-test; n=4 SW, n=3 UW;

CPPU treatment increased fruit FW in the non-girdled vines by about 30% (p<0.001; Table 8.3). Dry matter accumulation was also significantly increased by CPPU treatment by about 21 and 17% in UW and SW fruit respectively (p<0.002; Table 8.3). This smaller percentage increase in DW than FW resulted in significantly reduced DM% (UW p<0.02, SW p<0.001). Similar effects on 'Hayward' fruit FW and DW with CPPU have been widely reported (e.g., Costa et al. 1997; Famiani et al.

1997b). Surplus water also increased FW of CPPU treated fruit although the response was smaller than in the untreated fruit and not statistically significant. There might have been a small decrease (about 2%) in dry matter accumulation and because water influx had been increased by over 6%, DM% was significantly lower (p≤0.02). Although girdling increased FW of fruit from the CPPU treated UW vines, girdling appeared to have almost no effect in SW vines, thus there was no discernible response to surplus water in the CPPU treated fruit from girdled vines (Table 8.3). Girdling increased the average dry matter accumulation of CPPU-treated fruit from SW vines, although this increase was not statistically significant, and generally surplus water had little effect on dry matter accumulation in CPPU treated fruit as was the case in fruit not treated with CPPU (Table 8.3).

The main effect of surplus water and girdling was the same in CPPU and non-CPPU treated fruit. Both treatments (girdling and surplus water) increased fruit water influx by similar amounts and this suggests a common mechanism, namely, an increased hydraulic gradient to the fruit. However, while surplus water alters the gradient to the fruit by increasing vine water potential, girdling results in an accumulation of solutes, which lowers $\Psi_{leaf}$ (Table 8.2) and $\Psi_{fruit}$ relative to the rest of the vine (Patrick 1988; De Schepper and Steppe 2011). Water influx with both girdling and surplus water is limited by the capacity of the existing quota of fruit cells for expansion, i.e., by the extensibility of their walls (Patrick 1988). However, CPPU affects cell expansion of individual cells by increasing solute accumulation in the fruit (Antognozzi et al. 1996), which would lower $\Psi_{fruit}$ and thereby increase the hydraulic gradient. CPPU also acts hormonally to increase cell wall extensibility (Antognozzi et al. 1997; Patterson et al. 1993; Ron'zhina et al. 2003; Stoynova-Bakalova et al. 2011), which increases the fruit's capacity to respond to the increased hydraulic gradient. Other cytokinin-related effects probably contribute to the large CPPU response, such as the stimulation of cell division, which increases the fruit's quota of cells and thereby increases the total fruit capacity for water influx.

### 8.3.4 Wrapping fruit

Wrapping fruit during early fruit development (45 to 74 DAFB) to prevent transpiration caused a large reduction in fruit growth (Table 8.4). In the un-watered

vines by early harvest, water influx had been reduced by 23% resulting in a similar sized reduction in FW. However, dry matter accumulation was reduced by wrapping even more, with a 35% reduction in DW (Table 8.4). In the SW vines the effect of wrapping was less severe, with a 25% and 10% reduction in DW and water uptake respectively (Table 8.4). With wrapping, the fruit growth rate over the period of between 45 – 74 DAFB averaged about 0.56 g/fruit/week compared to the average over the same period for the unwrapped fruit of about 2.78 g/fruit/week (average of UW and SW).

Table 8.48 Effects of wrapping fruit during early fruit development (45-74 DAFB) on fresh weight (FW), dry weight (DW), water content (W), and dry matter concentration (DM%) of 'Hayward' fruit from un-watered vines (UW+Wrp) or vines given surplus water (SW+Wrp) compared (% difference) to un-wrapped fruit from un-watered control vines (UW) as measured at early harvest (74 days after full bloom).

| | UW | UW+Wrp | SW+Wrp | Difference (%) | |
| | | | | UW:UW+Wrp | UW:SW+Wrp |
|---|---|---|---|---|---|
| FW (g) | 69.0 (1.6)a | 52.2 (2.4)b | 61.2 (3.7)a | -24.3 | -11.3 |
| DW (g) | 7.1 (0.1)a | 4.6 (0.5)b | 5.4 (0.3)c | -35.4 | -24.8 |
| W (g) | 61.9 (1.6)a | 47.6 (1.9)b | 55.8 (3.4)a | -23.1 | -9.8 |
| DM% | 10.4 (0.2)a | 8.8 (0.6)b | 8.8 (0.2)b | -15.2 | -15.4 |

Different letters within rows denote significant differences, Fisher's protected LSD, $p < 0.05$.

The large reduction in DW in wrapped fruit may reflect the importance of fruit transpiration in regulating or stimulating phloem unloading (Morandi et al. 2010; Clearwater et al. 2012), although other effects such as a reduction in fruit temperature causing reduced starch synthesis cannot be discounted. Tombesi et al. (1993) reported wrapping kiwifruit berries in tinfoil reduced fruit temperatures by 4º to 5ºC, although it is unclear how or at what time they measured this. In their study, fruit remained wrapped in tinfoil until harvested mature at the end of the season and although DW was reduced, FW was not. This suggests that had we left the wrapped fruit until the final harvest, some recovery of FW may have occurred due to the capacity of fruit to eventually realise inherent growth potentials given sufficient time (Snelgar et al. 2012). High growth rates during early fruit development require water inputs from both xylem and phloem to sustain cell expansion, but during later stages of fruit growth the requirement for water can be met by the phloem (Clearwater et al. 2012;

Morandi et al. 2010). The smaller reduction in wrapped fruit growth in the SW vines demonstrates that the hydraulic gradient to the fruit can be regulated both by fruit transpiration and $\Psi_{stem}$. The higher $\Psi_{stem}$ in SW vines allowed the hydraulic gradient to the fruit to be better maintained in the absence of transpiration. Although there was there was no indication of reduced $\Psi_{leaf}$ in the UW vines (Table 8.2), it is possible there was some xylem backflow from the wrapped fruit in the UW vines due to lower $\Psi_{leaf}$ (Morandi et al. 2010). However, such backflows appear limited to kiwifruit growing in hot dry climates with high vapour pressure deficits such as California and are not considered important in typical NZ growing conditions (Clearwater et al. 2012).

### 8.3.5 Second water cycle

A second water cycle began at 135 DAFB and was maintained for 24 days. Five days after the water was turned off, all fruit from the vines was harvested (final harvest; 163 DAFB). At the final harvest, fruit from SW was 5.5% heavier and had accumulated 6.2% more water than fruit from UW vines. Although dry matter accumulation may have been slightly increased, it was not sufficient to prevent DM% being significantly reduced (Table 8.5). A reduction in DM% with surplus water was more pronounced in CPPU-treated fruit. Within this series, fruit from SW was 7.2% heavier than UW fruit due to an 8.1% increase in water uptake, but there was little change in dry matter accumulation (Table 8.5). Thus during the late stages of fruit development, CPPU-treated fruit appear to have retained a greater capacity to respond to an increased hydraulic gradient than untreated fruit, presumably due an increased capacity for cell expansion. However, at early harvest it was the untreated fruit that showed a greater response to increased $\Psi_{stem}$ than the CPPU-treated fruit. In this case the accumulation of solutes in CPPU fruit may have resulted in an increased hydraulic gradient and a better realisation of the potential for cell expansion regardless of $\Psi_{stem}$. In contrast, at final harvest, the CPPU treated fruit may have had a greater capacity for water uptake with increasing $\Psi_{stem}$ due to changes in membrane or cuticle permeability (Ghiani et al. 2011; Saladie et al. 2007), which would be consistent with their decreased firmness at harvest and during storage (Iwahori et al. 1988; Cruz-Castillo et al. 1999).

By final harvest, girdling had increased fruit FW by nearly 9% in the UW vines. The response to girdling was even bigger in the CPPU-treated fruit, where there was a 13.6% FW increase (Table 8.5). Dry matter accumulation in UW fruit was also increased by girdling (+8.0%) and the response was also larger when combined with CPPU (+14.6%) (Table 8.5). Thus girdling gave a more balanced stimulus to fruit growth by increasing both water and dry matter accumulation relatively equally. Where fruit had not been treated with CPPU, the FW response to surplus water was similar in girdled and un-girdled vines. However, in CPPU-treated fruit, although there was a similar response to surplus water in un-girdled vines as was found in non-CPPU treated fruit, there was little response to surplus water in the girdled CPPU-treated fruit, especially in respect to dry matter (Table 8.5). The results suggest that as long as there remains a capacity for water uptake (or cell expansion) in the fruit, it can be satisfied by either phloem or xylem inflow. If the hydraulic gradient favours xylem inflow, then the capacity for cell expansion might be realised quickly by xylem water. On the other hand, increased availability of assimilates, as occurs after girdling, increases the $\Psi$ differential between the leaves and fruit, favouring increased phloem flux. This also realises the capacity for cell expansion but simultaneously brings dry matter into the fruit. With the resultant increase in $\Psi_{fruit}$ the hydraulic gradient is flattened and this prevents further influx of water through the xylem.

Table 8.49 The effect of surplus soil water during early (45 – 66 DAFB) and late (135 – 158 DAFB) stages of fruit development on fresh weight (FW), dry weight (DW), water content (W), and dry matter concentration (DM%) of 'Hayward' fruit from un-watered control (UW) and surplus water (SW) vines, +/- girdle[1], and +/- CPPU as measured at final harvest (163 DAFB).

| | | -CPPU | | +CPPU | |
| | | -Girdle | +Girdle | -Girdle | +Girdle |
|---|---|---|---|---|---|
| FW (g) | UW | 104.5 (3.2)a | 113.8 (4.5)b | 134.3 (4.7)a | 152.6 (8.0)b |
| | SW | 110.3 (3.5)a | 119.7 (2.6)b | 144.0 (2.9)a | 155.3 (3.6)b |
| Difference (%) | | 5.53 | 5.21 | 7.18 | 1.83 |
| | p-value | 0.06 | 0.11 | 0.02 | >0.15 |
| DW (g) | UW | 17.5 (0.6)a | 18.9 (0.9)b | 20.0 (0.71)a | 23.0 (1.4)b |
| | SW | 17.9 (0.5)a | 19.1 (0.5)b | 20.4 (0.53)a | 22.8 (0.6)b |
| Difference (%) | | 2.05 | 0.76 | 1.75 | -0.65 |
| | p-value | >0.15 | >0.15 | >0.15 | >0.15 |
| W (g) | UW | 87.0 (2.6)a | 94.8 (3.7)b | 114.3 (4.0)a | 129.6 (6.6)b |
| | SW | 92.5 (3.0)a | 100.6 (2.2)b | 123.6 (2.4)a | 132.5 (3.1)b |
| Difference (%) | | 6.23 | 6.11 | 8.13 | 2.27 |
| | p-value | 0.04 | 0.06 | 0.01 | >0.15 |
| DM% | UW | 16.8 (0.18)a | 16.7 (0.25)a | 14.9 (0.15)a | 15.1 (0.20)a |
| | SW | 16.2 (0.15)a | 15.9 (0.14)a | 14.2 (0.17)a | 14.7 (0.20)a |
| Difference (%) | | -3.34 | -4.26 | -5.09 | -2.46 |
| | p-value | <0.01 | 0.01 | <0.01 | 0.07 |

Standard error in parenthesis; different letters within a row denote significant difference p<0.05, two-tailed T-test; p-value, two-tailed T-test; n=4 SW, n=3 UW; DAFB: days after full bloom. [1]Second trunk girdle applied at the start of the 2nd water cycle (134 days after full bloom).

The lack of any significant increase in DM% with girdling in this experiment is not unexpected because the timing of the girdles was intended to increase water uptake more than dry matter accumulation. The first girdle was applied at the normal time for a 'spring' girdle i.e., during the period of rapid cell expansion and the treatment did increase fruit water uptake more than dry matter (Table 8.3). Currie et al. (2003) found a similar effect with a spring girdle at applied at 28 days after mid bloom.

Although the spring girdle was applied considerable later than this at 45 DAFB, the effects were measured before the fruit was mature, whereas Currie et al. (2003) were reporting the results from measurements made on mature fruit. The main point to be extracted from the results in this respect, is that the spring girdle did not increase water uptake in SW more than UW; presumably because the capacity for water uptake had already been realised by the girdling, which had affected a 10% increase in water uptake (Table 8.3). The second girdle was applied late in fruit development and well outside the normal time for a 'summer' girdle (Currie et al. 2003). The overall response to both girdles did support the idea that increasing solute accumulation during early and late stages of fruit development could increase water uptake relative to dry matter if the girdling coincided with a period of increased soil water availability. With the combination of girdling and increased water availability at early and late stages of fruit development, fruit water uptake was increased by 15.6%. When these two treatments were combined with the hormonal effects of CPPU, acting both on cell wall expandability and solute accumulation, the increase in fruit water uptake was 52.3%. The overall response to CPPU was similar to those reported elsewhere for *A. deliciosa* (Woolley and Cruz-Castillo 2006; Famiani et al. 1997) and when applied at a similar stage of fruit development (Brown 2009). The experiment did not allow accurate differentiation of the effects of the two girdles or water cycles because fruit was not sampled before the start of the second water cycle and girdle. The fairly equivalent proportional increase in dry matter and water accumulation with girdling noted at final harvest may have reflected the longer lasting effect of the first girdle in providing increased assimilate availability extending during Stage 2 of fruit development.

## 8.3.6 Foliar-applied nitrogen

Four applications of 1% foliar-applied urea between 34 and 60 DAFB gave small FW and DW increases of about 3% over all the treatments at final harvest. Only within the girdled UW vines were the effects of foliar-applied urea larger and approaching or reaching statistical significance (Table 8.6). Although the effects of urea were probably greater in CPPU-treated fruit, the statistical significance was lower (Table 8.6). When the data for un-girdled and girdled vines was combined, the statistical significance of the effects of urea in CPPU-treated fruit was increased (FW and DM%: $p = 0.05$; DW $p = 0.03$; W $p = 0.06$).

Table 8.50 Effects of foliar urea sprays during early fruit development on fresh weight (FW), dry weight (DW), water content (W), and dry matter concentration (DM%) of 'Hayward' fruit at final harvest (163 DAFB) from girdled[1]. un-watered (control) vines.

| | -CPPU | | | | +CPPU | | | |
|---|---|---|---|---|---|---|---|---|
| | -urea | +urea | % diff | p-value | -urea | +urea | % diff | p-value |
| FW (g) | 110.6 | 117.0 | 5.9 | 0.31 | 142.7 | 162.4 | 13.8 | 0.07 |
| DW (g) | 17.9 | 20.0 | 11.8 | 0.06 | 21.5 | 24.6 | 14.8 | 0.73 |
| W (g) | 92.6 | 97.0 | 4.7 | 0.41 | 121.3 | 137.8 | 13.6 | 0.09 |
| DM% | 16.2 | 17.1 | 5.6 | <0.01 | 15.0 | 15.1 | 0.6 | 0.07 |
| Standard error | | | | | | | | |
| FW | 3.0 | 6.1 | | | 7.8 | 7.9 | | |
| DW | 0.6 | 1.1 | | | 1.3 | 1.4 | | |
| W | 2.5 | 5.1 | | | 6.6 | 6.6 | | |
| DM% | 0.3 | 0.2 | | | 0.1 | 0.3 | | |

p-value from two-tailed T-test, n = 10. [1.]Second trunk girdle applied at the start of the 2nd water cycle (134 days after full bloom).

The results suggest that the response to girdling and CPPU may be limited by N and that foliar-applied N may be useful in such situations for providing a supplemental N supply. It is unclear if urea stimulated the supply, transport, or utilisation of assimilates that resulted in the dry matter increases found. Nevertheless, the results do confirm that foliar urea sprays applied during early fruit development may improve both FW and DM% of kiwifruit as reported in Chapters 5, 6, and 7.

## 8.4 Summary and conclusions

The results show that surplus water during early and late stages of fruit development can reduce dry matter concentration of the fruit by increasing water uptake, and the effect may be greater when fruit has been treated with growth stimulants. This might be due to the increased capacity of such fruit to respond to an increased hydraulic gradient with increased rates of cell expansion and water uptake. The results are also consistent with earlier research on kiwifruit that has shown fruit development to be very sensitive to vine water status. Wrapping the fruit to prevent transpiration showed the importance of transpiration for regulation of both dry matter and water accumulation. The smaller effect of wrapping in vines given surplus water also showed the importance of vine water status in modifying the effects of transpiration on fruit growth.

The results also show that foliar urea sprays during early fruit development may be a useful technique for improving both fruit size and quality (dry matter concentration) of kiwifruit. The relatively large variability in the effects of urea might be reduced with more uniform spray application techniques and if the experiment was repeated on a larger scale using uniform vines.

# Contents

## List of Figures

# 9. General discussion

## 9.1 Retrospect

The research presented in this dissertation was initiated with the intention to address two problems facing contemporary kiwifruit production in New Zealand related to the inefficient use of fertiliser. In comparison with some other fruit crops, fertiliser inputs to kiwifruit orchards appeared higher than were necessary based on estimates of the actual demand for nutrients (see Ch.1, section 1.3.1, Table 1.3). The first problem created by this situation is the risk of adverse environmental effects caused by fertilisers when they leach or are washed out of the root-zone and into environment adjacent to the orchards. Agricultural industries are being increasingly compelled by various regulatory demands to be accountable for the environmental impact or 'foot print' of their production processes and to use resources efficiently in the interests of sustainability (section 1.3.1).

Either of the three macronutrients nitrogen (N), phosphorus, or potassium could have been selected for study, since all three were often applied in relatively large amounts to kiwifruit orchards (Table 1.3). However, it was decided to focus on N since this fertiliser nutrient is not only well known as a potential environmental contaminant, but could also be linked to the problem of impaired fruit quality due to reduced concentration of solids in the fruit (DM%) (sections 1.3.8, 1.3.9). A potential link between excessive N fertilisation and reduced fruit DM% was thought to exist due to certain properties of the nitrate ion ($NO_3^-$), which predominates and is present in elevated levels following N fertilisation (sections 1.3.4, 3.3.1; Prasad et al. 1988). Furthermore, selecting N fertilisation as a topic for research was supported by the wide divergence of advice offered to growers regarding the optimum rate and timing of N fertiliser to apply to kiwifruit (Sale 1997a, b; Perham 1990; Warren 1989; Sher 1991; Smith and Buwalda 1991; Buwalda et al. 1990; Vizzotto et al. 1999; Mills et al. 2009).

Application of N solutions directly to young 'Hort16A' fruitlets was also attempted during the method development stage of the research for this thesis, as a way of increasing the $NO_3^-$ content of the fruit. This had an unexpected result (data not presented) of increasing fruit

growth and dry matter content, particularly when the foliar N treatments were combined with a proprietary biostimulant (Benefit Kiwi [®]) and led to the broadening of the research project to include effects of foliar-applied nitrogen. Comparing foliar application with soil application of N then became a central theme of this thesis. On the one hand were the negative effects of soil-applied N fertiliser of reducing fruit DM% (albeit with some increase in fruit size) and generating excessive shoot growth (vegetative vigour), while on the other hand were the positive effects of foliar-applied N on improving fruit size and DM% without affecting vegetative vigour. Interactions with bio-stimulants (Benefit Kiwi [®] and seaweed extract) and plant growth regulators (CPPU) and various other treatments such as girdling and surplus water (section 8.2.1) were primarily intended to create a wide range of physiological conditions in the fruit so that observations could be made on the physiological basis for the regulation of dry mater and water influx to the fruit.

## 9.2 Vegetative growth

The most noticeable effect of soil-applied $NO_3^-$ fertiliser was to increase shoot growth by up to 150% (Figures 3.2, 3.3, 6.1, 7.2). Although increased shoot biomass is a common response to increased N supply, in other species, vegetative growth responses are generally smaller. For example, a 13% increase in the dry weight (DW) of leaves and primary shoots was found in apples with an increased N supply (Bar-Yosef et al. 1988). The large increase in shoot biomass in response to soil-applied $NO_3^-$ fertiliser is typical of the capacity of many woody lianas for rapid shoot growth in response to increases in resource availability, particularly of soil N (Dillenburg et al. 1993). Consistent with this, Conradie (2001) reported that in grapevines, another woody liana, an application of only 50 kg N ha^{-1} at bud-break increased shoot biomass by 70%.

Many lianas have indeterminate growth habits and are capable of sustaining high rates of vegetative growth over long periods. In kiwifruit, active vegetative growth can persist simultaneously throughout the time of fruit development, and the vines can be 'extraordinarily vigorous' (Ferguson et al. 1987). This is in contrast to tree crops such as apples, where the growth of fruit and shoots are temporally separate and more determinant (Palmer 2007). A single *Actinidia* vine in the wild can cover 300 square meters, which is

much greater than, for example, a wild apple tree (Li and Lowe 2007). Hence, the greater harvest index of apple compared to kiwifruit is not just because apples have been cultivated for a longer historical period and have been bred specifically for this characteristic, but is also attributable to a fundamental ecological and botanical difference between the two fruit species (Teramura et al. 1991; Schnitzer and Bongers 2002).

Storage or sequestration of excess N is a common feature of perennial plants (Millard 1995). The more indeterminate growth habit of a liana provides a large and expandable sink for excess nitrogen. In other plant species, indeterminate genotypes also respond to N fertilisation with vigorous shoot growth, in contrast to determinate ones which show little vegetative response (Wallace et al. 1990). In lianas, this response to N can be the basis for the competitive dominance of the soil and aerial environment by lianas (Dillenburg et al. 1993). Induction of growth responses by root contact with exogenous $NO_3^-$ is a competitive adaptive strategy that enables the vine to rapidly exploit and sequester resources against potential competitors (Millard and Grelet 2010; Schnitzer and Bongers 2002).

In the case of kiwifruit, the relative weakness of the fruit as a competing sink (Minchin et al. 2010) is another characteristic typical of lianas. Lianas generally allocate less resources to seed reproduction, i.e., fruit have lower sink priority, than in trees, possibly because of the multiple propagative strategies available to lianas (Schnitzer and Bongers 2002). For example, *Pueraria lobata* a common leguminous vine is estimated to dedicate only 1-3% of shoot dry weight to seeds compared to 5 to 20% considered normal for temperate woody perennials (Teramura et al. 1991). In kiwifruit, the relatively low priority of fruit compared to shoot sinks is evident in the absence of a clear relationship between crop loads and shoot growth (e.g., Burge et al. 1987; Famiani et al. 1997). This is different from other perennial crops such as apples (Cripps et al. 1981, or even grapevines (Edson et al. 1993), where crop load is inversely related to shoot growth. Nevertheless, *Actinidia* genotypes with high harvest index and in which vegetative growth is strongly repressed by high crop loads do exist and are likely to become important in future kiwifruit production systems. For example, the new yellow fleshed cultivar 'G3' shows much reduced vegetative vigour when allowed to carry high crop loads and is capable of very high levels of productivity (Anon 2010; Snelgar 2011). The priority in a liana can be more towards vegetative dominance of the available resources rather than seed production for reproduction (Schnitzer and Bongers 2002). The capacity of lianas for such strong vegetative growth is in fact a reproductive strategy (Schnitzer and

Bongers 2002). It could also be said that there is a trade-off between fruit and vegetative reproductive strategies regulated by N in vines such as kiwifruit, at least in the case of the vigorous *A. deliciosa* and *A. chinensis* cultivars that have dominated the New Zealand kiwifruit industry to date.

Other characteristics typical of lianas also help to explain how kiwifruit differ from perennial tree crops. For example, whereas Palmer (1988) found a root/shoot ratio of 0.22 before pruning with five-year-old 'Crispin' on the dwarfing M.27 rootstock, Clark and Smith (1992) reported a root/shoot ratio of 0.98, before pruning with six-year-old vines of 'Hayward'. Although Ferguson and Bank (1986) found a lower value of 0.78 with fifteen-year-old 'Hayward' vines, this is still much higher than observed with apples on dwarfing rootstocks. Roots are more important in lianas for storage of reserves (Teramura et al. 1991) and in kiwifruit, roots can contain 90% of total vine starch content in winter (Smith et al. 1992). This is consistent with the increases fruit dry matter accumulation obtained with girdling kiwifruit during the main period for fruit dry matter accumulation (Stage 2 of fruit development, Figure 1.1; Currie et al. 2008b), which coincides with the beginning of the main period of root growth (Buwalda and Hutton 1988). Kiwifruit roots are also the major site for storage of N and can contain more than 70% of the total dormant vine N content (Clark and Smith 1991; Kotz and de Villiers 1989). The emergence of new cultivars may in the future make kiwifruit behave more like apples in terms of the priority of sinks and the proportion of resources allocated to fruit.

In Chapter 3, induction of a strong vegetative growth response by N appeared to be a direct response to soil $NO_3^-$ rather than a reflection of vine N status. The similar shoot growth rate of vines given high rates of N fertiliser (HN) and vines given no N fertiliser (LN) in the previous two seasons as measured over a two week period before N fertiliser had been applied in Year 3 (Table 3.10) provided some evidence for this. Even a relatively moderate level of soil-applied N fertiliser (80 kg N ha$^{-1}$) induced a strong vegetative response (Figure 7.2).

The physiological basis for the vegetative response to soil-applied $NO_3^-$ probably involves a combination of several effects, of which the two main ones are (Figure 9.1):

(1) exposure of roots to $NO_3^-$ increases fine root proliferation with an associated increase in cytokinin synthesis and translocation to the shoots; in the shoot

meristems cell division is stimulated by the increased levels of cytokinins (van der Werf and Nagel 1996; Gebler et al. 2004; Sakakibara et al. 2006);

(2) exposure of membranes to $NO_3^-$ signals the activation of aquaporins, which combined with the osmotic effects of $NO_3^-$, its assimilation products, and accompanying cations, results in increased water uptake and hydraulic gradients that promote cell expansion (Okajima 1977; Guo 2007; McIntyre 1997).

Nitrate and the immediate products of its reduction are particularly effective in the stimulation of vegetative growth because they integrate the two primary drivers and components for growth – protein and water (McIntyre 1997); thus explaining the close synergetic relationship between water and N in the stimulation of growth (Jose et al. 2003; Benson et al. 1992).
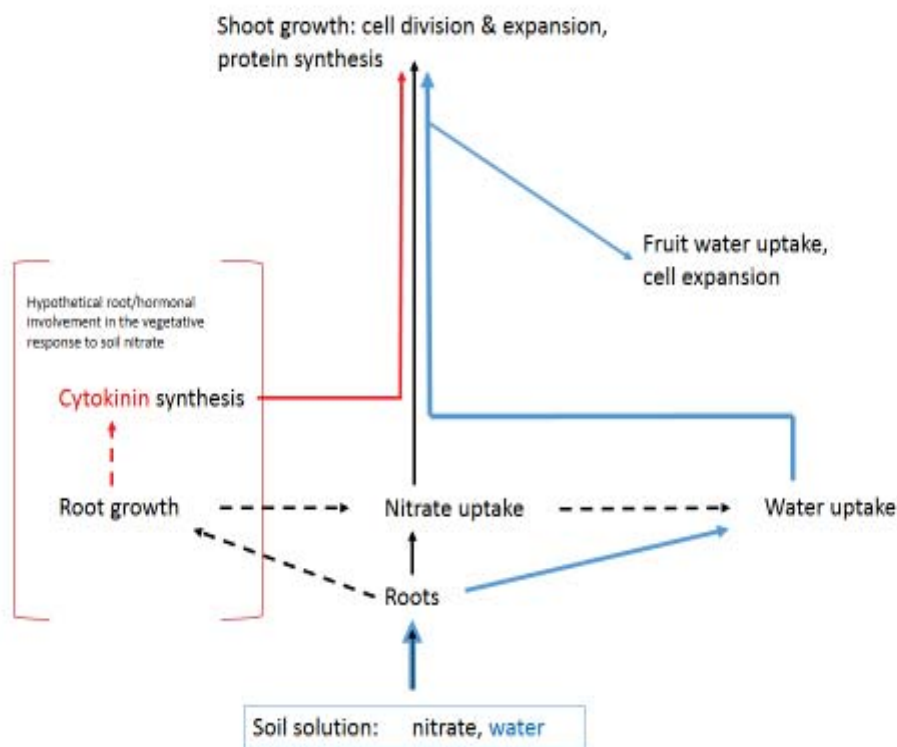


Figure 9.42 Schematic representation of the proposed effects of nitrate (black arrows) on vegetative and fruit growth of kiwifruit. Dashed arrow: stimulates target, solid arrows: flow towards. See text for details.

## 9.3 Fruit growth

The application of a nitrate-nitrogen fertiliser approximately every twenty days during the period of fruit development was intended to maximise the duration as well as the intensity of $NO_3^-$ related effects within the vine (Chapters 3, 4, and 6). It was originally hypothesised that maintaining a high level of $NO_3^-$ within the vine would reduce starch synthesis and thereby reduce fruit DM% (sections 1.3.9, and 3.1). Reduced starch synthesis with increasing levels of $NO_3^-$ was reported by Scheible (1997) and is consistent with the generally inverse relationship between carbohydrates and increasing levels of N nutrition (Marshner 2002). Despite the prolonged period of elevated $NO_3^-$ uptake and a much increased $NO_3^-$ content in the xylem sap induced by the fertiliser treatment, it had only small or moderate effects on fruit DM% ranging from a reduction in absolute terms of 0.3% in Year 1 to 6.0% in Year 4 (Table 3.20). Furthermore, reduced DM% was more the result of increased water influx to the fruit rather than reduced dry matter accumulation (Table 3.20).

Fruit growth was increased by high N supply due to increased fruit water uptake in two out of four of the experimental seasons (Year 2 and 4, Tables 3.17, 3.19, and 3.20). Increased water influx to the fruit suggests a steepened hydraulic gradient to the fruit was created by the increased vine water uptake and content induced by $NO_3^-$ (section 3.3.4; Chapter 8; Figure 9.1). The lack of a corresponding and simultaneous increase in dry matter accumulation is consistent with the separated regulation of water and dry matter influx in fruit (Figure 9.2; Han and Kawabata, 2002; Coombe 1976). Fruit water uptake is driven by hydraulic gradients generated in the fruit by cell turgor and solute accumulation (the growth induced water potential differential - Boyer 1988) and by the balance between vine water uptake, and canopy and fruit transpiration. In kiwifruit the sensitivity of fruit to vine water status is especially pronounced during early stages of fruit development (Judd et al. 1989) when both xylem functionality and fruit transpiration are highest (Morandi et al. 2012). In contrast to water, dry matter arrives at the fruit primarily via the phloem, which in kiwifruit, like apple, probably involves active phloem unloading and therefore depends more on the supply of assimilates from the leaves (Morandi et al. 2012; Patrick 1997). Although photosynthesis and the supply of assimilates can be increased by increasing N uptake and leaf N content, the relationship is curvilinear due to metabolic inefficiencies at higher leaf N levels (Manta et al. 2005). Thus increased leaf chlorophyll content found with higher rates of N fertilisation of

kiwifruit (Smith and Miller 1992) is likely to be associated with lower chloroplast efficiency (Jia et al. 1999; Bondada and Syvertsen 2003). Higher leaf N levels are also associated with lower Rubisco activation and increased respiration rates (Manta et al. 2005). The capacity of the developing fruit to attract a greater supply of assimilates in conditions of supra-optimal N supply is further limited by the associated increased competitive strength of the shoots.
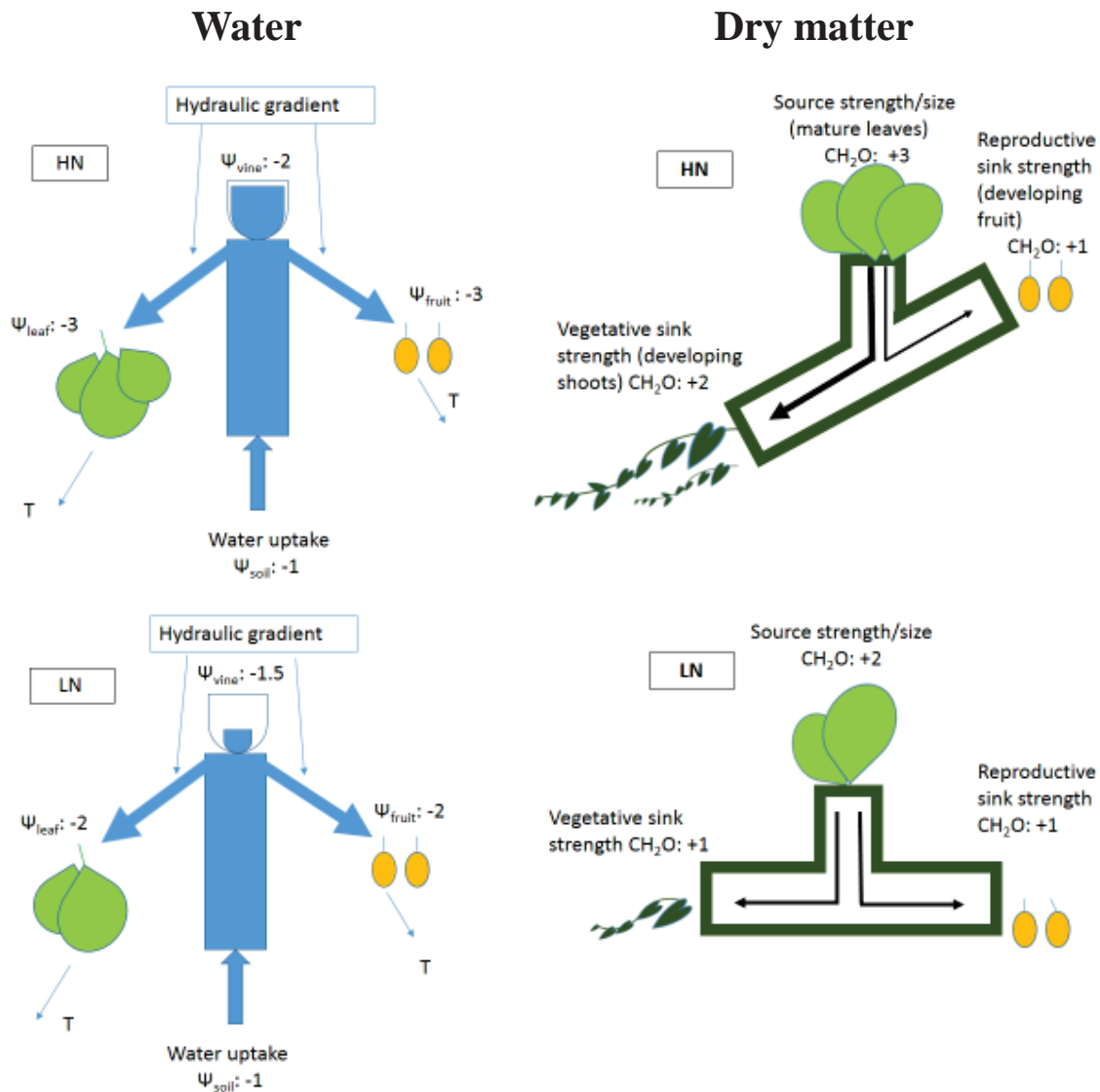


Figure 9.43 Schematic representation of the proposed separated regulation of water and dry matter accumulation by developing *Actinidia* spp. fruit showing the effect of competition for dry matter between shoots and leaves, and dependence of water uptake on hydraulic gradients that may or may not differ between vegetative and reproductive sinks and the way these relationships may change under conditions of high (HN) or nil (LN) rates of nitrogen fertilisation. Units are hypothetical only and represent relative increases or decreases in sink or source strength (source/sink defined in section 1.2.1); T = transpiration; $CH_2O$ = carbohydrate (assimilate); $\Psi$ = water potential. = leaf; = fruit; = shoot.

In contrast to the effects of soil-applied N on mature fruit, at an early stage of fruit development in Year 1, Year 2, and Year 4, fruit from 'Hayward' vines given high rates of soil-applied $NO_3^-$ fertiliser appeared to have stronger growth (i.e., increased fresh weight) and sink strength (i.e., dry matter accumulation) than fruit from unfertilised vines (Chapter 3, Tables 3.15, 3.17, and 3.19). In contrast, Minchin et al. (2010) found stimulation of shoot growth reduced fruit growth and dry matter accumulation, especially if shoot growth was stimulated in a period within about 10 days before fruit set. Although shoot growth was not recorded in Year 1 and 2, based on the consistent large vigour responses found in Year 3 (Figure 3.2), and two different experiments in Year 4 (Figures 6.1 and 7.2) it seems reasonable to assume that the increased dry matter accumulation in HN fruit at the earliest sampling times have occurred despite greater competition for assimilates from vegetative sinks. The study reported by Minchin et al. (2010) was undertaken by manipulating shoot growth of individual shoots and recording the effects on fruit on the same girdled shoots; whereas the effects on fruit reported in Chapter 3 integrated whole canopy effects and might have been due to an increased canopy photosynthetic rate and assimilate supply as a result of increased N availability as suggested by Buwalda and Meekings (1993). The sink strength and capacity for water uptake of the fruit might both have been increased by higher N availability. Nitrogen, and in particular $NO_3^-$, interacts strongly with the hormonal regulation of processes such as cell division and cell expansion (Sakakibara et al. 2006). Stimulation of fruit growth by increased N availability would be consistent with the increased responsiveness of fruit to the stimulation of cellular growth processes during Stage 1 (section 1.2.2) of fruit development (Famiani et al. 1997) as is found with the use of exogenous growth regulators and biostimulants (e.g., CPPU and Benefit Kiwi®). Additional beneficial effects of high N availability early in the season could include higher fruit numbers (Table 3.11) and reduced shoot abortion (section 3.3.3.2). Other studies with kiwifruit also reported N fertilisation improved yield due to increased bud-break and flower numbers (Smith and Miller 1992; Buwalda et al. 1990).

The 18% increase in fruit number/shoot between HN and LN could also represent a significant increase in yield and profitability (Table 3.11). Increases in kiwifruit yield associated with high rates of N fertilisation were attributed to increased fruit number by Buwalda et al. (1990) and Smith and Miller (1991). The significant increases in fresh weight (FW) with soil-applied N in some seasons (Figure 3.20) may represent another way of increasing orchard returns, especially if such increases could be obtained without significant

reductions in fruit quality. However, this approach could be limited by higher labour costs for management of more vigorous canopies (Figures 3.2 and 6.1).

In summary, the results suggest soil-applied N gives some advantage in terms of fruit size and yield but fruit quality can be reduced in terms of the fruit's dry matter concentration. The results also indicate that the timing and rate of N application may influence the balance between beneficial and adverse effects of N fertilisation.

### 9.3.1 Optimising nitrogen fertiliser application

To have the advantages of high N availability during spring but a less vigorous canopy during summer when fruit is developing would be ideal. To achieve this it would help to understand how persistent are the invigorating effects of $NO_3^-$ after a single application of N fertiliser. The use of the petiole sap nitrate testing procedure described in Chapter 2 (section 2.6) would make monitoring the time course of $NO_3^-$ uptake in relation to single soil applications at varying rates easy and quick. Similarly easy would be the measurement of shoot elongation rates which would allow the derivation of correlations between sap petiole $NO_3^-$ concentration and vegetative vigour.

Inorganic forms of N in the soil can change rapidly due to plant uptake, microbial activity, and the high mobility of $NO_3^-$ (Eagle and Matthews 1958). Following an application of N at a rate equivalent to 200 kg N ha, the $NO_3^-$ concentration in the soil solution was increased from a background level of about 5 mM up to 20 mM (Yanai et al. 1998). The effect persisted for up to 20 days after the application, but as plants absorbed the nitrogen, over the next 20 days levels fell back to become similar to those existing before the application (Yanai et al. 1998). If a similar efficient sequestering of mineral N from the soil solution occurs with kiwifruit, which seems likely given the characteristics typical of lianas (section 9.2) and from descriptions of kiwifruit root distributions (Gandar and Hughes 1988), the invigorating effect of $NO_3^-$ might be relatively short lived. Recommendations for N fertiliser use for kiwifruit usually include a split application, with one application made at bud-burst and another in November (i.e., close to anthesis) (Sale and Lyford 1990; Fletcher 1973; Smith et al. 1985). However, significant N uptake is unlikely to occur until the leaves have developed sufficiently to create a significant transpirational flux. This is because the majority of N uptake is by mass flow depending on water uptake and $NO_3^-$ concentration in the soil solution (Miller and Cramer 2004; Simunek and Hopmans 2009). The much lower $NO_3^-$

concentration in bleeding sap in N-fertilised vines at budbreak (Table 3.8) than by anthesis (Table 3.3) supports this conclusion. Delaying a single spring application until the canopy has developed would also reduce the risk of $NO_3^-$ being leached out of the rootzone since vine uptake and a more active microbial biomass at this time would ensure rapid immobilisation. Omission of a second N application around the time of anthesis might lead to a 'quietening' or reduced shoot vigour during the summer when fruit is developing. Therefore, a single N application after budbreak but not later than one month before anthesis is recommended to allow the early season benefits of higher N levels early in the season, avoid excessive vegetative vigour during the main period of fruit development, and minimise the risk of $NO_3^-$ leaching.

## 9.4 Foliar-applied nitrogen

Evidence presented in Chapters 5, 6 and 7 supports the use of foliar-applied N to supplement that supplied by soil applications of fertiliser or derived from the mineralisation of soil organic matter. Furthermore, foliar-applied N appears not to stimulate shoot growth in the same way as soil-applied N even when comparative amounts are applied (Figure 7.2). When applied during early fruit development it can specifically target and stimulate fruit growth and dry matter accumulation (Chapters 5 and 6).

### 9.4.1 Effects of foliar-applied N on fruit growth

In experiments with 'Hort16A' and 'Hayward' vines described in Chapters 5 and 6 respectively, foliar applications of N were made during mid and late Stage 1 of fruit development (Figure 5.2; Table 6.2). In the second season with 'Hort16A', earlier foliar applications were more effective compared to later ones to stimulate fruit growth and dry matter accumulation (Figure 5.2). In both seasons, seven applications were generally more effective than only three applications in 'Hort16A' (Figures 5.1 and 5.2). However, in experiments with 'Hayward' vines eleven applications spread over a longer period from before Stage 1 to the end of Stage 2 of fruit growth appeared to be no more effective in terms of stimulating fruit growth than four applications during Stage 1. In both cases fruit growth was increased by 8% (p<0.05) and average dry matter accumulation by about 5% (Figures 7.2 and 6.9).

The increases in fruit FW and DW found with foliar-applied N, especially in vines of low N status, as described in Chapters 5, 6, and 7, provides further evidence that increases in vine and/or fruit N content benefits fruit growth (section 9.3) and that both timing and number of applications can influence the response. Results also suggest that foliar-applied N, even when applied at the same N application rate as soil-applied N, is less stimulatory to shoot growth (Figure 7.2). The data presented provides some solid evidence that significant gains could be had in terms of orchard productivity if N status could be maintained in the vines without inducing excessive vegetative vigour.

Snelgar et al (2012) estimated a per hectare increase in orchard gross revenue of between $600 and $1000 per gram (FW) increase in fruit size. Based on this value, the FW increases obtained with foliar-applied N as reported in Chapters 5 and 6 could potentially represent an additional $3600 to $15,000 per hectare of income for the grower depending on size and yield. The cost of three foliar N applications is estimated to be between $150 - $200 per hectare (J. Evans personal comment).

Future trials should look at the effect of foliar-applied N applications on kiwifruit vines of varying N status (see section 9.9). Such work should also take into account run-off and ground water contamination resulting from soil applications of nitrogen compared to foliar applications.

## 9.5 Nitrogen reserves

Nitrogen taken up by a crop that is surplus to its immediate needs can be stored as $NO_3^-$ in cell vacuoles (Blom-Zandstra 1989), as amino or amide compounds, or as proteins such as Rubisco and other unspecified storage proteins in various tissues such as bark, leaves, and roots (Millard 1995; Millard and Grelet 2010). Remobilisation of stored or 'reserve' N in the spring of the following season often supplies much or all of the N demand for new canopy development of fruit trees during the first month after bud-break (Millard 1995). The remobilisation of stored N is not regulated by sink strength but rather by source strength – that is remobilisation is determined by the amount of N stored (Millard and Grelet 2010). Therefore, if the increased sap flow of the unfertilised LN vines (section 3.3.3) was related to

the de-polymerisation of storage compounds, it seems likely that it was the hydrolization of stored carbohydrates rather than N compounds that generated the increased root pressures indicated by the sap flow rates (Enns et al. 2000).

In some fruit crops, late-season N applications have been shown to increase N reserves, while being less stimulatory to vegetative vigour than equivalent applications made during the early season (Conradie 2001). The effects of late season N application on vegetative vigour in kiwifruit has received little attention. Clark and Smith (1992) reasoned there would be little purpose in late season applications because reserves in roots and aerial components were virtually restored by the end of the season without them. However, their study was conducted on well-fertilised vines given 200 kg N ha$^{-1}$ in spring. The restoration of reserves may be less assured with lower N fertiliser rates. Tagliavini et al. (2000) as cited by Boyd et al. (2007), found autumn-applied N was effectively absorbed by the roots and was remobilised to the shoots in the following spring, apparently without a stimulatory effect on vegetative vigour. Other experiments with late season N applications in kiwifruit have not reported effects on vegetative growth (Boyd et al. 2007; Marsh et al. 1993; Prasad and Spiers 1991). Rapid canopy development in spring is important to maximise photosynthetic capacity, which is relatively slow to develop in kiwifruit (Buwalda et al. 1991b). More research is needed to better understand the potential for late season N applications and other techniques, such as foliar-applied N, to increase vine N status and encourage the early development of high photosynthetic rates. The effects of vine N status rather than N fertiliser rate on photosynthetic rate and shoot vigour need better elucidation.

In macadamia (*Macadamia integrifolia*) vegetative growth was reduced but yields increased from N deficient trees given small monthly applications of just 4 kg N ha$^{-1}$ compared to the growth and yield of trees given 145 kg N ha$^{-1}$ in one application (Stephenson and Gallagher 1989a). The possibility of maintaining optimum vine N status by small but regular N applications should be investigated in future trials. The efficiency of uptake of N from small applications is likely to be higher than from larger application rates and it might be a promising approach to avoid activation of the vine's vigour response mechanisms. Late season foliar applications of N might also be useful to maintain vine N reserves. Efficient absorption and translocation to perennial storage organs of N from late season foliar application is likely (Swietlik and Faust 1984; Xia and Cheng 2004). Higher rates (e.g., >4% w/v urea) are sometimes used in autumn foliar applications since it is reasoned that leaves

will soon senescence anyway so phytotoxic effects are not important (Swietlik and Faust 1984; Weinbaum 1988; Boyd et al. 2007). However, buds could be damaged by high rates of foliar-applied N in autumn with adverse effects on canopy development in the following spring (Wood and Beresford 2000). Therefore a series of foliar N applications at conservative rates (e.g., <2% w/v of urea) in the immediate post-harvest period could be more effective. Future work needs to further explore the relationships between vegetative vigour and fruit growth, and vine N status (see section 9.9). For example, there appeared to be little difference between fertilised and unfertilised vines in early season shoot extension rates (Table 3.10). This suggests that N does not limit spring canopy development in fertile soils because vines can acquire sufficient N made available during the season from the mineralisation of soil organic matter to maintain their internal N reserves. A better understanding of the comparative vigour of vines given one or two relatively large N fertiliser applications compared to vines provided with soil conditions conducive to long term N acquisition is still needed.

## 9.6 Fruit firmness, water, and transpiration

Fruit from vines given high rates of soil-applied N fertiliser had generally lower levels of firmness at harvest and after cool-storage than fruit from unfertilised vines (Figure 4.2). Lower average firmness was also found in fruit given moderate rates of soil-applied N fertiliser (Figure 7.3). In some of the experiments, both the urea and $KNO_3$ forms of foliar-applied N also reduced fruit firmness compared to non-treated (control) fruit (Figures 6.4 and 7.3).

Fruit softening during maturation is due to a combination of cell wall disassembly and water loss (Rose and Bennett 1999; Ghiani et al. 2011; Saladie et al. 2007). While cell wall metabolism is mainly involved in ripening-related textural changes in the flesh, resistance to compression, as measured with the penetrometer, is mainly related to cell turgor (Ghiani et al. 2011). The main barrier to water loss from the fruit is the cuticle (Maquire et al. 2001), which is therefore much involved in the regulation of fruit water status and cell turgor (Ghiani et al. 2011).

Cuticle permeability can be increased by N fertilisation due to reduced density and altered morphology of the cuticular wax layer (Prior et al. 1997; Bondada et al. 2001; Chiu et al. 1992). In leaves, reduced thickness or density of the cuticular wax layer with N fertilisation might be related to the greater surface area of the leaves which the wax must cover (Prior et al. 1997). However, under N-limitation or drought stress, wax synthesis can be stimulated due to an accumulation of sugars, which are the necessary precursors (Prior et al. 1997). This is consistent with wax composition and quantity being actively regulated in response to environmental factors (Post-Beittenmiller 1996). It also offers an explanation for the reduced fruit firmness found in fruit from N fertilised compared to unfertilised vines (Chapter 4, Figures 4.1 and 4.2; Chapter 6, Figure 6.3; Chapter 7, Figure 7.3) and as reported elsewhere for kiwifruit in association with N fertilisation (Prasad and Spiers 1991; Vizzotto et al. 1999; Johnson et al. 1997).

There might also have been a connection between foliar-applied N and the fruit cuticle, leading to increased water loss and subsequent reduced firmness. Evidence for this, at least in the case of urea is found in the widely observed efficiency of urea absorption by leaves (Swietlik and Faust 1984) that is suspected of being due to a chemical interaction between urea and the cuticular membrane that loosens its bonds (Wojlik 2004; Yamada et al. 1965). This interaction not only facilitates efficient uptake of the urea but might also increase fruit transpiration and water losses.

The large reduction in firmness in fruit treated with CPPU might also have been related to an increase in fruit transpiration rate (Figure 6.4). Gas exchange is probably increased by lenticels in the fruit epidermis, which form as the periderm is torn during expansion growth (Hallett and Sutherland 2005). Considering the higher growth rate of CPPU-treated fruit, it seems reasonable to assume that the frequency of tearing and formation of lenticels would also be increased leading to the potential for greater rates of transpiration and water loss. Cuticle permeability can also increase with increasing water content (Kerstien 1996). The percentage water content of CPPU-treated fruit was significantly higher than fruit not treated with CPPU.

Preventing transpiration by wrapping the fruit during early fruit development had a large effect on fruit growth (Table 8.4). This suggests an important role for transpiration in determining growth rates of young kiwifruit. It might also be involved in the fruit growth

responses found with both soil and foliar-applied nitrogen and CPPU, whereby increases in cuticle permeability induced by nitrogen or CPPU resulted in increased fruit transpiration. Such a possibility deserves further research since it could improve our ability to optimise fruit growth rates (section 9.9).

## 9.7 Disease resistance

The increase in vine water content associated with $NO_3^-$ uptake has implications beyond the negative effects of fruit dry matter dilution noted in Chapters 3 and 6. These include the relationship between increased succulence (Chapter 3, section 3.3.4) and the susceptibility to diseases. For example, in stone fruit Vigouroux and Bussi (1999) found large increases in the development of bacterial canker (*Pseudomonas syringae*) were associated with differences in tree water content similar to those reported in Chapter 3 (Tables 3.12, 3.13). High susceptibility (+60% incidence and +205% severity) to foliar fungal disease (*Uncinula necator*) was also related to increased succulence induced by N fertilisation of grapevines (Keller et al. 2003). In their study, N fertilisation also reduced leaf phenolic compounds known to be active in disease resistance. In Chapter 4 higher levels of total reducing compounds (bound + free or 'total phenolics') in the pulp and skin of fruit from unfertilised vines than in fruit from N fertilised vines were reported (Table 4.15). Whether N fertilisation affects fruit resistance to infection by disease organisms by altering epidermal phenolic chemistry awaits further research.

Nitrogen fertilisation is associated with increased plant respiration rates (Manter et al. 2005). Higher respiration rates could have detrimental effects on cellular defence and repair mechanisms leading to increased susceptibility to disease (Manter et al. 2005).

Further research is needed to understand whether the lower oxalate and calcium levels found in N fertilised vines (section 4.3.2) could alter resistance to disease or insect pests, or influence the incidence of calcium-related physiological disorders and loss of firmness during storage (Boyd et al. 2004). Calcium oxalate synthesis may be an inducible defence response to tissue damage (Nakata 2003). As much as 50% and 80% of total calcium content of fruit and leaves respectively can be in the form of insoluble and presumably physiologically

inactive calcium oxalate crystals (Watanabe and Takahashi 1998; Clark et al. 1987). Therefore alterations in oxalate content and in the fruit oxalate:calcium ratio in response to N fertilisation may have implications for fruit quality during storage and for resistance to herbivore damage.

More research is needed to investigate relationships between soluble sugar contents of the fruit and foliar-applied nitrogen (section 5.3.6). Is this effect limited to the fruit or is the soluble sugar content of the leaves also reduced by foliar applications of nitrogen? Lower levels of soluble sugars could be advantageous in terms of disease and insect pest resistance since these compounds within can serve as food substrates for pathogen growth (Chaboussou 2004). A similar relationship with disease susceptibility might exist in the case of free amino compounds whose levels can increase in response to N fertilisation (Clark et al. 1992; Chaboussou 2004).

## 9.8 Conclusion

Over the four years of the experiments with soil-applied N fertiliser, vines that received no N fertiliser appeared not to be N-deficient in terms of leaf symptoms or leaf N content. However, fruit from unfertilised vines tended to be smaller than fruit from vines given high rates of N fertiliser. When the unfertilised vines (LN) were treated with foliar-applied N during early fruit development, fruit size was increased to equal that of fruit from the soil fertilised vines. Furthermore, whereas dry matter was not increased in fruit with soil fertilisation, it was increased by foliar-applied N in the unfertilised vines. This significant difference appears to be related to the different effect on vegetative vigour of foliar-applied N compared to soil-applied N fertiliser.

Generally, the results of the experiments with foliar-applied N provided evidence that this technique is a better way of improving fruit size and quality in terms of DM% than soil-applied nitrogen. Potassium nitrate appeared to be at least as, if not more, effective than urea for foliar application to increase fruit growth. However, if the main objective was to increase leaf and vine N content, then urea rather than $KNO_3$ would be the most suitable form of N, due to the difficulty of supplying an equivalent amount of N with $KNO_3$ without causing phytotoxicity. The foliar application of N also offers a way for improving the efficiency of fertiliser use in kiwifruit orchards in order to comply with societal and market demands for improved sustainability in agricultural production systems. The dual benefits of improved fruit growth and environmental outcomes strongly support the use of foliar-applied N in kiwifruit production.

The importance of hydraulic gradients between stem and fruit for maintaining the balance between water and dry matter accumulation by fruit was demonstrated by the experiment reported in Chapter 8. Results presented in Chapters 3, 5, and 6 showed soil-applied N and biostimulants can also alter the balance between water and dry matter accumulation by fruit in a manner consistent with interactions with hydraulic gradients within the vine. Taken together the results confirm the importance of both water and N fertiliser management for fruit quality in kiwifruit production. Furthermore, it is concluded that one of the main effects of high levels of $NO_3^-$ supply is to alter vine water relations, which in turn supports increased

vegetative vigour and fruit water uptake. Such effects need to be recognised and properly managed in order to optimise fruit quality and orchard productivity.

Levels of several secondary metabolites namely ascorbic acid, oxalate, and phenolic compounds, were significantly reduced in the fruit by high rates of soil-applied N fertiliser. Both the nutritional value of the fruit for the consumer and storability of the fruit could be adversely affected by reductions in levels of these compounds. However, $NO_3^-$ was not found to accumulate to any great degree in fruit (compared to levels known to accumulate in certain vegetables) even when vine uptake and content of $NO_3^-$ was much increased by high rates of N fertilisation. However, the increased water content or succulence of HN vines could increase their susceptibility to pest and diseases such as *Pseudomonas syringae* pv. *actinidiae* (PSA). Care needs to be excised when using N fertiliser to stimulate the rapid development of new canopies in orchards being converted to new cultivars that susceptibility to PSA infection is not inadvertently increased.

## 9.9 Future research

As with most research there always remains more to be known and certainly many new questions have been generated by the research presented in this dissertation. Some of the questions awaiting answers from future research include:

- What are the optimum timing, rates, and form of N to apply both for soil-applied N fertiliser and for foliar-applied N? (sections 9.3, 9.4, and 9.5).
- How do the findings presented here for 'Hayward' and 'Hort16A' apply to new cultivars such as 'G3'?
- Particularly in respect to new cultivars, what are the risks of phytotoxicity with foliar-applied N during early fruit development? (section 6.3.2.4).
- What are the relationships between vine water content and disease resistance and how can the growth of new canopies during orchard re-development be optimised without increasing susceptibility to PSA infection? (section 9.7).
- How does fertilisation with N, foliar N applications, or nutrition in general affect levels of secondary metabolites and soluble sugars, and amino acid composition, and how do such effects affect fruit quality and disease resistance? (section 9.7).
- What is the role of transpiration in the response of fruit to exogenous bio-stimulants or N fertilisation? (section 9.6).

Future research also needs to:

- Explore the relationships between vegetative vigour and fruit growth, and vine N status (section 9.4).
- Build a better understanding of the comparative vigour of vines given one or two relatively large N fertiliser applications compared to vines provided with soil conditions conducive to long-term N acquisition (section 9.5).
- The comparison needs to be extended to include the vegetative vigour responses of late season soil or foliar applications of nitrogen, and of small but regular N applications (section 9.5).

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322

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# Appendices

## Appendix 1. Published material

Citations for three publications based on the work contained in this thesis are listed below:

1. A paper presented at the 24th Annual FLRC Workshop held at Massey University, Palmerston North, New Zealand February 2011. The original publication is available at http://flrc.massey.ac.nz/publications.html

Morton, A. and Woolley, D. 2011. Foliar urea as a substitute for soil-applied N: effects on vegetative vigour and fruit quality of kiwifruit (*Actinidia deliciosa* cv. Hayward). In: Adding to the knowledge base for the nutrient manager. (Eds L.D. Currie and C L. Christensen). http://flrc.massey.ac.nz/publications.html. Occasional Report No. 24. Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand.

2. A paper presented at the 7th International Symposium on Kiwifruit held in Faenza, Italy from 12 to 17 September 2010 and which forms the basis for Chapter 8 of the thesis. The original publication is available at www.actahort.org

Morton, A. and Woolley, D. 2011. Manipulation of fruit water and dry matter content by treatments applied during early and late stages of fruit development in kiwifruit. Acta Horticulturae 913: 309-314.

3. An article summarising some of the key findings of the experiments with foliar applied N on 'Hort16A' and 'Hayward' vines and published in the NZ Kiwifruit Journal.

Morton, A. 2012. Early season foliar nitrogen can increase fruit size and improve environmental sustainability. New Zealand Kiwifruit Journal (May/June): 30-33.

# Appendix 2. Illustrations



Plate 1. Dosatrons in-line fertigation system for high and low nitrate-nitrogen supply in the pot-trial with 'Hort16A' vines in the 2006-07 season (Chapters 3 and 4).



Plate 2. Pot trial with 'Hort16A' vines 2006-07 season.

Plate 3. The T-bar 'Hayward' vines in the Massey University experimental orchard used for the experiments with soil-applied nitrogen fertiliser and some of the foliar-applied nitrogen experiments described in Chapter 3, 4, 6, 7, and 8.



Plate 4. The T-bar 'Hayward' vines in the Massey University orchard 19 January 2007

Plate 5. Hayward fruit approximately 30 DAFB the time of the second foliar N application (Chapter 6).



Plate 6. Experimental set up for the trial reported in Chapter 8.

Plate 7. Commercial 'Hort16A' orchard in Hawkes Bay used in two seasons for the foliar-applied nitrogen experiments reported in Chapter 5.