Preharvest Practices Affecting Postharvest Quality of 'Hayward' Kiwifruit

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Abstract

Repeat purchase of kiwifruit is primarily driven by consumer judgement of internal fruit quality attributes, including those affected by dry matter concentration (DMC) and mineral composition in fruit. This research investigated mechanisms affecting carbohydrate, mineral and water accumulation in 'Hayward' kiwifruit (*Actinidia deliciosa*), and related these to specific management practices. Canopy manipulation through pruning and treatments such as artificial pollination, defoliation, girdling, thinning and application of the auxin transport inhibitor TIBA, may affect fruit DMC and mineral composition.

Leaf photosynthesis and fruit dry matter concentrations (DMC) started to decline as leaf area index values increased above 3-4. In addition to reducing competition for carbohydrates between vegetative and reproductive growth, leader pruning probably increased DMCs of fruit in the leader zone by improving light interception. Photosynthesis was not affected by crop loads between 20-60 fruit m^{-2}, but was consistently higher on non-terminating (long) shoots than on terminating (short) shoots, as were fruit DMCs. Differences in photosynthetic rate of leaves on these two shoot types were attributed to differences in shoot exposure to the sun, and also to the greater demand for carbohydrate within long shoots.

Leaves subtending fruit may increase Ca, and to a lesser extent Mg, flow into fruit, however their accumulation was not affected by leaves outside the fruiting shoot. Xylem sap Ca and Mg concentrations were higher in shoots with a high rather than a low leaf: fruit (L:F) ratio and this may, at least partially, relate to the increase in shoot transpiration that occurs as shoot L:F ratios increase. Within vine variation in fruit Ca concentrations may reflect variations in xylem sap flow rates and Ca concentrations of xylem sap reaching fruit.

Calcium translocation may occur independently of ion movement in the transpiration stream. Timing and extent of vascular differentiation in flower and fruitlet pedicels, possibly regulated by auxin, may influence fruit Ca accumulation. It is likely that early differentiation of vascular tissue in flower and fruitlet pedicels influenced cell division and subsequent (carbohydrate) sink strength of fruit by determining availability of carbohydrate for partitioning into cell walls.

While growers have the potential to induce minor changes in fruit DMC, further increases will depend on the separation of carbohydrate and water accumulation. Further research is required to elucidate the mechanisms regulating phloem transport and unloading of sucrose in kiwifruit.

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List of Abbreviations

ABA	abscisic acid
ANOVA	analysis of variance
AuG	autumn girdling
a_w	water activity
BER	blossom end rot
CME	chloroflurenolmethylester
CP	conventionally-pruned
CPPU	N1-(2-chloro-4-pyridyl)-N3-phenylurea
CSA	cross-sectional area
DAFB	days after full bloom
DM	dry matter
DMC	dry matter concentration
DPFB	days prior to full bloom
DW	dry weight
FB	full bloom
FW	fresh weight
FZ	fruiting zone
GLA	gap light analyser
GLM	general linear model
Gs	stomatal conductance
HP	high crop load, pruned (vines)
HUP	high crop load, unpruned (vines)
IAA	indole-3-acetic acid
K_h	hydraulic conductivity
LAI	leaf area index
L:F	leaf: fruit (ratio)
LP	leader-pruned
LSD	least significant difference
LTB	low temperature breakdown
LUP	low crop load, unpruned (vines)
LwP	low crop load, pruned (vines)
LZ	leader zone
NAA	napthalene acetic acid
NPA	1-N-napthylphthalamic acid
NPQ	non-photochemical quenching

PAR	photosynthetically active radiation
PC	personal computer
PGR	plant growth regulator
PIX	1,1-dimethyl-piperidinium
Pn	Net photosynthesis
RH	relative humidity
rSSC	ripe soluble solids concentration
SA	surface area
SB	short-base (proximal cane end)
SE	short-end (distal cane end)
SLW	specific leaf weight
SmD	summer defoliation
SpD	spring defoliation
SpG	spring girdling
SSC	soluble solids content
TIBA	2,3,5-triiodobenzoic acid
VMC	ventromedian carpellary (vascular bundle)
VPD	vapour pressure deficit
WAFB	weeks after full bloom

1. General Introduction

1.1. Internal quality in kiwifruit: importance of high Ca and DM concentrations

In the last decade the New Zealand kiwifruit industry doubled in value and is currently the largest horticultural export earner in New Zealand (30% of total horticultural exports and 2.3% of total merchandise exports from New Zealand), with export earnings of NZD 659 million for the year ended 30 June, 2004 (Kerr *et al.*, 2004). New Zealand is the second largest kiwifruit exporter (33% of world kiwifruit exports), behind Italy. Last year, New Zealand supplied 0.25 million tonnes of kiwifruit to 42 different countries from a productive area of 10,580 ha, just over 80% of which is located in the Bay of Plenty.

As the industry is predominantly export based, further expansion in export sales returns is vital to the continued success of the industry. This must be achieved despite rapidly escalating international competition due to: (a) increasing global production, (b) greater product selection (including competition from other fruit and non-fruit products) and, (c) the introduction of technologies that allow fruit to be cool-stored for up to eight months, which has resulted in a greater overlap between southern and northern hemisphere marketing seasons (Belrose Inc, 2004).

In response to this escalation in competition and to declining returns in the 1990s, the New Zealand kiwifruit industry developed the ZESPRI™ brand in an attempt to position New Zealand kiwifruit as an upmarket fruit category and relieve price competition (Beverland, 2001). While branding may lead to price premiums, consumer and trade loyalty and co-operation, and joint marketing efforts (Aaker and Joachimsthaler, 2000), branding also increases supplier accountability as branded produce is easily identified. Now, more than ever before, quality is paramount to the success of the New Zealand kiwifruit industry.

Many factors contribute to fruit quality including the aesthetic characteristics of a fruit, taste and texture, postharvest quality (length of time a fruit can be stored without developing pathological, physiological or physical defects, such as *Botrytis cinerea*, pitting and excessive softening), health and safety factors and the sustainability of production practices used to produce fruit (Kader, 2001). These factors can be grouped into two categories, including the largely visual aesthetic attributes such as size, shape, colour and freedom from visible defects (external quality attributes), and quality attributes that can't be seen (internal quality attributes).

A recent study found that taste was the most important factor driving the repeat purchase of kiwifruit products (Harker, 2004). Australian consumers rated taste and health as the most important quality attributes when it came to selecting fruit and vegetable products (Pearson, 2000). Other studies also rated taste and health as key attributes in determining food preferences, in addition to convenience and cost (Steptoe *et al.*, 1995; Connors *et al.*, 2001; Harker *et al.*, 2003), and Ferguson (2004) suggested that pleasure, health and convenience would be the key requirements for new product development in the kiwifruit industry.

The above findings suggest that, when making repurchase decisions, consumers of fruit and vegetable products place more value on internal than on external quality attributes. This is probably due to two reasons. Firstly, fruit that fail to meet certain external quality benchmarks are usually identified and discarded prior to storage and transport, so consumers may not experience low external quality. Secondly, consumers can easily gauge whether external attributes of a fruit or vegetable product are acceptable or not (you get what you see), so are less likely to be disappointed with their purchases. In contrast, it is difficult to gauge whether internal attributes of a product are acceptable. As internal quality is not discernible at the time of purchase it is difficult to extract value from a market for superior levels of internal quality attributes (Banks, 2003).

Fruit internal quality is a function of fruit composition or the concentration and combination of various components, including carbohydrates, minerals, lipids, proteins, organic acids, vitamins and water, within a fruit (Kader, 2001). Excluding water, these components can collectively be combined under the heading 'dry weight (DW)'. Fruit dry matter concentration (DMC), which is the percentage of total fruit mass derived from solid material or the ratio of DW to fresh weight (FW), has been associated with kiwifruit flavour and postharvest quality (Feng *et al.*, 2003; Harker, 2004, and references herein).

Fruit DMC is not to be confused with the DW of the fruit (also called the fruit DM content), or the fruit soluble solids concentration (SSC, often described as the °Brix), which includes simple sugars, acids, salts and other soluble components in the cells. The term DM is used interchangeably in the literature to describe kiwifruit DM concentrations (Seager and Hewett, 1995; Maguire and Mowat, 2003; Thorp *et al.*, 2003a) and contents (fruit DW) (Smith *et al.*, 1995; Walton and Fowke, 1995; Richardson *et al.*, 1997). There also is evidence of the incorrect use of the phrase DM content, when the authors are actually referring to the DMC of fruit (Velemis *et al.*, 1997; McGlone *et al.*, 2002; Cheng *et al.*, 2004; Clark *et al.*, 2004). In order to avoid any ambiguity, the phrase DMC (dry matter concentration) will be used in this thesis to describe the ratio of FW to DW in the fruit, as described above. It is suggested that this terminology is adopted for use in future publications to avoid any vagueness surrounding the use of the phrase DM.

1.1.1. Fruit DMC and taste

While consumers will make a decision to purchase kiwifruit based on its external quality, their decision to repurchase is based on its taste or flavour (Anon, 2005). An Australian study has shown that following a bad eating experience, consumers were likely to stop purchasing a product, buy fewer of that product or switch to lower priced products (Batt and Sadler, 1998). Therefore every poor tasting fruit that enters the market has the potential to discourage kiwifruit consumption and ultimately reduce grower returns (Anon, 2005).

Unfortunately it is impossible to produce a kiwifruit with universal consumer appeal. Studies show that there is a group of consumers who like sweet, softer kiwifruit and a group who prefer tangy, firmer kiwifruit (Jaeger et al., 2003). However, in both groups of kiwifruit consumers there was a positive correlation between flavour acceptance and fruit DMC. Fruit with a higher DMC is perceived to have more flavour (Harker, 2004). However, the range in flavour liking for a given fruit DMC may vary, so that a difference of 2-4 %-units may be required between two fruit before consumers perceive a difference in their sensory quality (Burdon *et al.*, 2004). High DMC fruit tend to be very consistent in their flavour profile irrespective of how long they are stored, or how ripe they are (Harker, 2004).

Table 1.1. Concentration of soluble carbohydrates and starch in 'Hayward' kiwifruit

Carbohydrate	**Average concentration (g $100g^{-1}$ FW) 150 days after full bloom (around harvest)**			**Average concentration (g $100g^{-1}$ FW) in ripe fruit**
	A Italian Fruit	**B Californian fruit**	**C New Zealand fruit**	**D New Zealand Fruit**
Glucose	0.8-0.9	0.22-0.44	0.92-1.4	1.28-4.8
Fructose	0.9-1.0	0.33-0.74	0.94-1.4	1.3-5.5
Sucrose	0.08-0.14	0.09-0.2	0.07-0.16	0.01-1.65
Inositol	-	0.07-0.12	-	0.04-0.26
Starch	2.5-4.25	6.5-7.9	4.7-6.0	Negligible
°Brix	6.7-7.7	5.1-5.9	5.4-5.6	9-18

Data calculated from: column A (Ferrandino and Guidoni, 1998), column B (Walton and De Jong, 1990), column C (MacRae *et al.*, 1989), and column D (carbohydrates: Wright and Heatherbell, 1967; Patterson *et al.*, 1991; Brix: Jordan *et al.*, 2000).

Kiwifruit DW is predominantly composed of carbohydrate, in addition to the compounds mentioned previously, which are derived from carbohydrates produced in the leaves and mineral

ions taken up by the roots. Therefore, fruit DMC will be affected by both carbohydrate (transported to fruit from leaves via the phloem) and water (predominantly transported to the fruit via the xylem) accumulation. Table 1.1 illustrates the concentration of starch and the main soluble carbohydrates in kiwifruit.

Insoluble sugars include starch and structural carbohydrates, such as cellulose, pectins and other cell wall materials. Glucose, fructose, sucrose and inositol are the main soluble sugars and together, largely determine the fruit soluble solids concentration at eating ripeness (rSSC) (Okuse and Ryugo, 1981; MacRae *et al.*, 1989; Ferrandino and Guidoni, 1998; Han and Kawabata, 2002). Structural carbohydrates, soluble sugars and starch comprise 10-12%, 10-18% and 20-34% of the total DW at harvest, respectively, depending on the stage of fruit maturity and the efficiency of carbon partitioning to the fruit (Okuse and Ryugo, 1981; MacRae *et al.*, 1989; Han and Kawabata, 2002). Although there is considerable variation in the composition of kiwifruit reported in the literature, in general reported concentrations lie within the specific ranges given in Table 1.1.

Soluble sugars are responsible for creating the sweet flavours associated with kiwifruit (Heatherbell, 1975) and also promote cell enlargement by acting as an osmoticum (Boldingh *et al.*, 2000). Insoluble sugars are sequestered in vacuoles and other storage structures or are used to maintain the structural integrity of the fruit by inclusion in cell walls (Heatherbell, 1975). Starch, which is the principal storage carbohydrate in kiwifruit (Davison, 1990), has an important influence on the final flavour of fruit, since as fruit mature, starch is hydrolysed to form soluble sugars (MacRae et al., 1989). In addition to these carbohydrates, organic acids and volatiles influence the taste and flavour of the fruit (Heatherbell, 1975). More information on carbohydrate accumulation in kiwifruit can be found in Section 1.2.

The New Zealand kiwifruit industry have used the relationship between fruit DMC and consumer preference of kiwifruit, and consequently have introduced a payment system, based on fruit DMC at harvest, to encourage growers to produce high DMC fruit. Under this system, known as the Taste ZESPRI™ initiative, payments to growers are based on the mean and standard deviation of DMC values within an orchard maturity area (an area within an orchard recognised as producing fruit of similar quality, or fruit that are at a similar stage of ripeness: Anon, 2004). The standard deviation accounts for variation in fruit DMC that occurs within a maturity area. This variation, or lack of uniformity is a problem, because it limits the ability of the industry to segregate fruit according its DMC, and to use DMC as a marketing tool to increase profitability. For example, without consideration of the variation in fruit DMCs within an orchard block it is impossible to determine the proportion of fruit in a population that is above or below the lower threshold (taste)

limit of 14.5% DMC (the DM value above which fruit taste is perceived by consumers as being acceptable, Anon, 2004).

1.1.2. Postharvest quality, fruit mineral and DM concentrations

Although only a small component of fruit DW, inorganic nutrients may influence fruit quality in many ways (Ferguson and Boyd, 2001). In kiwifruit, several mineral ions, including Ca, Mg, K and P, have been linked to the formation of pathological, physiological and physical defects in storage (Prasad and Spiers, 1991; Ferguson *et al.*, 2003, and references herein). Increasing production (volumes of fruit) and longer selling seasons increase the risk of fruit loss due to the occurrence of these defects.

Table 1.2. Incidence of storage disorders in 'Hayward' kiwifruit from six different growing regions after 24 weeks storage at 0°C from April/May 2003.

Storage disorder (% incidence)	Auckland	Bay of Plenty	Nelson	Northland	Poverty Bay	Whakatane	Overall
Stem-end (botrytis) rot	0.2_a ***0.1***	0.4_a ***0.06***	0.1_a ***0.05***	0.0_a ***0.0***	0.5_a ***0.17***	0.1_a ***0.0***	0.3 ***0.0***
Wound rot	0.3_a ***0.19***	0.2_a ***0.05***	0.2_a ***0.06***	0.1_a ***0.06***	0.3_a ***0.06***	0.2_a ***0.09***	0.2 ***0.0***
Physiological pitting	0.3_a ***0.19***	0.6_a ***0.12***	0.4_a ***0.15***	0.2_a ***0.1***	0.4_a ***0.13***	0.9_a ***0.23***	0.5 ***0.09***
Shrivel	1.0_{ab} ***0.22***	1.5_{ab} ***0.16***	0.5_a ***0.19***	4.7_c ***0.79***	2.2_b ***0.61***	2.4_b ***1.06***	1.8 ***0.19***
Premature softening	12.9_a ***4.0***	11.0_a ***0.79***	3.4_b ***1.46***	13.7_a ***1.46***	10.5_a ***2.47***	14.9_a ***2.25***	11 ***0.67***
Side rot	3.7_a ***1.0***	3.3_a ***0.34***	1.0_a ***0.32***	4.0_a ***0.92***	1.9_a ***0.39***	3.6_a ***0.52***	3.1 ***0.25***
Number of orchards	3	46	6	5	5	7	72

Values are means for each region with standard errors shown in bold text. Values for each region followed by the same letter are not significantly different at $P < 0.001$. Least square difference values were 0.8 for the shrivel disorder and 6.3 for the premature softening disorder. Source Maguire and Mowat (2003).

In 2002/03 a survey of 72 orchards from six different growing regions in New Zealand, showed fruit losses of 17% as a result of storage disorders, such as pitting, softening and rotting after storage for 24 weeks at 0°C (Maguire and Mowat, 2003: Table 1.2). The most prevalent disorder was premature softening, followed by rot. The 2002/03 season was a low pit season, and pit incidence in this season averaged only 0.5% of all fruit (Table 1.2). However pitting incidence fluctuates seasonally, and is worse in years where large numbers of fruit are coolstored in air for more than 12-16 weeks (N. Lallu; pers. comm.). Pitting incidence also is elevated following a wet autumn, and tends to be higher in fruit from the Northland region of New Zealand. Pitting incidences of 30-40% have been reported in some lines of fruit (N. Lallu; pers. comm.).

Widespread evidence suggests that fruit Ca concentrations are positively correlated with fruit firmness and negatively correlated with rates of fruit softening in storage (Prasad and Spiers, 1991; Davie, 1999; Benge *et al.*, 2000; Xie *et al.*, 2004; Gerasopoulos and Drogoudi, 2005). This may be related to the inhibitory effect of Ca on polygalacturonase and pectin methylesterase activity, two enzymes involved in fruit ripening and cell wall degradation (Lee *et al.*, 2001). Calcium incorporated into the cell wall structure in linkages with pectins and hemicelluloses also may directly inhibit cell wall degradation, providing resistance to the action of some of the cell wall degrading enzymes, as occurs in grapes (Cabanne and Doneche, 2001) and apples (Roy *et al.*, 1994).

The ratio of Ca to other minerals also has been associated with fruit quality in storage. Generally, fruit with low Ca, Mg and P concentrations and high K concentrations are susceptible to rots (Maguire and Mowat, 2003) and softening (Feng *et al.*, 2003) disorders in storage. Ferguson *et al.* (2003) found that pitting incidence was enhanced in fruit with low Ca and Mg concentrations and high concentrations of K and P. Fruit Ca concentrations increased nearly 40% and fruit firmness was enhanced by 11% in fruit sprayed with $CaCl_2$ during development (Gerasopoulos and Drogoudi, 2005). Such fruit could be stored for 10-12 weeks longer than untreated fruit before postharvest quality was considered unacceptable (Gerasopoulos *et al.*, 1996; Gerasopoulos and Drogoudi, 2005).

Fruit DMC also affects fruit quality in storage. Feng *et al.* (2003) suggested that variations in fruit DMC could account for up to 73.8% of the variation in fruit firmness in storage. Low fruit DMC has been associated with pitting, softening and rot incidence in storage of 'Hayward' fruit (Ferguson *et al.*, 2003; Maguire and Mowat, 2003) and chilling-related rot injury in 'Hort16A' (*Actinidia chinensis*) fruit (Clark *et al.*, 2004).

There is considerable evidence to suggest that sub-populations of fruit exist within a vine that are prone to storage disorder development, and that different factors may be affecting fruit DM and mineral concentrations in fruit located in different parts of the vine (Smith *et al.*, 1994). For example, incidence of physiological pitting was more prominent in fruit from short, terminating shoots, which usually have lower DM, Ca, Mg concentrations and high K and P concentrations, than fruit from long, non-terminating shoots that usually have higher Ca, Mg and DM concentrations and lower K and P concentrations (Thorp *et al.*, 2003b). These findings were consistent across 18 orchards from the Bay of Plenty, New Zealand. Low DMCs are typically associated with fruit on canes that originate near the trunk, and increase as cane distance from the trunk increases (Smith *et al.*, 1994; Max, 2004). Fruit DMCs tend to decrease with increasing distance down the cane, so the highest DMCs are found in fruit that originate from the base of

canes arising from the distal end of the central leader (Smith et al., 1994). Variations in fruit SSC showed similar within-vine trends (Hopkirk *et al.*, 1986; Smith *et al.*, 1994; Pyke *et al.*, 1996). However, positional effects were not always consistent across orchards or between seasons (Smith *et al.*, 1994; Pyke *et al.*, 1996). Similarly, orchards or vines that produce fruit with a high DMC in one year will not necessarily produce fruit with a high DMC in the following year (Fig 1.1: K. Patterson, unpublished data).

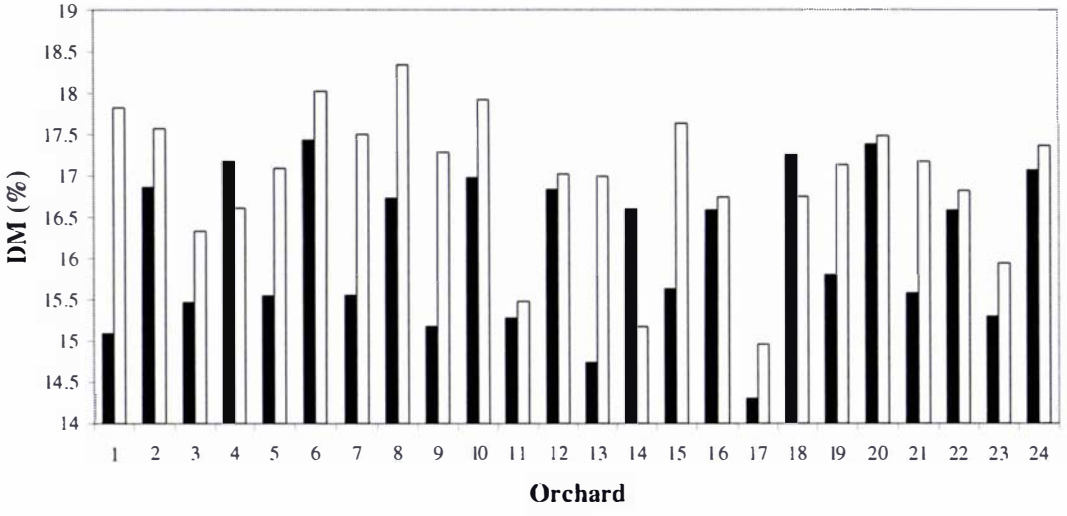


Fig 1.1. Average DMC of "Hayward' kiwifruit from 24 different orchards within New Zealand as measured in 2004 (black bars) and 2005 (white bars). Even numbers illustrate high DMC orchards, whilst odd numbers illustrate low DMC orchards. K. Patterson (unpublished data).

From industry data it is estimated that around 60% of the total variation in DMC in a kiwifruit crop occurs within vines (Max, 2004). Smith *et al.* (1994) found that differences between pergola-trained vines accounted for 5% of the total variation in DM, differences between canes within a vine accounted for 7%, differences between laterals within a cane accounted for 18%, and differences between fruit within a lateral accounted for nearly 70% of the total variation. These values were 2-11% (variation between vines), 18-20% (canes), 29-30% (laterals within a cane) and 39-50% (between fruit within a lateral) for mineral ions, the range depending on the ion concerned (Smith *et al.*, 1994).

1.1.3. Factors affecting fruit variability

As highlighted above there is a large inherent variation in fruit DMC and mineral contents within and between kiwifruit orchards. This is probably reflected in quality variation between different

lines of fruit arriving at the pack-house. Such variability affects the ability of industry to accurately predict quality changes during postharvest distribution under controlled conditions (Shewfelt, 1999). Even a low incidence of poor quality fruit in the market place can result in dissatisfied consumers, which in turn can have a significant impact on subsequent sales and the level of marketing effort needed to sustain ongoing sales volumes (Ehrenburg, 1998). However, while the importance of minimising fruit quality variability has been acknowledged, preharvest factors affecting fruit variability are poorly understood.

Kiwifruit composition has been found to vary in response to climatic (Snelgar *et al.*, 1993; Han and Kawabata, 2002; Richardson *et al.*, 2004), genetic (Fuke and Matsuoka, 1982; Klages *et al.*, 1998; Boldingh *et al.*, 2000), and orchard management factors. Typical fruit DM and mineral concentrations vary in fruit from different growing regions in New Zealand (Maguire and Mowat, 2003: Table 1.3). While genetics and climate are primary determinants of fruit composition (Gardiner and Max, 2004; Anon, 2005), there is a general belief in the New Zealand kiwifruit industry that fruit-to-fruit variability can be strongly influenced by the grower (Gardiner and Max, 2004). This is the reason for the Taste ZESPRI™ initiative, and for the recent innovation fora for growers, which largely focused on technologies to increase DMC and to reduce DMC variability of kiwifruit.

Table 1.3. Average fruit mineral and DM concentrations in 'Hayward' kiwifruit from six different growing regions after 24 weeks storage at 0°C from April/May 2003.

Fruit attribute	Auckland		Bay of Plenty		Nelson		Northland		Poverty Bay		Whakatane		Overall	
Ca (mg fruit^{-1})	29_{ab}	***4.88***	26_{a}	***0.55***	29_{ab}	***1.94***	32_{bc}	***2.4***	33_{b}	***1.78***	28_{ac}	***1.18***	28	***0.54***
Mg (mg fruit^{-1})	12_{a}	***0.43***	13_{a}	***0.14***	14_{a}	***0.47***	14_{a}	***0.47***	14_{a}	***0.53***	14_{a}	***0.53***	14	***2.12***
K (mg fruit^{-1})	258_{a}	***7.54***	283_{bc}	***2.36***	292_{bd}	***4.44***	273_{ac}	***6.49***	306_{d}	***9.57***	275_{ac}	***6.2***	283	***0.13***
P (mg fruit^{-1})	28_{a}	***1.77***	31_{ab}	***0.39***	32_{ab}	***1.55***	33_{bc}	***0.66***	36_{c}	***0.8***	30_{ab}	***1.09***	31	***0.36***
DM (%)	16.5_{ab}	***0.44***	16.2_{a}	***0.11***	15.8_{a}	***0.2***	17.2_{bc}	***0.26***	17.8_{c}	***0.44***	16.0_{a}	***0.19***	16.4	***0.1***
Number of orchards	3		46		6		5		5		7		72	

Values are means for each region with standard errors shown in bold text. Values for each region followed by the same letter are not significantly different at $P < 0.001$. Source Maguire and Mowat (2003).

A grower can influence fruit quality and variability indirectly through adoption of optimum nutrition, irrigation, pollination and pest and disease control strategies, or by direct manipulations of the plant itself (Table 1.4). Direct manipulations may be of two types: (a) removal of certain plant organs (e.g., pruning and fruit thinning), and (b) interference with mineral, photoassimilate or hormone translocation between organs (e.g., girdling). To offset the effect of poor growing seasons it is estimated that growers will require management techniques that can increase DMCs

by at least 0.6% (Gardiner and Max, 2004). Most of the technologies listed in Table 1.4 are believed to have the potential to increase DMCs between 0.15-1% (A. Mowat; pers. comm.).

Table 1.4. Kiwifruit vine characteristics affected by different orchard management strategies.

Management technology	Factors effected by this technology that may influence fruit variability and quality
CANOPY MANAGEMENT: Including timing and management of:	
Pruning (direct effect)	Leaf:fruit ratio, crop load, canopy vigour (wood type used), light exposure/canopy density, timing of flowering and leaf health and retention
Thinning (direct effect)	
Girdling (trunk and cane: direct effect)	
HiCane application (indirect effect)	
POLLINATION REGIMES (indirect effect)	Crop load and fruit sink strength (ability to attract carbohydrate through seed numbers)
FERTILISER AND IRRIGATION REGIME (indirect effect)	Canopy vigour and leaf health and retention
PEST AND DISEASE MANAGEMENT (indirect effect)	Canopy vigour, leaf health and retention and leaf:fruit ratio
ORCHARD MAINTENANCE (shelter-belts, training structures, mowing and weed control: indirect effect)	Light exposure, wind exposure, canopy vigour and leaf health and retention
REFLECTIVE GROUND COVERS (indirect effect)	Light exposure

Data summary from the New Zealand kiwifruit DMC field days and November 2004 innovation forum.

Partly because of large incentives now available in the fruit payment system for high DMC fruit, many growers are using some of these management techniques on their orchards, particularly those involving canopy management (Ferguson, 2004). However, there is a lack of information on the long-term effects of some of these technologies on fruit variability and fruit quality in relation to attributes such as fruit mineral concentration and storage behaviour. The ability of specific orchard management practices to reduce variability in fruit DM and mineral concentrations may vary from season to season and from orchard to orchard (A. Mowat: pers. comm.). In order to minimise fruit variability a greater understanding of factors affecting carbohydrate, mineral and water accumulation must be developed. This will enable the New Zealand kiwifruit industry to

identify and develop tools with the potential to minimise fruit variability, and to develop strategies to reduce the impact of unfavourable climatic conditions on export returns.

1.2. Carbohydrate, mineral nutrient and water accumulation in kiwifruit

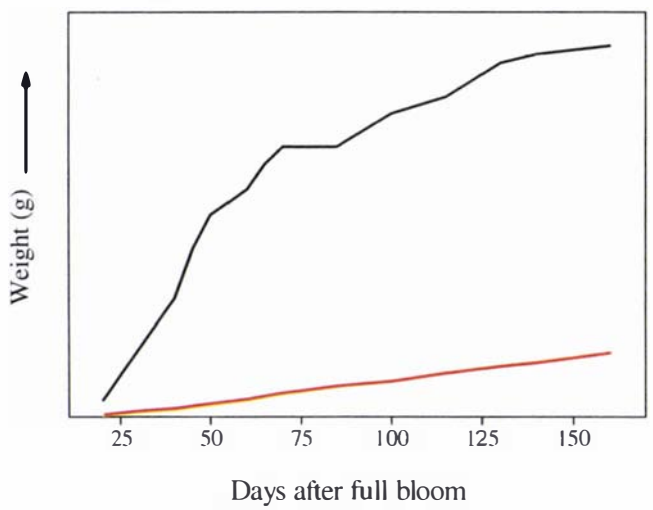


Figure 1.2. Fresh (black) and dry (red) weight accumulation in 'Hayward' kiwifruit. Data from Richardson et al., (1997).

In fruit crops orchard management tools are applied to manipulate patterns of carbohydrate, mineral and water accumulation in fruit, so that fruit composition at harvest is such that internal quality is maximised. An understanding of the patterns of carbohydrate, mineral and water accumulation in kiwifruit is the first step in understanding how and when specific management tools might be able to manipulate fruit internal quality.

1.2.1. Fruit growth

Actinidia deliciosa fruit growth, measured as fresh weight, has usually been shown to follow a double sigmoid curve (Fig 1.2: Hopping, 1976b; Smith *et al.*, 1995; Richardson *et al.*, 1997; Han and Kawabata, 2002), with a period of rapid growth following fruit set (phase I: cell division, 6-9 weeks), followed by a period of low or static growth (phase II: 3-5 weeks), and finally a second period of rapid growth until harvest (phase III: cell enlargement, 8-12 weeks). However, triple (Reid et al., 1982) and single (Walton and De Jong, 1990; Ferrandino and Guidoni, 1998) sigmoid growth curves have been reported.

In contrast to FW, DW is thought to increase in a linear (Hopping, 1976b; Okuse and Ryugo, 1981; Clark and Smith, 1988; Lai *et al.*, 1988), or almost linear fashion (Han and Kawabata, 2002: Fig 1.2) from 14-28 days after full bloom (DAFB) until harvest (around 160 DAFB). A linear increase in DW implies that the rate of carbohydrate partitioning to fruit is determined in the first

month after flowering and remains the same despite variations in the climate that may effect fruit FW (Richardson et al., 1997). It also suggests that DW accumulation is constrained by fruit sink strength (ability to obtain carbohydrate) rather than by the assimilate supply from leaves (source strength)

1.2.2. Phloem transport and carbohydrate accumulation

Carbohydrates, predominantly in the form of sucrose, are transported in living cells of the phloem. In leaves, or at various points along the pathway between source (leaves) and sink (organs that utilise carbohydrate), sucrose is actively (via apoplasmic pathways) or passively (via symplasmic pathways) loaded into the phloem (phloem loading). This causes water to move into the phloem, creating a positive internal pressure that induces a mass flow in the phloem to sites of lower positive pressure caused by the removal of solutes from the phloem (phloem unloading) (Patrick, 1997; Lalonde *et al.*, 1999). Specialised proteins known as sucrose carriers facilitate the process of sucrose loading and loading (Lalonde *et al.*, 1999). By adjusting turgor pressure, sucrose may act as a signalling molecule regulating the activity of these proteins and hence, assimilate flow between sources and sinks (Chiou and Bush, 1998). Endogenous plant hormones have been implicated also in regulation of assimilate transport between sources and sinks (Daie, 1989; Morris, 1996).

Flow rate and direction of flow in the phloem is closely related to the rate of release or unloading at the sink (Marschner, 1995). Rate of phloem unloading depends on the ability of a sink to utilise unloaded carbohydrate in metabolic reactions, to maintain cell structures or to remove carbohydrate from the symplast into specific organelles (physical compartmentation) (Hawker *et al.*, 1991; Patrick, 1997). Therefore, the ability of an organ to import assimilate or other solutes (its sink strength) may depend on the number and size of cells in a sink and the activity of key metabolic enzymes, such as acid invertases that trigger the chemical conversion of sucrose to other compounds (Ho, 1988a; Buwalda and Smith, 1990a). In kiwifruit, cell division is completed around 6 weeks after full bloom (WAFB), and rates of DW increase are linear from this time, which suggests that the cell number in kiwifruit may determine the capacity for the fruit to import carbohydrates.

Structural carbohydrate, starch and soluble sugar accumulation

In the first phase of kiwifruit fruit growth carbohydrates are utilised in biosynthesis of structural compounds, such as cell walls (Ferrandino and Guidoni, 1998), as starch concentrations in fruit are low. The total concentration of structural carbohydrates reaches a maximum around 35 DAFB

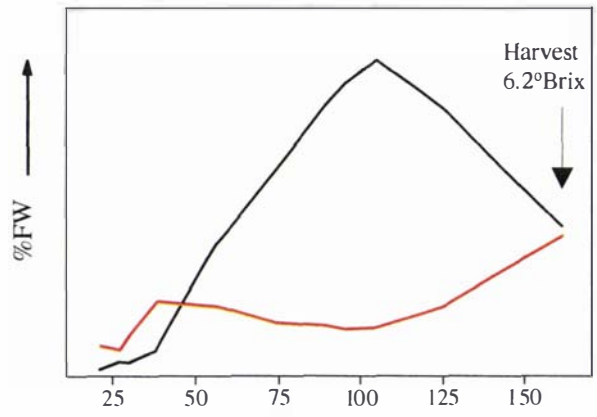


Fig 1.3. Starch (black) and soluble sugar (red) accumulation in 'Hayward' kiwifruit. Data from Okuse and Ryugo, (1981) Walton and De Jong (1990) and Klages et al., (1998).

(Han and Kawabata, 2002) after which time carbohydrates are stored as starch. Consequently, starch concentrations rise rapidly (42-63 DAFB), peaking approximately a month prior to harvest (at 18 ± 2 weeks after full bloom (WAFB)) when fruit attain a SSC of 6.2°Brix (Okuse and Ryugo, 1981; MacRae *et al.*, 1989; Stec *et al.*, 1989; Sawanobori and Shimura, 1990; Walton and De Jong, 1990; Smith *et al.*, 1992). During the month prior to harvest starch concentrations decrease rapidly as starch is hydrolysed to sucrose by invertase resulting in an inverse relationship between the soluble sugar content (SSC) and starch content (Fig 1.3: MacRae *et al.*, 1989).

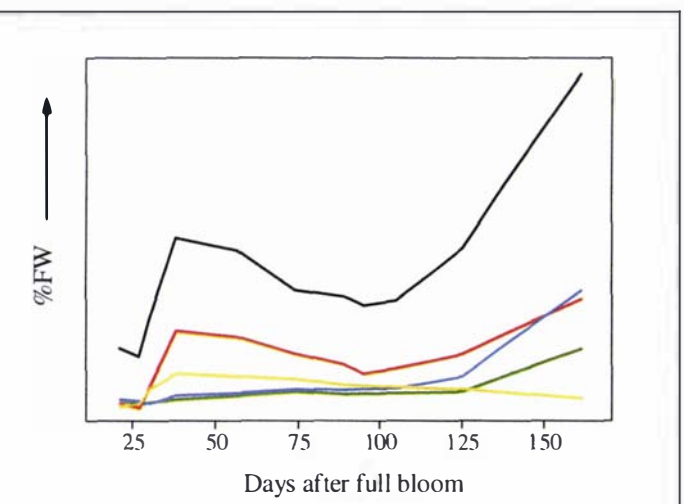


Fig 1.4. Soluble sugar accumulation in 'Hayward' kiwifruit. Total sugars (black), glucose (red), sucrose (green), fructose (blue) and inositol (yellow). Data from Okuse and Ryugo, (1981) Walton and De Jong (1990) and Klages et al., (1998).

Collectively, accumulation of glucose, fructose, sucrose, inositol and other solutes results in the curve for percent soluble solids content (SSC) that declines slightly immediately after fruit set, shows a transient rise, and then remains low and constant (≈ 5.5% FW) until between 126-140 DAFB when it rises rapidly (Okuse and Ryugo, 1981; MacRae *et al.*, 1989; Sawanobori and Shimura, 1990: Fig 1.4). Initially the SSC may decrease as the FW rapidly increases and the solute content in fruit is diluted. Once cell division is complete, the transient rise in glucose and inositol concentrations during

early development (Fig 1.4) may act as an osmoticum for cell expansion (Smith *et al.*, 1995; Boldingh *et al.*, 2000). Consistent with this hypothesis is the observation that peak glucose concentration coincided with a peak in fruit water content that occurs during the cell expansion phase (Smith *et al.*, 1995).

From 90-100 DAFB more than 85% of the carbohydrate transported to fruit is incorporated into the sugar fraction, and the proportion of carbohydrate transported to fruit declines once starch hydrolysis begins (MacRae and Redgwell, 1990). This suggests that the sudden rise in soluble sugar concentration, around 100 DAFB, is derived from current season's carbohydrate, which is converted directly into soluble sugars instead of being stored as starch (Reid *et al.*, 1982; MacRae and Redgwell, 1990; Ferrandino and Guidoni, 1998). The shift from incorporating sugars into starch to maintaining sugars in their soluble form may be triggered by an environmental stimulus, such as cooler temperatures and shorter days, as may the hydrolysis of starch to soluble sugars (MacRae and Redgwell, 1990; Seager *et al.*, 1996). This would explain why Hopkirk *et al.* (1989) reported kiwifruit maturation to be delayed in warmer climates. It is known that the climatic conditions in which a fruit matures can cause differences in the timing of starch and soluble sugar concentration changes, and carbohydrate composition in fruit at harvest and eating ripe maturity (MacRae *et al.*, 1989; Sawanobori and Shimura, 1990; Walton and De Jong, 1990; Ferrandino and Guidoni, 1998).

1.2.3. Xylem transport, water and mineral accumulation

In kiwifruit, as in apple (Failla *et al.*, 1990), peach (Lescourret *et al.*, 2001), citrus (Huang *et al.*, 1992) and tomato (Leonardi *et al.*, 1999) fruit, it appears that water is the main driver of fruit growth, as water accumulation closely matches the pattern of FW accumulation, tapering off towards the end of phase III fruit growth (Smith *et al.*, 1995; Han and Kawabata, 2002: Fig 1.2). Fruit water status is determined by entry of water via the xylem and phloem (Pate *et al.*, 1978; Ho *et al.*, 1987), accumulation in the fruit, losses due to either fruit transpiration (Syvertsen and Albrigo, 1980; Jones and Higgs, 1982; Ehert and Ho, 1986) and/or xylem back-flow to transpiring leaves or other plant sinks during periods of high potential evapotranspiration (Jones and Higgs, 1982; Lang and Thorpe, 1989).

Xylem water transport is driven by pressure gradients and accounts for most water accumulated in fruit (Marschner, 1995). These pressure gradients may be positive, for example, the hydrostatic pressure that is created by dissolved solvents in the apoplast of the stele pushing water towards the shoots (root pressure), or negative. Negative pressures result from evaporation of water from leaf

surfaces (transpiration), which creates a pressure differential inside xylem vessels that pulls water from the soil towards leaves. Negative pressures account for most of the water transport in plants, although root pressure may predominate during periods of low transpiration, such as at night time (Atwell *et al.*, 1999). During periods of rapid growth, water accumulation may be driven also by a growth-induced gradient in water potential in enlarging tissues. This gradient originates from the extension of cell walls, which prevents turgor from reaching a maximum, thereby creating a pressure gradient that causes water to move from the xylem at a rate that satisfies the rate of enlargement (Nonami and Boyer, 1987).

Pressure-driven mass flow in the xylem differs from that in the phloem (refer Section 1.2.2 for more information on phloem transport) in several ways (Marschner, 1995): (a) mineral ions are the dominant solutes in the xylem, whereas organic compounds are the dominant compounds in the phloem, (b) xylem transport is through non-living cells and is therefore driven purely by physical forces, whereas phloem transport is through living cells and may be driven by both active and passive forces, and (c) xylem transport is generally uni-directional in an acropetal (upwards) direction from the roots to the shoots, whereas the direction and/or rate of phloem transport is determined by the rate of phloem unloading at the sink. In xylem there is the possibility of reverse flow from fruit to shoot if the shoot water potential is less negative than the fruit water potential (Jones and Higgs, 1982; Lang and Thorpe, 1989).

In developing kiwifruit, water consumption of fruit varies between 0.1-0.6 m^3 ha^{-1} d^{-1}, with highest transpiration rates, and hence water consumption, occurring in the first 30 days after fruit set (Xiloyannis *et al.*, 1999). From 40 days after fruit set fruit transpiration rates are reduced as the skin permeance to water vapour declines (Smith *et al.*, 1995; Xiloyannis *et al.*, 1999). Furthermore, around this time the progressive decline in xylem functionality that occurs as vessels are stretched and broken during fruit expansion is more or less complete (Dichio *et al.*, 2003). Therefore, from 42-56 DAFB most of the water accumulating in fruit arrives via the phloem (Smith *et al.*, 1995; Xiloyannis *et al.*, 2001).

Xylem mineral transport

Most mineral ions are transported predominantly in the xylem transpiration stream and therefore their accumulation is often closely tied to water accumulation (Marschner, 1995). During xylem transport minerals may be taken-up (xylem loading) and released (xylem unloading) to the surrounding living cells, xylem parenchyma and phloem. Despite this loading and unloading of solutes along the xylem pathway in the stem, most of the solutes and water will be transported in the xylem vessels to leaves or other rapidly-transpiring organs (Marschner, 1995).

During xylem translocation polyvalent cations also may move by exchange via negatively charged sites along the walls of the xylem vessels towards growing tissues where new exchange sites are being synthesised (Van de Geijn *et al.*, 1979). Therefore, competition for Ca may occur between growing tissues and transpiring organs (Clarkson, 1984), the extent of competition being dependent on growing activity, transpiration rate of the various organs and Ca concentration in the xylem sap (Tromp and Van Vuure, 1993). As long, non-terminating kiwifruit shoots continue to produce new leaves until late in the season, new exchange sites will continue to be synthesised in these shoots. Conversely, on short terminating shoots (refer to Section 1.3.1 for more information on these shoot types), the full complement of leaves is usually produced shortly after flowering, so mineral movement by exchange may be less prominent than in long, non-terminating shoots, especially during periods of low transpiration.

Exchange adsorption of cations retards the rate of mineral translocation to a degree that depends on valency of the cation ($Ca^{2+} > K^{+}$), activity of the cation and competing cations, cation-exchange capacity (charge density of negative group) of the cell walls, pH of the xylem sap and diameter of the xylem vessels. Ions that are readily chelated or complexed are little affected by exchange adsorption and tend to travel through in xylem via mass-flow in the transpiration stream (Marschner, 1995).

Phloem mineral transport

Phloem mineral transport occurs in a bi-directional fashion, with the direction of transport being determined by solute demand (sink strength) of the various plant organs or sinks. Minerals may be loaded into the phloem in the roots, although it is more common for minerals to be loaded into the phloem in the stems and leaves of plants. Minerals also may be transferred from the xylem into the phloem, although there are few reports of transfer from the phloem to the xylem (Marschner, 1995). Due to the bi-directional nature of phloem transport, the phloem plays an important role in cycling mineral nutrients between various plant organs, and in transmitting signals between source and sink organs (Marschner, 1995).

Mineral ion mobility in the phloem varies; K and P are highly phloem mobile, while mobility of Ca is extremely poor, so Ca must travel almost exclusively in the xylem (Clarkson, 1984; Marschner, 1995). Mobility of Mg is intermediate between that of Ca and K (Marschner, 1995). Minerals travelling in the xylem are often directed towards organs with high rates of transpiration, whereas minerals travelling in the phloem tend to be allocated to organs on more of a per needs basis. Subsequently, a very small portion of the Ca taken up by a plant will end up in fruit. Most of the Ca will be in leaves, whereas much of the K and P will accumulate in fruit (Buwalda and Smith, 1987: Fig 1.5).

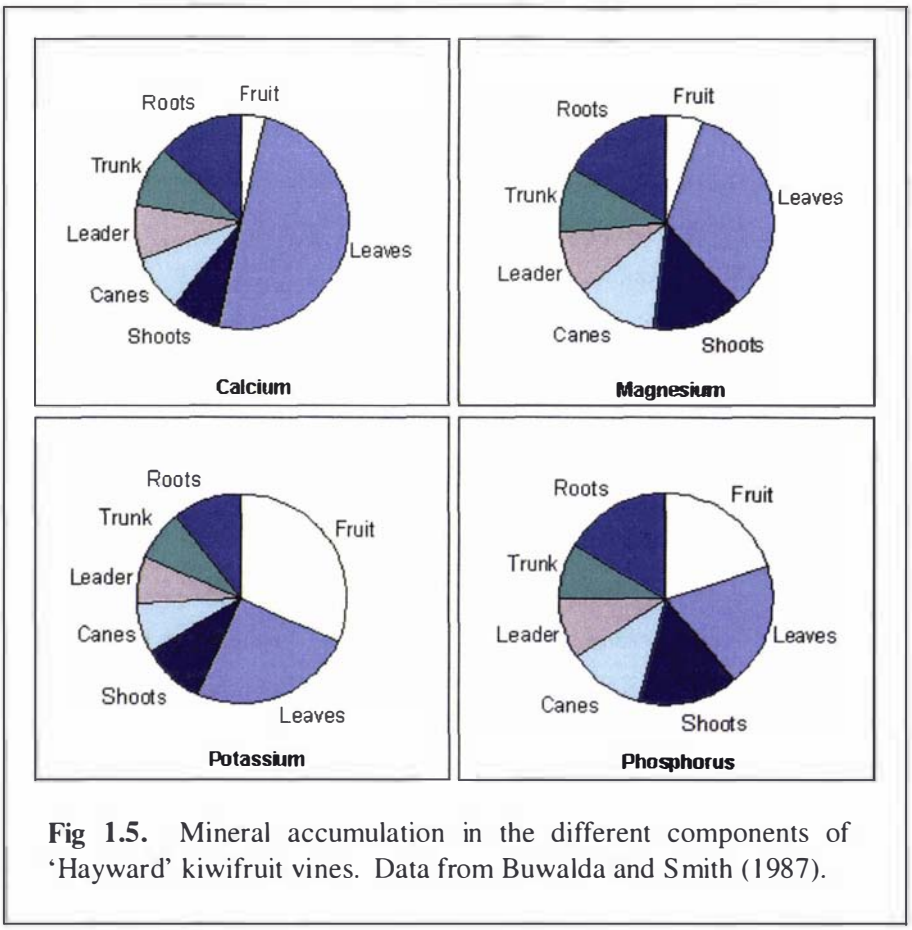


Fig 1.5. Mineral accumulation in the different components of 'Hayward' kiwifruit vines. Data from Buwalda and Smith (1987).

Non-vascular transport

A non-vascular component also may play a part in the fine regulation ingress of Ca into fruit, such as a cell-to-cell transport linked with a counter-transport of auxin (indolyl-3-acetic acid (IAA) and Ca (De la Fuente, 1984; Wand *et al.*, 1991a). Basipetal (downward) polar transport of IAA takes place most likely by H^+ -IAA symport at one end of the cell, and a Ca–regulated IAA efflux at the other (Banuelos *et al.*, 1987; Allan and Rubery, 1991). In addition to the interaction between Ca and IAA at the cellular level, there also is evidence that IAA regulates Ca allocation to developing tissues (Cutting and Bower, 1989). This is described in more detail in Section 1.6.5. As IAA efflux tends to be greatest from young, developing tissue (Banuelos *et al.*, 1988), IAA-Ca symport may play an important role in Ca accumulation in young fruit.

Mineral accumulation

Fruit Ca contents increase rapidly in the first eight weeks of kiwifruit growth (Fig 1.6) to around 70% of their final value at completion of cell division (Clark and Smith, 1988; Xiloyannis *et al.*, 2001). After this time, fruit Ca content increases slowly reaching a maximum value between February (Ferguson, 1980) and harvest in May (Clark and Smith, 1988). The decline in Ca

accumulation rates may occur because very little xylem water moves into fruit from eight weeks after anthesis (Xiloyannis *et al.*, 1999), or because xylem vessels in fruit become dysfunctional as fruit expand (Dichio *et al.*, 2003). This decline also could be due to a reduction in number of cation exchange sites formed in the apoplast (on cell walls) after the first phase of fruit growth, as cation exchange sites assist in xylem unloading by acting as sinks for Ca that accumulates at the end of xylem vessels (Marschner, 1995).

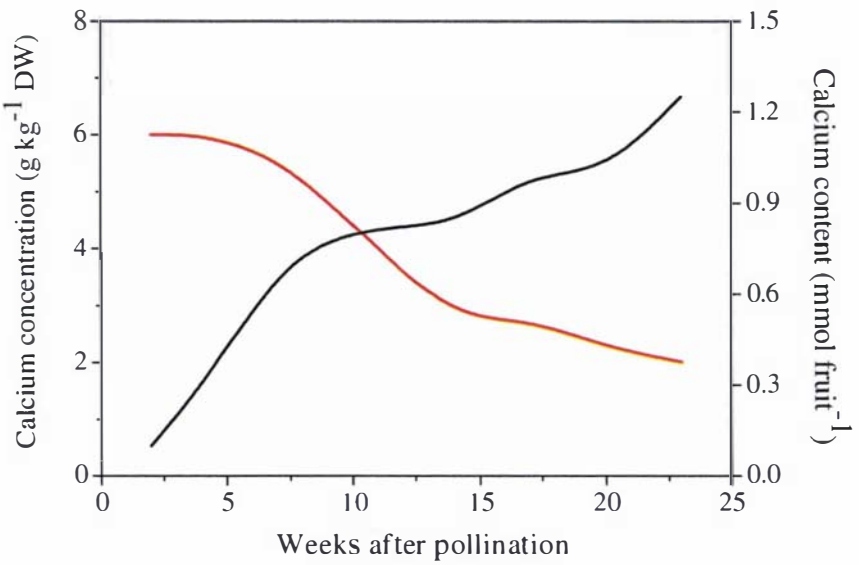


Fig 1.6. Fruit calcium contents (black) and concentrations (red) in 'Hayward' kiwifruit. Data from Clark and Smith (1988).

Fruit Ca concentrations decrease steadily during the season (Clark and Smith, 1988), whereas in leaves they increase linearly until leaf fall after an initial four week decline (Clark *et al.*, 1987). Since Ca cannot be transported in the phloem, it is not possible for Ca to be recycled from leaves to fruit (Buwalda and Smith, 1987). Leaves may compete with fruit for a supply of Ca when the plant is transpiring rapidly, as Ca is more likely to be directed towards high-transpiring organs. At night, when rates of plant transpiration are low, Ca accumulation in fruit may be driven by root pressure (Clarkson, 1984; Ho, 1988b; Huguet *et al.*, 1998), or Ca may be redistributed in the xylem from leaves back to the fruit (Lang and Volz, 1998: refer Section 1.3.2 for more information).

Accumulation trends for K and P are generally the opposite of those for Ca, whilst Mg shows a trend that is between that of Ca and K and P (Fig 1.7: Ferguson, 1980; Clark and Smith, 1988). This may reflect mobility of these ions in the phloem (Bangerth, 1979). Like Ca, the fruit content

of these three minerals increases until harvest, and although rates of accumulation are considerably slower than those for Ca during the first phase of fruit growth; they accumulate at faster rates later in the season (Ferguson, 1980; Clark and Smith, 1988). Concentrations of Mg in fruit decline throughout the season, albeit at a slower rate than Ca concentrations, whilst K and P concentrations decline during the first eight weeks of fruit growth, and then remain relatively steady until harvest (Clark and Smith, 1988).

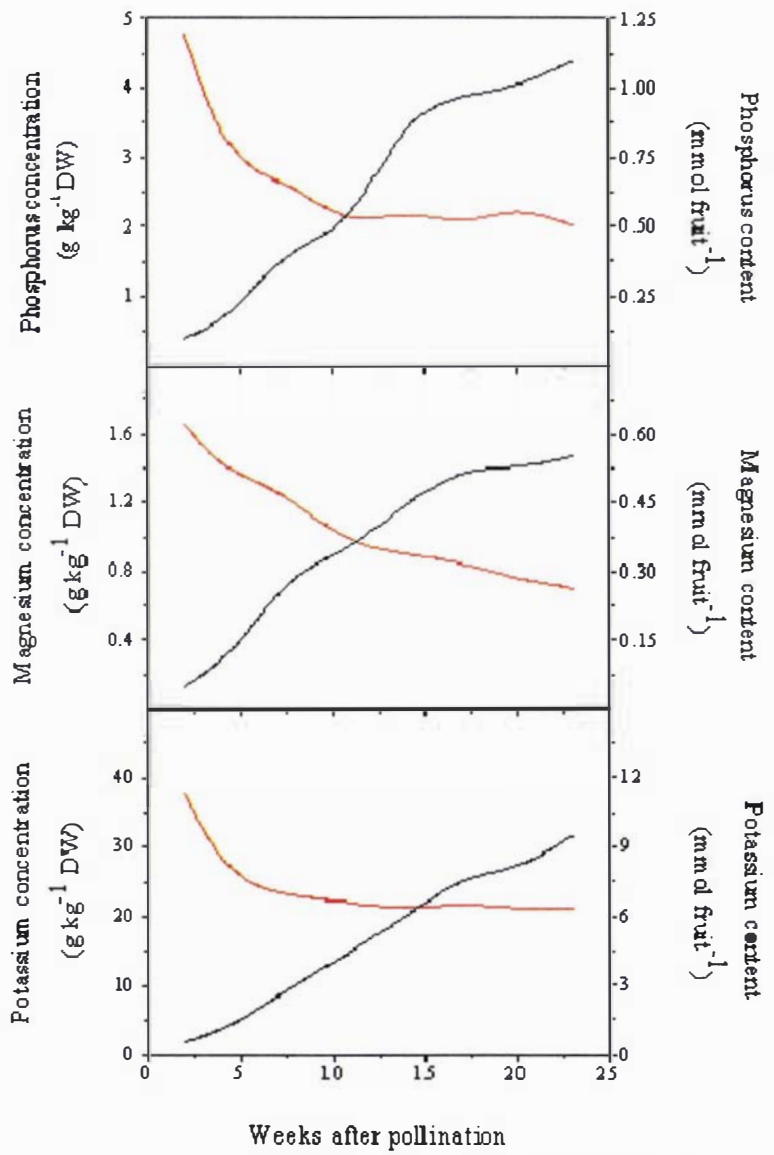


Fig 1.7. Fruit phosphorus, magnesium and potassium contents (black) and concentrations (red) in 'Hayward' kiwifruit. Data from Clark and Smith (1988).

As high fruit growth rates are often associated with high solute (sucrose) import via the phloem, the import of mobile mineral ions is more likely to keep track with fruit growth, whereas Ca concentrations will decline with fruit growth (Ho, 1988b). Furthermore, high rates of phloem unloading in fruit may depress the influx of xylem sap, or even reverse it (from fruit to shoot), especially if transpiration rates are low (Jones and Higgs, 1982; Lang and Thorpe, 1989), which would explain the decline in fruit Ca concentrations towards harvest.

Because of the greater phloem mobility of K, Mg and P, the ratio of these minerals to Ca will increase as fruit matures. Subsequently, there may be an imbalance of minerals in fruit, which could result in mineral disorders, such as pitting (Tadesse *et al.*, 2001; Thorp *et al.*, 2003b). This imbalance could be expected to be greater in fruit where there is a shift from a predominantly xylem supply of water to a predominantly phloem supply of water with increasing maturity (During *et al.*, 1987).

In summary, different mineral translocation mechanisms probably operate simultaneously in any fruit, the extent to which one translocation mechanism predominates over another depending on the: (a) fruit species and/or cultivar concerned, (b) climate in which the plant is growing; for example, during periods of low transpiration mineral translocation will be predominantly driven by root pressure or other mechanisms that are not regulated by transpiration (phloem transport, exchange adsorption, growth-induced mass flow, and non-vascular transport), (c) plant age (mineral accumulation in young plants that have a low surface and/or leaf area is little affected by transpiration, but may be strongly influenced by growth-induced mass flow), (d) concentration of mineral ions in the soil solution and/or xylem sap, and (e) type of solute transported (generally transpiration has a greater effect on uncharged molecules than on ions) (Marschner, 1995). In kiwifruit, the contribution that different mineral translocation mechanisms make to fruit mineral accumulation at different stages of fruit development is poorly understood. There also is a limited understanding of how the climate in which fruit develops may affect mineral accumulation in fruit.

1.2.4. Fruit DMC and its relationship to Ca

In New Zealand, patterns of fresh and dry weight accumulation in kiwifruit result in a DMC curve that declines during early growth due to a rapid influx in water, increases rapidly until the end of phase II fruit growth, and then increases more slowly until harvest as fruit DW continues to increase without a corresponding increase in water (Richardson *et al.*, 1997: Fig 1.8). In a study of Japanese fruit, this pattern differs slightly, as fruit DMCs drop slightly after phase II fruit growth before increasing slowly until harvest (Han and Kawabata, 2002).

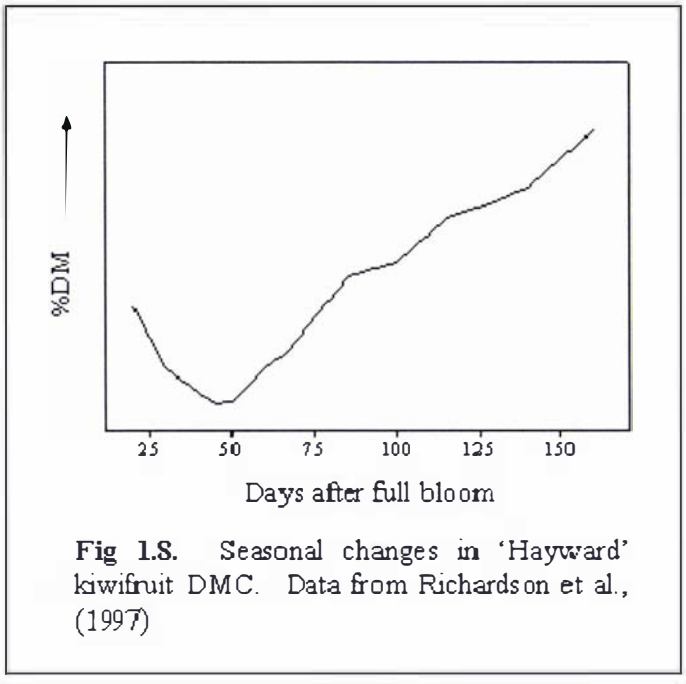


Fig 1.8. Seasonal changes in 'Hayward' kiwifruit DMC. Data from Richardson et al., (1997)

A simple positive linear relationship exists between fruit Ca and DM concentrations in 'Hayward' fruit sampled from within an orchard, and within a region. Although this relationship was not strong (R^2 = 0.57), it was consistent across orchards (Ferguson *et al.*, 2003). Within a vine, kiwifruit Ca concentrations were positively correlated with DMCs also, whilst P, K, and to a lesser extent, Mg concentrations were negatively correlated with DMCs (Smith *et al.*, 1994). Thorp *et al.* (2003b) found that Ca and DM concentrations in fruit on long, non-terminating axillary shoots were greater than those in fruit on short, terminating shoots of kiwifruit vines. This suggests that similar mechanisms control accumulation of these two fruit components, which is surprising as Ca and carbohydrates (the main component of fruit DW) are transported via different pathways (Clark and Smith, 1988). Furthermore, Ca is mainly accumulated during the first few weeks of fruit growth, whereas the DW of fruit increases almost linearly from fruit set.

It is possible that fruit DM and Ca concentrations are linked because the concentration of both of these components is affected by fruit water status. However, the relationship between Ca and DMC in kiwifruit has not been explored in detail, so this cannot be stated with any certainty. In papaya and bell-pepper fruit, increased DW accumulation lowered the water potential in fruit, which increased xylem flux into fruit and raised fruit Ca contents, although Ca concentrations declined as increases in Ca were not proportional to increase in FW accumulation (Qui *et al.*, 1995; Bar-Tal *et al.*, 1999).

Calcium is known to have important roles in signal transduction, cell division and membrane function, amongst other plant functions (Ferguson and Boyd, 2001). Therefore, it is possible that the inhibition of Ca accumulation may indirectly affect DW accumulation by disrupting these processes. For example, it has been proposed that the export of auxin from sink tissue, which is regulated by Ca (Section 1.2.3; non-vascular transport), might serve as a messenger to source (leaf) tissue to increase phloem loading (Daie, 1989). The remainder of this literature review is dedicated to a discussion on factors affecting fruit DM and Ca concentrations, with a focus on

kiwifruit. As both Ca and DMC are important quality determinants, an understanding of the factors affecting their physiology is important to kiwifruit fruit quality.

1.3. Canopy Management and Fruit Quality

1.3.1. Vine description

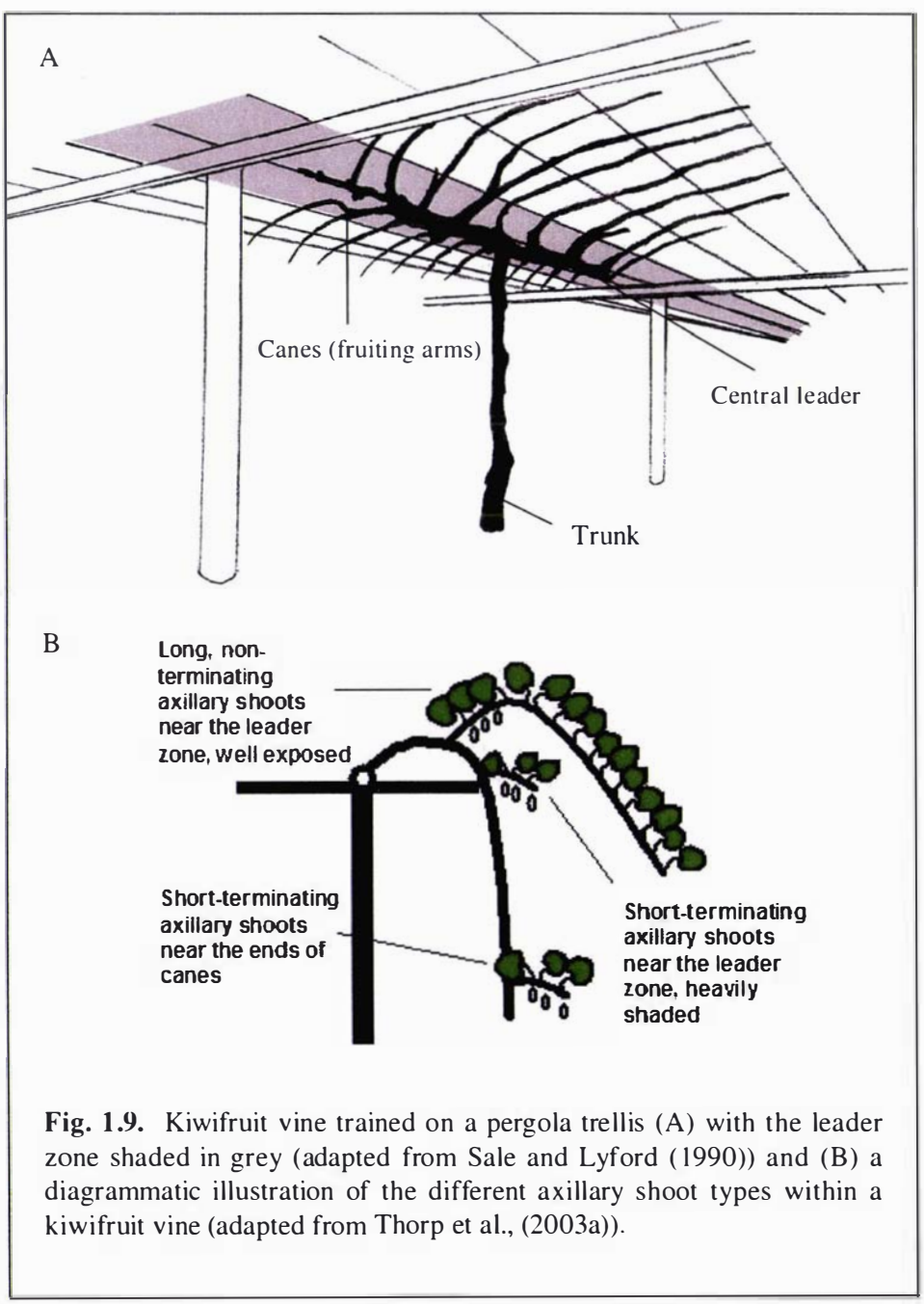


Fig. 1.9. Kiwifruit vine trained on a pergola trellis (A) with the leader zone shaded in grey (adapted from Sale and Lyford (1990)) and (B) a diagrammatic illustration of the different axillary shoot types within a kiwifruit vine (adapted from Thorp et al., (2003a)).

In New Zealand kiwifruit vines are trained on T-bar or pergola trellises, which support vine weight, enhance light interception by leaves, and provide a structure around which vines can easily be managed. Vines are trained as a single straight trunk, approximately 1.8 m high, which divides into two branches that run in opposing directions (Figure 1.9). These branches, known as the central or main leader, comprise the permanent wood. Current season's wood is comprised of fruiting arms, or canes, which grow at right angles to the central leader.

In spring bud-break occurs and axillary shoots, on which leaves and flowers will be borne, arise from the canes or parent canes as they are sometimes called (Sale and Lyford, 1990). As with the canes, these shoots may be terminating (short terminating) or non-terminating (long, non-terminating: Thorp *et al.*, 2003b), hereafter described as short and long shoots, respectively (Fig 1.9).

Short shoots cease extension growth in early summer and produce only a predetermined number of leaves preformed in the parent axillary bud. Long shoots produce more leaves than the number preformed in the parent axillary bud and continue extension growth until late summer (Selesnyova *et al.*, 2002). During summer, long shoots are normally pruned to three or four leaves past the last fruit, or if they aren't carrying fruit they are often removed to reduce competition with other shoots and fruit for carbohydrate and to improve light penetration in the canopy. Long shoots arising in the leader zone (LZ) of the vine, the zone that is about one metre wide and runs parallel to the central leader, may be left to grow to be tied down as 'replacement canes' for the following season.

On a whole vine basis, early season photosynthetic capacity is strongly related to leaf area development, although there are large day-to-day differences in photosynthesis associated with variation in light incidence and temperature. Maximum whole vine PS rates are obtained 3-5 months after leaf emergence when the leaf area index (LAI) usually exceeds 2.5 (Buwalda, 1994; Snelgar and Martin, 1997). However, an LAI of around 3.5 has been associated with maximum allocation of carbon to fruit growth and regeneration of reserves (Buwalda, 1991; Tombesi *et al.*, 1994; Snelgar and Martin, 1997). Carbohydrate is translocated bi-directionally from the leaves depending on the location and strength of sinks, including developing shoots and fruit (Lai *et al.*, 1988).

1.3.2. Canopy management

Canopy management strategies, including pruning, girdling and thinning are used to optimise light interception by leaves, and therefore production of photoassimilate, to enhance partitioning of this assimilate to fruit, and to replenish reserve carbohydrate pools within the vine. These strategies may affect fruit quality both directly and indirectly. Direct effects occur in response to the altered supply and demand for carbohydrate, disruption of phloem solute translocation and to changes in the mineral content and water status of fruit and leaves. Canopy management indirectly affects fruit quality by altering the canopy micro-climate, hormonal balance in the vine and wood type on which new shoots and fruit are borne (Mika, 1986; Saure, 1987; Chouliuras *et al.*, 1995).

Lack of, or inappropriate pruning leads to heavy shading, which may reduce vegetative growth (Grant and Ryugo, 1983; Morgan *et al.*, 1985), impair leaf health and hence photosynthetic efficiency (Biasi *et al.*, 1993; Smith *et al.*, 1994), and increase stomatal resistance (Chartzoulakis *et al.*, 1993b) and fungal disease incidence within the vine (Brigati *et al.*, 2003), the upshot of which is decreased carbon assimilation. In kiwifruit, shading has been shown to reduce vascular differentiation, especially xylem differentiation (Grant and Ryugo, 1983; Biasi and Altamura, 1996), and this along with increased stomatal resistance, may explain why a 50% reduction in photosynthetic photon flux density in one study reduced xylem sap flow by 55% and halved fruit Ca concentrations (Huang *et al.*, 2003). Vascular differentiation may be reduced in response to shading effects on auxin transport, as auxin efflux from fruit has been implicated in vascular development (refer Section 1.6 for more information).

Reported effects of shading on kiwifruit fruit development and quality are inconsistent and often small, but may include increased fruit drop and rot incidence, reduced fruit set, fruit size, firmness in storage, DMC, SSC and delayed maturity (Grant and Ryugo, 1983; Morgan *et al.*, 1985; Snelgar and Hopkirk, 1988; Snelgar *et al.*, 1991; Biasi *et al.*, 1993; Lionakis *et al.*, 1994; Snelgar *et al.*, 1998). Response of vines to shading may depend on interactions with crop load, the period over which vines are shaded, plant health and effects of temperature and other climatic factors on plant growth (Lionakis *et al.*, 1994; Snelgar *et al.*, 1998; Li *et al.*, 2003b). Shading effects may be more apparent when crop loads are high and/or temperatures are low, when carbohydrate supplies are already likely to be limited (Snelgar *et al.*, 1991; Li *et al.*, 2003b).

In addition to manipulating canopy light levels, pruning may alter the average cane age, angle, length and diameter as well as shoot lengths and diameter. On a pergola training system, fruit quality and fruit set are generally greater on large diameter (Inglese and Gullo, 1991; Volz *et al.*, 1991), horizontally growing canes (Snelgar and Thorp, 1988) that are initiated early in the season

(Snelgar *et al.*, 1992; Walton *et al.*, 2000). During early stages of fruit development more carbon may be partitioned towards fruit on terminated canes than towards fruit on growing canes, as fruit on the former do not have to compete with shoot elongation for a supply of photoassimilates (Amano *et al.*, 1998). Ultimately more carbon is partitioned towards fruit on large diameter canes than to fruit on small diameter canes (Thorp *et al.*, 2003a), although these large diameter canes are not usually terminated. Greater carbon partitioning to fruit on large canes and shoots may relate to the greater leaf area on these canes or to the increase seed number in fruit on large canes (Lai *et al.*, 1990; Volz *et al.*, 1991; Thorp *et al.*, 2003a).

As a result of the above research on the effects of shading and cane type, angle and age on fruit quality, the popularity of more open-style pruning systems that focus on utilising early season shoot growth and removing subsequent shoot growth, such as leader pruning (LP), is increasing (A. Mowat: pers. comm.). In comparison to the conventional (cane replacement) pruning systems (CP), LP has been shown to increase yields, mainly due to increases in fruit size, and fruit DMCs, especially DMCs of fruit in the LZ and/or of fruit on small diameter canes that normally have a reduced SSC (Miller *et al.*, 2001; Thorp *et al.*, 2003a). In addition, LP promotes leaf retention and delays leaf senescence on the vine. Fruit quality is not affected by the transition from CP to LP, and effects of LP may not become apparent until the year after transition to LP (Miller *et al.*, 1997). Although the effects of LP on mineral accumulation have not been investigated, regular summer pruning, which is a key feature of LP, has been shown to increase fruit Ca contents by 30% and this was associated with an increase in fruit firmness in storage (Gerasopoulos and Drogoudi, 2005).

Effect of leaves on mineral accumulation

Pruning and thinning may directly affect fruit Ca concentrations by altering the leaf:fruit (L:F) ratio on fruiting shoots. In apples, fruit Ca concentrations can be reduced by primary or bourse shoot removal, especially when applied at or soon after flowering. These positive effects of leaf area on Ca accumulation were noted soon after FB and continued until harvest (Ferree and Palmer, 1982; Proctor and Palmer, 1991; Volz *et al.*, 1994; Volz *et al.*, 1996). Fruit K and P concentrations were not affected by leaf area, while Mg concentrations tended to be slightly reduced by leaf removal, although not consistently (Proctor and Palmer, 1991; Volz *et al.*, 1996).

Jones and Samuelson (1983) concluded that apple fruit Ca accumulation was linked to leaf transpiration, since leaf bagging, which would not affect the exchange of hormones, carbohydrates or other substances between fruit and leaves, reduced fruit Ca concentrations. Since minimal Ca exchange occurs between leaves and fruit in the phloem (Jones *et al.*, 1983) it was proposed that leaves increase fruit Ca concentrations by promoting the flow of (Ca containing) xylem sap

between leaves and fruit (Lang and Volz, 1998). In one model Lang and Volz (1998) suggested that xylem sap, which contained low concentrations of Ca, may be drawn out of fruit towards transpiring leaves during day-time, and then later during the night is more than replenished by sap containing high Ca concentrations that flows from leaves to fruit. Consequently, fruit gain more Ca at night than they lose during the day. Factors that reduce leaf transpiration, such as defoliation or application of antitranspirants, lower leaf Ca concentrations, reduce the amount of Ca gained by fruit at night, and hence lower fruit Ca concentrations (Lang and Volz, 1998). It is possible that Ca drawn towards rapidly transpiring leaves accumulates or is sequestered in shoots, and is able to supplement Ca entering fruit in the transpiration stream as occurs in some legume species (Atkinson *et al.*, 1992).

Apart from apples, there is little information on the affects of leaf area on Ca accumulation in other crops. Calcium nitrate applied to leaves of capsicum fruit increased fruit Ca concentrations (Schon, 1993), suggesting sap flowed between leaves and fruit, similar to that observed by Lang and Volz (1998). Airflow over tomato canopies reduced fruit Ca and promoted blossom-end rot (BER), a Ca-deficiency related disorder, while airflow over fruit enhanced fruit Ca concentrations and reduced BER incidence (Wui and Takano, 1995). Anti-transpirants applied to potato leaves increased tuber Ca concentrations and reduced tuber necrosis (Win *et al.*, 1991). Therefore, unlike apple fruit, Ca accumulation in tomato and potato may be negatively related to leaf area and/or leaf transpiration rates, and therefore fruit and leaves may actually compete for a supply of Ca. Subsequently, in these crops fruit transpiration may be more important than leaf transpiration in promoting Ca accumulation.

In kiwifruit, Ca accumulation may be related to leaf area, as fruits with desirable mineral composition tend to be located in parts of the canopy where the leaf area is highest (Smith *et al.*, 1994). However, while fruit Ca concentrations increased with increasing leaf area on short shoots, increasing leaf area had no effect on the Ca concentration of fruit on long shoots (Thorp *et al.*, 2003b). This suggests that once the leaf:fruit ratio on a shoot exceeds a certain value, fruit Ca accumulation is no longer affected by further increases in leaf area, and other factors may be more important in determining fruit Ca concentrations. It is possible that leaves have a reduced effect on Ca accumulation from 8 weeks after full bloom (WAFB), as xylem tissues in the fruit may be largely dysfunctional from this time (Dichio *et al.*, 2003), so Ca could not move by exchange between fruit and leaves.

Crop load and fruit quality

In kiwifruit, fruit fresh and dry weights are negatively correlated with crop load. However, as crop loads increase, yields are increased also and consequently, there is a positive correlation between

crop load and the total amount of carbon partitioned to fruit (Richardson and McAneney, 1990; Richardson *et al.*, 1997: more details can be found on the specifics of crop loading in the following section). As less carbon is partitioned to fruit at low crop loads, fruit DMCs are only marginally (by less than 1%) increased by substantial reductions in crop load (Richardson *et al.*, 1997). In high yielding vines, where carbohydrate supplies may be limiting, the lack of reduction in fruit DMCs appears to be relate to the ability of kiwifruit vines to reduce carbohydrate partitioning to other non-fruit sinks, rather than to reduce carbohydrate allocation to fruit (Richardson and McAneney, 1990; Smith *et al.*, 1992). Ability of high crop-loading kiwifruit vines to partition more carbohydrate towards the fruit may relate to their higher daily carbon uptake, both per unit leaf area and in absolute terms, when compared to low-cropping vines (Vizzotto *et al.*, 2003) as fruit metabolic activity is little affected by crop load (Zhou *et al.*, 2000; Klages *et al.*, 2001).

The presence or absence of fruit has a major impact on photosynthetic performance and growth responses of apple trees (Wünsche and Ferguson, 2005). In low-yielding trees, vegetative vigour may initially be enhanced (Palmer, 1992), but once shoot growth ceases there may be an imbalance between carbohydrate metabolism and absorbed excitation energy (Wünsche *et al.*, 2005). This may directly reduce leaf photosynthetic efficiency by: (a) increasing non-radiative thermal dissipation, in response to accumulation of xanthophyll cycle carotenoids in leaves, to redistribute energy away from photosynthesis (Wünsche *et al.*, 2005), and (b) altering leaf morphology to increase the path length for CO_2 diffusion in the mesophyll (Wünsche and Palmer, 1997). Leaf photosynthetic efficiency also may be indirectly reduced at low crop loads if starch accumulates in leaf chloroplasts, which may: (a) prevent absorbed light from reaching thylakoids, thus inhibiting the light-dependent stage of photosynthesis, (b) reduce Rubisco activity and leaf chlorophyll concentrations (Wünsche *et al.*, 2005), and (c) physically damage the chloroplast (Wünsche *et al.*, 2000). Furthermore, stomatal apertures may be reduced, in response to increasing intercellular CO_2 concentrations, in order to control rates of carbon uptake (Lenz, 1978; Mansfield *et al.*, 1981; Giuliani *et al.*, 1997). Recently, it was proposed that leaf carbohydrate accumulation stimulates leaf hormone accumulation, and that this induced stomatal closure (Wünsche *et al.*, 2005).

In kiwifruit, rates of leaf photosynthesis also are reduced at low crop loads (Vizzotto *et al.*, 2003) and carbohydrate may accumulate in leaves causing end-product or feedback inhibition of photosynthesis, similar to that in apples (Currie, 1997). However, transpiration rates of individual leaves are little affected by crop load, and may even be higher in low-yielding vines (Vizzotto *et al.*, 2003). This suggests that stomatal aperture is not affected by leaf carbohydrate accumulation and, therefore, one might expect little effect of crop load on the accumulation of minerals, such as Ca, that travel by mass flow in the transpiration stream. This would contrast to the situation in

apples, where stomatal closure reduces leaf transpiration at low crop loads (Wünsche *et al.*, 2000). Coincidently, fruit Ca concentrations, and to a lesser extent fruit Mg concentrations are reduced at low crop loads, and incidence of bitter pit and other Ca-related storage disorders in apples is enhanced (Fallahi and Simons, 1993; Volz *et al.*, 1993; Volz and Ferguson, 1999). Accumulation of K, which travels predominantly in the phloem (Lang, 1990), was enhanced at low crop loads (Fallahi and Simons, 1993; Volz *et al.*, 1993).

Effects of crop load on mineral accumulation have never been investigated in kiwifruit. However, it has been found that to increase fruit size the best time to thin is at or very soon after flowering, as this increases the carbohydrate availability for subsequent fruit growth (Richardson and McAneney, 1990; Richardson *et al.*, 1997). Alternatively, thinning 3-4 weeks after flowering has been shown to have a bigger impact on kiwifruit DMCs than thinning at flowering, as thinning at this time produces smaller fruit, but still increases carbohydrate availability, thereby increasing the ratio of DW:FW (Currie *et al.*, unpublished data). The crop load that can be supported by a vine will depend on its leaf area, climate for growth and the management practices (i.e. to increase fruit size or fruit DMC) of the grower (Cooper and Marshall, 1991; Tombesi *et al.*, 1994).

Girdling and fruit quality

Girdling is the interruption of phloem transport between the canopy and roots, in order to manipulate photoassimilate, mineral nutrient and plant hormone distribution within the plant. This is achieved by removing a part or complete cylinder of all tissues external to the secondary xylem from a branch or trunk unit in order to block translocation into or out of that unit, thereby isolating it from the remainder of the plant (Noel, 1970; Goren *et al.*, 2004). The effect of girdling depends on the width and extent of the girdle (bark removed), timing of girdle application and the L:F ratio on the isolated unit.

L:F ratios, girdling and fruit quality

Girdling is often used as a tool to examine the effects of specific L:F ratios on fruit quality. In girdling studies on isolated shoots of kiwifruit a L:F ratio of 2:1 was required for a shoot to support fruit growth without having to import carbohydrate from elsewhere (Lai *et al.*, 1989a; Seager and Hewett, 1995; Snelgar *et al.*, 1998). Fruit on girdled shoots with a L:F ratio of less than two had a lower FW, DW, and ripe soluble solids content (rSSC) than fruit on un-girdled shoots. Fresh weight, DW and rSSC of fruit on girdled shoots with a L:F ratio of more than two was higher than in fruit on un-girdled shoots, and increased in a curvilinear manner in response to increasing leaf area. Although FW continued to increase above a L:F ratio of 4:1, fruit DW did not (Famiani *et al.*, 1997).

In contrast to girdled shoots, fruit on un-girdled shoots were not affected by changes in leaf area, as carbohydrate could be transported considerable distances from parts of the vine where there was an excess of carbohydrate, to shoots where there was a shortage of carbohydrate (Lai *et al.*, 1989b; Lai *et al.*, 1989a; Tombesi *et al.*, 1993). However, when leaf area was severely reduced, leaves closest to a fruit provided the most important source of carbohydrate, and fruit on shoots with insufficient carbohydrate to support their growth tended to abscise or were very small (Buwalda and Smith, 1990b; Tombesi *et al.*, 1993)

Lai *et al.* (1989a) attributed increases in FW and DW of fruit on girdled shoots with a high L:F ratio to the greater availability of carbohydrate in these shoots, as they found that leaf photosynthesis and stomatal conductance were not affected by girdling. Furthermore, they found no effect of crop load on photosynthesis of individual intact shoots. Their results were based on "one-off" measurements of photosynthesis on shoots with varying L:F ratios. When leaf photosynthesis was measured on a daily basis, leaves subtending fruit had a lower rate of photosynthesis than leaves of vegetative shoots and hence produced less carbohydrate over the growing season (Greer, 1999). While these results both suggest that photosynthesis in kiwifruit is not feedback inhibited, they differ from each other, and from the results of Vizzotto *et al.* (2003) who measured leaf photosynthesis on a whole vine basis and found that rates of leaf photosynthesis were higher in high-yielding vines than in low yielding vines.

In other crops, the affect of girdling on shoot photosynthesis and transpiration are equivocal also. Photosynthetic rates of apple leaves were reduced when girdles were applied at petal fall, but not when applied later in the season (Ferree and Palmer, 1982). Branch girdling in sweet cherry had no effect on rates of photosynthesis and transpiration (Roper *et al.*, 1987). Rates of photosynthesis in girdled nectarine branches were lower than those in un-girdled branches from 16 DAFB, as were rates of transpiration (Di Vaio *et al.*, 2001). It is likely that the response of individual shoots to photosynthesis differs according to factors such as the shoot L:F ratio, whether shoots are terminating or not, and the demand for assimilates from nearby shoots. In addition, leaf photosynthesis is likely to change in response to climatic changes and leaf maturation.

Leaf transpiration rates were reduced in girdled nectarine branches, as were leaf K, Ca and P concentrations (Day and De Jong, 1990; Di Vaio *et al.*, 2001). Due to the relationship between leaf transpiration and fruit Ca concentrations (described above), it might be expected that girdling would reduce the Ca concentration of nectarine fruit. Currie *et al.* (unpublished data) found that girdling had no effect on kiwifruit mineral concentrations, which suggests that transpiration may not be affected by girdling in this fruit crop. This agrees with the results of Vizzotto *et al.* (2003) the leaf transpiration is not affected by crop load.

Girdling effects on shoot hormone concentrations and hydraulic conductivity

In addition to altering the carbohydrate balance in a shoot, girdling also may affect fruit quality by altering a shoot's hormone balance (Dann *et al.*, 1985; Cutting and Lyne, 1993; Currie, 1997). Girdling increased IAA accumulation in kiwifruit shoots when applied at full bloom and this was thought to affect seed development as the average fruit on a girdled shoots had nearly 400 fewer seeds than control fruit (Currie, 1997). Girdling increased fruit IAA accumulation and reduced Ca accumulation during the second phase of nectarine growth, but increased Ca accumulation when applied during the final stages of nectarine growth so that fruit from girdled trees had a higher Ca concentration at harvest than fruit from un-girdled trees (Wand *et al.*, 1991b). Girdling reduced final fruit Ca concentrations in certain apple cultivars, and this was attributed to the blocking of auxin transport (Tomala and Dilley, 1990).

Effects of girdling may depend on fruit seed numbers. For example, FW of seedless grape fruit was increased by girdling after fruit set, whereas response of seeded varieties to girdling was inconsistent (Carreño *et al.*, 1998). Girdling caused IAA to accumulate in fruit (Dann *et al.*, 1985), which may explain why development of fruit with few or no seeds that produce little of their own IAA, is enhanced by girdling. In this respect, kiwifruit from long shoots, with plenty of seeds may respond less to girdling than fruit from short shoots that have fewer seeds (Lai *et al.*, 1990).

Finally, there is evidence that girdling may indirectly reduce (xylem) hydraulic conductance by lowering the xylem sap osmotic potential (Zwieniecki *et al.*, 2004), and this could affect Ca translocation to fruit. Pectins, known as hydrogels, which are located in bordered pit membranes, swell and shrink in response to changes in xylem sap osmolarity, thereby affecting xylem conductivity (Zwieniecki *et al.*, 2001).

Timing of girdling

Currie (1997) found that the best time to girdle to increase fruit size was 21 DAFB (spring-girdling). However, fruit on canes/shoots girdled at this time had a lower DMC than control fruit. Girdling in February (autumn girdling) had little effect on the FW of the fruit, but increased the DMC of fruit on the girdled cane/shoot when compared to the control. Cane girdling in both spring and autumn produced fruit that had both a higher FW and DMC than control fruit at harvest (Thorp *et al.*, unpublished data). In grapes spring trunk girdling increased fruit size, while autumn girdling increased grape SSC, and double girdling (in both spring and autumn) increased both the FW and SSC of fruit (Carreño *et al.*, 1998).

Interestingly, when kiwifruit shoots were girdled at 14 DAFB the DMC and FW of fruit on those shoots was lower than in control fruit at harvest (Currie, 1997). Girdling impeded auxin export from the shoot (Currie, 1997) and as rates of auxin release and vascular development are maximal around this time (see Section 1.5 for more information), girdling at this time may inhibit vascular development and restrict subsequent carbohydrate and mineral accumulation.

1.4. Fruit water loss and kiwifruit internal quality

Ca is primarily transported through plants in the transpiration stream (refer Section 1.2 for more information). Accordingly, an organ's Ca content will be related to the volume of water moving into it (Bangerth, 1979), which is largely determined by its transpiration rate. Transpiration of fruit involves diffusion of water vapour from fruit to the surrounding environment. In any given environment, rate of fruit water loss (fruit transpiration) can be calculated using the steady state solution of Fick's first law of diffusion (Equation 1.1: Nobel, 1991):

$$r'_{H_2O} = P'_{H_2O} \times \Delta p_{H_2O} \times SA \qquad (1.1)$$

where:

r'_{H_2O} = Rate of water loss (mol s^{-1})

P'_{H_2O} = Permeance of the fruit surface to movement of water vapour under prevailing conditions (nmol m^{-2} s^{-1} Pa^{-1}) or the ease with which water can escape from the fruit.

Δp_{H_2O} = Difference in partial pressure of water vapour between the environment and inside the fruit, (Pa) or driving force for water loss.

SA = Surface area of the fruit (m^2)

The driving force for water loss is determined by the water vapour partial pressure difference (VPD) between the environment and the inside of the fruit. Partial pressure is determined by both temperature and relative humidity (RH) of the environment. Water activity (a_w) is a measure of the partial pressure of vapour in the intercellular spaces, and in kiwifruit this ranges between 0.986-0.990 for a range of 13.8° – 15.7°Brix (Garcia-Martinez *et al.*, 2002). When air RH is less than the a_w water will move from the fruit into the surrounding environment, or from a high to a low concentration according to the laws of diffusion (Nobel, 1991). The greater the difference between a_w and RH of the air, the greater the water loss or fruit transpiration.

Effects of fruit transpiration on mineral accumulation may depend on: (a) stage of fruit maturity, (b) mobility of the mineral ion being measured in the phloem (refer Section 1.2.3 for more information), (c) dependence of fruit mineral accumulation on translocation by mass flow in the xylem transpiration stream (refer Section 1.2.3 for more information), and (d) the climate in which fruit are grown. A positive correlation exists between fruit transpiration and Ca accumulation in mango (Shivashankara and Mathai, 1999), grape (During and Oggionni, 1986), and paprika (Mix and Marschner, 1976) fruit. In kiwifruit (Xiloyannis *et al.*, 2001), papaya (Qui *et al.*, 1995) and beans (Mix and Marschner, 1976) the greatest rate of Ca uptake into fruit occurred when fruit transpiration rates were greatest; reduced fruit Ca concentrations later in the season were attributed to reductions in fruit transpiration. Conversely, a poor relationship was found between fruit Ca absorption and transpiration in grape (Boselli and Di Vaio, 1996) tomato (Mulholland *et al.*, 2000) and apple (Failla *et al.*, 1990) fruit.

In contrast to Ca, changes in the rate of fruit transpiration generally have little affect on the more phloem mobile nutrients, K, Mg and P (Mix and Marschner, 1976; During and Oggionni, 1986; Tromp and Van Vuure, 1993; Li *et al.*, 2003a). Exceptions include the increase in P concentrations in the peel of muskmelon fruit at low humidity (Combrink *et al.*, 1995) and the increase in K concentrations in apple fruit bagged two months after full bloom (Fallahi *et al.*, 2001).

In conditions of high humidity (low driving force for water loss) some fruit may have the ability to utilise alternative pathways to import Ca and other minerals that do not rely on translocation by mass flow in the transpiration stream. Strawberry plants appear to regulate Ca concentrations in the xylem sap independent of fruit transpiration (Choi *et al.*, 1997). In tomato, root pressure may be a major factor promoting transport of Ca into tomato fruit under the low transpirational flux induced by high humidity (Bradfield and Guttridge, 1984). In mango fruit Ca concentrations were reduced when fruit were bagged for less than 56 days, whereas in fruit bagged for longer than 82 days Ca concentrations were the same as for control fruit (Hofman *et al.*, 1997). Mango plants exposed to humid conditions for extended periods of time may utilise alternative pathways to import Ca to the fruit that do not rely on rate of fruit transpiration (Hofman *et al.*, 1997).

Long-term exposure to high humidity may also prompt changes in the cuticle structure that increase permeance to water vapour (Lendzian and Kerstiens, 1991; Combrink *et al.*, 1995; Hofman *et al.*, 1997; Beasley *et al.*, 1999). In addition, vascular differentiation may be enhanced at high humidity and this could increase the capacity of fruit to accumulate Ca and other xylem mobile nutrients. Li *et al.* (2003a) found that the number of vascular bundles in plants grown at high (60-70%RH) and ambient (30-40% RH) humidity were increased by 14.6 and 11.4%

respectively. The response of kiwifruit transpiration rates to changes in RH and the effect of changes in humidity on Ca accumulation in fruit are not known. It is possible that kiwifruit Ca concentrations could be affected by the conditions in the external environment during fruit development.

1.4.1. Fruit skin structure and water loss

The cuticle is the outermost layer of the skin of mature fruit, and provides a barrier to prevent excessive water loss from a fruit to its environment (Holloway, 1982). Cuticle structure influences the conductance, or permeance of a fruit skin to water vapour. Fruit permeance to water vapour varies considerably according to species, cultivar and stage of development (Holloway, 1982). Smith *et al.* (1995) defined three stages for changes in fruit permeance in kiwifruit; an initial rapid decline in the first two weeks after anthesis, followed by a less rapid decline for eight weeks as the suberised periderm develops, and finally a stage of relatively constant conductance. Similar findings have been reported in apple (Jones and Higgs, 1982; Woods, 1990), grape (Ollat and Gaudillere, 1998) and papaya (Qui *et al.*, 1995) fruit. However, during the initial rapid decline in surface conductance, conductance of kiwifruit is up to 10 times greater than that of apple (Smith *et al.*, 1995). Therefore, kiwifruit may have a greater potential than apple fruit to accumulate Ca in the early stages of fruit development when most Ca is accumulated in the fruit. This may explain why final Ca concentrations in kiwifruit are greater than those in apple (Kirkby and Philbeam, 1984).

At harvest, water vapour permeance measurements of 'Hayward' kiwifruit were 23.7 $nmol.s^{-1}.m^{-2}.Pa^{-1}$, which was slightly lower than that of Zespri Gold™ fruit (31.5 $nmol.s^{-1}.m^{-2}.Pa^{-1}$; D. Green: unpublished data). This compares to average permeance values for apple of 31.6 $nmol.s^{-1}.m^{-2}.Pa^{-1}$ (Maguire *et al.*, 1999a). Fruit permeance values of a batch of 80 Hayward fruit ranged from 16 to 35 $nmol.s^{-1}.m^{-2}.Pa^{-1}$, a 2-fold difference (D. Green: unpublished data). By contrast, permeance of 100 Braeburn apples harvested from the same orchard could vary up to 30-fold (Maguire *et al.*, 1999a). These variations may relate to differences in the structure of individual fruit skins, which change as fruit develop, and may be influenced by climate and cultural management practices (Lescourret *et al.*, 2001).

Fruit skin structure

The decline in permeance as fruit mature has been attributed to changes in the soluble cuticle lipid component of the fruit skin that occur during fruit development (Woods, 1990). Skin permeance also may decline as fruit mature when wax crystals in the fruit skin are degraded by a combination

of weathering by wind, solar radiation and mechanical abrasion after wax production has ceased (Jenks and Ashworth, 1999). In apple, permeance is drastically increased by development of small micro-cracks on the cuticle surface (Maguire *et al.*, 1999b). In 'Golden Delicious' apples these micro-cracks were caused by uneven rate of development of epidermal and cuticle layers, and were extended and widened by enlargement of the fruit as it neared maturity (Meyer, 1944). Fruit growth has been implicated also with development of micro-cracks and enhanced skin conductance of peach (Huguet *et al.*, 1998) and capsicum (Aloni *et al.*, 1998) fruit. Micro-crack development also may occur in response to sudden changes in climate and may be affected by management practices, such as spraying, thinning and pruning (Opara *et al.*, 1997).

In kiwifruit, permeance is thought to decline in response to structural changes in the epidermal cell layers (Xiloyannis *et al.*, 2001; Hallett and Sutherland, 2005). Permeance also may be affected by trichome structure and density. Trichomes are extensions of the epidermal cells and 'Hayward' kiwifruit are covered with two types of trichomes, large multiseriate trichomes and small, two-celled uniseriate trichomes (White, 1986a). Trichome initiation begins around 21 days after vegetative bud burst, when the ovary is at an early stage of development and the epidermis is actively dividing, and ceases 12-13 weeks later, although trichomes may continue to grow in length after this time. In small and developing fruit there are more trichomes per unit surface area than in large fruit. Trichome density is reduced as fruit expands, because trichome initiation does not keep track with fruit growth. Trichome abundance does not differ significantly from stalk to stylar ends of fruit (White, 1986a; White, 1986b).

Trichomes create an uneven or rough surface around fruit in which humid air that is lost from fruit may be trapped. This boundary layer of still, humid air reduces the VPD between the fruit and its environment and hence the driving force for water loss. Permeance of the boundary layer to water vapour is inversely related to its thickness or width. Boundary layer thickness is in turn positively correlated with trichome density, as air is more likely to get trapped between densely packed trichomes than between sparsely packed trichomes (Perez-Estrada *et al.*, 2000; Lenssen *et al.*, 2001).

In young kiwifruit trichomes are still living, so water may be lost from their surface (Smith *et al.*, 1995). As trichome density is very high in young fruit this may represent a significant increase in surface area for water loss and may explain why initial water loss from kiwifruit is higher than that of apple fruit that have no trichomes. At maturity, cells of kiwifruit trichomes are non-living (Schmid, 1978), therefore they are unlikely to contain water unless it is pulled into trichomes by capillary action (Smith *et al.*, 1995). Consequently, these trichomes are unlikely to be transpiring, although they will increase the boundary layer resistance to water loss. The importance of

boundary layer resistance in mature fruit will depend on the wind-speed around fruit, as layers of still air around fruit are broken down as the air velocity increases, and hence thickness of the boundary layer is reduced (Nobel, 1975). In high winds, the boundary layer will provide minimal protection against water loss, meaning that the cuticle will provide the most important barrier to water loss.

Permeance of *A. chinensis* 'Hort16A' kiwifruit at maturity is higher than that of very immature fruit, which have more trichomes than 'Hayward' fruit. This implies that the boundary layer may reduce permeance in the latter. Other studies also have shown that water loss is negatively correlated with trichome density and uniformity of trichome distribution (Perez-Estrada *et al.*, 2000; Bandyopadhyay *et al.*, 2004). Multi-celled and branched trichomes also are more effective at preventing water loss than uniseriate trichomes that are less elongated and less complex in structure (Bandyopadhyay *et al.*, 2004).

In addition to differing between fruit species and stage of fruit maturity (Bandyopadhyay *et al.*, 2004), trichome density may be affected by day-night temperature differences and changes in photoperiod (Gianfagna *et al.*, 1992; Perez-Estrada *et al.*, 2000). In tomato, trichome density was lower during short days than during long days, and was greatly influenced by temperature during winter (short days) (Gianfagna *et al.*, 1992). Sun exposed *Wigandia urens* plants had 3 and 28 times more trichomes than shaded plants in the dry and wet seasons, respectively. Futhermore, leaf trichome densities could be reduced by applying irrigation and/or shading to these plants (Perez-Estrada *et al.*, 2000).

Variations in fruit permeance

Despite the effects of temperature and light on cuticle permeance, Maguire *et al.* (1999a) could find no difference in permeance between the sun and shade sides of apple fruit. However, in grapefruit the sun-exposed surface of fruit from exterior canopy positions had a lower diffusive resistance to water vapour than the shaded surface of the same fruit (Purvis, 1984). Apple fruit from the inner canopy had mean water vapour permeance values 57% greater than those from the outer canopy (Maguire *et al.*, 1999a).

In kiwifruit it might be expected that fruit on long shoots would have a greater permeance than fruit on short shoots, because the former are borne on shoots in more exposed positions and tend to be larger, so are more likely to develop micro-cracks. Differences in the extent of micro-cracking between fruit have been implicated as the reason for variability in fruit permeance values found between fruit of the same cultivar, grown in the same environment (Leonardi *et al.*, 1999; Maguire *et al.*, 1999b). However, Smith *et al.* (1995) found little difference in permeance of fruit from the

lower (0.5-1 m above ground) and upper (1.5-2 m above ground) canopies. They did not measure differences in permeance between fruit on long and short shoots, but these data suggested that fruit exposure has little effect on permeance, although it did affect fruit water status, because fruit from the lower canopy had a higher water content than fruit from the upper canopy (Smith *et al.*, 1995). It is possible that differences in permeance of kiwifruit from different positions may be related to the structure and density of trichomes on fruit.

1.4.2. Fruit surface area and water loss

In beans (Grusak and Pomper, 1999) and eggplants (Diaz-Perez, 1998), transpiration rates, and fruit Ca content, were inversely related to fruit diameter. Alfalfa stem diameter had a greater impact on rate of water loss (drying rate) than trichome density (Lenssen *et al.*, 2001). In small eggplant fruit (<100g) transpiration was reduced significantly with minor increases in fruit size, although increases in fruit size resulted in little reduction in transpiration rates of larger (>100g) fruit (Diaz-Perez, 1998). Reductions in rate of water loss with increasing pod/stem diameter may be due to a reduction in the surface area (SA):volume ratio over which water can be lost, or to a change in properties of the cuticle occurring during pod/stem development. Ca influx may be unable to keep pace with increases in pod diameter (mass gain) as competition between fruit and leaves for water will be enhanced as more vegetative growth develops (Qui *et al.*, 1995). Leaves may attract greater volumes of water, and hence Ca, because their high SA: volume ratio means they transpire at an increased rate. In crops that rely on Ca accumulation by mass flow in the transpiration stream, fruit Ca concentrations may be reduced when rates of leaf transpiration are high. In tomato, evaporation per unit SA is little affected by fruit weight, which suggests that other characteristics of the fruit, such as the extent of cuticle development, play a greater role in determining fruit transpiration rates (Leonardi *et al.*, 1999).

1.5. Vascular tissues and their development in the fruit stalk

1.5.1. Effects of vascular capacity on carbohydrate and mineral accumulation

The fruit stalk is the final link between the vegetative organs of the plant and the fruit. Vascular capacity of the stalk therefore, may be vital in determining a fruit's potential to accumulate minerals and carbohydrates. The amount and distribution of vascular tissue within an organ may bear a direct relationship to its final size (Nii, 1980b; Nii, 1980c; Nii, 1980a; Bustan *et al.*, 1995). However, it is questionable whether vascular development increases proportionally in response to

tissue growth or vice versa. García-Luis *et al.* (2002) observed that differences in the rate of formation of conductive tissue in the fruit stalk of the developing citrus fruitlets followed rather than preceded changes in growth rate. They concluded that fruit growth and/or dry matter accumulation in citrus was not limited by vascular capacity of the phloem, but that rate of ovary development or sink potential at flowering and during early stages of fruitlet development determined subsequent vascular formation to ensure that forthcoming transport and mechanical requirements of the fruit were met. Other studies in strawberry (Darnell and Martin, 1987), citrus (Guardiola *et al.*, 1993) and maize (Salvador *et al.*, 1994) crops provide substantial evidence to support this conclusion.

While the above information suggests that phloem transport capacity does not restrict solute movement in the phloem, solute movement may be restricted by the xylem transport capacity (Ho *et al.*, 1993; Dražeta, 2002). Grape varieties with distinct constriction zones were more susceptible to bunch stem necrosis, a symptom of localised Ca deficiency, than varieties without constriction zones (Lee, 1989; During and Lang, 1993). Tomato fruit Ca concentrations were positively related to the extent of secondary xylem differentiation in fruit tissues (Belda and Ho, 1993; Ho *et al.*, 1993). In kiwifruit, Ca and Mg concentrations increased, and vascular differentiation was enhanced, in light-grown fruit when compared to (bagged) fruit grown in the dark (Biasi and Altamura, 1996). This may be because light-grown fruit were more exposed, and therefore transpired more rapidly than bagged fruit. Xylem dysfunction in the pedicel of 'Cox's Orange Pippin' apples is greater than in the pedicel of 'Royal Gala' apples and the former also tend to have lower fruit Ca concentrations and are more susceptible to bitter pit, a Ca deficiency related storage disorder (Lang and Ryan, 1994).

1.5.2. Xylem morphology

Xylem transport occurs in the lumen of a series of axially (longitudinally) connected cells (tracheary elements), after the cells have died and their protoplasts have been autolysed (Table 1.5). These cells are elongated with lignified walls that contain a variety of pits (tracheids and vessels) or perforations (vessels) to allow water and solute movement from cell to cell, or solute transfer to the phloem or surrounding tissues (Esau, 1960; Cordon, 1992). Vessels are more complex cells than tracheids, and are more efficient conductors of water, since water in vessel elements flows through perforations rather than diffusing through pits in the cell wall (Milburn, 1979). Parenchyma and fibre cells that are adapted to mechanical and storage functions, and do not transport water or solutes, run parallel to the tracheary elements. A radial or ray (transverse)

system of parenchyma cells is packed in around the axial system of cells to provide additional support.

Table 1.5. Cell types of the secondary xylem of kiwifruit

Cell Types	Principal Function
Axial system	
Tracheary elements	Conduction of water
Tracheids	
Vessel members*	
Fibers	
Fiber-tracheids	Support; sometimes storage
Libriform fibers	
Parenchyma cells	
Ray system	Storage and translocation of ergastic (non-protoplasmic) substances
Parenchyma cells	

Table adapted from Esau (1960 p 78) using data from Ferguson (1984) and Cordon (1992). * The operating units of the xylem are vessels, not vessel members. Vessels are made up from a series of vessel members connected end-to-end by perforation plates (Zimmerman, 1983).

Vascular tissues may be classified developmentally, into primary and secondary tissues depending on when they differentiate. Primary vascular tissues differentiate during ovary formation in the flower and are further developed during the activity of the apical meristem (procambium). Secondary vascular tissues are developed from a different meristem, the cambium, during cell division. Primary xylem contains the same basic cell types as the secondary xylem (Table 1.5), however it is organised into an axial system, and contains no rays (Esau, 1960).

Once walls of secondary xylem become lignified they are less extensible (De Boer and Volkov, 2003) and hence are frequently stretched and broken during periods of rapid fruit growth (Lang and Ryan, 1994; Malone and Andrews, 2001; Dichio *et al.*, 2003). In apple fruit, this xylem dysfunction coincides with the cessation of cambial activity (Dražeta *et al.*, 2004b), suggesting that cambial activity may be required for the repair and maintenance of xylem tissues.

1.5.3. Hydraulic conductance

Measurements of the fruit stalk (xylem) hydraulic conductance may provide an indication of the capacity of the stalk to transport Ca into fruit. Hydraulic conductance can be estimated using

Hagen-Poiseuille's law for fluid flow through a bundle of perfectly cylindrical pipes. This law has been modified by Tyree and Ewers (1991) and Equation 1.2 provides an estimate of conduit (xylem) hydraulic conductance:

$$k_h = (\pi\rho / 128\eta) \sum_{i=1}^{n} (d_i^4) \qquad (1.2)$$

Where:

k_h = hydraulic conductivity (conductance per unit pressure gradient of a bundle of pipes of differing diameters - kg s^{-1} m^{-1} MPa^{-1})

ρ = density of the fluid (sap) kg m^{-3}

η = dynamic viscosity of the fluid MPa s^{-1}

d = diameter (m) of the ith pipe

n = number of pipes in the bundle.

In kiwifruit, as in other vine crops, vessels are very wide (mostly between 0.12-0.5 mm in diameter) compared to those found in other non-vine species and provide a very low resistance pathway for water movement within the vine (k_h values of between 2.7-7.2 x10^{-7} m^2 Pa^{-1} s^{-1}, and positive root pressures of up to 20 kPa have been recorded: McAneney and Judd, 1983). Consequently, conductance values are likely to be very close to values estimated by the Hagen-Poiseuille law (Equation 1.1: Zimmerman, 1983) and there is a strong relationship between vessel size and density and k_h (Dichio *et al.*, 1999). Measurements of tracheary element diameters may therefore, provide a good indication of k_h for comparison between shoots or fruit stalks of fruit from different parts of the vine.

In kiwifruit it is known that k_h varies between different plant parts (i.e. between the roots and the shoots: Cordon, 1993), but there does not appear to be any information on k_h variation between the same plant part in different positions (i.e. long and short shoots). In chrysanthemum, there was considerable variation in k_h in different parts of the plant. Variability was attributed to differences in: diameter and length of xylem conduits (vessels and tracheids); number of conduits in a cross-sectional sample; extent of inter-conduit connections, including perforation plates and bordered pit pairs (Nijsse *et al.*, 2001). If similar variations in hydraulic conductivity were found in kiwifruit fruit stalks from different parts of the canopy, this could help to explain the large within vine variation in fruit mineral concentrations.

1.5.4. Phloem morphology

Like the xylem, kiwifruit phloem is comprised of several cell types (Table 1.6) that can be separated into primary (proto and metaphloem stages) and secondary phloem stages (Esau, 1960). Sieve elements, the conductive tissues of the phloem, are arranged into axial and radial systems in secondary phloem, and into axial systems in primary phloem (Beauvisage, 1920; Ferguson, 1984; Cordon, 1992). Unlike the xylem, the phloem of kiwifruit contains no fibrous tissue (sclerenchyma or collenchyma cells) (Chesnais, 1941; Schmid, 1978), although parenchyma cells are still present. As phloem tissues are still living at maturity they are often modified in relation to changes that occur during fruit development and so tend to be less sclerified and less persisting than xylem tissues (Esau, 1960) and are less likely to be effected by fruit growth (Dražeta *et al.*, 2004b). Areas of pores, known sieve areas, exist within the walls of sieve elements. These pores are penetrated by cytoplasmic strands that connect protoplasts of adjoining cells to enable solute movement between cells. Sieve areas are often found at the interface between sieve elements and their associated companion cells, and at the interface between two sieve elements.

Table 1.6. Cell types of the secondary phloem of kiwifruit.

Cell Types	Principal Function
Axial system	
Sieve elements*	Conduction of photosynthates and other solutes
Sieve cells	
Sieve tube members (with companion cells)	
Parenchyma cells	Storage and translocation of photosynthates, support
Ray system	
Parenchyma cells	

Table adapted from Esau (1960 p 123) using data from Beauvisage (1920), Ferguson (1984), and Cordon (1992). * A sieve tube is a series of sieve tube members connected end-to-end by sieve plates (Esau, 1960).

1.5.5. Vascular development in the fruit stalk

In apple, pedicel development begins around three weeks prior to fruit set and is completed around two weeks after flowering, coinciding with a plateau in the rate of vascular differentiation (Privé *et al.*, 1988; Dražeta *et al.*, 2004a). By contrast, pear fruit pedicel diameter increased rapidly during the first six weeks of fruit development, in conjunction with an increase in phloem and xylem area, then increased slowly until harvest (Nii, 1980b). Pedicel length increased more rapidly than

pedicel diameter, but maximum pedicel diameters may not be attained until after elongation has ceased (Privé *et al.*, 1988). Lang and Ryan (1994) found that pedicel diameter was not a good indicator of the vascular area (conductive or total) in apple fruit. However, in kiwifruit fruit size is highly correlated with pedicel diameter (Lai *et al.*, 1990), which suggests that stalks of larger fruit may have a greater vascular capacity than stalks of smaller fruit and hence, carbohydrate and mineral accumulation may be greater in these fruit.

The kiwifruit fruit stalk is comprised of a peduncle bearing a pedicel and its associated terminal flower (Fig 1.10). The junction between peduncle and pedicel is marked by two slightly juxtaposed bracts that occasionally develop into pedicels with associated lateral flowers (Brundell, 1975). Internal structure of the kiwifruit fruit stalk is very similar to that of the stem (Beauvisage, 1920). The young fruit stalk is quite flexible, but as it matures it becomes inflexible and cork-like as suberized cell layers form in the phellogen. Fruit stalk strengthening also may occur as layers of sclerified tissue form in the periderm from crushed phloem bundles that have been isolated from the conductive tissue (Beauvisage, 1920), although Schmid (1978) did not observe this.

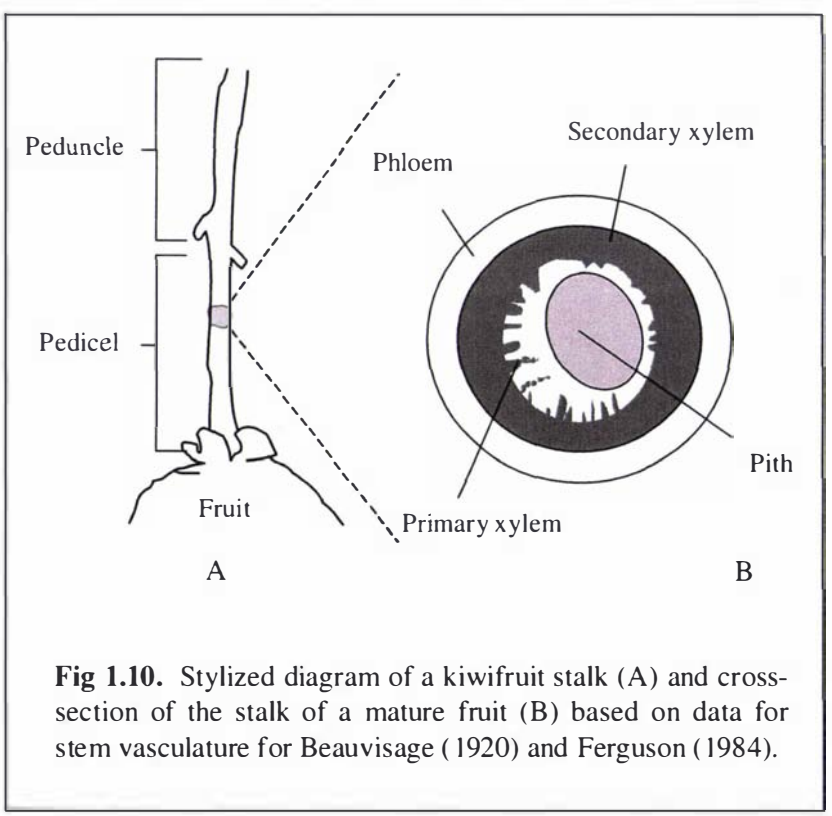


Fig 1.10. Stylized diagram of a kiwifruit stalk (A) and cross-section of the stalk of a mature fruit (B) based on data for stem vasculature for Beauvisage (1920) and Ferguson (1984).

The stele of kiwifruit fruit stalks contains roughly eight collateral vascular bundles (Schmid, 1978), compared to the stele of pome fruit stalks which contain around ten (Nii, 1980b; Dražeta *et al.*, 2004a). These bundles separate in the receptacle and spread throughout the fruit flesh (Schmid, 1978). Xylem parenchyma are abundant and form in rays and axial files around the vessels, which are relatively sparse in number and small in diameter (Beauvisage, 1920). These parenchyma cells extend from the perimedullary zone, which is a zone of the pith containing sclerified parenchyma cells. The central portion of the pith comprises of non-sclerified parenchyma cells that are crushed during development (Beauvisage, 1920).

Information regarding vascular differentiation in kiwifruit fruit stalks is limited. Early vascular development may be extremely important for final fruit quality by providing the 'networks' to enable mineral, carbohydrate and water accumulation in fruit. Biasi and Altamura (1996) noted that secondary xylem tissue was already present in the fruit stalk four weeks after anthesis. In the fruit stalk, phloem development precedes xylem development, although the vascular cambium usually produces more xylem than phloem tissue (Schmid, 1978; Biasi and Altamura, 1996), and thus the xylem:phloem ratio increases as kiwifruit develop and mature.

In apple (Barden and Thompson, 1963; Privé *et al.*, 1988; Dražeta *et al.*, 2004a), pear (Nii, 1980b), and grape (Theiler and Coombe, 1985) fruit, primary vessel differentiation begins 2-3 weeks prior to anthesis, and is largely completed by anthesis. On completion of primary vessel differentiation there was a plateau in apple vessel production that coincided with flowering. Approximately one week after flowering there is a marked increase in number (and hence conductance) of vessels with a diameter $\geq$ 10 μm, which probably marks the transition from procambial to cambial differentiation or the start of secondary vascular differentiation (Barden and Thompson, 1963; Privé *et al.*, 1988; Dražeta *et al.*, 2004a).

In apple fruit the cambium is active from around one week after full bloom until 5-6 weeks after full bloom, although secondary vessel differentiation may not cease until 1-2 weeks after the cambium becomes inactive. From this time vascular area only increases very gradually due to fibre and parenchyma development (Barden and Thompson, 1963). In studies on pome fruit, vessel k_h did not increase much from 3-4 weeks after flowering (Nii, 1980b; Dražeta *et al.*, 2004a), which coincides with the time when activity of the vascular cambium starts to slow and the first formed secondary xylem vessels started to mature (Barden and Thompson, 1963). In contrast to these findings, vessels in citrus fruit were still differentiating, and the xylem cross sectional area (CSA) continued to increase until 93 days (13 weeks) after anthesis (DAA). Cambial activity was noted after this time, but increases in vascular CSA were minimal (García-Luis *et al.*, 2002).

In apple, patterns of phloem differentiation parallelled those of xylem differentiation, except in the 12 days immediately after full bloom where increases in xylem area were minimal, and the phloem area increased rapidly. The first-formed secondary sieve elements matured around 10 days prior to the first formed secondary xylem vessels (Barden and Thompson, 1963). In citrus maximum phloem CSA was obtained (at 78 DAA) around two weeks prior to when the maximum xylem CSA was attained (García-Luis *et al.*, 2002). In apple (Barden and Thompson, 1963) and citrus fruit (García-Luis *et al.*, 2002), phloem conductance did not increase during the latter part of fruit growth, although fruit DW increased linearly until maturity, suggesting that at no stage during fruit development was phloem conductance limiting. By contrast, phloem cells in pears, especially the

sieve tubes, were very small at anthesis and differentiation was slight. Two weeks after anthesis phloem differentiation increased rapidly, before slowing during the second phase of fruit growth (stone cell development), and then increasing at a steady rate until harvest (Nii, 1980b).

In apples, vessel numbers were constant along the length of the fruit stalk, although there was a marked reduction in vessel conductance towards each end of the fruit stalk, especially the spur end (Dražeta *et al.*, 2004a). This reduction in conductance indicates a trend for vessel size to reduce towards the fruit and spur ends of the fruit stalk; this may constrict xylem sap translocation to the fruit (Dražeta *et al.*, 2004a). In other fleshy fruit 'constriction zones' have been found in the peduncles and fruit stalks or at the peduncle-fruit stalk interface (Barnell, 1939; Lee, 1989; During and Lang, 1993; Van Leperen *et al.*, 2003). In tomato (Lee, 1989) and apple (Barden and Thompson, 1963) fruit there was an increase in the number of sieve tubes distal to the constriction zone, while the number of xylem vessels remained low in the distal portion of the stem. It was suggested that constriction zones may divert water to the phloem, thereby allowing tomato fruit to accumulate both water and DW at the same time (Lee, 1989). As Ca is largely phloem immobile, these constriction zones may restrict Ca translocation to the fruit (Dražeta *et al.*, 2004a).

Constriction zones are often the site of abscission in mature fruit (Lee, 1989; Van Leperen *et al.*, 2003; Dražeta *et al.*, 2004a). Studies investigating the formation of a distinct fruit abscission zone in kiwifruit have so far been inconclusive. Fruit left on the canes tend not to abscise, but rot on the vine, suggesting that the fruit stalk may not have a constriction zone (Sharrock and Hallett, 1992: unpublished data). Therefore, Ca accumulation may be less restricted in kiwifruit, which could explain the high Ca concentration found in kiwifruit when compared to other fruit, such as apples (Kirkby and Philbeam, 1984). Schmid (1978) noted that sclerenchyma were absent in kiwifruit fruit, except in pith at the extreme base of a fruit, where they may conceivably be related to fruit abscission.

1.5.6. Factors affecting vascular development

It is widely accepted that vascular differentiation is controlled by auxins (Aloni *et al.*, 2000 : Refer to Section 1.6 for more information on the role of auxins in vascular differentiation). Other factors that may influence vascular development, possibly by altering the metabolism and/or distribution of auxins or other hormones, include: water availability (Lovisolo and Schubert, 1998); light levels in the canopy (Schultz and Matthews, 1993; Biasi and Altamura, 1996); shoot orientation (Schubert *et al.*, 1999; Lovisolo *et al.*, 2002), salinity of the soil water (Belda and Ho, 1993); temperature (Wilson *et al.*, 1991; Gorsuch and Oberbauer, 2002); endogenous sucrose (Uggla et

al., 2001), Ca (Soumelidou et al., 1994), B (Walker, 1944) and Mg (Hannick et al., 1993) concentrations; and tissue pH (Khan and Chauhan, 1993).

In kiwifruit vines light availability and shoot orientation vary considerably. This could affect vascular development in fruit stalks and shoots from different positions in the vine and might have a profound influence on carbohydrate and mineral translocation. For example, new shoots continue to be initiated until late summer and often shade shoots from the initial flush of vegetative growth that occurs prior to flowering. The climbing (vine) nature of kiwifruit means that some shoots assume a downward growth orientation, especially when laden with fruit (e.g., short shoots), whilst others will grow in an upwards direction (e.g., long shoots). There do not appear to be any studies investigating the relationship between vascular development and fruit composition in fruit and fruit stalks from different positions in the vine.

1.6. Auxins and Fruit Quality

A link between endogenous auxin concentrations and fruit quality has long been established. In addition to the effect of auxin on acropetal Ca transport (refer Section 1.2.3 (non-vascular transport) for more information), basipetal IAA transport out of fruit may influence mineral, carbohydrate and/or water accumulation indirectly by (a) stimulating vascular differentiation (Aloni and Zimmerman, 1983; Aloni et al., 2000), cell division (Gamburg, 1982) and elongation (Evans, 1985), (b) regulating activity of key enzymes involved in sugar metabolism, such as acid invertase (Lee *et al.*, 1997a), (c) enhancing mobilisation of photoassimilate (Cole and Patrick, 1998), and (d) regulating dominance phenomena between competing fruit sinks (Bangerth and Ho, 1984; Bangerth, 1989), and between competing fruit and vegetative sinks (Cutting and Bower, 1989).

It is generally accepted that auxins are synthesised in fruit seeds (Nitsch, 1950; Tamas *et al.*, 1986; Stephenson *et al.*, 1988; Gruber and Bangerth, 1990; Kojima *et al.*, 1994) and shoot and root meristems (Ljung *et al.*, 2005). However, auxin is still found in naturally occurring and hormone-induced parthenocarpic grape berries, and these berries are able to develop normally (Wang et al., 1993). This suggests that either auxin is transported to fruit, or that other tissues in fruit can synthesise auxins, possibly the nucellus (Nitsch, 1970). Following synthesis, auxins are transported basipetally, in an energy requiring manner through living tissues (Kaufman et al., 1995) to 'target tissues' where they take effect (Bennett et al., 1998).

This section will discuss auxin affects on the above plant processes. Auxins tend to work in conjunction with other plant hormones, and therefore their ability to regulate these processes is likely to depend on the presence or absence of other hormones (Ye, 2002). For example, cytokinins, gibberellins, ethylene, and abscisic acid have been implicated in differentiation of xylem and phloem elements (Aloni et al., 2000; Yamamoto et al., 2001). These hormones are likely to have a supportive role, increasing sensitivity to auxin, rather than stimulating vascular differentiation *per se* (Lachaud, 1989; Aloni *et al.*, 2000). In kiwifruit, a mixture of auxin, cytokinin and gibberellin is the most effective for promoting fruit growth (Hopping, 1976a; Lawes *et al.*, 1990), presumably by enhancing fruit cell differentiation. Fruit growth will not be promoted by any of these hormones alone. In addition, seeds must be present for this mixture of hormones to have any effect on fruit growth, suggesting the presence of another, unknown seed factor that is required for growth promotion. Phosphoinositides (Ye, 2002), sugars (Uggla *et al.*, 2001), polyamines (Clay and Nelson, 2005) and sterols (Carland *et al.*, 2002) have all been implicated in auxin signal transduction pathways for vascular development and potentially could be the seed factor.

1.6.1. Auxins and vascular differentiation

Auxins are known to regulate vascular cell differentiation, control cell dimensions in vascular tissues and promote vascular continuity (Aloni, 1987; Mattsson *et al.*, 1999; Ye, 2002). In older tissues auxins do not appear to affect vascular patterning, but may still alter vessel dimensions and alignment (Mattsson *et al.*, 1999). Sachs (1981) suggested that basipetal auxin diffusion through undifferentiated cells resulted in vascular strands that became the preferred path for auxin flow. An increase in vessel size and a decrease in vessel density along the plant axis is believed to be associated with a gradual decline in auxin concentration that occurs as auxin is carried from its site of synthesis (Aloni and Zimmerman, 1983). Evidence for these ideas include the development of vascular strands in response to local auxin applications; effects of impaired auxin transport on vascular patterns; and suggestive phenotypes of *Arabidopsis* auxin response mutants, such as auxin over-producing transgenic plants that show enhanced vascular differentiation (Berleth *et al.*, 2000; Ye, 2002; Mattsson *et al.*, 2003; Clay and Nelson, 2005).

Phloem elements will differentiate at low auxin concentrations, often in the absence of xylem differentiation, whilst xylem differentiation will only proceed at higher auxin concentrations in the presence of sieve elements (Aloni, 1987). In young fruit that have not yet begun to export substantial amounts of auxin, sieve element differentiation is detected a day or more before tracheary element differentiation can be observed. Differentiation of secondary phloem also may

precede that of secondary xylem by several weeks (Evert, 1963). This suggests that xylem differentiation may be more sensitive than phloem differentiation to management practices, such as girdling, that may alter a plant's hormone balance.

1.6.2. Auxins and cell division and elongation

Fruit size is a function of cell number, size and density (Ho, 1992). The number of cells in a fruit is important for determining a fruit's capacity to increase in volume and to import photoassimilates (Ho, 1992), although this capacity may not always be met if assimilate availability is limited (Currie, 1997; Zhang *et al.*, 2005). Fruit cell number is related to fruit size in a diverse range of fruit including, apple (Harada *et al.*, 2005), pear (Zhang *et al.*, 2005), tomato (Bertin, 2005), olive (Rapoport *et al.*, 2004), cherry (Yamaguchi *et al.*, 2004) and blueberry (Swain and Darnell, 2002). In kiwifruit, N1-(2-chloro-4-pyridyl)-N3-phenylurea (CPPU) enhances fruit size, cell division (particularly in the core area) and cell enlargement (Cruz-Castillo *et al.*, 2002).

In peach (Miller *et al.*, 1987; Miller *et al.*, 1989), strawberry (Archbold and Dennis, 1985) and tomato (Kojima *et al.*, 1994) fruit, auxin activity increases sharply during periods of rapid fruit growth. IAA was present in ovaries of 'Hayward' kiwifruit at fairly high concentrations before flowering and promoted division of the zygote and primary endosperm, although IAA concentrations did not peak until 7 DAFB (Tao *et al.*, 1994). The initial period of kiwifruit growth, between 10-40 DAFB, was closely correlated with the increase of cytokinin (ZR) and IAA in the fruit pulp and a decrease in ABA. However, the second period of fruit swelling between 80-100 DAFB was not related to the IAA concentration in the fruit pulp (Tao *et al.*, 1994). In melon, mesocarp IAA concentrations peaked 0-5 DAFB and then remained constant, but the rate of cell division and elongation slowed as fruit matured (Lee *et al.*, 1997a). Sensitivity of citrus fruitlets to auxin decreased with age, and fruit were no longer responsive to auxin at 30 DAFB (Guardiola *et al.*, 1993). This suggests that either stimuli responding to auxin become insensitive to auxin during the later stages of fruit development, or that a certain amount of auxin is required to trigger a chain of reactions leading to fruit expansion. Once triggered these reactions will proceed independent of auxin.

1.6.3. Auxins and carbohydrate accumulation within the fruit

Auxins may stimulate carbohydrate transport to and within developing sinks by acting directly on plasma membrane transport processes mediating phloem unloading (Cole and Patrick, 1998). Subsequently, turgor pressure of the sink would be lowered and this may stimulate the bulk flow

of phloem sap into the developing sink. Auxin applied to flag leaves or detached ears of wheat plants stimulated photoassimilate transport to and within developing grains for up to 30 days after anthesis (during the grain filling growth phase), but did not alter rates of flag leaf and ear photosynthesis or relative rates of carbohydrate export from flag leaves. Moreover, IAA application did not alter transport properties of the axial phloem or change phloem sap sucrose concentrations or osmolarity (Cole and Patrick, 1998).

Others suggested that IAA enhanced carbohydrate uptake by regulating the activity of sugar metabolising enzymes. For example, in melon and eggplant, IAA efflux from seeds to the mesocarp stimulated cell wall bound acid soluble invertase activity, which in turn stimulated sucrose unloading by triggering conversion of sucrose to fructose and glucose. This stimulation of acid soluble invertase activity by IAA was higher at 25 DAFB than 35 DAFB (Lee *et al.*, 1997a; Lee *et al.*, 1997b). In eggplant, IAA was able to trigger sucrose uptake, probably by stimulating activity of membrane bound H^+-ATPase thereby increasing extra-cellular proton concentrations and facilitating sucrose transport across the membrane by co-transport (Lee *et al.*, 1997b). This carrier-mediated system of sucrose uptake only operated at low sugar concentrations, such as those found in young fruit. In older fruit a simple diffusion uptake system was responsible for sugar accumulation and that was not influenced by IAA (Ofosu-Anim *et al.*, 1998).

1.6.4. Auxin application and the effect of seeds

Seed number per fruit is related to the number of pollen grains deposited on stigmatic surfaces during pollination of kiwifruit flowers (Hopping and Jerram, 1979). Pollination is normally achieved using honeybees that are brought into the field during flowering, and occasionally by artificial pollination. The importance of seeds in normal growth of kiwifruit is well documented. However, while seeds may set the potential for fruit growth, final fruit size is determined by a complex number of factors that may affect a fruit's ability to respond to its seed complement, including those that influence carbohydrate availability (e.g., crop load: refer Section 1.3.2 (crop load and fruit quality), girdling: refer Section 1.3.2 (girdling and fruit quality) and climatic conditions), and carbon partitioning to fruit (Hopping and Hacking, 1983; Clinch, 1984; Grant and Ryugo, 1984b; Grant and Ryugo, 1984a; Pyke and Alspach, 1986; Ho *et al.*, 1987; Lai *et al.*, 1989b; Lai *et al.*, 1989a; Lawes *et al.*, 1990; Currie, 1997). Often high-seeded kiwifruit exhibit dominance over low-seeded fruit by restricting their size, even when ample supplies of carbohydrate are available (Lai *et al.*, 1990). This may relate to the ability of high seeded fruit to enhance seed abortion in low-seeded fruit, which may depress fruit development by reducing auxin synthesis (Currie, 1997)

In kiwifruit, FW has been reported to increase both linearly (Grant and Ryugo, 1983; Hopping and Hacking, 1983; Clinch, 1984; McAneney *et al.*, 1984; Vaissiere *et al.*, 1991), and curvilinearly (Hopping, 1976b; Pyke and Alspach, 1986; Lawes *et al.*, 1990) with increasing seed number. It has been suggested that seed weight might be a better indicator of the potential fruit size a fruit may attain, because as the seed number increases, the weight of each successive seed is reduced, and smaller seeds are less effective at promoting fruit growth than larger seeds (Lawes *et al.*, 1990; Currie, 1997).

Fruit on long axillary shoots tend to be larger (109 g average) than fruit on short shoots (97 g average), primarily due to the greater seed number in fruit on long shoots (1052 average) when compared to fruit on short shoots (819 average: Lai *et al.*, 1990). Therefore, vascular pathways to fruit on long shoots might be more developed, cell division and elongation might be more pronounced, and sugar metabolism enhanced, all of which could increase DW accumulation in long shoots. Currie (1997) found that fruit with >400 seeds had a 32% greater DW than fruit with <400 seeds. In grape berries seed number was directly related to accumulation of C14 photosynthate and berry fresh and dry weights. Prior to veraison fruit SSC was not affected by seed number, but after veraison the SSC was inversely related to seed number (Cawthon and Morris, 1982), suggesting that FW increased more than DW. Vaissiere *et al.* (1991) found no relationship between seed number and sugar content in kiwifruit.

Fruit seed number also may influence mineral accumulation (Bangerth, 1976). Efflux of IAA from parthenocarpic tomato, apple, and pear fruit can be lower than that of seeded fruit and these fruit also contained less Ca and developed more Ca-deficiency induced disorders, such as bitter pit and blossom end rot, than did seeded fruit (Sjut and Bangerth, 1984; Banuelos *et al.*, 1987). In grape berries, the duration over which Ca, and to a lesser extent Mg, could accumulate in fruit was greater in fruit with high seed numbers than in fruit with low seed numbers (Boselli *et al.*, 1995). In grapes xylem may become dysfunctional around veraison (Findlay *et al.*, 1987; Creasy *et al.*, 1993; During and Lang, 1993; Greenspan *et al.*, 1994) and the presence of seeds may prevent or delay this dysfunction, so that Ca and Mg can continue to accumulate in grape fruit during the later stages of development, as is known to occur in pistachio (Polito, 1999).

Despite the extended duration over which Ca and Mg accumulation could occur in high-seeded grape fruit, there was no correlation between grape berry seed number and fruit Ca, Mg or K concentrations (Boselli *et al.*, 1995). Similarly, the Ca concentration of 'Spartan' apples was not related to seed number (Tomala and Dilley, 1990). However, seed number did affect the Ca concentration of 'McIntosh' and 'Braeburn' apples (Tomala and Dilley, 1990; Brookfield *et al.*,

1996). The discrepancy between these results may relate to the stimulatory effect of seeds on fruit growth. Fruit weight was positively correlated with seed number in 'McIntosh' but not 'Spartan' apples (Tomala and Dilley, 1990), and it has been suggested that fruit mineral concentrations may be 'diluted' as fruit FW increases (Winkler and Williams, 1936; Olmo, 1946; Cawthon and Morris, 1982; Boselli *et al.*, 1995).

1.6.5. Effect of auxin transport inhibitors on mineral and carbohydrate accumulation

Application of the auxin transport inhibitors 2,3,5-triiodobenzoic acid (TIBA), 1-N-naphthylphthalamic acid (NPA) or chloroflurenolmethylester (CME) reduced fruit Ca concentrations in vegetative and/or reproductive organs of numerous crops including kiwifruit (D. Woolley and S. Lawes: unpublished data), tomato (Bangerth, 1976; Banuelos *et al.*, 1987; Hamamoto *et al.*, 1998) pome (Benson and Stahly, 1972; Bangerth, 1976; Raese *et al.*, 1995), avocado (Cutting and Bower, 1989) and stone (Wand *et al.*, 1991a; Wand *et al.*, 1991b) fruit. On the other hand 1,1-dimethyl-piperidinium chloride (PIX), an auxin transport stimulator, enhanced auxin transport and acropetal movement of Ca (Banuelos et al., 1987). The prime effect of TIBA is to reduce basipetal transport of IAA and acropetal movement of Ca, by saturating sites on a proposed IAA-efflux carrier, thereby restricting basipetal movement of IAA and subsequent acropetal movement of Ca (Hertel, 1983; Muday and De Long, 2001).

TIBA did not influence K, P or Mg movement into fruit (Marschner and Ossenberg-Neuhaus, 1977; Banuelos *et al.*, 1987) or transport of Rb^{+}, and only slightly influenced transport of Sr^{2+} compared to its effect on Ca^{2+} transport. Furthermore, TIBA did not affect the acropetal transport of labelled water into fruit, or the cation-exchange capacity of fruit (Banuelos et al., 1987). This indicates that the effect of TIBA on Ca accumulation was specific, and was independent of any treatment affect on evapotranspiration and/or Ca movement by exchange in the xylem.

In addition to influencing fruit Ca concentrations, NPA application to kiwifruit stalks shortly after full bloom reduced IAA efflux from fruit by 97%, decreased fresh and dry weights by up to 50% and reduced the total seed weight (Currie, 1997). Effects on fruit DMC were inconsistent between seasons indicating that other factors were involved in the response of fruit DMC to NPA application. In addition to reducing FW, TIBA and NPA application to kiwifruit stalks reduced fruit stalk diameter (D. Woolley and S. Lawes, unpublished data). They proposed that IAA efflux from fruit favoured import of Ca and carbohydrate into fruit, but it also is possible that IAA efflux stimulated vascular differentiation and that this enhanced the capacity of fruit to import Ca and carbohydrate.

In kiwifruit the greatest response to NPA application was observed when applied 7-14 DAFB, and there was little effect when applied more than 21 DAFB (Currie, 1997). Similarly, tomato (Banuelos *et al.*, 1987; Hamamoto *et al.*, 1998) and peach (Wand *et al.*, 1991a) fruit Ca concentrations and seed weight were decreased more by TIBA application 7-10 DAFB than by earlier (five DAFB) or later (15-21 DAFB) applications. In apple fruit TIBA application at two weeks after anthesis resulted in the greatest reduction in fruit Ca concentrations and the highest bitter pit incidence when compared to later applications (Benson and Stahly, 1972). These findings suggest that IAA is not essential for fruit growth at all times, and may only effect young, developing organs in which fruit and vascular cells are rapidly dividing. Greatest reductions in fruit Ca concentrations were observed when auxin transport inhibitors were applied during the peak diffusible IAA efflux from fruit (Banuelos *et al.*, 1987). It is possible that application of TIBA to kiwifruit fruit at this time would allow conclusions to be drawn on the role of auxins in promoting vascular development in the stalks and carbohydrate, mineral and water accumulation in fruit.

1.7. Problem statement and objectives

1.7.1. Statement of the problem

There is an increasing shift in the kiwifruit industry towards basing fruit quality not only on external, but also on internal (sensory and storage) attributes. It has been recognised that internal quality is more important than external quality in driving repeat sales particularly in quality sensitive markets such as Japan. Fruit DM and mineral concentrations are known to influence kiwifruit internal quality, yet there is limited understanding of the vine factors affecting mineral, carbohydrate and water movement within the vine and accumulation within the fruit. There is considerable within-vine variation in fruit DM and mineral concentrations and this variability is reflected in lines of fruit arriving at the packhouse. As fruit with poor internal quality are not readily identifiable at harvest or during fruit segregation in the packhouse, this variation restricts the ability of industry to segregate and extract value from fruit that have high inherent internal quality. Moreover, there is a risk that kiwifruit will be exported that does not meet consumer expectations and this may discourage repeat purchases. A better understanding of the physiological factors affecting carbohydrate, mineral and water accumulation should allow development of vine management practices that will assist growers to reduce variation in fruit internal quality and raise fruit internal quality. This will ensure that the kiwifruit industry extracts

value from high quality lines of fruit and expands sales, both of which will ultimately increase grower returns and enhance profitability to the entire supply chain.

1.7.2. Objectives

The overall objective of this thesis was:

"To investigate reasons for within vine and between vine variation in fruit DM and mineral concentrations"

In particular, this research investigated how specific grower management practices may affect carbohydrate, mineral and water accumulation and subsequent sensory and postharvest quality of fruit from long (long, non-terminating) and short (short terminating) axillary shoots. Fruit from these shoots were chosen as they differ in their ability to attract carbohydrates and minerals; long shoots tend to bear fruit that have high Ca and DM concentrations, while short shoots bear fruit that have low Ca and DM concentrations (Thorp *et al.*, 2003b); this suggests that different factors may be affecting carbohydrate, mineral and water accumulation in these two shoot-types.

In the work reported in Chapters 3 and 4, treatments were applied at the whole vine level. Chapter 3 reports on an investigation on the effect of two different canopy management regimes, based on leader and conventional style pruning regimes, on fruit internal quality over a three-year period. Chapter 4 reports on the effect of crop load on photosynthesis and transpiration in leaves from long and short shoots, and subsequent effects on carbohydrate and mineral accumulation in fruit from those shoots. In the experiments outlined in Chapters 5-9, treatments were applied to individual shoots and their affects on phloem and xylem translocation and subsequent fruit internal quality are reported. In Chapter 5, results are presented on manipulation of phloem translocation by girdling and of xylem translocation by defoliation. The affects of fruit transpiration (Chapter 6) and vascular development (Chapters 7 and 8) on fruit Ca (xylem), carbohydrate (phloem) and water (phloem and xylem) accumulation are reported. Vascular differentiation was manipulated by applying 2,3,5-triiodobenzoic acid (TIBA), an auxin transport inhibitor, to fruit and by reducing fruit seed numbers (Chapter 8). Finally, mineral ion compositions were measured in xylem sap collected non-destructively from fruit stalks on long and short shoots (Chapter 9). Specific research questions addressed in each of the experimental chapters were as follows:

Chapter 3: Can the adoption of canopy management practices of one grower, who is regarded in the kiwifruit industry as an 'expert in orchard management (Buxton, 2001),

induce perceivable differences in fruit DMC and general fruit quality within the three-year period of this study?

Chapter 4: What is the affect of crop load on photosynthesis and transpiration of leaves on long and short shoots? Is there a relationship between rates of leaf photosynthesis and transpiration and fruit internal quality?

Chapter 5: Are the factors influencing carbohydrate, mineral and water accumulation in fruit similar, and if not, how do they differ? How do the factors affecting carbohydrate, mineral and water accumulation in fruit from long and short shoots differ?

Chapter 6: Does fruit transpiration affect mineral accumulation in kiwifruit?

Chapter 7: What are the seasonal patterns of phloem and xylem differentiation in kiwifruit?

Chapter 8: Does TIBA application and poor pollination affect vascular development in kiwifruit and subsequent fruit internal quality?

Chapter 9: Are there differences in the mineral ion composition of xylem sap collected from fruit stalks on long and short shoots?

As the 'Hayward' cultivar accounts for 95% of all commercial trade and 85% global plantings (Ferguson, 2004), this research focuses on 'Hayward' kiwifruit. The physiology of *A. chinensis* ('Hort 16A') is quite different to that of the green fruit, and therefore the results reported here are not necessarily applicable to this or any other *Actinidia* species.

2. General Methods

The following methods apply to all experiments completed as part of this study unless otherwise stated in the methodology section of individual chapters. Methodologies that are specific to individual experiments are described in the individual chapters. All experiments were carried out on green kiwifruit [*Actinidia deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson var. deliciosa 'Hayward'] grown in various locations from Te Puke, Bay of Plenty (37° 49' S, 176° 19' E) to Clevedon, Auckland (37° 15' S, 174° 42' 59" E), NZ, as stated in the individual chapters. Vines ranged in age from 20-35 years and were all managed for commercial production on a pergola-trained system. All vines were growing on open-pollinated 'Bruno' seedling rootstocks.

2.1. Chemicals

Appendix II contains details of all the chemicals utilised in fruit assessment.

2.2. Fruit selection

Fruit were selected from long, non-terminating (long) and short-terminating (short) axillary shoots produced on the current season's canes (refer Section 1.3.1). Short shoots were selected from anywhere along the cane, whereas long shoots were selected from within the leader zone of the vine near the base of their parent cane. All selected shoots had more than two fruit with either the second or third fruit being sampled.

2.3. Field preparation of fruit and fruit stalks for assessment

Fruit were harvested with their fruit stalks attached. Fruit were weighed in the field using battery-operated scales (Sartorius AG, Göttingen, Germany), and their soluble solids concentrations (SSC) (where measured) were determined from a combined juice sample from either end of the fruit using an Atago digital PR-refractometer (Tokyo, Japan) (Lallu et al., 1989). Following this, an equatorial slice (5-6mm) was cut, weighed and placed in a small sealable plastic bag for DMC assessment on return to the laboratory. A cross-sectional slice (2-3mm) also was taken from either side of the equatorial slice for analysis of fruit mineral concentrations. Although equatorial slices

do not account for longitudinal gradients in mineral distribution within a kiwifruit, they account for variations in mineral composition between tissues and give a closer representation of the true fruit mineral content than flesh plug samples (L. Boyd 2005: unpublished data). Slices for mineral analyses were weighed, placed into small sealable plastic bags and stored at –20°C awaiting assessment.

In the field, fruit stalks were separated from the fruit, and digital callipers (Mitutoyo, Tokyo, Japan) were used to measure length and minimum and maximum diameters of the fruit, and fruit stalk length and diameter at the fruit-, mid- and shoot-ends of the fruit stalk. Fruit stalks were transferred to individually labelled containers, so that they could be traced back to the fruit from which they were taken, and fixed in 4% paraformaldehyde in 0.1M Sörensen Phosphate Buffer (Glauert and Lewis, 1998) pending microscopic examination.

Fruit and fruit stalk sample numbers differed depending on the experiment in question, and are detailed in each chapter. Time constraints often restricted the number of samples that could be analysed. This was taken into consideration when interpreting differences between treatments and/or shoot types.

2.4. Secondary vascular measurements

Fruit stalks were removed from the phosphate-buffered 4% paraformaldeyhde solution and washed in distilled water. A 200-400 µm thick cross-section was cut 30% of the way along the fruit stalk, as measured from the fruit end. Sectioning was done by hand using a single-edged microtome blade, except in very young fruit stalks (< 4 weeks from anthesis) that had not lignified and were too flexible to cut. These fruit stalks were embedded in a piece of carrot for support (O'Brien and McCully, 1981) and cut with a microtome (Vibratome 1000 (Technical Products International, St Louis, Missouri)) to a thickness of 100-150 nm.

Fruit stalk cross-sections were placed in the centre of a glass slide and soaked for 30 s in 2-3 drops phloroglucinol solution (2.5g in 50ml of 70% v/v ethanol) before being flooded with concentrated HCl (95% w/w). This procedure resulted in xylem strands (lignified tissue) being stained red so that they were easily distinguishable from the relatively transparent parenchyma cells (Dražeta *et al.*, 2004b). The phloroglucinol and acid were subsequently absorbed with a paper tissue, the fruit stalk cross-section mounted in 80% w/w glycerine and examined with a compound light microscope (Olympus Vanox AHT3 (Olympus Optical Co Ltd., Tokyo, Japan)).

Phloem and xylem area, and the size and frequency of xylem vessels within any size class, were assessed on the day of slide preparation since the phloroglucinol stain fades after a few hours. The image from a compound light microscope (Olympus Vanox AHT3 (Olympus Optical Co Ltd., Tokyo, Japan) coupled to a CoolSnap (RoperScientific Ltd, Tucson, Arizona) digital camera was projected on a computer screen using RSImage software (RoperScientific Ltd, Tucson, Arizona) and the magnification recorded. Based on the method described by Lang and Ryan (1994) internal diameters of the vessels were estimated by eye using custom mouse-controlled software that presented a moveable bitmap overlay image on the screen. This image comprised a set of five circles of increasing diameter (given the labels 1, 3, 5, 7, and 9) corresponding to diameters of 3, 7, 12, 16 and 21 μm. By interpolation, estimations were made of four intermediate-sized circles (2, 4, 6, and 8) with corresponding diameters of 5, 9, 14, and 18 μm. The diameter range of these nine circles was equivalent to the range of vessel diameters commonly found in kiwifruit. Subsequently, the diameter of each vessel could be compared against the bitmap image circles to find the circle that best represented, or matched, the diameter of the vessel. The number of this circle was then recorded using custom software that converted the PC's numeric keypad into a nine-channel tally counter. An estimated conductance value (C) was calculated for each size class of vessel using Equation 2.1 which is based on the Hagen-Poiseuille law (Nobel, 1999).

$$C = \sum nd^4 \tag{2.1}$$

This equation states that C (arbitrary units) is equivalent to the number of xylem vessels of a particular size class (n, circles sizes 1-9) multiplied by the fourth power of their internal diameter (d). A total estimated C was then calculated for each fruit stalk by summing the conductance values for each size class. As calculated conductance values tended to be very large (>10,000) they were divided by 1000 to make them easier to work with. It should be noted that C is an arbitrary value calculated in order to make comparisons between treatments. Actual xylem hydraulic conductance (distance of sap movement per unit time) will depend on xylem sap viscosity, vessel length, and the pressure difference across the vessel (Nobel, 1999) and on characteristics of the xylem (Tyree and Ewers, 1991).

Images collected using the CoolSnap camera were saved and imported into ImageJ (Natural Institutes Health, USA) for assessment of vascular area. Using this software a line was drawn around the xylem/phloem/pith perimeter, the number of pixels enclosed within that line calculated, and this number converted to an area measurement based on a calculation of the number of pixels in a known area for that particular magnification.

2.5. Mineral analysis

A (3-4 g) sub-sample was taken from each pair of cross-sectional fruit slices and each sub-sample was separately digested in $HNO_3/HClO_4$ (both 70% w/w). These digestates were transferred to a 20 ml volumetric flask and made up to volume with $LaCl_3$ solution (5.34 g L^{-1} distilled water), which prevents phosphate interference during Mg and Ca determination. From these volumetric flasks a sub-sample was removed and further diluted with the same $LaCl_3$ solution, this time containing 2-3 drops of octylphenol ethoxylate (Triton^{TM}X) surfactant per litre of solution to help generate an aerosol after aspiration (L.M. Boyd, pers. comm.). The extent of dilution depended on the maturity of fruit at harvest, and on detection limits of the mineral being assessed. Samples were analysed for K, Ca and Mg using atomic absorption spectrophotometry (GBC Avanta, Australia). Using the same digestates, P concentrations were measured colormetrically by the molybdate blue reaction (refer Section 2.1. for a list of the reagents used) method (Turner et al., 1977).

2.6. Dry matter concentration

Each equatorial slice was oven-dried to a constant weight at 60-65°C, and the dry weight of the slice recorded and expressed as a percentage of the fresh weight (DMC).

2.7. Xylem functionality

2.7.1. Dye infiltration

Fruit were harvested, with fruit stalks attached, before dawn when their water potential was close to zero. Whilst in the orchard each fruit stalk was cut under water to prevent embolisms developing and fruit were promptly placed in a polyethylene bag that was sealed to prevent fruit transpiration. Fruit were then transported back to the laboratory and prepared for analysis. Six to eight fruit were selected, depending on their size and a ring of vaseline was put around the distal end of each fruit to prevent surface capillary movement of dye solution throughout the fruit (Dichio *et al.*, 2003). Fruit stalks were again cut under water, just above the previous cut, with a sharp blade to expose a clean, uncrushed surface. Fruit were then immediately placed (upside down) with their stalks in a 1.5 ml Eppendorf tube filled with a 0.1% w/v toluidine blue O dye solution made up to volume with deionised water (Fig 2.1, Van Leperen *et al.*, 2003). Each

Eppendorf tube fitted into a rack that was placed for 60 minutes in wind tunnel equipment that provides standard and high transpiration conditions (Fig 2.1, Dichio *et al.*, 2003), as verified by vane anemometer measurements (wind speed 3 m s^{-1}).

Toluidine blue easily passes via vessels and through pit membranes (Van Leperen *et al.*, 2003) and was translocated into fruit in the transpiration stream, staining the cell walls of water-conducting vessels as it moved. This procedure was repeated until each fruit had been analysed, taking care to ensure that a few fruit from each treatment were assessed at each time.

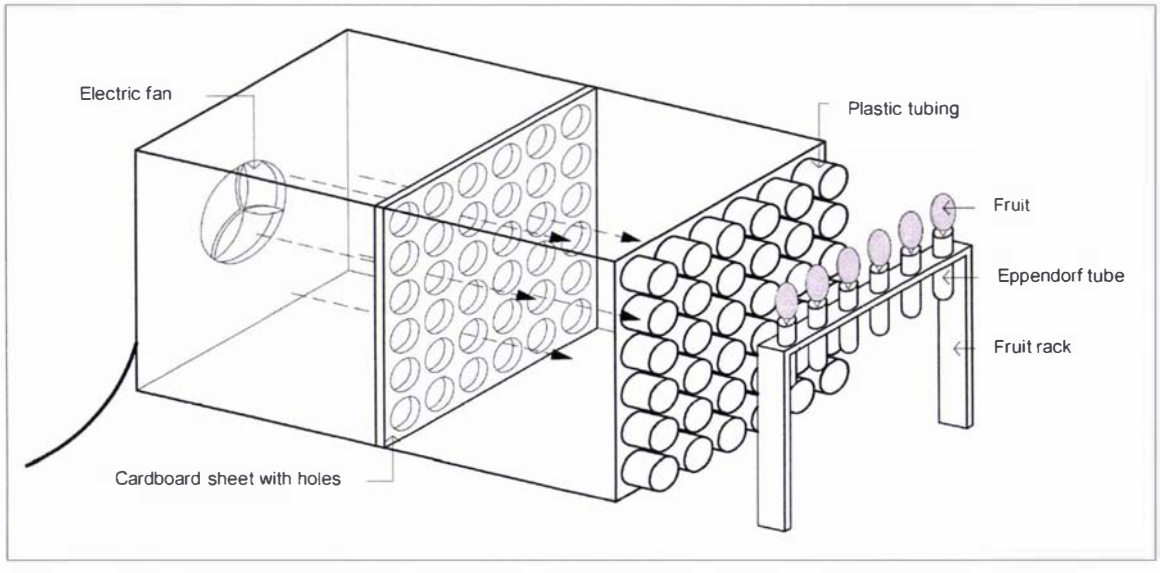


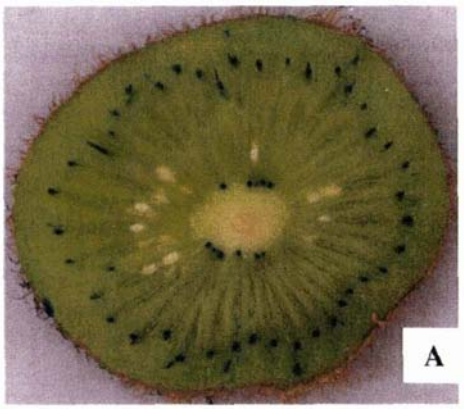
Fig 2.1. Wind tunnel design for measuring xylem functionality. Air passed through the tunnel and out of the plastic tubing in the direction indicated by the arrows. As it passed through the various obstacles within the tunnel, air turbulance was greatly reduced and this ensured that fruit were all subjected to high and uniform wind speeds.

2.7.2. Determining xylem functionality

After 1 h fruit were removed from the dye solution and their stalks were removed. Ten percent of the way along each fruit, as measured from the stylar end, a transverse cut was made to split each fruit in two. The total number, and number of stained ventromedian carpellary (VMC) bundles, was determined using a Leica MZ FLIII stereomicroscope (Leica Microscopy Systems Ltd., Heerbrugg, Switzerland) and RSImage software (RoperScientific Ltd, Tucson, Arizona: Figure 2.2).

Xylem functionality was calculated as a percentage using Equation 2.2 (Dichio *et al.*, 2003).

$$\% \text{ xylem functionality} = 100 \text{ x } \frac{\text{number stained VMC bundles}}{\text{total number VMC bundles}} \quad (2.2)$$



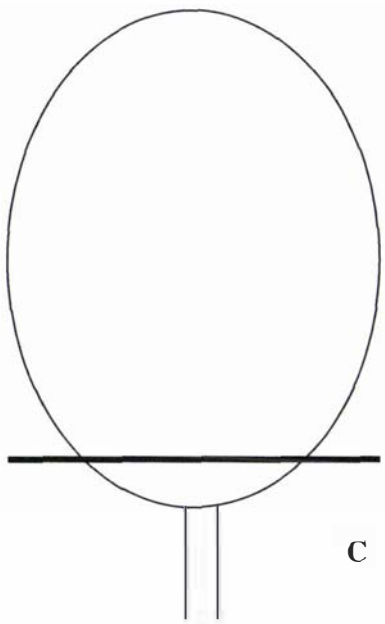


Fig 2.2. A transverse kiwifruit section illustrating the stained and unstained ventromedian capellary (VMC) bundles (A and B; close-up of the VMC bundles shown in A). Toluidine blue dye moved into fruit in the transpiration stream via the VMC bundles. Sections were cut as indicated in C and percentage xylem functionality was calculated according to the methods of Dichio *et al.*, (2003).

2.8. Harvest Dates

Fruit were harvested while immature, in January, and again in April or May, just prior to commercial harvest. Two harvest dates were chosen as it was believed that vascular area and conductance data in January might provide a better reflection of the relationship between vascular capacity and carbohydrate and mineral accumulation. Where experiments were completed over a period of more than one season efforts were made to harvest fruit at an equivalent maturity each

year. However, this was not always possible, as experimental harvests had to be completed before the commercial harvest. The maturity of fruit at harvest was considered when interpreting results in relation to treatment differences between years.

2.9. Fruit storage assessment

Fruit harvested for assessment after storage was packed into modular bulk packs, each containing 10 kg fruit (corresponding to 76-127 fruit depending on their count size). Plastic polyliners lined the boxes and were folded over the fruit before transferring them to 0°C on the day of harvest, or following grading and packing. At approximately four-week intervals from 12 weeks after harvest, fruit were assessed for:

1. Firmness: peel was removed from two opposing sides of the fruit with a slicer, blade height 1mm, and firmness was measured on the two sides using a 0-5 kgf (0-49 N) Effegi fruit tester with a 8mm head (Lallu et al., 1989); firmness was calculated as the average of the measurements from the two sides of the fruit.

2. Percentage soluble solids (SSC) was measured using a hand refractometer as described in Section 2.3 (Atago, Tokyo, Japan: Lallu *et al.*, 1989).

3. Presence or absence of rots and physiological pitting was determined using visual assessment (refer to Ferguson et al., (2003) for a more detailed description of physiological pitting).

At final assessment, around 26 weeks after harvest or when fruit had attained an average firmness of 0.4-0.8 kgf (3.9-7.8 N, eating ripe firmness), in addition to assessing the above, 20% of the fruit in each pack were also assessed for low temperature breakdown (slight: flecking observed around the perimeter of the fruit; severe: water-soaked flesh). This equated to 15 (count 27) to 25 (count 42) fruit depending on their count size. Where significant differences in DM and/or SS concentrations were noticed at harvest and/or during storage, fruit DMC also was re-measured using the methods described above.

2.10. Data handling and statistics

Statistical methodologies that are specific to a specific experiment are described in the relevant chapters, while more general statistical methodologies are described here.

2.10.1. Data handling

As far as possible, data collected were entered directly into a spreadsheet to reduce the possibility of transcription errors. The number of significant digits for each variable was fixed according to the precision of the measuring device before being sorted into numerical sequence and visually assessed for recording and transcribing errors. In addition, during 'data exploration', scatterplots were made to compare treatment differences and/or relationships between meaningful pairs of variables, such as vascular conductance and fruit Ca concentrations, and obvious outliers were checked and removed if thought to be impossible or very unlikely, such as a 300 g kiwifruit. Finally, residual plots were plotted and inspected for outliers.

2.10.2. Experimental design and statistical analysis

Most experiments were based on a randomised complete block design according to Mead *et al.*, (1993) with individual vines used as blocks in order to remove any effects due to vine differences. Experimental units consisted of shoots within a vine, and these were grouped by position and length to ensure treatment balance with shoot type.

Box-plots and/or scatterplots were used to inspect datasets for skewness, and where required, data were transformed according to the method of Fernandez (1992) in order to conform to assumptions underlying analysis of variance (ANOVA: Cochran, 1947). ANOVA was undertaken using the general linear model procedure in MINITAB® Release 14.11 (MINITAB® Inc, Pennsylvania, USA) and residual plots were used to check data for normality. In addition, an Anderson-Darling test (Stephens, 1974) was used to check the hypothesis that data were normally distributed while a Levene's test (Levene, 1960) was used to test for equal variances between variables. Data were transformed back to their original scale for presentation purposes.

Where P-values indicated significant differences between means ($P < 0.05$), treatment means were compared using the following methods:

1. The Bonferroni method was used for pre-planned pair-wise comparisons of individual treatment means. As this method is very conservative, it was often followed up with one of the following multiple comparison techniques, which are less conservative (Hsu, 1996).

2. Fisher's protected least significant difference (LSD) procedure was used for multiple comparisons between treatments from balanced datasets (Saville, 1990).

3. In the case of unbalanced datasets, population marginal means were used. Population marginal means are based on expected treatment averages if there were equal numbers of variables for each treatment, and avoids means being weighted towards treatments with fewer missing data (Searle *et al.*, 1980).

MINITAB® Release 14.11 also was used to assess correlations between variables using simple linear regression and to conduct other statistical analyses as described in individual chapters.

3. Kiwifruit Pruning Systems and Fruit Quality

3.1. Introduction

The New Zealand kiwifruit industry recognises the importance of producing fruit with a high DMC. Some growers have developed specific management practices to raise the DMC of their fruit (Buxton, 2001; Ferguson, 2004), some of which have been individually evaluated for their effects on fruit quality. In general horizontally trained (pergola), large diameter canes initiated early in the season are associated with increased fruit set, fruit size, fruit soluble solids at eating ripeness (rSSC) and fruit DMCs (Snelgar and Hopkirk, 1988; Inglese and Gullo, 1991; Volz *et al.*, 1991; Snelgar *et al.*, 1992; Walton *et al.*, 2000). Fruit rSSC and DMCs can be enhanced by avoiding excessive shading within the canopy (Grant and Ryugo, 1984b; Snelgar *et al.*, 1991; Lionakis *et al.*, 1997), while regular summer pruning may increase fruit Ca concentrations and maintain fruit firmness in storage (Gerasopoulos and Drogoudi, 2005). Consequently, it has been recommended that New Zealand kiwifruit growers attempt to increase fruit DMCs by adopting canopy management strategies that ensure rapid canopy development in spring and avoid excessive shading as the canopy develops (A. Mowat: pers. comm.). Subsequently, there has been an increase in the use of open-style pruning strategies, such as leader pruning (LP).

Leader pruning aims to reduce vine vigour and promote development of the current crop and of less vigorous replacement canes to carry next season's crop, while maintaining a framework of main and secondary branches (Miller *et al.*, 2001). Vigorous shoots growing close to the central leader zone are removed as they emerge, and non-fruiting canes and shoots are removed from the fruiting zone (FZ) of the vine. Only a few slow growing, self-terminating canes (about 3.3 canes m^{-2} leader) are retained as next years fruiting cane (Miller *et al.*, 2001). This ensures that vine vigour is continually suppressed leading to increased carbohydrate partitioning to fruit, especially to those within the leader zone (LZ) (Miller *et al.*, 2001; Thorp *et al.*, 2003a). Leader pruned vines are generally more open (less dense) than conventionally pruned (CP) vines, and much of the crop is borne on thin canes with short internodes that have a tendency to self-terminate and thus require less pruning (Miller *et al.*, 2001; Thorp *et al.*, 2003a). Such canes in LP vines are just as productive as vigorous, large diameter canes in CP vines, but as LP also slightly increases the productivity of large diameter canes, it is recommended that these are selected in preference to thin, low-vigour canes (Thorp *et al.*, 2003a).

Miller *et al.*, (2001) and Thorp *et al.*, (2003a), are the only available reports that compare the effects of leader and conventional pruning and cane size on shoot growth, productivity and fruit DMCs in kiwifruit. The affects of these pruning systems on fruit mineral concentrations were not investigated, despite the recognition that, in addition to DMC, a fruit's mineral composition at harvest is known to affect its quality in storage (Ferguson *et al.*, 2003). As fruit Ca concentrations tend to be positively correlated with fruit DMCs (Thorp *et al.*, 2003b), it is possible that LP also positively influences Ca accumulation. However, it has been suggested that practices such as LP, that increase fruit size, may reduce fruit Ca concentrations also (Ferguson and Boyd, 2001).

The affect of two pruning systems on shoot growth, fruit DMC and mineral accumulation were compared. Treatment one involved conversion of a CP orchard to an LP orchard and subsequent management under that system, undertaken by a recognized 'expert in orchard management' (Buxton, 2001). Treatment two was a CP system. The 'expert' believed that using his LP system, he could raise the DMC of fruit from the orchard used in this experiment within the three-year experimental time frame without reducing total yields. This claim was investigated. Results of this study comparing the pruning systems of two growers are compared with affects of individual practices, such as girdling and defoliation, on DM and mineral concentrations, which are discussed in Chapter 5. Mechanisms by which the pruning systems might be influencing fruit quality are discussed.

3.2. Materials and Methods

3.2.1. Experimental design

This experiment was run over three seasons from October 2001 to October 2004 using vines within a mature orchard located in James Rd, Te Puna, Tauranga, New Zealand. Two adjacent blocks were selected, one with seven rows of vines, 'Block C' and one with six rows of vines, 'Block D'. Each block was divided into four areas numbered one to eight, each with 30-38 female vines and 5-11 male vines. Half of these areas were pruned using a LP system (treatment one) and half were pruned using a CP system (treatment two) (Fig 3.1: refer to Section 3.2.2 for a description of leader and conventional pruning). Within each of these areas three vines were selected (vines 1-24: Fig 3.1) and their growth characteristics were monitored throughout each season to determine the affect of each pruning system on vine architecture, canopy density and cane fertility as described in Section 3.2.3. One to two days prior to commercial harvest fruit were harvested from: (a) individual areas (ten fruit from each area) to determine the effect of each

pruning system on fruit quality and yield and to assess how consistent the effects of each pruning system were between areas; (b) individual vines to determine if yield and fruit quality, either across the vine or in specific zones within the vine (as described in Section 3.2.3), changed in response to the different pruning systems over the three years.

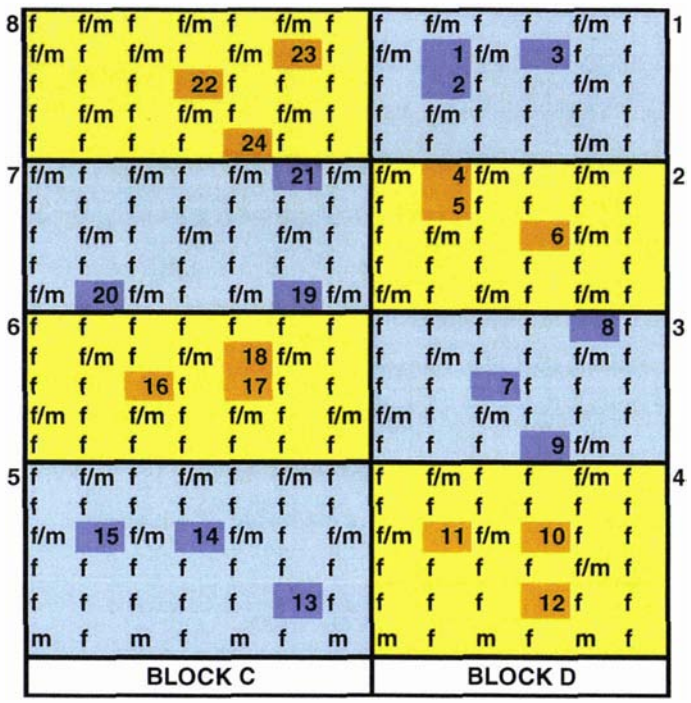


Fig 3.1. Design of the pruning systems experiment. Two blocks, C and D, were divided into eight areas, as numbered, and half of these were managed using a conventional pruning style regime (highlighted in yellow), whilst the other half were managed using a leader pruning style regime (highlighted in blue). In each area three vines were selected for monitoring (numbered 1-24). The letters 'f' and 'm' refer to female and male vines, respectively.

3.2.2. Pruning strategies

The LP system was based on the following key practices:

a) Early season pruning was targeted towards achieving a full canopy as soon as possible after growth commenced and subsequent (summer) pruning was used to ensure that initial (first-developed) leaves were always in the sun. The number of vigorous, late season, non-fruiting, upright-growing shoots developing in the canopy was minimized, especially those arising from the LZ (described as the area 0.75 m out from the leader and running

the length of the leader). Vines were pruned every 2-4 weeks over the summer months (from flowering until late February), depending on canopy density.

b) Replacement canes were kept only if required in the following season or to fill gaps in the canopy.

c) Medium diameter shoots with short spaces between internodes that had a tendency to self-terminate were selected as next years fruiting canes. Generally these shoots were the first to develop in the previous season (i.e. the initial wood), as indicated by the presence of old fruiting stalks. Thin, weak shoots and large shoots with a vigorous growing habit were avoided.

d) Pruning decisions were based on the L:F ratio on a fruiting shoot, and not the whole vine leaf to fruit ratio; an optimum L:F ratio was 3:1-4:1 (about 6 leaves past the last fruit). Terminated shoots were not pruned.

e) Vines were not treated with naphthalene acetic acid (NAA-pruned), as the 'expert' believes that this enhances vine vigour.

f) Standard winter pruning practices were used, aiming for 40 buds m^{-2} canopy, and 30 cm spacing between canes (16-17 canes on each side of the leader).

This differed from the CP system in the following ways:

a) Growth in the LZ was left virtually untouched, and shoots were kept as potential 'replacement canes' for the following season. Consequently, more potential replacement canes were carried in the LZ of the vine than were required in the following season.

b) Vegetative shoots were removed from the FZ (described as the proportion of the canopy beyond the LZ), even those that were non-terminating, and shoots were pruned to 3-4 leaves past the last fruit, even if they were terminated.

c) Just prior to flowering vines were NAA-pruned to restrict growth early in the season and subsequent pruning was used to prevent tangling and to avoid excessive shading. Vines were pruned 4-6 times per season as required to maintain the desired level of control over the canopy.

d) Winter pruning targets were similar to those for LP vines. Large diameter, vigorously growing canes were selected as next years fruiting wood in preference to small or medium diameter terminated canes.

Many in the New Zealand kiwifruit industry consider the canopy management practises of the 'expert in orchard management' to be innovative (Buxton, 2001), and the apparent fruit quality benefits attained from using such practises are a major factor in the impetus to use 'open-style' canopies to raise fruit DMCs. Like the 'expert in orchard management', the grower managing the

CP vines had been in the industry for many years, and is known for consistently producing high-yields of good-sized kiwifruit. He based his canopy management on a conventional cane replacement system that he found provided the best returns with minimal effort to the grower.

3.2.3. Seasonal analyses

Season one (October 2001 to October 2002)

Winter vine measurements: On 15 November 2001, prior to establishing the experiment, the number of buds and spurs (short terminated shoots less than 0.75 m long) in both the leader and fruiting zones (LZ and FZ, respectively) were counted on one half of each vine. In addition on the same half of the vine, the number of 1 year-old canes per metre of leader, the length and basal diameter of each cane and their growth habit (terminating or non-terminating) were recorded. The same procedure was used for 2 year-old canes and in addition, a record was made of whether canes had been fruiting or non-fruiting in the previous season.

Yield components: On 2-3 December 2001, two canes were selected, one from each side of the vine, and the number of winter buds on each cane were counted in addition to the number of flowers per winter bud. This information was used to calculate the percentage floral bud-burst and the average flower number per winter bud, or the fertility of each cane.

Canopy light transmission: The percentage of light passing through the canopy (canopy light transmission) was calculated using the point-grid method (Wünsche *et al.*, 1995), whereby a 1 m^2 sheet of white plastic ruled up into 0.1 m^2 squares is put under the canopy and the number of line intercepts (grid points) in sun flecks are counted. The percentage of canopy light transmission was estimated from the percentage of grid points in the shade during each sampling period. Though not as accurate as other methods for measuring light transmission (estimates of light transmission are slightly ($\approx$ 15%), but not significantly, lower than those obtained using hemispherical photography, light sensors and ceptometers (Wünsche *et al.*, 1995)), on clear days this method allows rapid estimates to be made on large numbers of plants. Measurements were made on 30 January 2002, approximately 25 days after both treatments were pruned, and 4 April 2002. All measurements were made between 11:00 and 13:00 h.

Fruit data: Ten fruit were randomly selected from each experimental area (Fig 3.1) on 3 May 2002 and five fruit were randomly selected from each vine on 5 May 2002 and their fresh weight, DMC, SSC, and mineral concentrations were determined as described in Chapter 2. In addition,

the lengths and diameters of stalks from two fruit per area and two fruit per vine were measured. In total this equated to 120 fruit and 48 fruit stalks being sampled from the monitored vines and 80 fruit and 16 fruit stalks being sampled from the remaining vines. On 10 May 2002 (178 DAFB), the remaining fruit from each of the monitored vines were harvested and fruit from individual vines was later run over a grader to determine the average size of fruit on each vine and the total vine yield. A day later fruit from the remaining vines (i.e. each experimental area) were harvested and commercially graded, as a bulked sample, to determine the total area yield, % class one and class two fruit, and size profiles for class one fruit. Both class one and class two fruit must be free from pathological, physiological, and physical damage and quarantine pests (dependent on the market) and must weigh more than 70 g (count 42). In addition, class one fruit must have no cosmetic defects (for example, dropped shoulder, 'Hayward' mark, and water stain). Tolerance of these defects is greater for class two fruit.

Postharvest vine measurements: On 17 May 2002 (185 DAFB), the number of leafless shoots and potential replacement canes (current season's axillary shoots greater than 0.75 m long arising from the LZ) in each monitored vine were counted. The number of dead leaves in a 1 m^2 quadrant placed (a) on the ground and (b) in the FZ of the canopy, 0.5 m out from the leader also were counted. After leaves had been collected and counted from the ground, the percentage grass cover was estimated in the same 1 m^2 quadrant. This information was used as an indication of the vine density in which fruit had developed.

Storage attributes: Two polylined boxes of 100 fruit were put into coolstorage (at 1-3°C) from each vine. Where possible, efforts were made to select count 36 fruit (average weight 100 g). In addition, nine boxes were selected from each experimental area, 3 boxes of count 42 fruit (127 fruit per box, average weight 79 g), 3 boxes of count 33 fruit (92 fruit per box, average weight 109 g), and 3 boxes of count 27 fruit (76 fruit per box, average weight 132 g). Each box contained polyliners and was put into a commercial cool store at 0°C. One box of fruit from each size class was selected from each experimental area (three boxes per area) to be used in sensory analysis (see below). The SSC, firmness, and incidence of pitting, rots, and low temperature breakdown (LTB) of fruit from the remaining two boxes from each experimental area, in addition to the two boxes selected from each of the vines, were assessed 12, 17, 24, 26 and 28 weeks after harvest as described in Section 2.9. At each of these times, five fruit were selected for assessment from one of the vine boxes and five fruit were selected from one box for each of the different size classes, for each experimental area. Fruit from the remaining boxes were quickly assessed visually for rot incidence whilst in the coolstore, except at the final assessment time (28 weeks after harvest) when all the boxes were removed from the coolstore for assessment.

Sensory assessment: On 20 June 2002, approximately 6 weeks after harvest, one box of fruit from each size class from each area (i.e., three boxes per area) was removed from storage and placed in a ripening room at 20°C to soften to eating ripeness (3.9-7.8 N) according to Stec *et al.*, (1989). Once softened, fruit were stored at 1-3°C awaiting sensory assessment. Sensory assessment was conducted over a 3-day period from 1-3 July 2002 using 15 and 16 year-old children from varied ethnic backgrounds including European Pakeha, Chinese, Indian and Polynesian using a 'triangle test'. Over these three days 87 children were available to sample fruit. The children were selected as sensory panellists as there was a relatively large number of them and they were available for the whole three day period. Triangle tests determine whether a perceptible sensory difference exists between samples of two products, in this case fruit from the two different pruning systems. This test method is applicable when the nature of the difference between the samples is unknown. It does not determine the size or the direction of the difference and the attribute(s) responsible for the difference are not identified (Frazer *et al.*, 2001). To conduct this test each panellist received three coded fruit, two from one treatment and one from another treatment. On each day fruit from different count sizes were used and fruit were selected to ensure that even numbers of fruit were used from each of the eight experimental areas (Fig 3.1). Each panellist was asked to measure the firmness of their fruit using a 0-5 kgf (0-49 N) Effegi fruit tester with a 8mm head as described in Section 2:10, and to record measurements on a provided 'answer' sheet. Their fruit was then swapped for another panellist's fruit and they were told to cut a small slice from each end of their fruit. They were then asked to taste all three fruit and to select the one that they thought was the least like the other two, using water crackers and water to cleanse their palettes if desired. This information was recorded on their answer sheet, in addition to any comments on the reason(s) for their selections. Their sheets were collected before they asked to assess the SSC of each of their fruit using a refractometer as described in Section 2:10. In this way data was collected on the firmness and SSC of each tasted fruit, and on the affects of each pruning system on fruit flavour.

Seasons two (October 2002 – October 2003) and three (October 2003 – October 2004)

Winter vine measurements (12 August 2003, 14 August 2003), yield component calculations (14 November 2002, 1 December 2003), post-pruning vine measurements (21 May 2003 (175 DAFB), 18 May 2004 (172 DAFB)), and sensory evaluation (5-7 July 2004) were conducted as for season one (above). Canopy light transmission was determined on 5 December 2002, 22 January 2003, 16 February 2003 and 8 April 2003 in season two, using the point-grid method as in season one. In season three, canopy light transmission was estimated from LAI and canopy openness data on 18 December 2003, 10 and 20 February 2004 (post-pruning) and 22 March 2004, using a digital camera (Coolpix 990, Nikon) with a fish-eye lens converter (FC-E8, Nikon, Japan: field view 183°) to take a black and white hemispherical photo of the canopy (2048 x 1536 pixels: Fig 3.2), as viewed from the orchard floor (Rich *et al.*, 1993). The camera was mounted at a height of 0.3

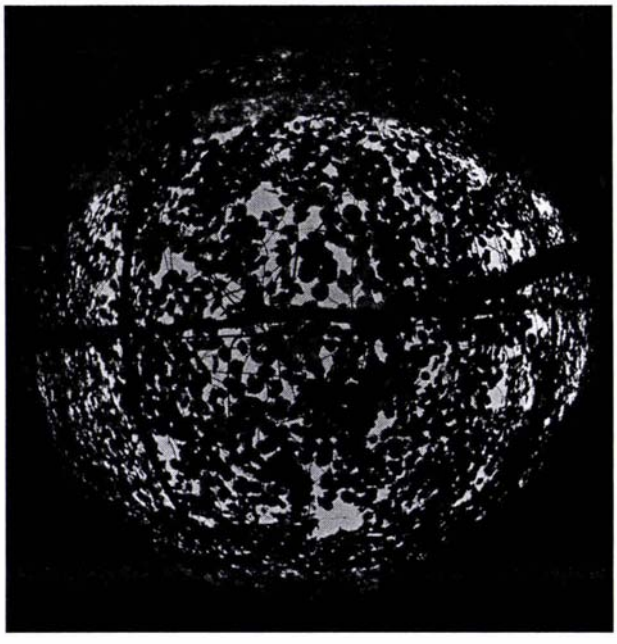


Fig 3.2. A digital hemispherical photo taken from underneath the canopy of a kiwifruit vine.

cm above ground using a tripod with a bubble level attached to ensure level and constant positioning of the camera from photograph to photograph. Photos were taken directly under the central leader, 0.5 m out from the trunk, with the camera orientated so that the top of the photo always faced true north. All photos were taken in cloudless (at dawn or dusk) or overcast conditions to ensure even backlighting and to avoid bright reflections being mistaken in analysis for areas of open sky (Frazer *et al.*, 2001). To set exposure the camera was put in automatic mode with the exposure compensation set at minus one and pointed at the open sky, the exposure reading was noted and fixed in manual mode. Photos were taken with the image quality set on 'fine'. Once the photo had been taken, the photograph's quality and contrast (between the sky and the canopy) were assessed by putting the camera into play mode, and if necessary another photo was taken (Frazer *et al.*, 1999). Digital images were analysed using Gap Light Analyzer (GLA) version 2.0 software© (Stenberg *et al.*, 1994). Percentage canopy openness (percentage of the sky area without canopy leaves, branches, or other plant material between the sky and the point of observation, as seen from beneath the canopy) values were determined in addition to LAI values (Frazer *et al.*, 2001). Canopy openness measurements potentially range from 0% (no portion of the sky is visible) to 100% (no plant material obscures the sky).

Fruit assessments

A ten fruit sample was taken from each experimental area for analysis (23 April 2003, 2 May 2004), and the all areas were harvested individually (3 May 2003 (157 DAFB), 7 May 2004 (161 DAFB)) as described in season one (above). Harvest dates depended on when contractors were available to assist with the picking and transporting of fruit. In season three nine boxes of fruit from each area were put aside for sensory and storage analyses, as in season one. However, it was decided that sensory experiments would only be conducted in the first and final years to see how fruit taste and flavour were affected by each pruning system. Therefore, in season two only six

boxes of fruit (2 from each count size (42, 33 and 27)) were collected from each experimental area for storage assessment, as described in season one. One box of fruit from each size class, from each area (24 boxes in total), was removed from the coolstore 6 weeks after harvest. Sensory assessment was conducted from 30 June 2004-2 July 2004, as described for season one. Fifty-five children were available to sample fruit in season three. Storage trial fruit were assessed after 12, 16, 20 and 26 weeks at 0°C in season two and after 12, 16, 21 and 26 weeks at 0°C in season three, as described for season one.

In the 2nd and 3rd years of the experiment, the canopy of each monitored vine was segregated into two zones, the LZ and the FZ. Three fruit were selected from each zone within each of the monitored vines (6 fruit in total per vine) and analyses were conducted as for season one (above: 24 April 2003, 1 May 2004). The diameter, length, xylem and phloem area and estimated secondary xylem conductance of one fruit stalk from each zone, from each of the monitored vines also was determined. Fruit from the leader and fruiting zones were harvested and run over the grader separately (2 May 2004, 5 May 2004), and one box of 95-100 fruit (average count size = 36 (100g)) was taken from each zone and stored at 1-3°C. In the second year it was not always possible to collect 100 fruit from the LZ of each monitored vine as fruit numbers were reduced (see following section for more information). Fruit were assessed after 12, 16, 21 and 26 weeks in storage.

3.2.4. Experimental problems

In order of occurrence:

1. Vines already had received one light prune by the time the experiment commenced on 1 October 2001, prior to assessing the winter canopy characteristics (winter vine measurements). Consequently, bud and spur numbers in the FZ may be slightly lower, and cane lengths slightly shorter, than in succeeding years.
2. Hydrogen cyanamide (HiCane®; a chemical used to enhance fruit set and promote bud-break uniformity in kiwifruit (McPherson *et al.*, 2001)) was applied too early in the second year, and consequently fruit set was reduced (see results). In addition, shortly after flowering, vines were affected by high winds, which blew out many canes, and frost, both of which further reduced fruit numbers and left gaps in the canopies of some vines. Leaf health and physiological balance within vines both may have been affected. Leader pruned monitored vines appeared to be more affected than CP vines, and consequences of this are discussed in the results.

3. In the final year, every cane with a diameter exceeding one finger width (approximately 15 mm) wide was girdled (in all experimental areas). Girdling affects fruit size and DMCs in kiwifruit (Currie, 1997), and may also affect mineral accumulation (refer Chapter 5 for more information). Therefore it is difficult to determine whether canopy management had any effect on fruit FW, or DM and mineral concentrations between years two and three of the experiment.

3.2.5. Statistical analysis

An ANOVA GLM was used to identify mean differences between fruit from different pruning systems, areas, seasons, vines and zones within a vine. Means separation was conducted as described in Section 2.10. Simple linear regression was used to establish the significance of relationships between pairs of variables, such as the vine crop load and total fruit DW concentration.

3.3. Results

3.3.1. Canopy attributes

In the first season there were no differences in any of the canopy attributes measured prior to the experiment being implemented. In the second season there was 16% more 2nd year wood in LP vines than in CP vines ($P < 0.05$). This 2nd year wood was smaller in diameter in LP vines than in CP vines ($P < 0.05$). There were no other effects of the pruning systems on canopy attributes when vines were assessed in the first winter after treatments commenced.

Leader pruned vines were more open than CP vines (Tables 3.1 and 3.2), especially in the second and third seasons. In the first season the LZ also was more open than the FZ in both LP and CP vines, but these differences were not found in the second season (Table 3.1). In LP vines, canopy light transmission was higher in the FZ in the second season than in the first season. Light transmission was reduced in the LZ of CP vines over the first two years of this experiment.

Table 3.1. Canopy light transmission in the leader and fruiting zones (LZ and FZ) of leader (LP) and conventionally pruned (CP) vines, as measured in the first (2001/2002) and second (2002/2003) seasons using the point-grid method.

Light passing through the canopy (%)				
Season	Zone	LP system	CP system	*P-value* (pruning system differences)
1	LZ	28_a	19_a	*
1	FZ	9_b	7_b	*ns*
2	LZ	25_a	9_{bc}	***
2	FZ	23_a	13_{ac}	*

Stars indicate significant differences between treatments (rows) at * P <0.05, *** P < 0.001, ns = not significant. For each treatment (columns) values followed by different letters are significantly different at P <0.01.

Table 3.2. Canopy openness and leaf area index of leader (LP) and conventionally pruned (CP) vines, as measured using hemispherical photography in the 2003/2004 season.

Pruning system	% canopy openness		LAI	
LP	2.95	(0.271)	3.69	(0.077)
CP	2.39	(0.259)	4.27	(0.142)
P-value	*0.05*		*0.01*	

Data are mean values (± s.e.)

At the end of the growing season there were 46, 27 and 42% more potential replacement canes in CP vines than in LP vines in the first, second and third seasons, respectively (P <0.001). This equated to 12, 10 and 13 more canes per vine in CP vines in the first, second and third seasons, respectively. In season two, there were 10-13 (37-48%) more replacement canes in LP vines and 7-11 (19-30%) more in CP vines than in seasons one and three (P <0.01). There was no effect of pruning system on the percentage grass cover under the canopy, number of leafless shoots, or number of dead leaves in the canopy and on the ground (data not shown). Grass cover under the vines averaged 75% during the three years of the experiment, whilst the number of leafless shoots in the canopy never exceeded four in any one vine. In the second season there were 52-53% more dead leaves (including those in the canopy and on the ground) than in the first and third seasons (about 42 leaves).

3.3.2. Yield components

In season two, flower bud-burst was approximately 30% less than in seasons one and three (Fig 3.3). In LP vines the flower number per winter bud was reduced by 54 and 63% in season two compared to seasons one and three, respectively. These values were 32 and 46%, respectively, in

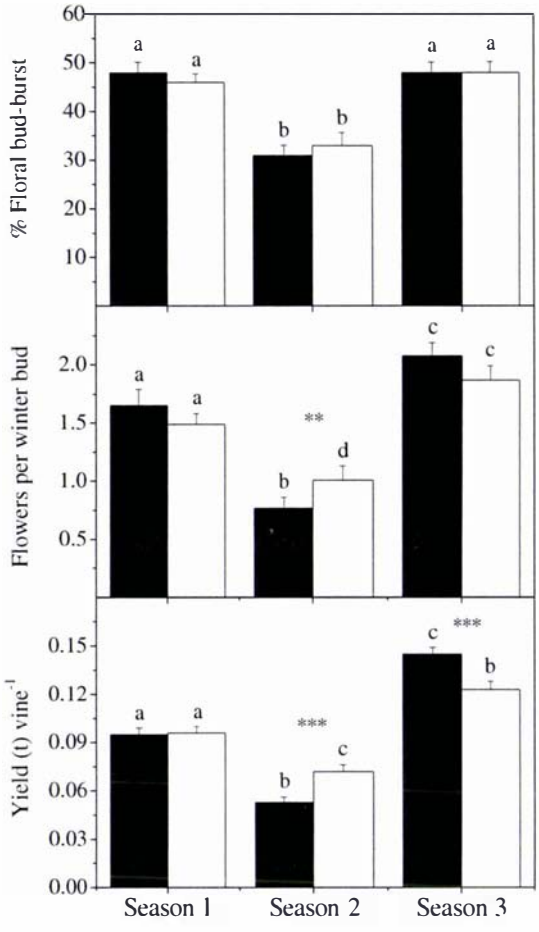


Fig 3.3. Flowering and yield in monitored leader (black bars) and conventionally pruned (white bars) kiwifruit vines, as measured over three seasons. Bars are mean values (± s.e.). Bars followed by the same letter are not significantly different at P <0.001 and indicate significance between seasons. Stars ** P <0.01, *** P <0.001 indicate significance between the two canopy management regimes.

CP vines. The flower number per winter bud was greater in CP vines than in LP vines in the second season. These trends were reflected in the average yield per (monitored) vine (Fig 3.3) and total yield (yield from all experimental areas; Fig 3.4). In season two yield per vine did not differ between treatments in the LZ of monitored vines (data not shown). However, yields were 21% lower (0.01 t per vine) in the LZ of CP vines than in the LZ of LP vines in the final season (P <0.001). The yield per vine was 31% (0.02 t per vine) lower in the FZ of LP vines than in the FZ of CP vines in season two, whilst in season three LP vines had 11% (0.01 t per vine) more fruit in the FZ than CP vines (P <0.001).

3.3.3. Fruit data

Fresh weight

Over the three seasons, the average FW of fruit from LP vines did not change, but CP fruit increased in size (Table 3.3). Initially LP fruit were larger than CP fruit. However, by season three, pruning systems had no effect on fruit size when fruit were considered on a vine basis. When samples from each experimental area were considered, fruit from CP vines were slightly larger than those on LP vines. Fruit FW was always greater in the FZ than in the LZ.

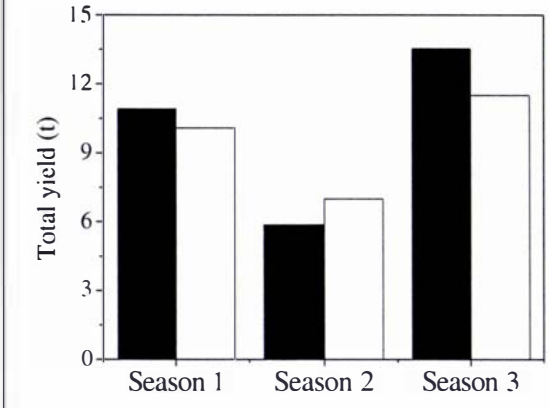


Fig 3.4. Total yield from leader (black bars) and conventionally (white bars) pruned vines.

Table 3.3. Fresh weight of fruit from the leader (LZ) and fruiting (FZ) zones of leader (LP) and conventionally (CP) pruned vines.

Fresh weight (g)				
Season	Zone	LP system	CP system	*P-value* (pruning system differences)
1	all	101_a	97_a	***
2	all	105_a	99_a	***
3	all	105_a	105_b	*ns*
2	LZ	102_a	96_a	**
2	FZ	109_b	102_b	**
3	LZ	103_a	103_b	*ns*
3	FZ	107_b	107_c	*ns*

Stars indicate significant differences between treatments (rows) at ** P <0.01. *** P <0.001. ns = not significant. Letters indicate significant differences between seasons (top columns) or zones (bottom columns) for each treatment. Values followed by a different letter are significantly different at P <0.01. Fruit samples were collected from the individual monitored vines.

Dry matter concentration

Fruit DMCs decreased steadily as the experiment progressed in both LP and CP vines (Fig 3.5). In the first two seasons the DMC of fruit from LP vines was higher than that of fruit from CP vines, but by the third season fruit DMC differences were no longer significant. Fruit from the LZ of LP vines always had a higher DMC than fruit from the LZ of CP vines (Table 3.4). There was no

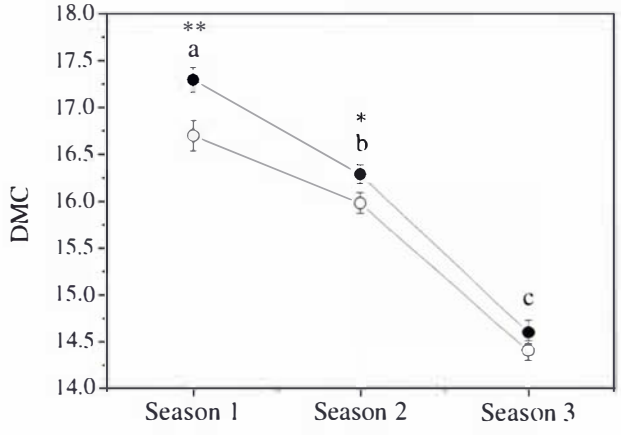


Fig 3.5. DMC of fruit from leader (closed circles) and conventionally pruned (open circles) vines, as measured over three seasons. Data are mean values (± s.e.). Values with the same letter are not significantly different at P <0.001 and indicate significance between seasons. Stars * P <0.05, ** P <0.01 indicate significance between the two canopy management regimes.

effect of pruning system on the DMC of fruit from the FZ. In season two, DMCs were higher in the LZ of LP vines than in the FZ, but this was not the case in CP vines. In season three, fruit DMCs were higher in the LZ than in the FZ regardless of the canopy management system used. When ten fruit were sampled from individual areas the seasonal decline in fruit DMC was still apparent, but this sampling technique was not sensitive enough to pick up differences between treatments.

Table 3.4. DMC of fruit from the leader (LZ) and fruiting (FZ) zones of leader (LP) and conventionally pruned (CP) vines, as measured in season two (2002/2003) and three (2003/2004).

		DMC		
Season	Zone	LP system	CP system	*P-value* (pruning system differences)
2	LZ	16.6_a	16.1_a	*
2	FZ	15.9_b	15.9_a	*ns*
3	LZ	15.2_c	14.7_b	*
3	FZ	13.9_d	14.0_c	*ns*

Stars indicate significant differences between treatments (rows) at * P <0.05, ns = not significant. For each treatment (columns), values followed by a different letter are significantly different at P <0.001.

Though differences in the DMC of fruit harvested from different areas and different vines were always highly significant (P <0.001), these differences were not consistent from season to season. For example, fruit from vines 17, 10 and 18 and areas 4, 8 and 7 had the lowest DMC in seasons one, two and three, respectively. This variation could not be attributed to differences in the crop load on individual vines in the current season, as the relationship between (vine) crop load and

fruit DMC was never significant (Fig 3.6). However, fruit DMCs were positively correlated with canopy density (Fig 3.7).

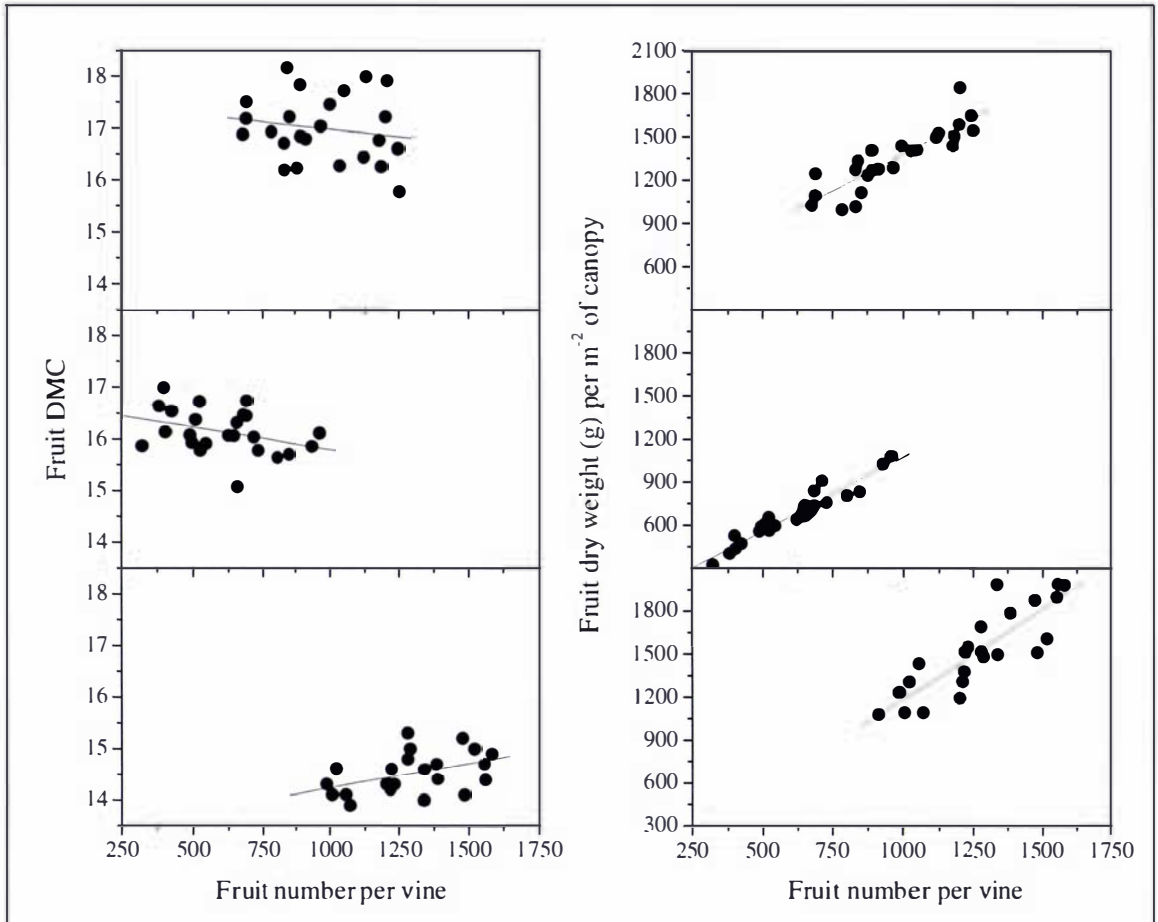


Fig 3.6. Relationship between crop load and fruit DMC (left) and fruit dry weight per m^2 of canopy (right) in seasons one (top), two (middle) and three (bottom). The relationship between crop load and fruit DM was never significant at $P < 0.05$. R^2 values and equations for the correlation between fruit DW per m^2 of canopy and crop load were (season one) $Y=1.01X+371$; $R^2 = 0.75$, (season two) $Y = 1.03X+47$; $R^2 = 0.91$, (season three) $Y=1.26X-86$; $R^2=0.71$. These correlations were significant at $P < 0.001$.

A linear relationship existed between fruit DW per m^2 canopy area (g m^2) and crop load per vine (Fig 3.6), indicating that as the crop load increased the total amount of carbohydrate partitioned into the fruit biomass increased also. This relationship was consistent between seasons.

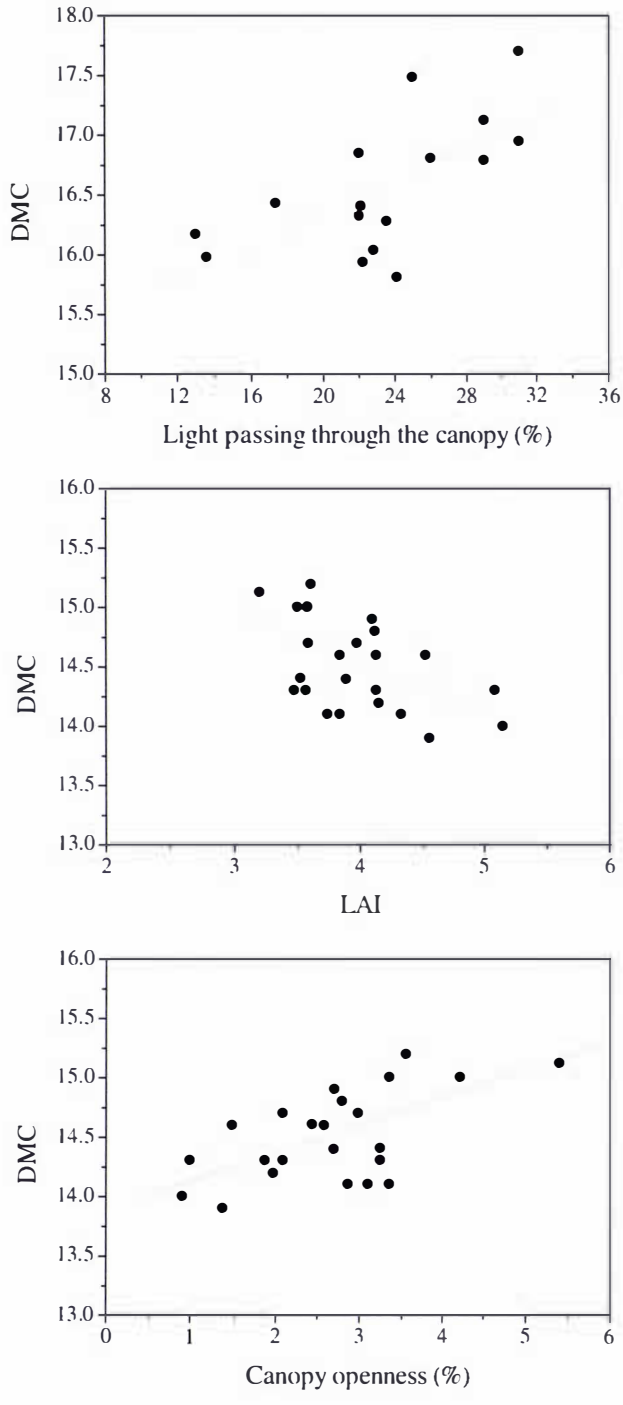


Fig 3.7. Relationship between fruit DMC and canopy density in seasons one and two (top: DMC =0.067X+15, R^2=0.38, P<0.01) and season three (middle: DMC =-0.385X+16, R^2=0.23, P <0.05; bottom: DMC =0.216X+14, R^2=0.32, P <0.01).

In seasons one, two and three fruit were harvested when their SSC was 7.5-7.9, 5.5-5.7 and 6.2–6.3 °Brix, respectively. The main crop is normally harvested when fruit reach 6.2 °Brix, therefore in season one fruit were harvested late, whilst in season two they were harvested early. Differences in the maturity of fruit at harvest may therefore explain why fruit DMCs dropped by between 5-6% between seasons one and two, but could not explain the 10% drop in fruit DMCs between seasons two and three.

In the first season, CP fruit had a 5% higher SSC at harvest than LP fruit, however, this finding was reversed in the second season and in the final season there were no significant differences between treatments. Fruit SSC was 4-5% higher (0.3° units) in the LZ of LP vines than in the FZ, but did not differ between these zones in CP vines.

Dry weight

Treatment affects on fruit DW accumulation were virtually identical to those for fruit DMC. The average fruit DW decreased by 18% and 11% (3.29 and 1.78 g) between seasons one and three in LP and CP vines, respectively. Fruit DW was greater in LP vines than in CP vines in seasons one (1.55 g, P <0.01) and two (0.88 g, 0.05), but not in season three. Similar findings were observed in the LZ, whilst canopy management

practices never had any effect on fruit DW in the FZ (data not shown). In LP vines fruit from the LZ had a higher DW than fruit from the FZ in both seasons two (0.86 g, P <0.05) and three (1.32 g, P<0.01). However, fruit DW never differed between zones in CP vines.

Minerals

Fruit K and Mg concentrations decreased from season one to three, whilst P concentrations increased over this period in both LP and CP vines (Fig 3.8). Fruit Ca concentrations did not change during the course of the experiment. Fruit from CP vines always had a higher Mg concentration than fruit from LP vines. The fruit Ca and K concentrations of CP vines also were higher than those of LP vines, but differences were only significant in the first (Ca) and third (K) seasons. There was no affect of pruning system on fruit P concentrations.

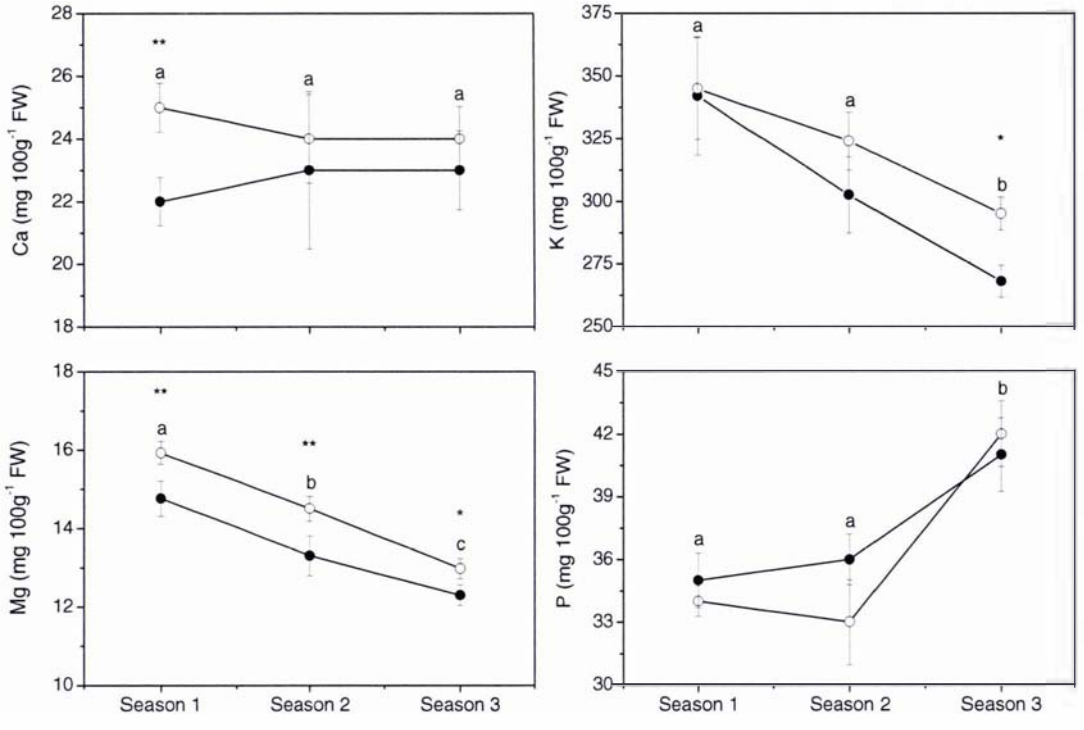


Fig 3.8. Fruit Ca, Mg, K and P concentrations in leader (closed circles) and conventionally pruned (open circles) vines, as measured over three seasons. Data are mean values (± s.e.). Values with the same letter are not significantly different at P <0.05 and indicate significance between seasons. Stars * P <0.05, ** P <0.01 indicate significance between the two pruning systems.

Fruit K concentrations decreased (by 36-49 mg $100g^{-1}$ FW) between seasons two and three in the LZ, but not in the FZ (P <0.05). Between seasons two and three, fruit Mg concentrations decreased in both the LZ and FZ (by 1.1-1.6 mg $100g^{-1}$ FW, P <0.05), whilst fruit P concentrations increased in both the LZ and FZ (by 3.8-9.3 mg $100g^{-1}$ FW, P <0.05). Fruit Ca concentrations did

not change in either the LZ or FZ between seasons 2-3. Fruit Ca concentrations also were identical in the LZ and FZ, as were fruit K and Mg concentrations (data not shown). Fruit P concentrations in the FZ of LP vines were higher than in the LZ. Fruit mineral concentrations varied from vine to vine, and between areas, but as with fruit DMCs these variations were not consistent between seasons (data not shown). Moreover, there was no relationship between canopy openness of LAI and fruit K, Mg, Ca or P concentrations.

Fruit stalks

Length and diameter of fruit stalks varied between growers in seasons one and two, but not in season three. Differences were not consistent between seasons or between data analysed on a vine and area basis, and appeared to relate more to the effects of fruit size on fruit stalk growth than to any direct effect of canopy management on fruit stalk development. When data from the first two seasons were combined, smaller fruit had significantly longer stalks (R^2 = 0.13, P <0.05), whilst large fruit had wider stalks (R^2 = 0.38, P <0.001). Pruning systems had no effect on xylem, phloem or pith areas or estimated secondary xylem conductance (data not shown).

3.3.4. Storage and sensory data

Storage

Treatment affects on fruit rSSCs were similar to those for fruit DMCs. Like fruit DMC, the rSSC of fruit declined between seasons one and three, irrespective of whether data were analysed on a vine or area basis (Table 3.5). Only in season two did the pruning system used have any affect on fruit rSSC, the greatest effect being in fruit from the LZ. In this season, fruit rSSC was higher in LP vines than in CP vines. Fruit from the LZ of LP vines, but not CP vines, always had a higher rSSC than fruit from the FZ. The greatest differences between the rSSC of fruit from LP and CP vines were observed in count 42 fruit (on average 5.5% greater than in count 33 and 27 fruit, or 0.76° units). Count 42 fruit had, on average, a 5% lower rSSC than count 33 and 27 fruit in CP vines (P <0.05), but rSSC was not affected by count size in LP vines. These trends changed very little during 12 weeks at 0^{o}C.

Fruit firmness was highest in the second season, irrespective of count size or treatment (P < 0.001, Table 3.6). Overall fruit from CP vines tended to be firmer than fruit from LP vines, but differences were only significant in count 27 fruit in the first and third seasons and in count 33 fruit in the final season. There was no effect of pruning system on fruit firmness when fruit were analysed on a vine basis (data not shown). Effects of count size on fruit firmness were inconsistent between seasons (Table 3.6). When data from each season were combined, there was

no effect of count size on fruit firmness (data not shown). As with the rSSC, these findings were apparent after 12 weeks in storage at 0-3°C.

Table 3.5. Ripe soluble solids concentration of fruit from the leader (LZ) and fruiting (FZ) zones of leader (LP) and conventionally (CP) vines.

rSSC				
Season	Zone	LP system	CP system	*P-value* (pruning system differences)
1	all	14.9_a	14.7_a	*ns*
2	all	14.3_b	14.0_b	**
3	all	13.1_c	13.1_c	*ns*
2	LZ	14.8_a	13.8_a	***
2	FZ	13.9_b	14.2_b	*ns*
3	LZ	13.5_c	13.3_c	*ns*
3	FZ	12.8_d	12.9_d	*ns*

Stars indicate significant differences between treatments (rows) at ** P <0.01. *** P <0.001, ns = not significant. Letters indicate significant differences between seasons (top columns) or zones (bottom columns) for each treatment. Values followed by a different letter are significantly different at P <0.01. Fruit samples were collected from the individual monitored vines.

In season one, pit incidence was higher in count 42 fruit than in count 33 and 27 fruit, irrespective of the pruning system used (Table 3.7). Incidence of stem-end (Botrytis) rot, wound rot, and side rot was low (<3%), and therefore these categories were combined under the heading 'rot incidence'. Rot incidence was higher in count 27 fruit than in count 33 and 42 fruit from LP vines, and LTB was higher in count 27 fruit from CP vines than in count 33 and 42 fruit. LTB was higher in count 27 fruit from CP vines than in count 27 fruit from LP vines. When data from each count size were combined, pitting, rot and LTB incidence did not differ between treatments or areas in any season. In the second and third seasons pitting and rot incidence never exceeded 3.6%. However, in the second season a blemish developed on fruit that looked like the initial stages of pitting, except that affected areas were not sunken. Incidence of this blemish was 63% higher in LP fruit than in CP fruit (6.2% incidence as opposed to 2.3% incidence).

Sensory

No difference was perceived between the flavour of fruit from LP and CP vines or between fruit from the different areas. In both seasons one and three, fruit SSC could be used to explain 80-82%

of the selections (i.e., 80-82% of fruit selected as the odd fruit out were either the sweetest or the sourest fruit) and fruit firmness an extra 4-5%. In season one, descriptors such as "not as sweet" and "less flavoursome" were 37% more likely to be used to describe fruit from CP vines than fruit from LP vines, whilst panelists were 38% more likely to describe fruit from LP vines as being sweeter than that from CP vines. However, in season three panelists were 25% more likely to describe CP fruit as more flavoursome (either sweeter or tangier) than LP fruit.

Table 3.6. Firmness of fruit from three different count sizes, from leader (LP) and conventionally pruned (CP) vines after 26 weeks in storage at 0°C.

		Firmness (N)		
Season	Count size	LP system	CP system	*P-value* (pruning system differences)
1	42	10.11_{a}	10.54_{a}	*ns*
1	33	10.61_{a}	10.90_{a}	*ns*
1	27	9.55_{a}	11.21_{a}	***
2	42	15.81_{a}	16.82_{a}	*ns*
2	33	17.24_{a}	16.28_{ab}	*ns*
2	27	16.90_{a}	14.71_{b}	*ns*
3	42	9.44_{ab}	8.40_{a}	*ns*
3	33	9.33_{a}	10.78_{b}	**
3	27	10.37_{b}	11.51_{b}	**

Stars indicate significant differences between treatments (rows) at ** P <0.01, *** P <0.001, ns = not significant. For each season, values for each treatment (column) are significantly different at P <0.001 when followed by a different letter, and indicate differences between fruit from different count sizes.

Table 3.7. Storage disorder incidence in fruit from three different count sizes from leader (LP) and conventionally pruned (CP) vines, as measured on 16 May 2002 (season one).

% incidence				
Disorder	Count size	LP system	CP system	*P-value* (pruning system differences)
Physiological pitting	42	15.3_a	12.0_a	*ns*
	33	4.3_b	5.8_b	*ns*
	27	3.8_b	4.3_b	*ns*
Rot	42	3.5_{ab}	3.6_a	*ns*
	33	2.3_a	3.0_a	*ns*
	27	7.1_b	5.9_a	*ns*
Low temperature breakdown	42	11.8_a	8.3_a	*ns*
	33	17.8_a	16.5_{ab}	*ns*
	27	14.3_a	23.3_b	*

Stars indicate significant differences between treatments (rows) at * P <0.05. ns = not significant. For each disorder, values followed by the same letter are not significantly different at P <0.01.

3.4. Discussion

3.4.1. Fruit quality: physical attributes

Fruit DMC was consistently higher in the LZ of LP vines than in the LZ of CP vines, with greatest differences occurring in seasons one and two. It was not expected that differences between the DMC of fruit from the different treatments would decrease as the experiment progressed when data were considered on a whole vine basis. Moreover, it was not anticipated that fruit DMC would decrease in both treatments over the three year experimental period. Instead, it was anticipated that differences between vines in the two treatments would increase as the experiment progressed, as fruit DMC increased in the LP treatment.

Fruit DMCs were not affected by the crop load on the vine, or the yield per area in any of the three seasons (see Chapter 4 for a more detailed discussion on the effects of crop load on fruit DMCs). However, a negative correlation was found between canopy density and fruit DMCs, which suggests that DMCs were higher in fruit from LP vines than in CP vines, because the former were not as dense, especially in the LZ. A similar negative correlation between fruit DMCs and the amount of shading within a canopy has been found by Snelgar *et al.* (1998). Leaf photosynthetic

efficiency and stomatal conductance were reduced as canopy density increased and light levels in the canopy were lowered, and as a result photoassimilate production was reduced (Biasi *et al.*, 1993; Chartzoulakis *et al.*, 1993b; Smith *et al.*, 1994). Shaded leaves may even draw on other carbohydrate supplies if they use more carbohydrate (in respiration and structure maintenance) than they produce (Grant and Ryugo, 1984b). Carbohydrate availability is likely to be less in densely shaded canopies than in open canopies and carbon partitioning to fruit may be reduced in the former. Increased light exposure also may affect kiwifruit quality directly (Biasi *et al.*, 1993), by enhancing vascular development within the fruit, thereby increasing the capacity of fruit to import carbohydrates and minerals (Biasi and Altamura, 1996).

By removing vigorous shoot growth from the LZ, LP practices may have altered carbohydrate partitioning in favour of the fruit, particularly those in the LZ (Miller *et al.*, 2001). In kiwifruit, shoot growth is known to be a stronger sink for carbohydrate than fruit growth (Buwalda and Smith, 1990b). Carbohydrate availability in the LZ has a strong impact on growth of fruit in both the LZ and the FZ, return bloom and root growth, whereas carbohydrate availability in the FZ has little effect on the growth of fruit outside of that zone, or on return bloom or root growth (Buwalda and Smith, 1990b). Therefore, shoot pruning in the LZ is likely to have more of an impact on carbon partitioning to fruit than shoot pruning in the FZ, which is the focus of the CP pruning system.

The pruning systems, as applied in this experiment, had less of an affect on fruit DMCs than other studies comparing the effects of LP and CP (Miller *et al.*, 2001; Thorp *et al.*, 2003a), particularly in the final season. There are several possible reasons for this, including the (a) reduction in magnitude of difference between the two pruning strategies when compared to other studies, (b) affects of adverse climatic conditions and poor timing of HiCane® application, and (c) effects of cane girdling on the response of vines to canopy management. Differences in the canopy attributes of LP and CP vines were negligible, and a lot smaller than those typically found in a comparison between LP and CP systems (Table 3.8: Miller *et al.*, 2001; Thorp *et al.*, 2003a). For example, although CP vines still had more replacement canes, they were not longer, larger in diameter or less likely to terminate than in LP vines. Moreover, the ratio of first year to second year wood did not differ in LP vines and CP vines in the first and third seasons. In the second season there was 16% more 2-year-old wood than 1-year-old wood in LP vines than in CP vines, as opposed to 79% more in the study of Miller *et al.*, (2001). Therefore, it is possible that the vine differences between the two pruning systems used here were not large enough to have significant effects on fruit quality.

Table 3.8. Attributes of leader (LP) and conventionally pruned (CP) vines; a comparison between studies

Years after transition	Vine attribute	Vine pruning system (This experiment)			Vine pruning system (Miller *et al.*, 2001; Thorp *et al.*, 2003a)		
		LP	CP		LP	CP	
2	Spurs per vine	21.3	19.4	n.s.	15.1	11.4	n.s.
3	Cane length (m)	1.68	1.79	n.s.	1.3	1.51	***
2	Diameter 1yr-old canes (mm)	14.5	15.2	n.s	13.7	15	*
3	Diameter 1yr-old canes (mm)	13.5	14.0	n.s.	14.1	15.3	***
2	Self-terminated canes (%)	37	41	n.s.	35.1	22.8	n.s.
3	Percentage of canes with fruit stalks	25	11	n.s	27.1	5.4	***
3	Number of potential replacement canes (per m on each side of leader)	3.6	6.2	***	3.0	6.5	***
3	Dead leaves (under the canopy)	17	23	n.s.	14.2	36.8	*
3	Dead leaves (in the canopy)	1	1	n.s.	5.2	20.2	***
3	Grass cover (%)	61	76	n.s.	26.7	5	**

Data are mean values from 24 vines (observations). Within a row values for each experiment are significant at * P <0.05, ** P <0.01 and *** P <0.001. Not significant = n.s.

In the second year of the experiment, HiCane® was applied too early, and consequently floral bud-burst was reduced with fewer flowers per winter bud. Vines also were affected by high winds and frost around flowering. As a consequence, yields were significantly reduced, and many vines had large gaps in the canopy. It is possible that leaf health was affected, as there were more dead leaves in the canopy and on the ground in the second year of the experiment than in both other years despite fruit being harvested relatively immature in season two (leaves were counted 185,

175 and 172 DAFB in seasons one, two and three, respectively). In response to these factors, the 'orchard management expert' deliberately left more shoots in the leader zone and, to a lesser extent, the fruiting zones to 'fill-in' gaps in the canopy and to promote production of photoassimilate to minimize likely effects on flower development and shoot production in the following year. While this may have enhanced floral bud-burst and flower numbers in the final year of the experiment when compared to those in CP vines, this may have reduced the positive benefits that can be attained by adopting a LP strategy, where replacement canes are normally removed from the LZ.

Flower numbers per winter bud, yields per vine and total yields, fruit K concentrations, and fruit DWs were reduced more in LP vines than in CP vines in season two. Blemish incidence was higher in LP vines than in CP vines in this season. This suggests that LP vines were more affected by the poor HiCane® application and adverse weather conditions than CP vines, and may explain why differences in the DMC of fruit from LP and CP vines decreased as the experiment progressed. It is possible that the more open nature and reduction in leaf area in LP vines made them more susceptible than CP vines to these factors, as the leaves on the vines would be more exposed and there may be less stored reserves in the former. Stored reserves could be used as an alternative carbohydrate source when leaf net assimilation rates are low.

Finally, LP may have had little impact on fruit quality in the final year of the experiment because all the canes (in every area) larger than ≈15 mm in diameter were girdled at full bloom. LP had the greatest effect on quality of fruit from small diameter canes, such as those that were not girdled, presumably because it enhanced carbohydrate partitioning to these canes, which would otherwise be too 'weak' to compete with more vigorous shoot growth for a supply of carbohydrate (Thorp *et al.*, 2003a). Girdling would prevent the movement of carbohydrate out of the remaining (i.e., those that were not removed as part of LP system) canes and into the thin, weak canes. Currie (1997) noted that mean FW of fruit on non-girdled canes was lowered when the percentage of girdled canes in a vine was increased from 50-100%, suggesting that carbohydrate partitioning to these fruit was negatively affected.

Cane girdling also may have reduced the flow of carbohydrates to roots, and hence root development could have been impaired, or the movement of hormones, such as auxin and cytokinins, between roots and shoots may have been restricted (Currie, 1997). Both of these factors could affect root function and subsequently the transport of water and mineral nutrients to the shoots. This could explain the sharp reduction in fruit DMCs that occurred between the second and third seasons in both LP and CP vines. Within the New Zealand kiwifruit industry there is

concern amongst growers that the excessive use of trunk, and/or cane girdling (applied close to the central leader) will impact on vine productivity and fruit DMC (A. Mowat: pers. comm.).

Altered root function also may explain why fruit Mg and K concentrations were reduced in the final season, whilst P concentrations were enhanced. Miller *et al.*, (1997) found that leaf K concentrations were reduced in the transition year from CP to LP, and this effect may be similar to that occurring in fruit in this experiment. It is interesting that Ca concentrations were not affected, especially if water uptake was enhanced, because Ca is thought to travel predominantly by mass flow in the transpiration stream (Clarkson, 1984; Clark and Smith, 1988). This result suggests that Ca concentration in fruit is not totally dependent on fruit transpiration as believed, an idea that is explored in more depth in Chapters 5, 6 and 8).

Fruit K, Mg and Ca concentrations were always slightly higher in CP vines than LP (although differences were not always statistically significant). In the case of Ca, this may be because of the greater leaf area in CP vines when compared to LP vines, as fruit Ca concentrations are reduced by shoot defoliation (refer to Chapter 5 for more information). However, the effect of whole vine leaf areas was small compared to the effect of individual shoot leaf areas, which suggests that the leaves subtending fruit may be more important for promoting mineral accumulation in fruit than those further away. In kiwifruit, fruit Ca concentrations were positively correlated with shoot leaf numbers in fruit from short shoots (Thorp *et al.*, 2003b), and leaves subtending fruit influence Ca accumulation in apple fruit (Volz *et al.*, 1995). The whole vine leaf:fruit (L:F) ratio may also affect fruit mineral concentrations by determining the extent of basipetal auxin transport towards the roots, and hence, root development and water and mineral ion uptake. Auxins affect many aspects of root development, including initiation and emergence of lateral roots, patterning of the root apical meristem, gravitropism, and root elongation (Ljung *et al.*, 2005).

3.4.2. Fruit quality as perceived by the consumer

The pruning system used had little effect on fruit postharvest quality during storage at $0^{O}C$ or sensory attributes as perceived by the consumer, despite at times influencing fruit DMCs, rSSC and mineral concentrations. This suggests that other factors, aside from the fruit DMC and mineral composition influence keeping quality and fruit sensory attributes, or that the effects of canopy management practices on fruit mineral and DM concentrations were not large enough to have a perceivable affect on fruit quality.

Burdon *et al.*, (2004) found that discrimination among sensory attribute scores was only achieved for broad ranges in DMC. For example, a statistical difference was found between the overall liking of fruit with a DMC of less than 14-15% and a DMC greater than 18-19%, but within the range 14-21% DMC values had no affect on overall liking. At the low DMC range (<15%), smaller changes in DMC (2-3%-units) were able to influence flavour preference, but at the high DMC range (>16%) differences of more than 4%-units were required before consumers could perceive differences in the flavour intensity. Only for one sensory attribute, sweetness intensity, was the DMC difference required to cause a perceivable difference in taste (2-4%-units) consistent over the entire DMC range (12-20+). It was concluded that any segregation system to remove fruit with a DMC of less than 14-15% (roughly 11% rSSC), might reduce the risk of poor tasting fruit, but would not guarantee improved sensory quality, in terms of consumer perception. This agrees with results for Californian fruit showing that flavour acceptance is likely to be reduced in fruit with a rSSC of less than 11.6% (Crisosto and Crisosto, 2001).

Despite the lack of significant effects of treatments on sensory discrimination of fruit, fruit from LP vines were generally described as being more flavoursome than CP fruit. It is useful to compare this result with a previous study, completed in 1997 using fruit from various growing regions within New Zealand that compared sensory attributes of fruit from LP and CP vines. The results showed that fruit from a LP orchard had a stronger flavour intensity after 10 and 18 weeks in storage than fruit from a CP orchard (Richardson-Harman *et al.*, unpublished data), and after 18 weeks in storage consumers also preferred the flavour of fruit from the LP orchard. However, there was little affect of pruning system on the flavour preference liking after ten weeks in storage or on the overall flavour liking after 10 and 18 weeks in storage, confirming the results in the present study.

The effect of varying mineral concentrations on maintaining quality at 0°C is less easy to establish, because often it is not one mineral, but the ratio between minerals that is important in determining fruit quality (Ferguson and Boyd, 2001). For example, in kiwifruit, physiological pitting is associated with low fruit Ca and Mg concentrations and high concentrations of P and K, often in addition to low fruit DMCs (Ferguson *et al.*, 2003). Moreover, certain levels of disorder incidence must be present before relationships with fruit mineral concentrations can be established. Sub-populations of fruit within a vine may be susceptible to a disorder, whilst other fruit may be less susceptible. In kiwifruit, fruit on long shoots are less likely to develop pitting than fruit on short shoots (Thorp *et al.*, 2003b), as are fruit that are more mature at harvest (Boyd *et al.*, 2004).

In season one, physiological pitting incidence ranged from 3.8-15.3% and was between 52-75% more prominent in count 42 than in count 33 and 27 fruit. This may relate to the lower rSSC in

these fruit, because there is a positive linear relationship between fruit SSC and fruit DMCs (Burdon *et al.*, 2004), and fruit that have a low DMC are thought to be more susceptible to pitting (Ferguson *et al.*, 2003). Fruit DMCs could be low in count 42 fruit because they are poorly pollinated (refer to Section 8.3.3 for more information). Fruit DMCs also have been linked to fruit firmness in storage (Feng *et al.*, 2003; Maguire and Mowat, 2003), which may explain why in the final year of this experiment, when fruit DMCs were the lowest, fruit firmness after 12 and 26 weeks in storage also was the lowest in all fruit except count 27 fruit. As larger fruit contain more seeds, and as fruit DMCs are positively correlated with fruit seed number (refer to Section 8.3.3 for more information), these fruit may still have been able to obtain a sufficient DMC to maintain a higher level of firmness than count 42 and 33 fruit. Others also have found that fruit quality in storage is affected by fruit size, with smaller fruit softening at a faster rate than larger fruit (Crisosto *et al.*, 1999). Again this could relate to the lower DMCs found in smaller fruit.

Fruit DMCs were not increased by the pruning systems investigated in this chapter. In the following chapters effects of specific management practices on fruit DMCs are investigated, and alternative mechanisms by which fruit DMCs may be affected are described.

3.5. Conclusions

On a whole vine basis, LP was not able to increase fruit DM during the three-year experimental period. Moreover, LP did not consistently raise the average fruit DM within the vine when compared to CP. Therefore, the outcome of this experiment was not as anticipated. However, the results here allowed a number of other conclusions to be made.

1. Leader pruning consistently increased DM content of fruit from the LZ. It is suggested that this is because light transmission was increased within this zone. This would have enhanced photosynthetic efficiency of the leaves, and reduced competition for carbohydrate between the shoots and fruit within this zone. The latter outcome could have increased the overall carbohydrate availability within the vine, particularly within the LZ, and hence the amount of carbohydrate able to be partitioned to fruit. Different factors may affect carbohydrate partitioning to fruit in different parts of the vine. This may affect the response of fruit from different zones or plant parts to different canopy management practices.

2. The canopy management strategies investigated in this experiment had little affect on fruit mineral accumulation, although K, Mg and Ca concentrations appeared to be slightly

higher in CP than in LP vines. It is postulated that the whole vine L:F ratio has little affect on mineral accumulation, but that the number of developing shoots within the canopy may affect the amount of carbohydrate transported to the roots. This may affect root growth and functioning and hence the amount of water and inorganic mineral ions taken up by the vine, and possibly their distribution within the vine.

3. Carbohydrate, water and mineral accumulation are not only affected by environmental factors and management practices used in the current season, but may also be affected by the environmental factors and management practices used in the previous season. This 'carry-over' effect may explain why fruit mineral concentrations and fruit DM concentrations vary from year to year and are not always related to the existing canopy attributes in a vine.

4. The pruning systems investigated had no perceivable affect on fruit sensory attributes or quality in storage. This may be because bigger changes in fruit mineral and DM concentrations are required before fruit internal quality is affected.

4. Crop Load Effects on Photosynthesis and Transpiration and Subsequent Effects on Fruit Quality

4.1. Introduction

Thinning has long been used as a tool to adjust fruit size and productivity in commercial fruit growing and has potential as part of the management system for kiwifruit (Richardson and McAneney, 1990; Richardson *et al.*, 1994, and references herein). Reductions in crop load have been shown to increase both fresh and dry weight accumulation, the extent of increase depending on thinning severity, and complex plant *x* environment *x* crop management interactions (Cooper and Marshall, 1991; Tombesi *et al.*, 1994; Richardson *et al.*, 1997). Recently, the New Zealand kiwifruit industry has become interested in using thinning as a tool to increase fruit DMCs (Currie *et al.*, unpublished data). Thinning 'Hayward' vines shortly after FB increased the proportion of carbohydrate partitioned to fruit. However, as fruit fresh weight (FW) tends to increase with reduced crop load, large reductions in crop load are required before thinning has any appreciable affect on fruit DMCs. For example, reducing fruit numbers by 60%, from 50 to 20 fruit m^{-2}, only increased fruit DMCs by 0.4-0.5%-units (Richardson *et al.*, 1997 and Currie et al., unpublished data). This intrinsic link between DW and water accumulation may relate to the fact that phloem translocation is driven by changes in turgor pressure (Marschner, 1995).

Large reductions in crop load also may fail to elicit any significant change in fruit DMCs, because at low crop loads the proportion of total carbohydrate partitioned to fruit may be reduced (Richardson *et al.*, 1997). Vegetative vigour is increased in 'Hayward' vines thinned shortly after full bloom (Burge *et al.*, 1987; Vizzotto *et al.*, 2003). Conversely, in high-yielding vines the total amount of carbohydrate partitioned to the fruit sink (calculated as the average fruit DW multiplied by the yield) is increased (Richardson *et al.*, 1997), often at the expense of carbohydrate allocation to reserve pools. This may have negative consequences for return bloom in the following season (Richardson and McAneney, 1990; Smith *et al.*, 1992).

In papaya (Zhou *et al.*, 2000) and apple (Klages *et al.*, 2001) fruit metabolic activity, as determined by enzymatic activity, is little affected by crop load, which suggests that the ability of high-yielding plants to partition more carbohydrate to reproductive sinks may not be related to characteristics of the fruit. Greater carbohydrate partitioning to fruit at high crop loads may, therefore, relate to characteristics of the leaves. In apple, rates of leaf photosynthesis were up to 60-65% higher in high-yielding trees than in non-yielding trees, when photosynthesis was

measured after shoot development ceased and fruit DW was increasing at maximum rates (Palmer *et al.*, 1997; Wünsche *et al.*, 2005). At this time, leaf photosynthesis showed a curvilinear response to crop load (Palmer *et al.*, 1997). In kiwifruit, leaf photosynthetic efficiency is increased slightly (by less than 30%) at high crop loads (Currie, 1997; Greer *et al.*, 2003; Vizzotto *et al.*, 2003). However, other results suggest that fruit do not affect leaf photosynthetic efficiency in kiwifruit (Lai *et al.*, 1989a), or that they may reduce rates of leaf photosynthesis (Greer, 1999). In grape, a crop with similar growth habit, rates of leaf photosynthesis also are little affected by crop load (Chaumont *et al.*, 1994; Rubio *et al.*, 2004). Leaf photosynthesis in kiwifruit may only be down-regulated in low crop load vines when rates of shoot extension slow towards the end of the growing season. Prior to this time there may be no affect of crop load on rates of leaf photosynthesis and this may explain why some researchers have failed to detect a relationship between crop load and photosynthesis in kiwifruit.

Like photosynthesis, apple leaf transpiration is reduced in low crop load trees once rates of shoot growth start to decline, as leaf stomatal aperture is reduced (Giuliani *et al.*, 1997; Wünsche *et al.*, 2000). Decreased stomatal aperture may reduce the flow of high Ca-containing sap from leaves to fruit (Lang and Volz, 1998: refer to Section 1.3.2 for more information), which in turn would reduce fruit Ca concentrations in low-yielding apple trees compared to high-yielding trees (Volz and Ferguson, 1999; Wünsche *et al.*, 2000). Effects of crop load on fruit Mg concentrations were similar, but less pronounced, to those for Ca, whilst fruit K concentrations were increased at low crop loads (Fallahi and Simons, 1993; Volz *et al.*, 1993).

In kiwifruit there is little information on the effects of crop load on leaf transpiration. On a whole vine basis, transpiration was greater in low-yielding vines than in high-yielding vines, by virtue of greater leaf area, but on a per leaf basis transpiration was only slightly higher in the former (Vizzotto *et al.*, 2003). This response is the opposite of that found in apples and suggests that stomatal aperture, and Ca and Mg accumulation may be less affected by thinning in kiwifruit than in apples. Kiwifruit Ca concentrations may be reduced in low crop load vines if fruit size is increased resulting in a dilution of fruit Ca concentrations. At present there is no information on the effect of crop load on mineral accumulation in kiwifruit to verify these statements.

Feedback inhibition of photosynthesis and reductions in stomatal aperture may be more prominent in leaves on short shoots than in those on long shoots, as there are no developing leaves on the former, and therefore no alternative sinks to which 'excess' carbohydrate can be partitioned. Much of the carbohydrate produced in the fruiting zone (FZ), where short shoots tend to arise, is used within this zone, whereas carbohydrate produced in the leader zone (LZ), where long shoots tend to arise, is exported out of this zone where it is used in root growth, to promote development

of the current season's vegetative and reproductive growth and the following season's floral buds, and to replenish reserve pools (Buwalda and Smith, 1990b). From this data it is postulated that reductions in crop load are less likely to enhance fruit DMCs and are more likely to reduce Ca accumulation in fruit from short shoots than in fruit from long shoots.

This chapter comprises results from an investigation on crop load induced regulation of transpiration and photosynthesis in leaves on long and short shoots. Effects of crop load on mineral and carbohydrate accumulation in fruit from these shoots were also investigated. The working hypothesis was that substantial reductions in crop load would not elicit any change in kiwifruit DMCs, as excess carbohydrate would not be partitioned into fruit, but may instead: (a) be partitioned towards new developing vegetative sinks, or (b) accumulate in mature leaves causing end-product or feedback inhibition of photosynthesis and reducing stomatal aperture. The latter may indirectly affect Ca accumulation in fruit by reducing the movement of xylem sap between fruit and leaves.

4.2. Materials and methods

4.2.1. 2002/2003 Season

Sixteen mature vines from an orchard on Number 3 Road, Te Puke, New Zealand were used in this experiment. On 12 December 2002 (13 DAFB), eight of the vines were uniformly thinned to obtain yields of five fruit m^{-2} (low crop load treatment; actual range 3.5-6.2 fruit m^{-2}) and the remaining eight vines were left un-thinned (high crop load treatment). High crop load vines had an average yield of 35 fruit m^{-2} (range 26-62 fruit m^{-2}), which is relatively low compared to those normally obtained in a commercial situation (40-50 fruit m^{-2}), but was still, on average, seven times greater than yields in the low crop load treatment. Half of the high crop load, and half of the low crop load vines (four vines each) were left unpruned. The remaining vines were pruned using a standard leader-pruning strategy (refer Chapter 3 for details), which involved removing and weighing all secondary regrowth at three-weekly intervals from 21 November 2002 until 6 March 2003, after which time there is little shoot growth. Regrowth was removed to examine the effects of pruning, at different crop loads, on leaf photosynthesis and transpiration.

Canopy openness, canopy light transmisison, leaf area index (LAI), stomatal conductance and rates of leaf photosynthesis were measured as described in the following section. In addition, the specific leaf weight (SLW = leaf area (cm^2) divided by leaf FW (g)) was measured on 21

December 2002 and 6 May 2003. After harvest, on 24 May 2003, a count was made of the number of leafless shoots in each vine and of the number of dead leaves in a 1 m^2 quadrat placed in and under the canopy of each vine, as described in Section 3.2.3.

On 29 April 2003, six fruit were harvested from each vine (with attached stalks), three each from long and short shoots, and their FW, DMC and mineral composition were measured as described in Chapter 2 (24 fruit in total per crop load *x* pruning treatment). In each vine, lengths and diameters, vascular (pith, xylem and phloem) areas and estimated secondary xylem conductance of four fruit stalks (two from fruit on long shoots and two from fruit on short shoots) were determined as described in Chapter 2 (16 stalks per crop load *x* pruning treatment or 64 stalks in total). On 30 April 2003, whole vines were harvested, separating fruit that came from the leader and fruiting zones. Fruit were run over the grader at the Te Puke Research Centre, and a total yield and average fruit size were calculated for each vine and for each zone of each vine. A box of up to 100 fruit was collected from each zone and stored at 1-3°C for 20 weeks. On average there were 100 fruit per box in the leader and fruiting zones of high crop load vines and 35 (range 14-58) and 100 fruit per box in the leader and fruiting zones of low crop load vines, respectively. After 24 weeks at 1-3°C, fruit firmness and SSC were measured, and rots, LTB and pitting incidence were assessed as described in Section 2.9. Quick visual assessments also were made after 12, 16 and 20 weeks in storage and rot incidence was recorded before discarding any rotten fruit.

Canopy measurements

Photosynthesis, transpiration and stomatal conductance

Prior to crop load establishment (11 December 2002), and at five further times during the season (21 December 2002, 28 January 2003, 31 March 2003, 28 April 2003 and after harvest on 24 May 2003), six leaves were selected from near the base of three long and three short shoots in each of the vines (24 leaves per crop load *x* pruning treatment or 96 leaves in total at each measuring date). Stomatal conductance, and rate of photosynthesis were measured on each leaf using the Li-6400 open photosynthesis system (LiCor Inc, Ne, USA) fitted with a red-blue light source and a CO_2 mixer. Fully expanded, intact leaves were enclosed in the chamber and measurements were recorded after leaves reached a constant rate of photosynthesis at 1300 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR) and a CO_2 concentration of 375 µmol mol^{-1} at the leaf surface. Leaf temperatures and vapour pressure deficits (VPD) at the leaf surface averaged 23°C (range 18-31°C) and 1.5 kPa (range 0.8-2.9 kPa), respectively.

The light source was used to provide constant ambient conditions, thereby eliminating the variation likely to occur if leaf photosynthesis was measured using ambient light when it may be affected by cloud cover, and shading from the canopy and sensor head. Light levels were set at 1300 μmol m^{-2} s^{-1} PAR as this is above the light saturation point for this species (Buwalda *et al.*, 1992; Greer and Halligan, 2001), but below the radiation level at which photoinhibition is likely to occur given the range of leaf temperatures experienced during the course of this experiment (Buwalda and Smith, 1990a; Greer and Laing, 1992).

Canopy openness, light transmission and LAI

As described in Section 3.2.3, the point grid method was used to calculate canopy light transmission in each vine on 12 December 2002, 24 March 2003 and 30 April 2003. Hemispherical photos also were taken of each vine on 5 February 2003, 2 April 2003 and 7 May 2003 to obtain canopy openness and LAI.

4.2.2. 2003/2004 Season

This experiment was conducted in conjunction with an experiment by Currie *et al.* (unpublished data). Details of the experimental design, including vine details and methods used to ensure vines were accurately thinned to target levels are described in their report. Currie *et al.* provided technical assistance in setting up the experiment, however the data collected here is independent of that collected by Currie *et al.* (unpublished data).

Experimental design

Mature vines from Greenways Orchard on Te Matai Rd, Te Puke, New Zealand were used in this experiment. Ten vines each from a single orchard block were thinned to 20, 35 and 50 fruit m^{-2} (30 vines in total) 23 DAFB (19 December 2003) as per Currie *et al.* (unpublished data). All vines were leader pruned (refer Chapter 3 for details). Within each vine four shoots were selected, one long and one short shoot from each side of the vine (40 shoots for each crop load treatment). These shoots were marked and their characteristics were measured as described below. One fruit was harvested (with stalks attached) from each of these shoots on 27 April 2004 and analysed as in Section 4.2.1 (120 fruit in total). In addition, two fruit stalks were selected from each vine (20 per crop load treatment), one from a long shoot and one from a short shoot, from alternating sides of the vines, and these were analysed as described above (Section 4.2.1). On 5 May 2004, 50 fruit were randomly selected from each vine and put into storage for assessment of the firmness, SSC, and LTB, pitting and rot incidence 25 WAFB. A quick visual assessment also was made after 12 weeks at 1-3^{O}C and rot incidence was recorded before removing rotten fruit from the boxes.

Shoot characteristics

On 5 January 2004, the length and diameter of each of the monitored shoots, and the canes from which they arose were recorded. In addition, the L:F ratio was determined for each of the monitored shoots. Leaf stomatal conductance and rates of leaf photosynthesis were measured on one leaf from each of the monitored shoots on 16-18 December 2003 (prior to crop load establishment), and 13-14 January, 9-10 February, 14-15 March and 22-23 April 2004, as described in Section 4.2.1. A note was made of whether leaves were shaded or whether they were in sunlight. Hemispherical photos were taken of each vine on 20 December 2003, 15 February 2004, 18 March 2004 and 29 April 2004 as described in Section 3.2.3.

4.2.3. Statistical analysis

Mean differences between fruit from different crop load and/or pruning treatments, and between fruit from different shoot-types or zones were determined as described in the general methods using an ANOVA general linear model (Section 2.10). Where the F-stat indicated a significant difference between treatments, Fisher's protected least significant difference (LSD) procedure was used to establish significance between treatment means. Relationships between variables were plotted and curves were fitted to data using Origin 7.5 (Massachusetts, USA). Where relationships between variables were linear, simple regression was used to establish R^2 and significance values for curves fitted to the data. Where non-linear curves were fitted to the data, an F-stat was calculated for each curve based on R^2 values given by Origin 7.5 and the degrees of freedom for each curve (based on the number of parameters used to fit each curve and the total number of observations).

4.3. Results

4.3.1. Canopy density

In 2002/2003, canopy light transmission did not differ between high and low crop load vines when data from pruned and unpruned vines were combined. Low crop load vines from the unpruned treatment (LUP) had a lower light transmission than high crop load vines from this treatment (HUP) (Fig 4.1). There was no difference in light transmission between high and low crop load vines that had been pruned (HP and LwP, respectively). Canopy light transmission was always greater in pruned vines than in unpruned vines (Fig 4.1). Greatest differences between pruned and unpruned vines were observed in December and March, and by April differences in light

transmission between pruned and unpruned vines were not significant. Differences also were only significant when light transmission was measured in the fruiting zone (FZ) of the vines, and not when measured in the leader zone (LZ).

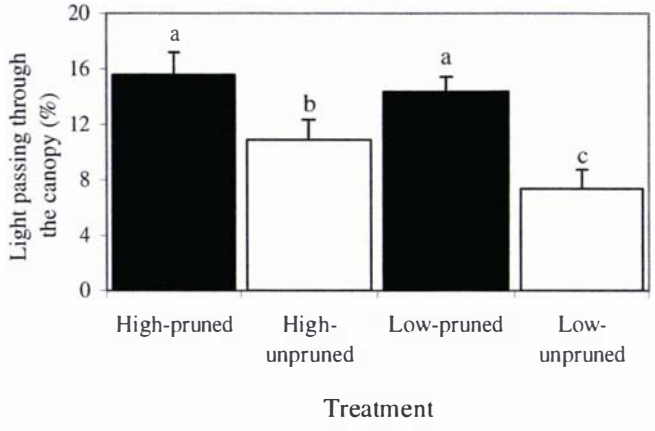


Fig 4.1. Canopy light transmission in high and low crop load, pruned and unpruned vines as measured by the point grid method between 12 December 2002 and 30 April 2003. Bars are mean values (± s.e.) and are not significantly different at P <0.05 when labelled with the same letter.

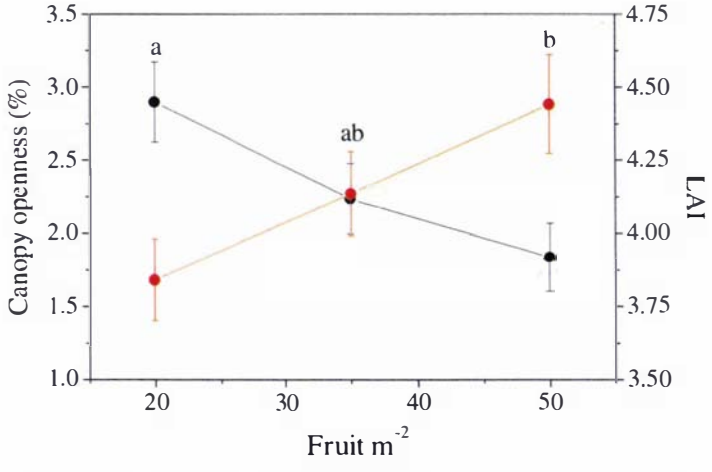


Fig 4.2. Canopy openness (black line) and LAI (red line) as measured at three different crop loads between 20 December 2003 and 29 April 2004. Data points are mean values (± s.e.) and are not significantly different at P <0.05 when labelled with the same letter.

In both seasons the extent of light transmission in vines was reflected in canopy openness and leaf area index (LAI) data (Fig 4.2 and 4.3). In the 2003/2004 season there was a clear difference between the canopy openness and LAI of high and low crop load vines (Fig 4.2). These differences were apparent in data collected in March and April, but not in data collected prior to this time. There was never any effect of crop load on canopy openness in the 2002/2003 season, but in this season the LAI of LUP vines was higher than that in HUP vines when data from each measurement date were combined. In this season canopy openness was always higher in pruned vines than in unpruned vines, and LAI lower, regardless of crop load or measurement date.

Canopy density differences between vines were reflected also in the amount of vegetative regrowth removed from vines during the growing season, and in the number of leafless shoots and dead leaves in and underneath the canopy in May. Over the

2002/2003 growing season, 41% more regrowth was removed from low crop load vines than from high crop load vines (every three weeks on average 2.78 ± 0.51 and 1.65 ± 0.43 g regrowth was removed per m^2 cane, in low and high crop load, pruned vines respectively). There were 84% more leafless shoots in unpruned vines than in pruned vines (55 and 9 shoots, respectively), 67% more leaves on the ground under the canopy (19 and 57 leaves, respectively), and 50% more dead leaves in the canopy (13 and 26 leaves, respectively).

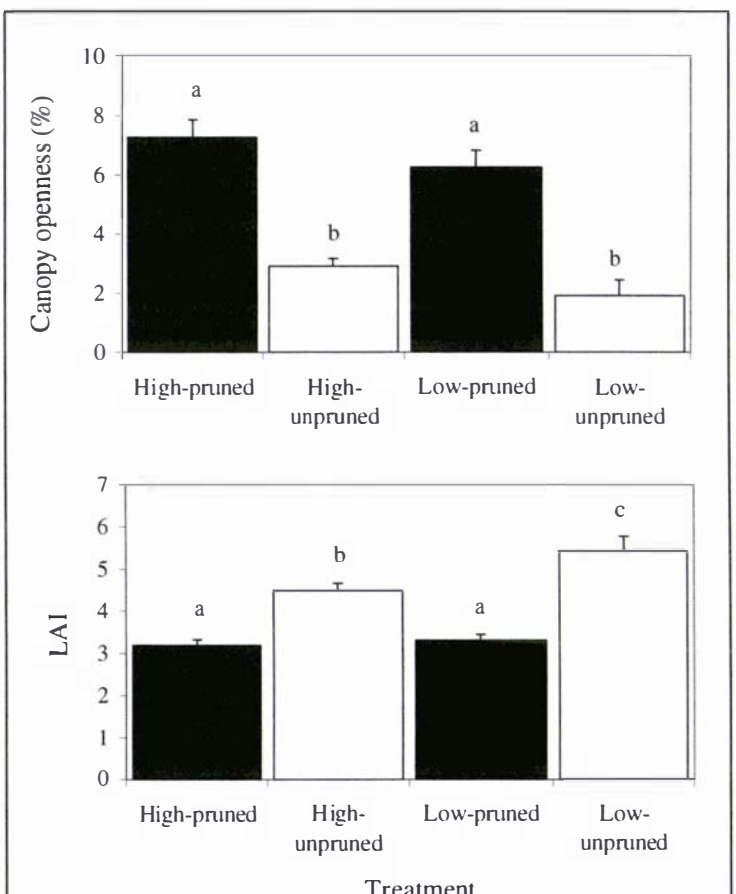


Fig 4.3. Canopy openness and LAI in high and low crop load, pruned and unpruned vines as measured between 5 February and 7 May 2003. Bars are mean values (± s.e.) and are not significantly different at P <0.05 when labelled with the same letter.

4.3.2. Fruit and fruit stalk data

FW, DW and fruit DMCs

In the 2002/2003 and 2003/2004 seasons fruit FW decreased as the crop load increased (by 20% and 17%, respectively, P <0.001; Fig 4.4 and 4.5), regardless of whether or not vines were pruned

(2002/2003 season). Fruit DW and water content also decreased with increasing crop load, and closely matched the trends for FW (data not shown). Fruit DM and SS concentrations (data not shown) at harvest were not affected by crop load or pruning treatments.

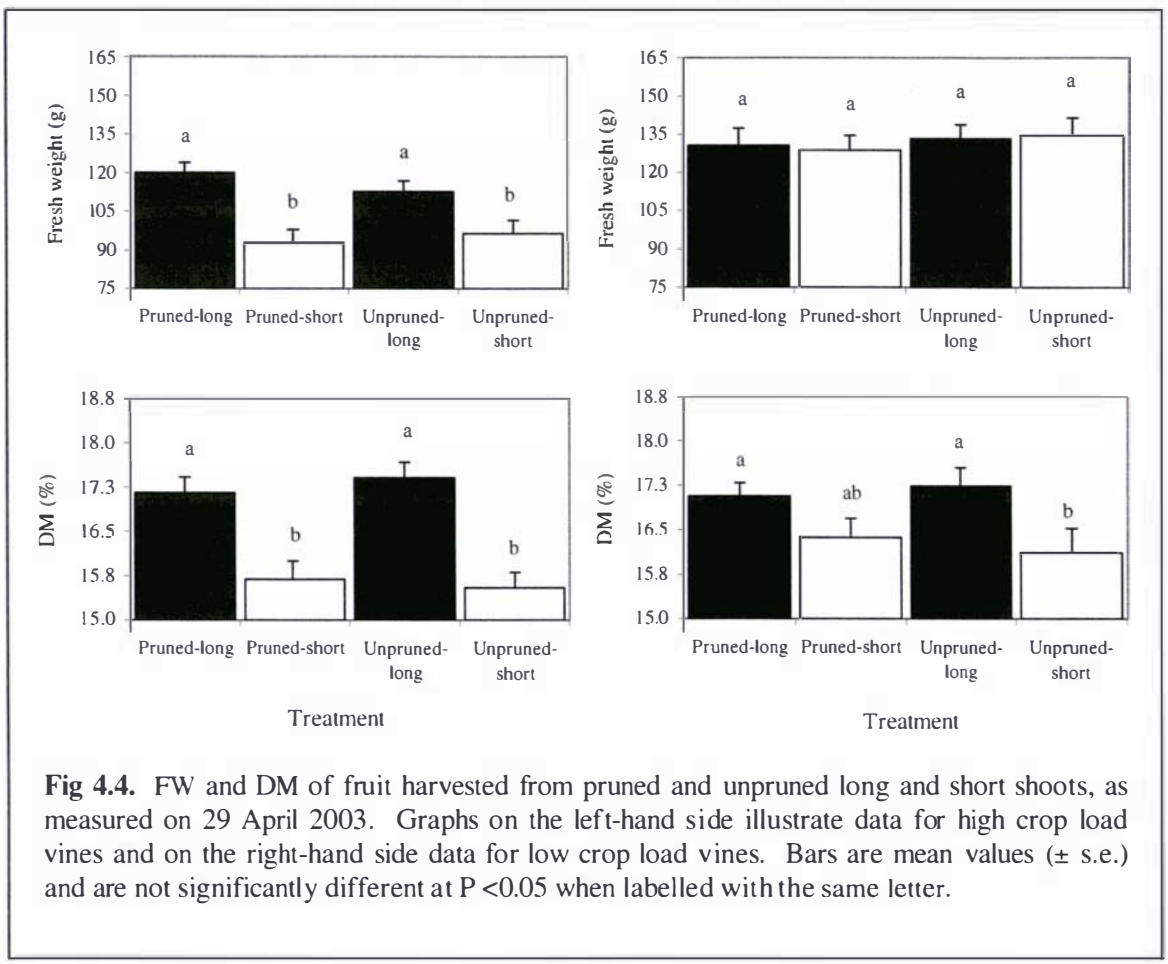


Fig 4.4. FW and DM of fruit harvested from pruned and unpruned long and short shoots, as measured on 29 April 2003. Graphs on the left-hand side illustrate data for high crop load vines and on the right-hand side data for low crop load vines. Bars are mean values (± s.e.) and are not significantly different at $P < 0.05$ when labelled with the same letter.

In the 2003/2004 season, FW of fruit from long shoots was the same as that of fruit from short shoots (Fig 4.5, Table 4.1). However, in the 2002/2003 season, in high crop load vines, fruit from long shoots had a greater FW than fruit from short shoots (Fig 4.4, Table 4.1). There were no differences between the FW of fruit from long and short shoots in low crop load vines. In both the 2002/2003 and 2003/2004 seasons, fruit DMCs were 0.6-1.7%-units (4-10%) higher in fruit from long shoots than in fruit from short shoots, depending on the crop load (Fig 4.4 and 4.5). In the 2002/2003 season, differences between the DMC of fruit on long and short shoots decreased as the crop load was reduced, especially in pruned vines (Fig 4.4, Table 4.1). Soluble solids concentration differences between fruit on long and short shoots also decreased as crop load decreased in the 2002/2003 season ($P < 0.01$). However, in the 2003/2004 season the most significant differences between the DMC of fruit on long and short shoots were found in the 35 fruit per m^2 treatment (Fig 4.5).

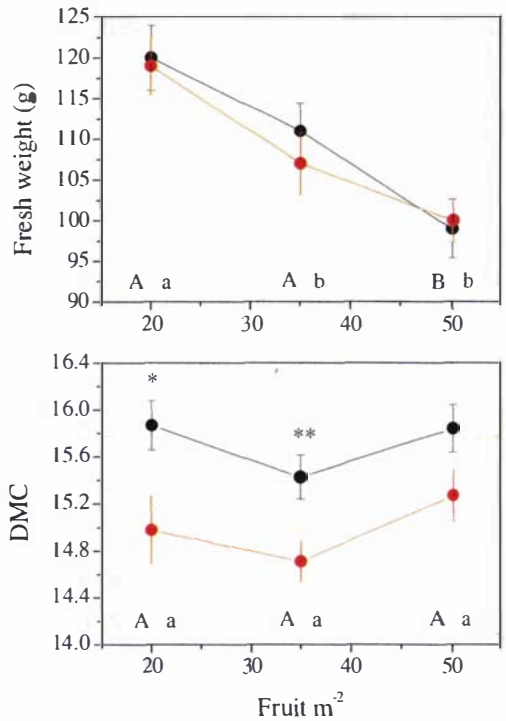


Fig 4.5. FW and DMC of fruit from long (black) and short (red) shoots, as measured at three different crop loads on 27 April, 2004. Data points are mean values (± s.e.). For each shoot type, data points labelled with the same letter are not significantly different at P <0.001. Capital and lower case letters indicate significant differences between crop loads for fruit from long and short shoots, respectively. Stars indicate significant differences between shoot types at * P <0.05 and ** P <0.01.

In the 2002/2003 season, total yields ranged from 114-205 fruit in low crop load vines (0.015-0.027 t per vine), and from 845-2038 fruit in high crop load vines (0.093-0.22 t per vine) with average crop loads of 159 and 1140 fruit per vine (0.021-0.12 t per vine) in low and high crop load vines, respectively. There was no effect of pruning on crop load per vine either on a fruit number or fruit weight basis. Yields were on average 78 and 81% higher in the FZ than in the LZ of low and high crop load vines, respectively (0.0039 t in the LZ against 0.017 t in the FZ in low crop load vines and 0.019 t in the LZ against 0.10 t in the FZ of high crop load vines). As fruit number per vine increased, the total amount of carbohydrate partitioned to fruit also increased, as indicated by the increase in fruit DW per m^{-2} at higher crop loads (Fig 4.6). In the 2002/2003 season this increase occurred in both high and low crop load vines.

In 2002/2003 fruit were harvested at 6.1 °Brix, whilst in 2003/2004 they were harvested at 6.0°Brix. Average DMCs at harvest were 16.6 and 15.4%, respectively in the 2002/2003 and 2003/2004 seasons.

In the 2002/2003 season a weak positive linear correlation existed between fruit DMC and cane length ($P < 0.017$, $R^2 = 0.04$), cane diameter ($P < 0.003$, $R^2 = 0.07$) and shoot diameter ($P < 0.001$, $R^2 = 0.14$). A weak positive linear correlation also existed between fruit DW and shoot diameter ($P < 0.02$, $R^2 = 0.04$). As the leaf:fruit (L:F) ratio on short shoots declined, fruit fresh ($P < 0.003$, $R^2 = 0.07$) and dry weights ($P < 0.041$, $R^2 = 0.03$) also declined. L:F ratio had no effect on the FW, DW or DMC of fruit on long shoots.

Table 4.1. Attributes of leaves, fruit stalks and fruit on long, non-terminated and short terminated shoots, as determined from data collected in the 2002/2003 and 2003/2004 seasons.

Plant organ	Attribute	2002/2003					2003/2004				
		Long		Short			Long		Short		
Leaf	Net assimilation rate (μmol CO_2 m^{-2} s^{-1})	13.83	*0.25*	6.70	*0.25*	***	11.39	*0.33*	6.07	*0.29*	***
	Stomatal conductance (mol H_2O m^{-2} s^{-1})	0.42	*0.01*	0.17	*0.01*	***	0.36	*0.01*	0.13	*0.01*	***
Fruit stalk	Phloem area (mm^2)	2.05	*0.09*	1.73	*0.09*	**	1.89	*0.09*	1.62	*0.07*	**
	Xylem area (mm^2)	2.04	*0.09*	1.49	*0.09*	***	2.03	*0.12*	1.59	*0.10*	***
	Estimated secondary xylem conductance (arbitary units)	59	*6.48*	43	*6.45*	*ns*	46	*8.34*	36	*6.66*	*ns*
Fruit	Fresh weight (g)	124	*2.81*	113	*3.92*	***	110	*2.35*	109	*2.19*	*ns*
	Dry weight (g)	21	*0.51*	18	*0.75*	***	17	*0.40*	16	*0.37*	*ns*
	DMC	17.2	*0.13*	15.9	*0.17*	***	15.8	*0.10*	15.1	*0.13*	**
Fruit	Ca (mg $100g^{-1}$ FW)	26	*1.01*	21	*0.98*	**	29	*1.24*	26	*1.20*	*ns*
	Mg (mg $100g^{-1}$ FW)	15.6	*0.41*	14.7	*0.39*	*ns*	11.9	*0.26*	11.7	*0.23*	*ns*
	K (mg $100g^{-1}$ FW)	287	*8.59*	318	*10.18*	**	294	*6.31*	293	*8.92*	*ns*
	P (mg $100g^{-1}$ FW)	24	*1.12*	28	*1.72*	*	30	*0.86*	30	*0.80*	*ns*

Within a season shoot-type differences are significant at * P <0.05, ** P <0.01, *** P <0.001 or not significant (ns). Data are mean values ± *s.e.*

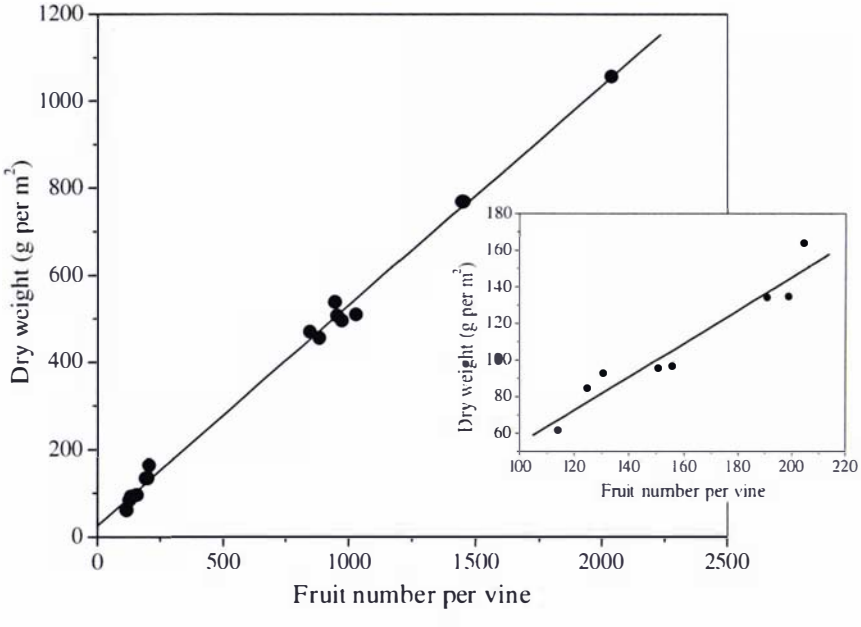


Fig 4.6. Relationship between crop load and fruit DW (g per m^2), with data for low crop load vines inset (P <0.001, R^2 = 0.995 (total data) and 0.92 (low crop load vines only)). Fruit were harvested on 29 April 2003.

Fruit minerals

In 2002/2003, fruit K and P concentrations increased with decreasing crop load, regardless of whether vines were pruned or not (Fig 4.7). In unpruned vines fruit Mg concentrations increased with decreasing crop load, but were not affected by crop load in pruned vines. Fruit Ca concentrations were not affected by crop load. Calcium concentrations were numerically higher in unpruned vines than in pruned vines, although differences were not statistically significant. In the 2003/2004 season fruit Ca, K, Mg and P concentrations were not affected by crop load (data not shown).

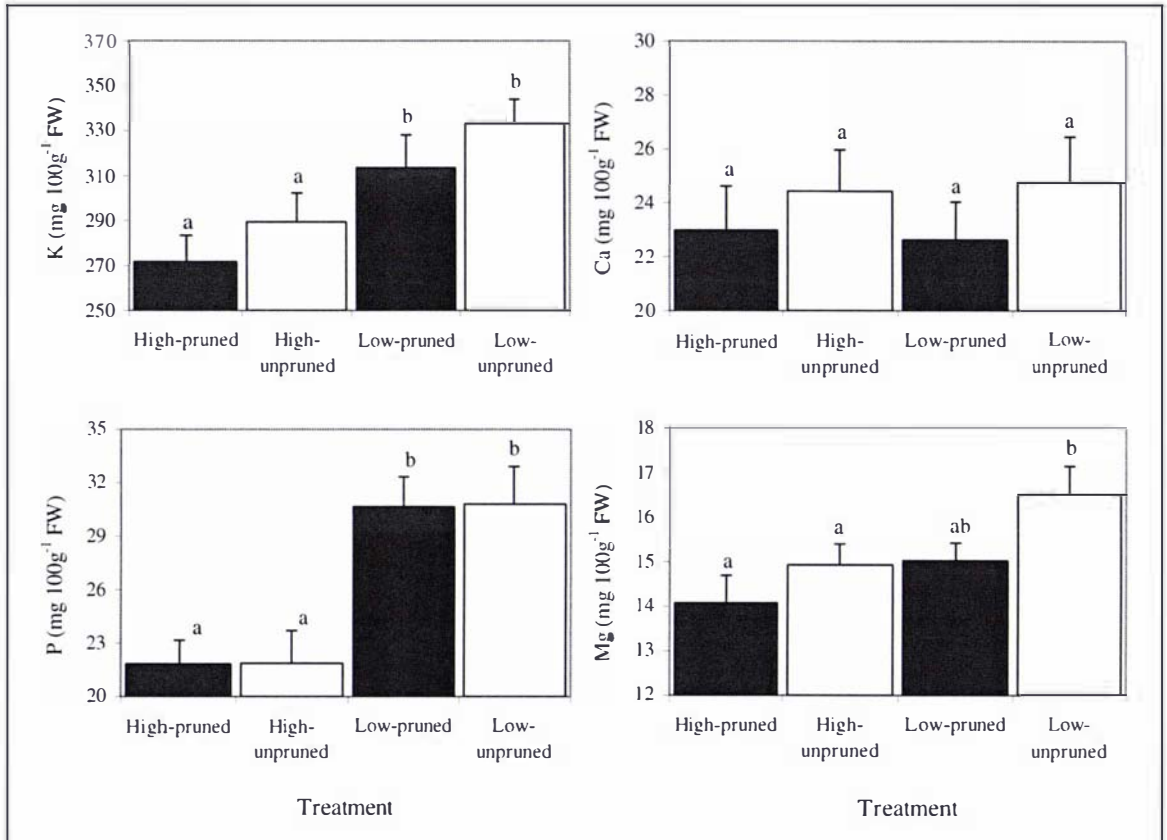


Fig 4.7. Potassium, P, Ca and Mg concentrations in fruit from high and low crop load, pruned and unpruned vines, as measured on 29 April, 2003. Bars are mean values (± s.e.) and are not significantly different at P <0.05 when labelled with the same letter.

In 2002/2003, fruit K and P concentrations were generally higher in short shoots from low crop load vines than in long shoots from these vines. These differences were most apparent in low crop load, pruned vines in the case of K, and in low crop load, unpruned vines in the case of P (Fig 4.8). In the 2003/2004 season there was no effect of shoot-type on fruit K or P concentrations at P <0.05 (Table 4.1). In 2002/2003, in high crop load vines fruit Ca concentrations were generally higher in fruit from long shoots than in fruit from short shoots, especially in vines that had not been pruned (Fig 4.8). In the 2003/2004 season, fruit Ca concentrations were only higher in fruit from long

shoots than in fruit from short shoots in the highest crop load treatment (50 fruit per m^2, $P < 0.007$; 29 mg $100g^{-1}$ FW and 21 mg $100g^{-1}$ FW in long and short shoots, respectively). Fruit Mg concentrations did not differ between shoot-types in the 2002/2003 season (Fig 4.8, Table 4.1).

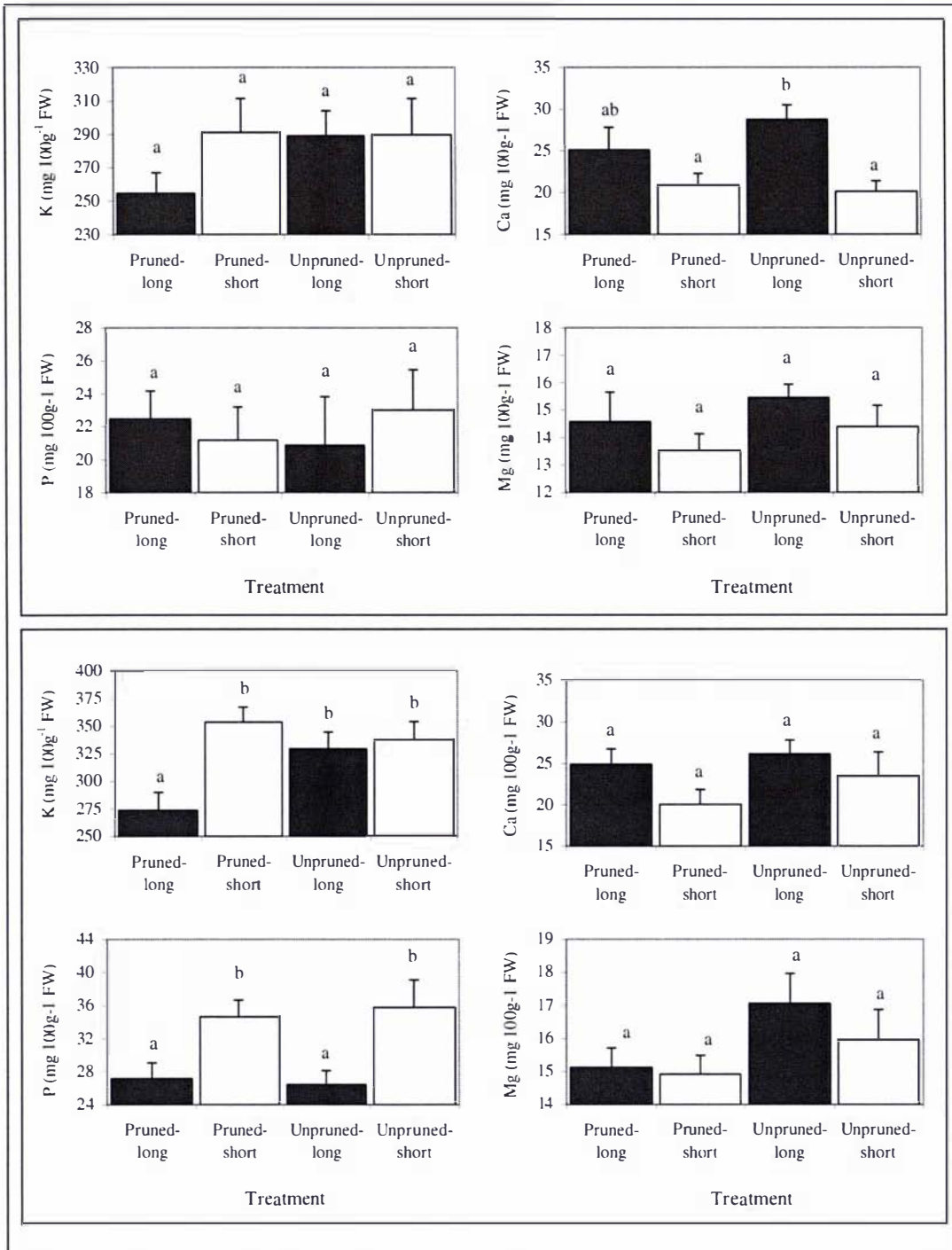


Fig 4.8. Potassium, P, Ca and Mg concentrations in fruit harvested from pruned and unpruned long and short shoots from high (top) and low (bottom) crop load vines. Bars are mean values (± s.e.) and are not significantly different at $P < 0.05$ when labelled with the same letter. Fruit were harvested for assessment on 29 April 2003.

When fruit mineral concentrations from the high crop load treatment (average 35 fruit per m^2) in 2002/2003 season and the medium crop load treatment (35 fruit per m^2) in 2003/2004 season were compared, fruit K and Mg concentrations were virtually identical (less than 1% difference). Fruit Ca concentrations were 6% higher in the 2003/2004 season than in the 2002/2003 season, although this difference was only significant at the 10% level ($P < 0.075$, 25.2 and 23.7 mg $100g^{-1}$ FW, respectively). Fruit P concentrations were 33% higher in the 2003/2004 season than in the 2002/2003 season ($P < 0.001$, 33 and 22 mg $100g^{-1}$ FW, respectively).

A weak positive linear correlation existed between fruit K concentrations and cane diameter ($P < 0.03$, $R^2 = 0.07$). Fruit concentrations of other minerals were not correlated with parent cane diameter or length and shoot diameter or length. In short shoots, there was a linear correlation between fruit Ca concentrations and the shoot L:F ratio ($P < 0.02$, $R^2 = 0.018$), however this correlation was not found in long shoots, or for any of the other minerals (data not shown).

In the 2002/2003 season, fruit K and P contents, like fruit K and P concentrations, increased as crop loads declined, regardless of whether vines were summer pruned or not ($P < 0.003$). Phosphorus contents also increased as the crop load decreased in the 2003/2004 season ($P < 0.001$), but K contents were unaffected by crop load. In high crop load vines, fruit from long shoots had higher K ($P < 0.023$, 18% higher) and P ($P < 0.038$, 21% higher) contents than short shoots. This differs from the concentration trend for these elements, where short shoots had higher K and P concentrations than long shoots. In low crop load vines P contents were still higher in short shoots than in long shoots ($P < 0.012$, 22% higher). Potassium contents were 19% higher in short shoots than in long shoots ($P < 0.019$) in low crop load vines (20 fruit per m^2) in the 2003/2004 season. Phosphorus contents never differed between shoot-types in this season.

Fruit Ca and Mg contents increased as crop loads decreased in both the 2002/2003 and 2003/2004 seasons as did fruit FW (refer Fig 4.4). In high crop load vines fruit Ca contents were 40% greater in long shoots than in short shoots, whilst Mg contents were 28% greater ($P < 0.001$). These shoot-type differences were more noticeable in vines that had been pruned. In low crop load vines no significant differences existed between Ca and Mg contents in long and short shoots.

In both seasons fruit mineral contents were strongly correlated with FW $P < 0.001$ (2002/2003: $R^2 = 0.67$, 0.70, 0.42 and 0.52 for K, Mg, Ca and P, respectively and 2003/2004: $R^2 = 0.51$, 0.70, 0.21 and 0.39 for K, Mg, Ca and P, respectively). Strongest correlations were always found between fruit Mg contents and FW, and weakest correlations were always found between fruit Ca contents and FW.

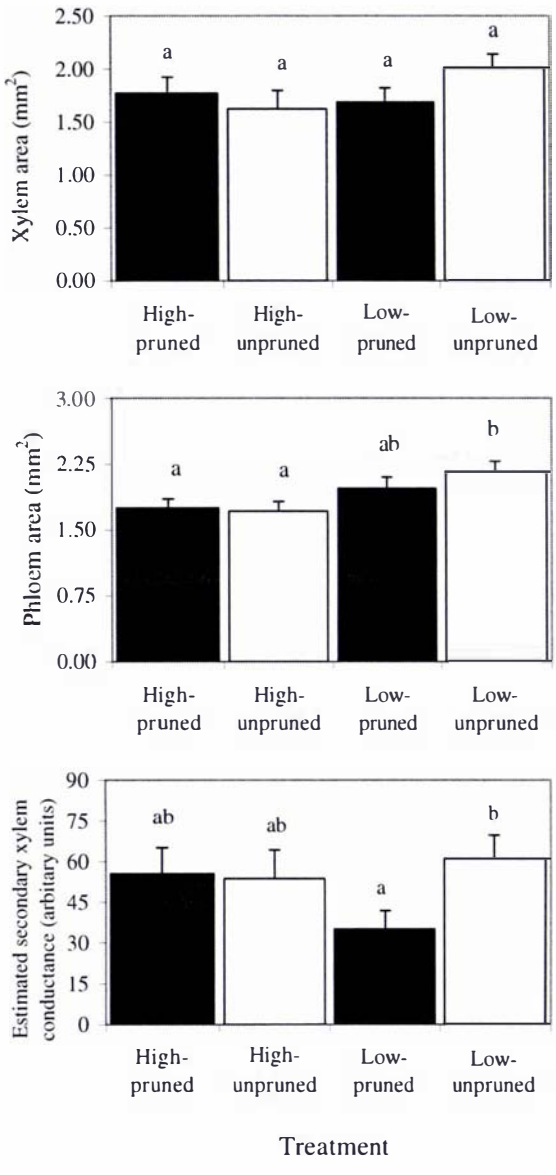


Fig 4.9. Xylem and phloem area and estimated secondary xylem conductance in the stalks of fruit from high and low crop load, pruned and unpruned vines, as measured on 29 April, 2003. Bars are mean values (± s.e.) and are not significantly different at $P < 0.05$ when labelled with the same letter.

Fruit stalk data

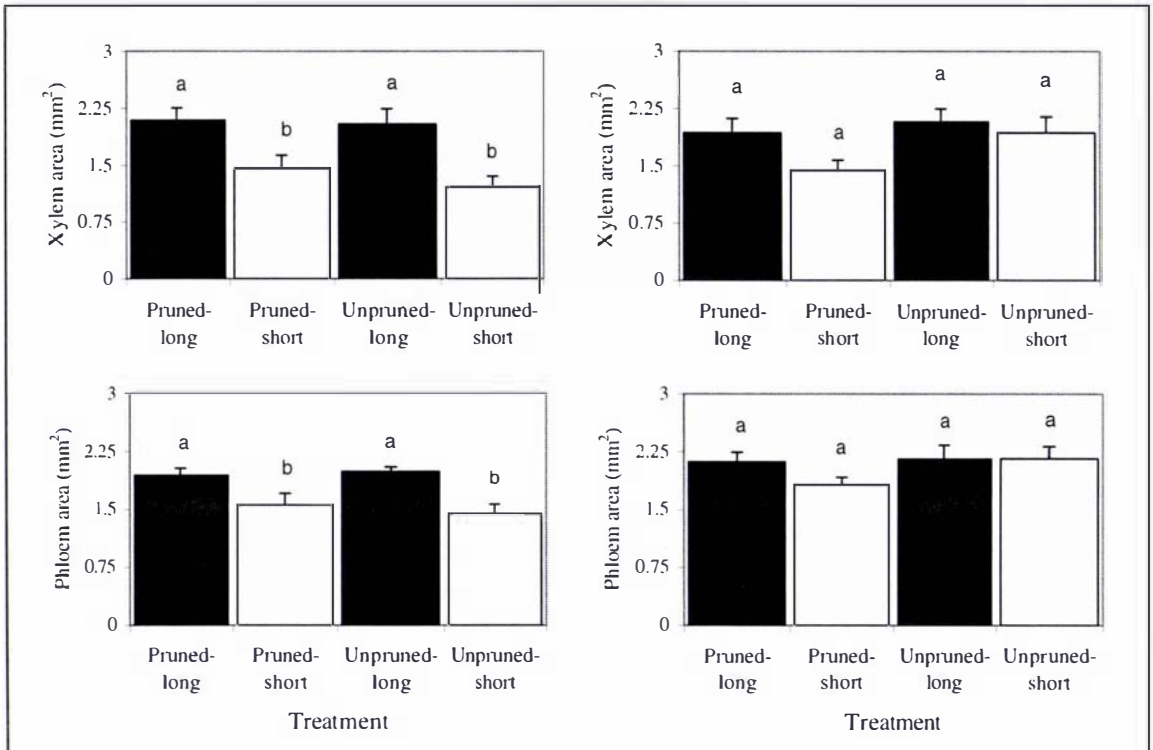


Fig 4.10. Phloem and xylem area in stalks of fruit from pruned and unpruned long and short shoots, as measured on 29 April 2003. Graphs on the left-hand side illustrate data for high crop load vines and graphs on the right-hand side illustrate data for low crop load vines. Bars are mean values (± s.e.) and are not significantly different at $P < 0.05$ when labelled with the same letter.

Fruit stalk diameter was positively correlated with FW in both seasons ($P < 0.001$, $R^2 = 0.32$ and 0.43, respectively), and responded similarly to pruning and crop load treatments. Fruit stalk length was not affected by pruning, crop load or shoot-type (long or short) in either season, and was not correlated with FW (data not shown). In both the 2002/2003 (Fig 4.9) and 2003/2004 seasons, xylem area and estimated secondary xylem conductance were not significantly affected by crop load. However, phloem area was 16% lower in the HUP vines than in LUP vines in the 2002/2003 season and was 17% lower in high crop load vines than in low crop load vines in the 2003/2004 season ($P < 0.05$). There was no difference in phloem area between HP and LwP vines. There also was no effect of pruning on xylem area or phloem areas in the 2002/2003 season. However, LUP vines had a greater estimated secondary xylem conductance than LwP vines (Fig 4.9). Pith area was never significantly affected by pruning or crop load treatments (data not shown).

In both the 2002/2003 and 2003/2004 seasons, phloem and xylem areas were greater in stalks of fruit from long shoots than in stalks of fruit from short shoots when data from high and low crop

load treatments were combined (Table 4.1). However, when considered in relation to crop load and summer pruning treatments, these differences were only significant in high crop load vines (Fig 4.10). In the 2003/04 season, estimated secondary xylem conductance was the same in fruit from long and short shoots, regardless of crop load (Table 4.1). In the 2002/2003 season differences between shoot-types were only significant at the 0.1% level ($P < 0.088$) when data from all treatments were combined. When considered in relation to crop load and summer pruning treatments, there also was no effect of shoot-type on secondary xylem conductance in the fruit stalk (Fig 4.11). However, in short shoots estimated secondary xylem conductance was greater in unpruned low crop load vines than in pruned low crop load vines. There were never any differences in pith area between fruit from long and short shoots regardless of crop load or summer pruning (data not shown).

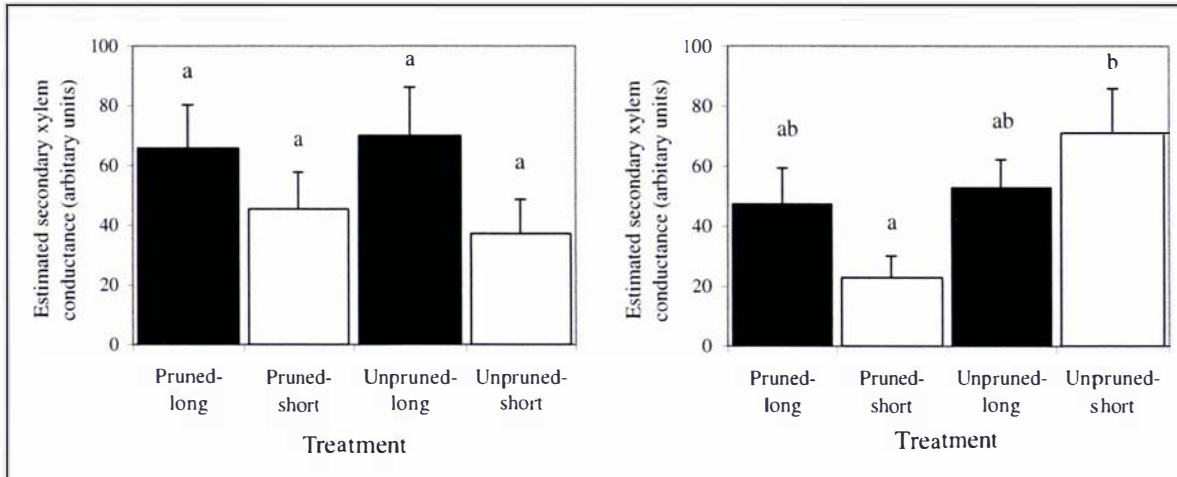


Fig 4.11. Estimated secondary xylem conductance in stalks of fruit from pruned and unpruned long, non-terminating and short, terminating shoots, as measured on 29 April 2003. Graphs on the left-hand side illustrate data for high crop load vines and on the right-hand side data for low crop load vines. Bars are mean values (± s.e.) and are not significantly different at $P < 0.05$ when labelled with the same letter.

In the 2002/2003 season, FW was positively correlated with fruit stalk phloem and, to a lesser extent, xylem area (Fig 4.12). There was no correlation between pith area or estimated secondary xylem conductance and FW. Fruit DMC was positively correlated with phloem area, as was fruit DW. A weaker correlation existed between fruit DMCs and xylem area. A positive linear correlation also existed between fruit Ca concentrations and xylem area (Fig 4.13). Fruit Mg concentrations were more strongly correlated with phloem area than xylem area ($R^2 = 0.30$ against 0.22), whilst no correlation existed between fruit K and P concentrations and phloem or xylem areas. Due to the correlation between FW and xylem and phloem areas (Fig 4.12), fruit mineral contents also were correlated with phloem ($P < 0.002$), and to a lesser extent xylem ($P < 0.049$) areas. A positive linear relationship existed between estimated secondary xylem conductance and

fruit Ca concentration (Fig 4.14) and contents ($P < 0.001$, $R^2 = 0.27$). However, there was no correlation between the fruit concentration or content of Mg, K or P and estimated secondary xylem conductance. Estimated conductance was not correlated with FW, DW, fruit water content or fruit DMCs (data not shown).

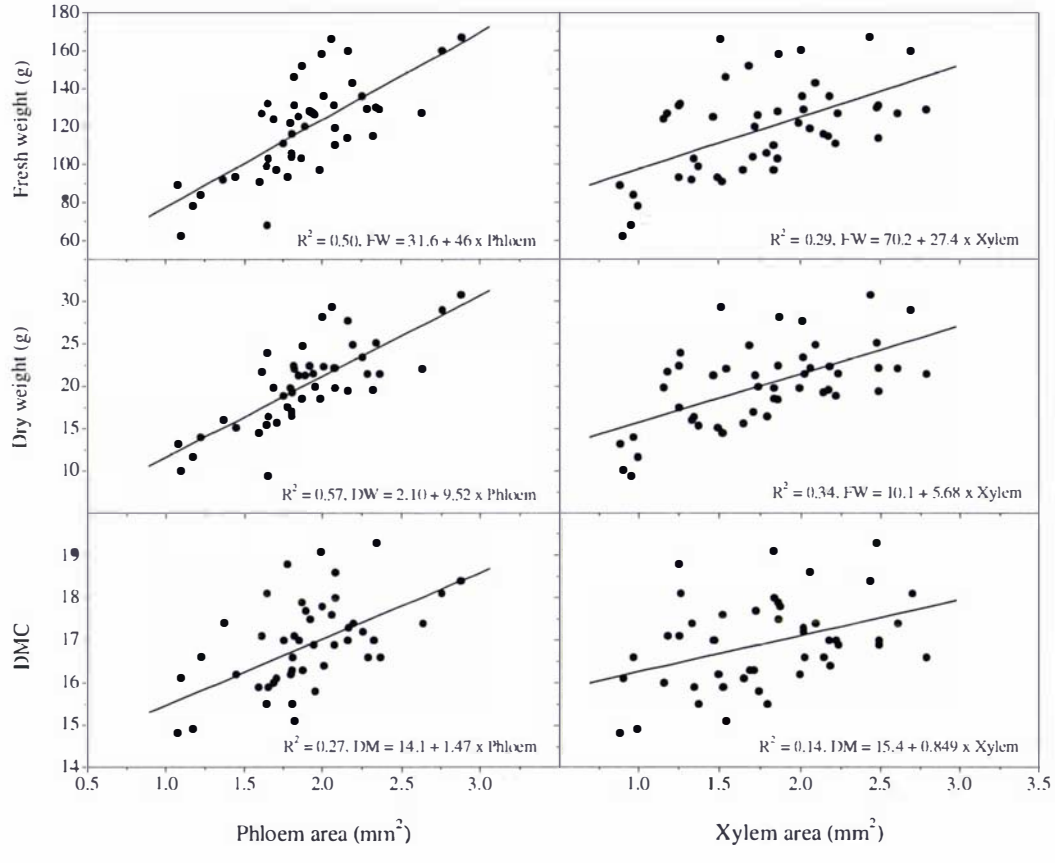


Fig 4.12. Relationship between fruit fresh weight, dry weight, DMC and fruit stalk phloem (left-hand side) and xylem (right-hand side) area. Fruit were sampled on 29 April, 2003. Correlations are significant at $P < 0.006$.

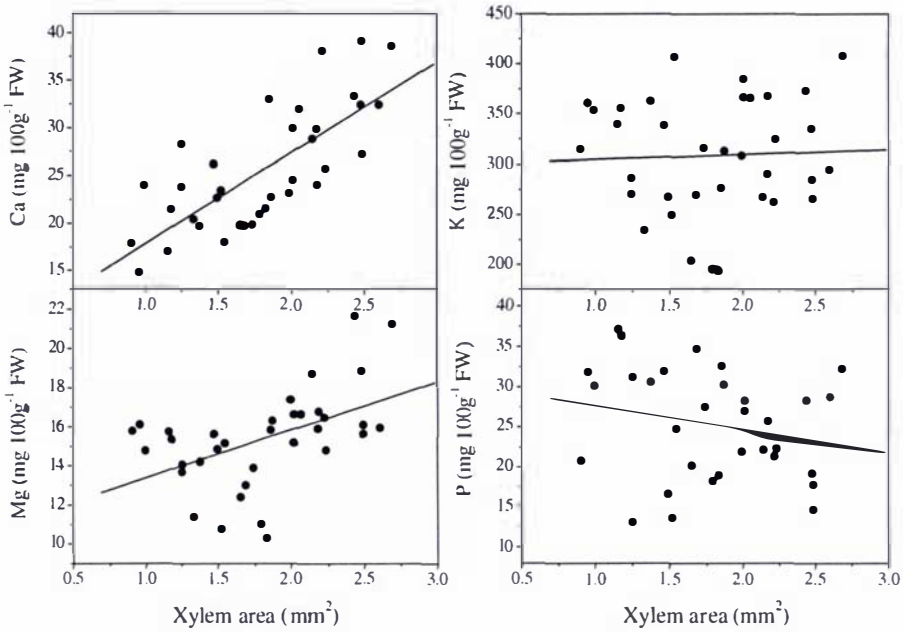


Fig 4.13. Relationship between fruit Ca, Mg, K and P concentrations and fruit stalk xylem area. Fruit were sampled on 29 April, 2003. Ca = 8.3 + 9.54 x Xylem area ($P < 0.001$, $R^2 = 0.55$), Mg = 10.9 + 2.46 x Xylem area ($P < 0.003$, $R^2 = 0.22$), K (not significant) and P (not significant).

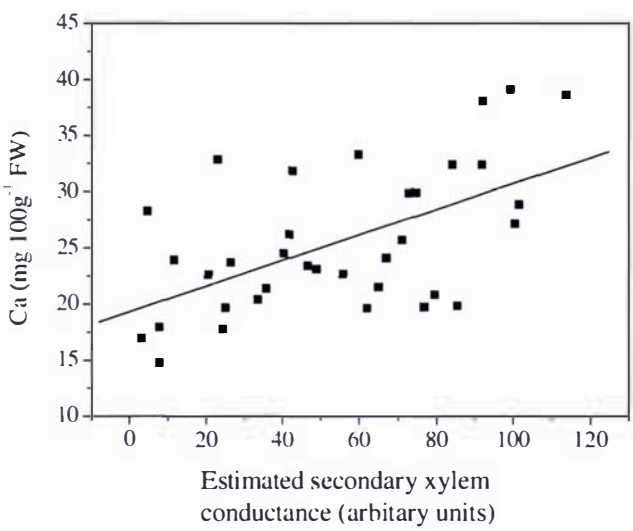


Fig 4.14. Relationship between estimated fruit stalk secondary xylem conductance and fruit Ca concentration (Ca = 19.3 + 0.114 x Xylem area; $P < 0.001$, $R^2 = 0.30$). Fruit were sampled on 29 April, 2003.

Similar findings were obtained in the 2003/2004 season, except that correlations were not as strong. Phloem area was correlated with FW ($P < 0.004$, $R^2 = 0.43$), but xylem area and estimated conductance were not. Fruit DW ($P < 0.003$, $R^2 = 0.26$) and DMCs ($P < 0.004$, $R^2 = 0.24$) also were positively correlated with the fruit stalk phloem area, however these variables were not correlated with the fruit stalk xylem area or estimated secondary xylem conductance. Mineral contents or concentrations were not correlated with xylem or phloem area or estimated secondary xylem conductance.

4.3.3. Photosynthesis and stomatal conductance

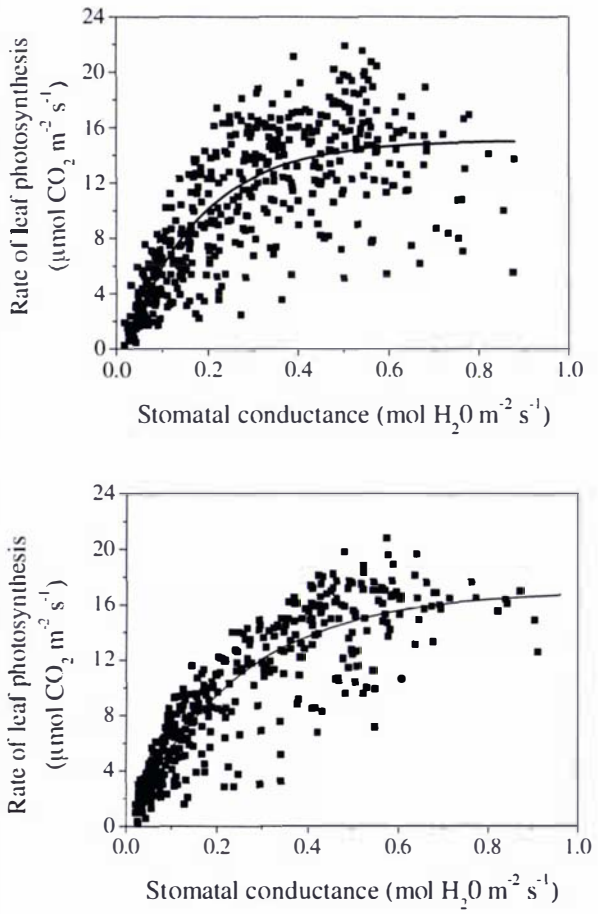


Fig 4.15. Relationship between leaf stomatal conductance (Gs) and leaf photosynthetic rates (Pn) in the 2002/2003 season (top: $P < 0.002$, $R^2 = 0.65$; Pn = 15.10-16.82*0.002*Gs) and 2003/2004 season (bottom: $P < 0.001$, $R^2 = 0.82$; Pn = 17.02-17.10*0.016*Gs).

Leaf photosynthesis (Pn) and stomatal conductance (Gs) were correlated in both the 2002/2003 season and the 2003/2004 season (Fig 4.15). Therefore, leaf Pn and Gs responded similarly to crop load, pruning and shoot-type effects. Leaf Pn rates were higher in pruned vines than in unpruned vines from 28 January 2003 until 28 April 2003, regardless of crop load (Fig 4.16). However, pruning effects were more noticeable in low crop load vines. From 31 March 2003 until the 28 April 2003, rates of leaf Pn were higher in high crop load vines than in low crop load vines that had not been pruned. Stomatal conductance was higher in pruned vines than in unpruned vines from 31 March 2003 until 24 May 2003, however, these differences were only significant in low crop load vines on 31 March and 24 May 2003 (Fig 4.16). On 28 April 2003, Gs also was higher in unpruned, high crop load vines than in unpruned, low crop load vines. In contrast to the 2002/2003 season, there

were never any effects of crop load on leaf Pn, or Gs in the 2003/2004 season. SLW was not affected by crop load or pruning in the 2002/2003 season.

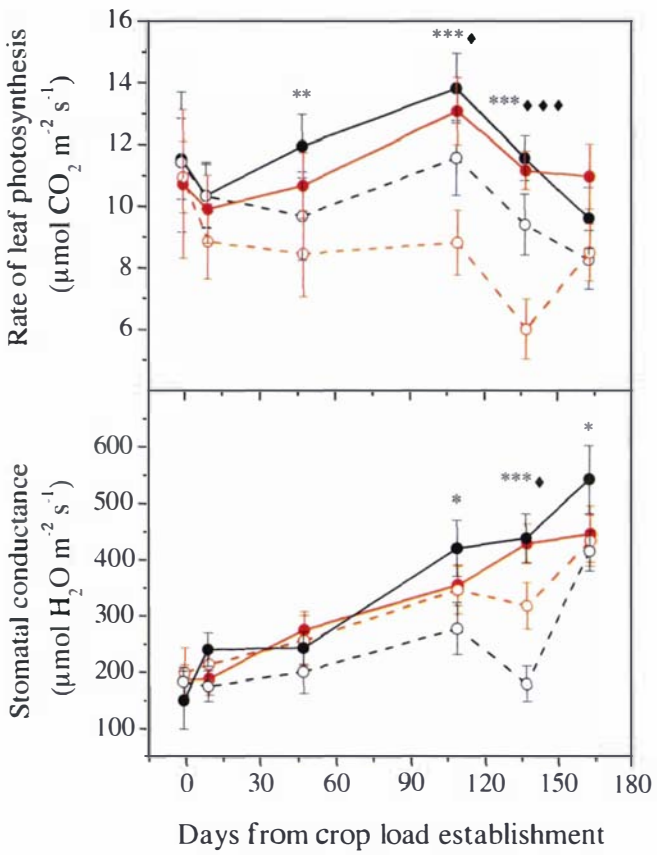


Fig 4.16. Photosynthesis and stomatal conductance in leaves from high crop, pruned (black, closed circles) and unpruned (black, open circles) vines, and low crop load, pruned (red, closed circles) and unpruned (red, open circles) vines as measured on 11 and 21 December, 2002, and 28 January, 31 March, 28 April and 24 May, 2003 (-1,9,47,109,137,163 days from crop load establishment (12 December, 2002), respectively). Data points are mean values (± s.e.). Stars indicate dates at which significant different differences were found between pruned and unpruned vines at * P <0.05, ** P <0.01 and *** P <0.001. Diamonds indicate dates at which significant differences were found between high and low crop load vines at ♦ P <0.05 and ♦♦♦ P <0.001. These differences are described in more detail in the text.

Leaf Pn and Gs were higher in leaves from long shoots than in leaves from short shoots at each sampling up until harvest, regardless of season, crop load or pruning regime used (Fig 4.17, Table

4.1). After harvest (24 May 2003), rates of leaf Pn were not significantly different in pruned long and short shoots from low crop load vines, whilst leaf Gs did not differ between unpruned long and short shoots from low crop load vines. As noted in Figure 4.16, differences between the rate of leaf Pn and Gs of pruned and unpruned vines were most apparent in March and April, and were more apparent in low than high crop load vines, when considered on a shoot-type basis.

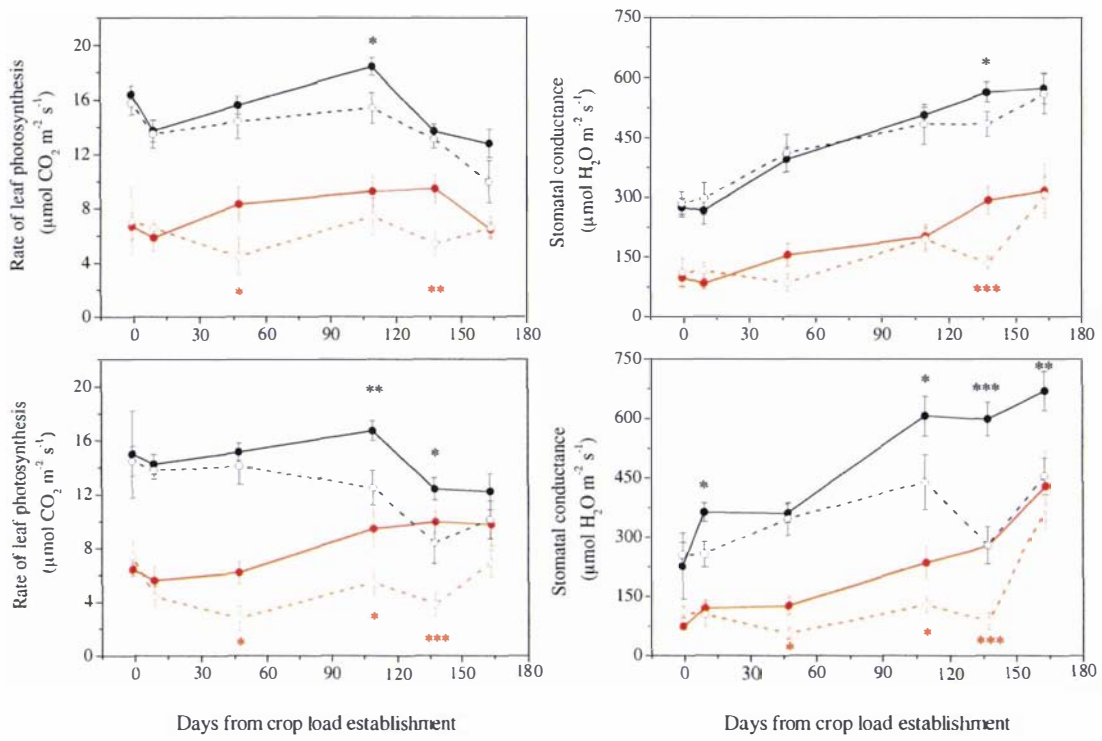


Fig 4.17. Photosynthesis and stomatal conductance in leaves from long, pruned (black, closed circles) and unpruned (black, open circles) shoots, and short, pruned (red, closed circles) and unpruned (red, open circles) shoots as measured on 11 and 21 December, 2002, and 28 January, 31 March, 28 April and 24 May, 2003 (-1,9,47,109,137,163 days from crop load establishment (12 December, 2002), respectively). The top graphs are data from high crop load vines, whilst bottom graphs are data from low crop load vines. Data points are mean values (± s.e.). Shoot type differences were significant ($P < 0.001$) at all dates, except the final sampling date in low crop load vines (see text for details). Stars indicate significant differences between pruned and unpruned vines for long (black stars) and short (red stars) shoots at * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

In both long and short shoots, leaf Pn and Gs also were higher in sun leaves than shade leaves at each sampling date in the 2003/2004 season (on average 56 and 71% higher over the entire season, respectively, $P < 0.001$). However, sun and shade effects could not explain shoot type differences, because long-shade leaves still had higher photosynthetic rates and Gs than short-shade leaves and long-sun leaves had higher photosynthetic rates and Gs than short-sun leaves ($P < 0.01$). At the start of the 2002/2003 season, leaves from long shoots had a 29% higher SLW (31 against 22) than

leaves from short shoots (P <0.001). By the end of the season this difference had increased to 59% (39 and 16, respectively; P <0.001).

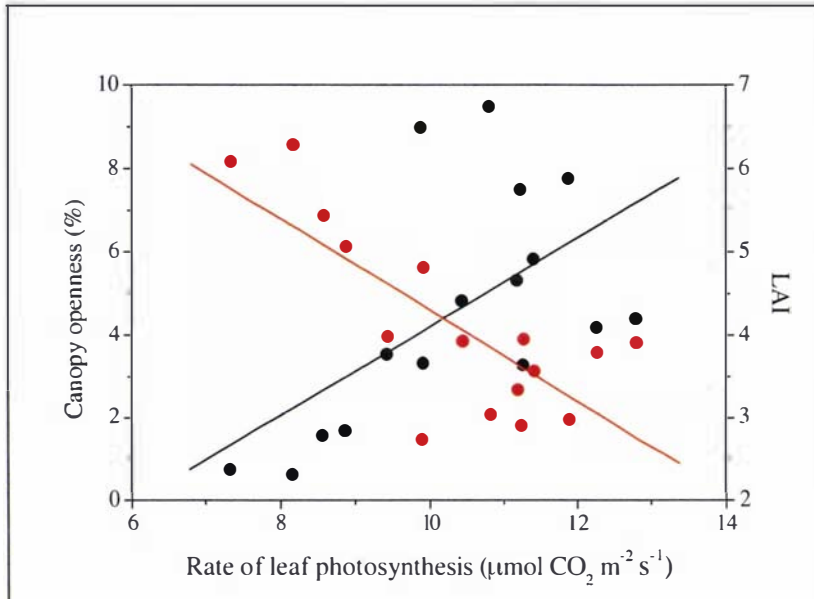


Fig 4.18. Relationship between leaf photosynthesis (Pn), canopy openness (Black: Pn = 8.83+0.331*openness; P <0.05, R^2 = 0.31) and LAI (Red: Pn = 14.7-1.06*LAI; P <0.001, R^2 = 0.55) in the 2002/2003 season.

In the 2002/2003 season, a positive correlation was found between average leaf Pn rates and canopy openness, whilst a negative correlation existed between LAI and leaf Pn rates (Fig 4.18). Similar correlations were found between leaf Gs and canopy openness (P <0.05, R^2 = 0.18) and LAI (P <0.004, R^2 = 0.42). However, in the 2003/2004 season there was no correlation between leaf Pn rates or Gs and canopy openness or LAI, despite differences in the Pn rate and Gs of sun and shade leaves.

In the 2003/2004 season, average leaf Gs values were determined for each shoot (data collected from the entire season) and were weakly correlated with fruit Ca concentrations (Fig 4.19). There was no relationship between leaf Pn rates and/or Gs and K, Mg or P concentrations. Regardless of shoot type, shoot leaf Pn rates were positively correlated with fruit DMCs (Fig 4.20). A slightly weaker correlation also existed between shoot Gs and fruit DMCs (P <0.001, R^2 = 0.027). Shoot leaf Pn rates and Gs did not affect fruit FW or DW. When average vine Pn rates and Gs were calculated and compared with average fruit DM, K, Mg, Ca and P concentrations for each vine no significant relationships were found (data not shown).

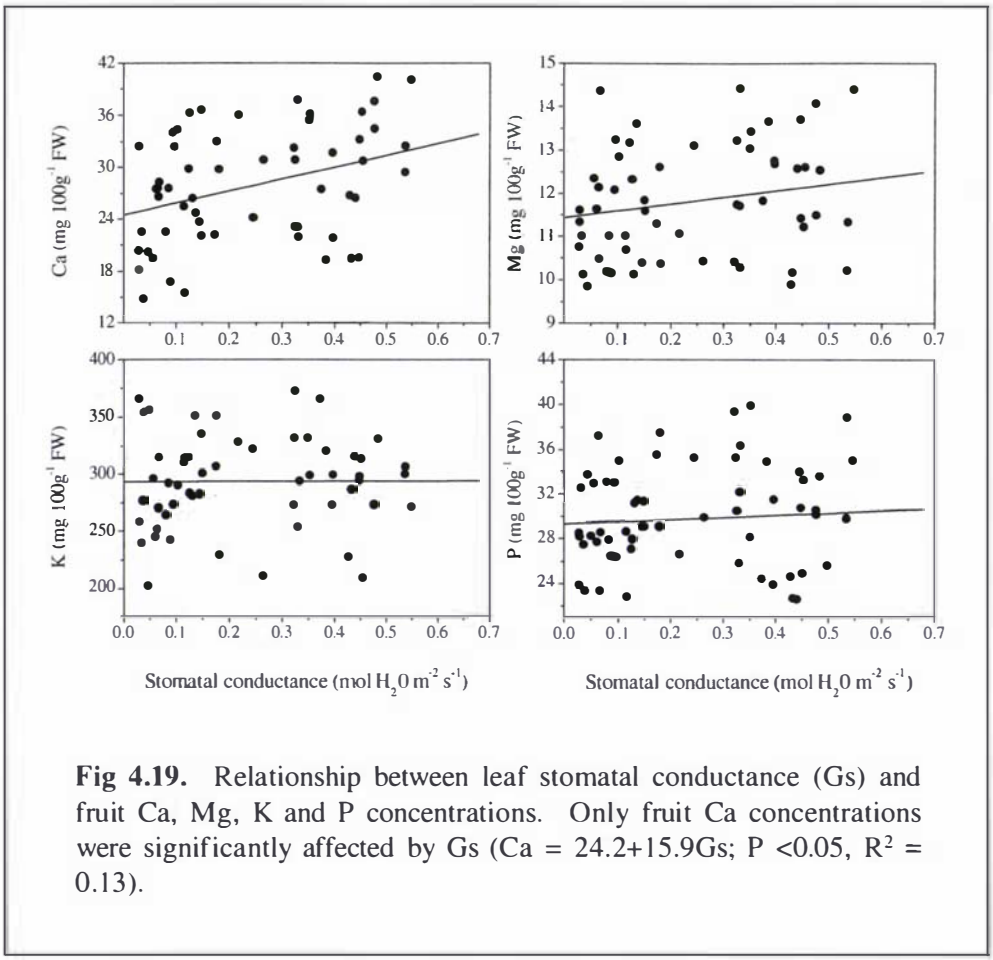


Fig 4.19. Relationship between leaf stomatal conductance (Gs) and fruit Ca, Mg, K and P concentrations. Only fruit Ca concentrations were significantly affected by Gs (Ca = 24.2+15.9Gs; $P < 0.05$, $R^2 = 0.13$).

4.3.4. Storage trial results

Fruit quality was very poor in the 2002/2003 season. At the final assessment date, after 24 weeks at 1-3°C, 20% of the fruit had softened below acceptable eating ripeness (3.9 N) and only 9% of fruit had a firmness above acceptable eating ripeness (7.8 N (average firmness was 5.4 N)). Many of the softer fruit developed a pale yellow colour in storage and almost every fruit showed signs of LTB, with 30% of fruit showing signs of severe LTB (water soaking). Thirteen percent of fruit developed a skin blemish that looked like physiological pitting, except that affected areas were not sunken. For each vine the average rot incidence was 21%, with a range of 2-54%.

Fruit firmness was higher in low crop load vines than in high crop load vines, especially in vines that had not been pruned (Fig 4.21). Fruit firmness also was higher in unpruned vines than in pruned vines, especially in low crop load vines. There was never any difference in firmness of fruit from the leader and fruiting zones of the vine. Despite there being no effect of pruning or crop load on fruit DM or SS concentrations at harvest, rSSC was higher in fruit from low crop load vines than in fruit from high crop load vines, especially in vines that had been pruned (Fig 4.21).

In low crop load vines rSSC also was higher in pruned vines than in unpruned vines. Fruit from the LZ always had a higher rSSC than fruit from the FZ (P <0.001).

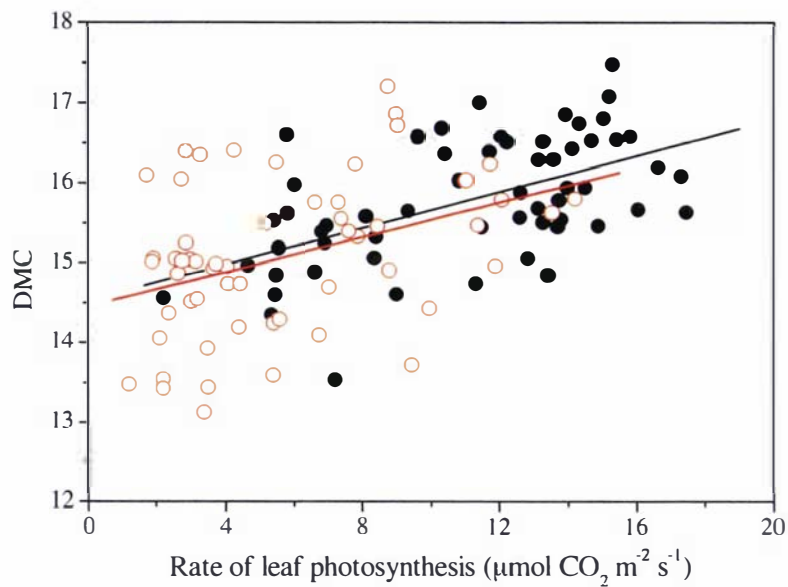


Fig 4.20. Relationship between shoot leaf photosynthetic rates (Pn) and fruit DMCs in short (red) and long (black) shoots. DMC (long) = 14.5+0.113Pn (P <0.001, R^2 = 0.29) and DMC (short) = 14.5+0.108Pn (P <0.01, R^2 = 0.13).

In the 2002/2003 season, fruit firmness was positively correlated with FW (P <0.007, R^2 = 0.37) and DW (P <0.022, R^2 = 0.27), which is why fruit were firmer in low crop load vines. In addition, a positive correlation was found between fruit firmness and fruit K (Firmness (N) = 0.017K+0.320; P <0.03, R^2 = 0.24) and P (Firmness (N) = 0.099P+2.798; P <0.29, R^2 = 0.25) concentrations. Due to the strong correlation between FW and fruit K, Mg and P contents (refer Section 4.2.2: fruit minerals), and to the correlation between fruit firmness and FW, firmness was strongly correlated with fruit K, Mg and P contents also (P <0.001). Firmness was not correlated with fruit Ca or Mg concentrations, fruit Ca contents or fruit rSSC (data not shown).

Using the equation for the correlation between fruit firmness and fruit P concentrations (Firmness (N) = 0.099P+2.798; P <0.29, R^2 = 0.25) found in the 2002/2003 season and average fruit P concentrations for the 2002/2003 and 2003/2004 seasons, it is estimated that fruit firmness would be 21.6% lower in the 2002/2003 season than in 2003/2004 season. In actual fact, it was 21.7% lower, a value that is extremely close to the estimated value.

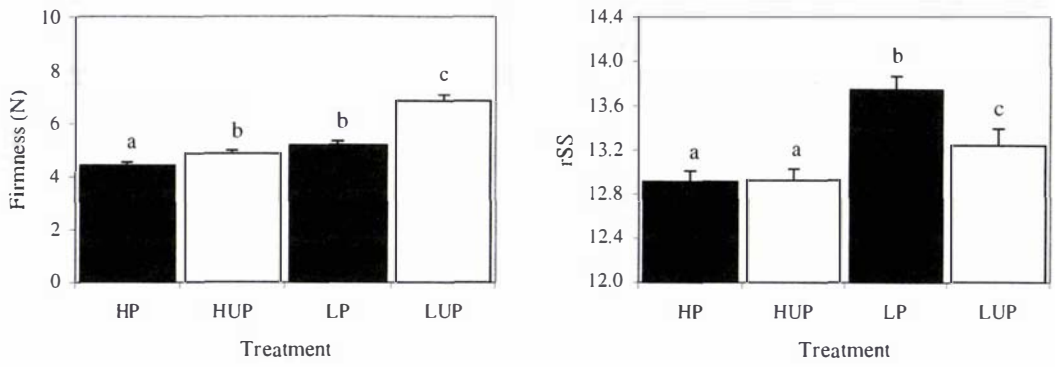


Fig 4.21. Fruit firmness and ripe soluble solids concentration in high and low crop load, pruned and unpruned vines after 24 weeks at 1-3°C (2002/2003 season). Bars are mean values (± s.e.) and are not significantly different at P <0.05 when labelled with the same letter.

Severe LTB incidence (signs of water soaking present) was higher in fruit from high crop load vines than from low crop load vines regardless of whether or not they were pruned (47.6 and 11.6%, respectively, P <0.001). LTB incidence did not differ in pruned and unpruned vines, or between zones (data not shown). Blemish incidence was not affected by crop load or pruning, but was higher in the FZ of low crop load vines than in the LZ of these vines (2.5% against 33.1% in the LZ and FZ of low crop load vines, respectively, P <0.01). Rot incidence also was higher in the FZ than in the LZ (on average 17 of the fruit in the FZ and 4% of the fruit in the LZ were rotten, P <0.001), and was not affected by crop load and pruning. Zone differences were more noticeable in low crop load vines than in high crop load vines for both stem-end (*Botrytis cinerea*) rot and side rot incidence (data not shown).

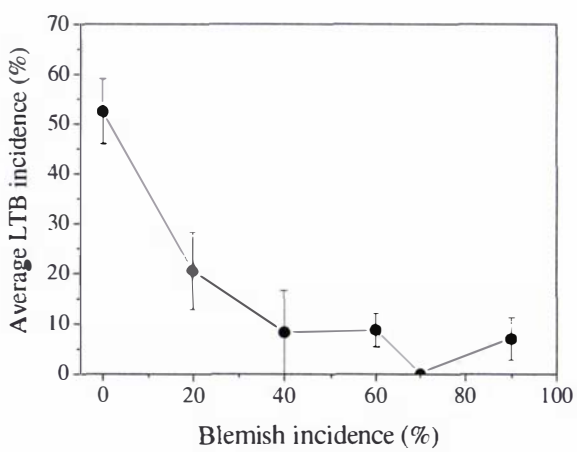


Fig 4.22. Relationship between LTB incidence and blemish (see text for details) incidence in the 2002/2003 season after 24 weeks at 1-3°C (P <0.001, R^2 = 0.39).

Severe LTB incidence increased as fruit firmness decreased and was therefore negatively correlated with fruit fresh (P <0.002, R^2 = 0.47) and dry (P <0.002, R^2 = 0.49) weights and fruit K (P <0.018, R^2 = 0.29) and P (P <0.035, R^2 = 0.23) concentrations. In addition, LTB incidence was negatively

correlated with the SSC at harvest and the rSSC at eating ripeness ($P < 0.05$, $R^2 = 0.31$ for both variables). There was no correlation between rot incidence or blemish incidence and any of the fruit variables assessed. However, blemish incidence was increased as LTB incidence declined (Fig 4.22).

Fruit quality was much improved in the 2003/2004 season, despite lower fruit DMCs (15.4%) in this season when compared to 16.6% in the 2002/2003 season. There was no effect of crop load or shoot type on rSSC (average 13.3), or LTB (no more than 1% incidence), pitting (no more than 0.1% incidence) and rot incidence (no more than 0.1% incidence) after 25 weeks in storage at 1-3°C (data not shown). Fruit firmness also did not differ between treatments or shoot types and averaged 6.89 N. At this firmness softening curves for the treatments may have started to coalesce, so treatment and/or shoot type affects that were present earlier in the season may not have been detected.

4.4. Discussion

4.4.1. Crop load effects on photosynthesis and stomatal conductance

This trial was set up to investigate effects of crop load on leaf photosynthesis (Pn) and stomatal conductance (Gs) and subsequent carbohydrate and mineral accumulation in kiwifruit. At the whole vine level, there was no effect of commercially viable crop loads on Pn or Gs of kiwifruit leaves, although Pn and Gs were reduced in unpruned vines between March and April when crop loads were exceptionally low (average 5 fruit m^{-2}). Fruit DM and calcium concentrations were never affected by crop load, even at extremely low crop loads. Some interesting results were obtained when Pn and Gs were analysed in relation to the L:F ratio on a fruiting shoot. Photosynthesis and stomatal aperture may be regulated by the demand for assimilates within a fruiting shoot. Sink demand for carbohydrates is determined by the L:F ratio on the fruiting shoot and may be increased by pruning, which stimulates shoot growth. Pruning also may enhance leaf photosynthetic efficiency by enhancing light interception in the canopy. It is suggested that optimum leaf photosynthetic rates will be attained at a LAI of between 3 and 4. These factors are discussed in more detail in the following section. In addition, the response of kiwifruit to declining crop loads is compared with the response of other crops, such as apple, in which photosynthesis is feedback inhibited at low crop loads.

At the whole vine level

Differences between the leaf Pn rate and Gs of high and low crop load vines were only observed in unpruned vines when fruit and shoot growth rates slowed and the demand for carbohydrate dropped (in April and May). Following bud-break in September, vines experience a carbohydrate deficit (negative carbon balance) that continues until 3-8 weeks after bud-burst, depending on spring temperatures (Piller and Meekings, 1997; Greer and Jeffares, 1998; Greer, 2001). At this time, leaves have a low photosynthetic efficiency and high capacity for non-photochemical quenching (NPQ - they can increase thermal radiation for the dissipation of excess energy, Greer and Halligan, 2001). As the vine is source-limited at this stage, any photoassimilate that is produced is partitioned to developing shoots and/or fruit or is used to maintain existing structures. Around 3-5 months after anthesis vines loose their capacity for NPQ and photosynthetic efficiency is improved as more energy is dissipated in photochemical reactions (Greer and Halligan, 2001). The demand for carbohydrate also drops around 3-5 months after anthesis, depending on the environmental conditions in spring (Greer, 1999), as rates of shoot and fruit growth start to decline (Buwalda *et al.*, 1991; Greer and Jeffares, 1998). Therefore, most shoots will produce a surplus of carbohydrate from this point in time (Greer, 2001).

Under normal circumstances this carbohydrate is exported from the shoots to fruit and other sinks within the vines (Lai *et al.*, 1988). However, in low crop load vines there are a reduced number of sinks into which carbohydrate can be loaded. Consequently, there may be an imbalance between carbohydrate metabolism and absorbed excitation energy, which could lead to a decline in sucrose translocation and hence its accumulation in the leaves (Wünsche and Ferguson, 2005). This can reduce leaf photosynthetic efficiency and may reduce stomatal aperture (Wünsche and Ferguson, 2005) and could explain why Pn and Gs were higher in leaves from high crop load, unpruned vines than in leaves from low crop load, unpruned vines in April and May.

In the 2002/2003 season, carbohydrate accumulation in leaves of low crop load vines may have been particularly high, as mean daily temperatures in March and April (2003) were 1.6°C higher than those in March/April 2004 (W. Snelgar: unpublished data). Shoots grown at 17/12°C (day/night temperature) had a total carbohydrate surplus of 1.4 g over 5 months, whereas shoots at 28/22°C accumulated 46 g of carbon at this time (Greer and Jeffares, 1998). It also is possible that treatment differences were more apparent in the 2002/2003 season due to the extreme reductions in crop load in this season (only 5 fruit per m^{-2}) when compared to the low crop load treatment in 2003/2004 (four times more fruit than in the 2002/2003 season).

There was no effect of crop load on leaf Pn and Gs in pruned vines. Pruning may prevent or delay sucrose accumulation in leaves and this could alleviate the negative effects of crop load on leaf photosynthetic efficiency and transpiration (as regulated by Gs). In this experiment, the carbohydrate deficit period may have been prolonged when vines were pruned shortly after anthesis. This may have restricted carbohydrate partitioning to roots and reserve pools, which are weaker sinks for carbohydrate than developing shoots and fruit at this time (Buwalda and Smith, 1990b; Greer *et al.*, 2003). Subsequently, in low crop load, pruned vines there may have been more potential to partition surplus carbohydrate towards the roots, reserve pools, and possibly fruit and shoots later in the season when vines became sink limited than in low crop load, unpruned vines. Furthermore, pruning may have stimulated shoot growth and this may have prevented large carbohydrate surpluses from accumulating in leaves and causing the feedback inhibition of photosynthesis. These findings also could explain why leaf Pn and Gs were higher in pruned vines than in unpruned vines in the latter half of the growing season, and why leaf photosynthesis was negatively correlated with canopy density.

In apples, crop load had the greatest effect on leaf Pn rates and Gs towards the end of the growing season, when rates of shoot and fruit growth slowed, but rates of photosynthesis were still relatively high (Palmer *et al.*, 1997; Wünsche *et al.*, 2000; Wünsche *et al.*, 2005). However, in apples crop load effects on photosynthesis and Gs were more apparent than in kiwifruit and were still observed in pruned vines. Apple fruit comprise 60-70% of the total plant biomass (Palmer *et al.*, 2002), compared to 11-18% for kiwifruit (Greer *et al.*, 2003) in New Zealand growing conditions. Therefore, the source-sink balance within the apple plant would be more affected by fruit thinning than in kiwifruit. The more vigorous, vegetative growth habit of kiwifruit suggests that there is more potential to partition surplus carbohydrate into shoot development than in apple. In this study, 41% more vegetative regrowth was removed from low crop load (average 5 fruit m^{-2}) vines than from high crop load (35 fruit m^{-2}) vines. Greer *et al.* (2003) found that young vegetative vines had up to 50% more leaf area than fruiting vines at the same developmental stage. In kiwifruit, leaves on shoots with a high L:F ratio (4:1) had a greater individual area and higher Pn (when measured 6 WAFB) than leaves on shoots with a L:F ratio < 4:1 (Lai *et al.*, 1989a). Compared to apple trees, kiwifruit vines produce significantly more leaf area than is required to support the crop, suggesting that in kiwifruit the vegetative sink may be stronger than the fruit sink (Greer *et al.*, 2003). This differs from the situation in apples where fruit are the stronger sink (Palmer *et al.*, 2002).

At the shoot level

The affects of shoot type on Pn and Gs were more apparent, regardless of time in the season, than whole vine crop load and pruning effects. Long shoots are more likely to develop in the sun than

short shoots, and are therefore likely to have the higher Gs and Pn (Lionakis *et al.*, 1997; Xiloyannis *et al.*, 2000). The finding that rates of leaf photosynthesis were higher in pruned vines, which had improved light transmission, supports this assumption. However, shoot-type differences in light transmission were not entirely accountable for shoot-type effects on photosynthesis and Gs. Greer and Halligan (2001) noted that kiwifruit leaves have the ability to acclimatise to a wide range of light conditions in order to maximise carbon gain in different light conditions. They showed that specific leaf characteristics have more of an effect on rates of photosynthesis than responses of vines to varying light environments.

Differences between the Pn rates and Gs of leaves on long and short shoots also may relate to the rate of shoot development and position of these shoots within the canopy, which would effect their demand for carbohydrate. Rates of leaf area development are higher in long shoots than short shoots and long shoots are more likely than short shoots to arise from the LZ of the vine. In this zone demand for carbohydrate may be high, as a large amount of vigorous shoot growth develops in the zone (Miller *et al.*, 2001). Leaves on faster growing (long) shoots had a higher maximum and average rate of photosynthesis than leaves on slow growing (short) shoots when measured between bud-burst and anthesis (Piller and Meekings, 1997). Rates of photosynthesis also were a lot higher in leaves from shoots that arose near the basal end of parent canes (i.e. the long shoots used in this study) than those that arose nearer the distal end (i.e. the short shoots used in this study). Fruiting shoots also had a higher rate of photosynthesis than vegetative shoots early in the season (Greer, 1999). This suggests that in the early stages of the growing season rates of leaf photosynthesis are regulated by carbohydrate demand; long shoots have a greater demand for carbohydrate than short shoots, and hence a greater NAR.

It also has been suggested that leaf Pn rates are influenced by carbohydrate demand later in the season (Greer, 1999). Equivalent fruit numbers were attained on long and short shoots used in this study (average three fruit), although the long shoots had more leaves and continued to grow in length until later in the season. Therefore, respiratory losses from long shoots would be higher than in short shoots and they would have a greater maintenance cost, so would have a greater demand for carbohydrate. Greer (1999) suggested that demand for carbohydrate later in the season would be higher on long shoots than in short terminating shoots. Currie (1997) found that as the L:F ratio on girdled shoots decreased, rates of leaf photosynthesis were enhanced. It is likely that carbohydrate demand increased as fruit numbers on the shoots increased, especially as the carbohydrate could not be exported from the shoot to alternative sinks if carbohydrate was not able to be utilised by the vegetative sinks within the shoot. Greer *et al.* (2003) found that rates of photosynthesis were, on average, 17-21% higher in fruiting vines than in vegetative vines.

The greater demand for carbohydrate in long shoots than in short shoots may prevent carbohydrate accumulation in leaves inhibiting photosynthesis later in the season when carbohydrate supply often exceeds demand. The greater SLW of leaves on short shoots may be evidence of greater starch deposition in these leaves than in leaves on long shoots. Leaf anatomy also may have been altered in response to the carbohydrate accumulation on short leaves. In apples, cells in leaves on low crop load vines were more densely packed, and it was suggested that this reduced intercellular CO_2 concentrations and hence photosynthetic efficiency (Wünsche and Palmer, 1997).

4.4.2. Crop load effects on fruit properties

Leaves subtending fruit provide the majority of their carbohydrate (Lai *et al.*, 1988; Lai *et al.*, 1989a) and have a strong influence on Ca accumulation in fruit (refer Chapter 5 for more information). Therefore, it is not surprising that differences in the NAR and Gs of long and short shoots were reflected in fruit quality. Fresh and dry weights, DM and Ca concentrations were generally higher in fruit from long shoots than in fruit from short shoots (Table 4.1), especially in high crop load vines and in the 2002/2003 season, when whole vine carbohydrate supplies may have been limited by high crop loads and/or a low LAI. Under conditions of limited assimilate availability kiwifruit vines have a limited capacity to partition carbohydrate towards areas of the vine where there is a shortfall of carbohydrate, so fruit growth must be supported by leaves within the shoot (Lai *et al.*, 1989a). Correlations between the Pn and Gs of leaves on a shoot and the DM and Ca concentration of fruit on that shoot are further evidence that fruit DM and Ca concentrations are influenced by rates of transpiration from, and rates of photosynthesis in, subtending leaves.

Minerals

Fruit Ca concentrations were never affected by crop load when data were analysed at the vine level. This may be because crop load effects on leaf Gs and hence, water movement to the shoot in the transpiration stream, were not apparent until after the majority of Ca is accumulated in the fruit (Clark and Smith, 1988). Shoot type differences in leaf Gs were apparent from an early stage of fruit development and tended to be higher in leaves from long shoots than in leaves from short shoots (Table 4.1). Fruit Ca concentrations were higher in fruit from long shoots than in fruit from short shoots. This suggests that stomatal conductance of leaves within a fruiting shoot may influence fruit Ca concentrations by regulating the rate of xylem sap accumulation in fruit. It is possible that the transpiration rate of leaves outside of the fruiting shoot has little affect on fruit Ca, which would also explain why there was no effect of crop load on fruit Ca concentrations at the vine level.

In contrast to Ca, fruit K and P concentrations, and to a lesser extent Mg concentrations, increased with decreasing crop load, especially in the 2002/2003 season, when greatest differences were noted between the Pn and Gs of individual leaves on high and low crop load vines. Later in the growing season, when the stomatal conductance of leaves on low crop load vines is reduced, K and P are predominantly transported to fruit via the phloem (Clark and Smith, 1988). Therefore, it appears that under conditions of low transpiration (reduced stomatal conductance), phloem translocation to fruit may be enhanced to compensate for reductions in xylem translocation. The greater phloem area in low crop load vines than in high crop load vines may be evidence of this occurring, and could explain why fruit K and P concentrations were negatively correlated with crop load. As Mg is not as mobile as these minerals in the phloem (Clark and Smith, 1988), this would explain why fruit Mg concentrations were only marginally affected by crop load.

During early stages of fruit development, like Ca, Mg is predominantly transported in the xylem and fruit Mg concentrations also are greater in long shoots than in short shoots (Table 4.1). Therefore, it appears that fruit Mg accumulation also is affected by the transpiration rate of leaves within a fruiting shoot, although the effect of leaf transpiration is not as strong as for Ca. In contrast to Ca and Mg, fruit K and P concentrations tended to be higher in fruit from short shoots than in fruit from long shoots, especially in low crop load vines that have reduced stomatal conductance. This confirmed findings at the vine level, that accumulation of these minerals in fruit is not totally dependent on translocation in the transpiration stream. It also confirms suggestions that fruit K and P accumulation may be enhanced under conditions of reduced evapotranspiration. Alternatively, K and P may be actively partitioned towards fruit on short shoots in the xylem sap. This possibility is discussed more in Chapter 9.

Fruit Ca concentrations and contents were positively correlated with estimated secondary xylem conductance, whereas concentrations or contents of K, Mg and P were not. This may be further evidence that fruit K, P, and to a lesser extent Mg, accumulation is less dependent on transpiration, whereas fruit Ca accumulation is dependent on translocation by mass flow in the xylem transpiration stream. In keeping with FW differences between fruit on long and short shoots, xylem and phloem areas were greater in stalks of fruit from long shoots than in stalks of fruit from short shoots (Table 4.1).

Carbohydrates and water

Dry weight accumulation was reduced at high crop loads, because although more carbohydrate was partitioned towards the fruit, this was not enough to accommodate the increase in fruit numbers. Richardson *et al.* (1997) noted that rates of fruit DW accumulation were reduced at high crops from the very early stages of fruit development, and remained constant over the entire

season. These results suggest that rates of DW partitioning are set during the very early stages of flower/fruit development, and that the potential to manipulate DW partitioning in kiwifruit is greatly reduced as fruit mature.

In kiwifruit, cell division is largely complete by 3-4 weeks after flowering (Hopping, 1976b), after which time there appears to be little potential to alter rates of carbohydrate partitioning to fruit (Richardson *et al.*, 1997). Therefore, the number of cells in a fruit may determine the potential for a fruit to accumulate carbohydrate. Piller *et al.* (1998) found that early season fruit set and development was very sensitive to carbohydrate limitations. Rates of cell division may be positively correlated with early season carbohydrate availability, and once the number of cells in the fruit is set, a certain proportion of the total carbohydrate supply (dependent on the crop load at that time) may be partitioned towards fruit to support cell elongation and to maintain cellular structures. As early season rates of photosynthesis are higher in long shoots than in short shoots (Piller and Meekings, 1997), rates of cell division also may be higher in fruit on long shoots, and subsequently rates of DW accumulation may be higher in these fruit than in fruit on short shoots (Table 4.1).

If this hypothesis is true, then it is not surprising that (a) fruit fresh and/or dry weights are little affected by late season changes in crop load (Currie *et al.*, unpublished data) and (b) fruit DMCs are little affected by large changes in crop load. The amount of carbohydrate partitioned to a fruit is likely to be directly proportional to the amount of carbohydrate accumulating in that fruit. Unless this carbohydrate is sequestered away or converted into other substances, it will lower the water potential of the fruit, thereby promoting water movement into the fruit and subsequent cell enlargement. This has the effect of increasing fruit FW, the extent of FW increase being directly proportional to the amount of carbohydrate accumulating in a fruit. This would explain why increases in fruit FW were almost identical to increases in fruit DW for a 30 fruit m^{-2} reduction in crop load (FW increased by 20 and 17% and DW increased by 21 and 16% in the 2002/2003 and 2003/2004 seasons, respectively). Variations in fruit DMC may depend on the amount of carbohydrate sequestered into the vacuole and other cellular organelles or on the rate of enzymatic conversion of soluble carbohydrates into other plant substances.

The increase in fruit FW at low crop loads was correlated with the increase in phloem area (R^2 = 0.50). This suggests that the phloem has the capacity to increase in area in response to the demands of the fruit, or that fruit volume (DW and FW) will increase in response to increasing phloem capacity. However, the potential for xylem area to increase in response to increases in fruit volume appears limited. A weak correlation existed between fruit FW and xylem area (R^2 = 0.29), and FW accumulation was not related to estimated secondary xylem conductance.

Moreover, Ca was only weakly correlated with fruit FW when compared to the other more phloem mobile minerals. Estimated secondary xylem conductance was higher in unpruned than pruned vines, which suggests that some substance produced in developing shoots influences the rate of estimated secondary xylem conductance. In Chapters 5 and 8 the possibility that this substance is an auxin is explored.

4.4.3. Crop load effects on postharvest fruit quality

In 2002/2003 fruit postharvest quality was very poor compared to that in 2003/2004. This did not relate to differences in fruit DMC, because average fruit DMCs were 1.2%-units higher in 2002/2003 than in 2003/2004, when vines with similar crop loads were compared. Moreover, differences in fruit quality in storage between the two seasons could not have been related to differences in fruit maturity at harvest, as in both years fruit were harvested at between 6-6.1 °Brix. In the 2002/2003 season fruit Ca concentrations were 6% lower than the 2003/2004 season, whilst fruit P concentrations were more than 30% lower. Storage disorder incidence did not appear to be correlated with fruit Ca concentrations, or the K:Ca ratio in fruit, but this may relate to how fruit were sampled. Relationships between fruit mineral composition and disorder incidence are often lost when data are analysed on a whole vine basis, because often there are sub-populations of fruit within a plant that differ in susceptibility to disorder incidence (Ferguson and Boyd, 2001).

Fruit firmness was positively correlated with fruit K and P concentrations while LTB incidence was negatively related to fruit K and fruit P concentrations. It is unclear as to whether this is an effect of these minerals on fruit firmness per se, or whether these minerals are simply related to firmness because they tend to increase as crop loads decline. Fruit from low crop load vines also had a higher FW and DW content, and fruit fresh and dry weights were correlated with fruit firmness (positive correlation) and LTB incidence (negative correlation). Fruit P concentrations also were negatively correlated with low temperature and senescent breakdown in apples (Yogaratnam and Sharples, 1982), although no explanation was given for this relationship. These relationships may relate to the suppressing effects of P on fruit respiration (Knowles *et al.*, 2001). This suggests that fruit P concentrations may have a direct influence on fruit firmness, a finding that warrants further investigation.

4.5. Conclusions

1. In well managed kiwifruit vines with a commercially viable crop load it is unlikely that fruit numbers on the vine will influence rates of leaf photosynthesis or transpiration, as surplus carbohydrate appears to be utilised in shoot growth at low crop loads or is partitioned into developing fruit. This means that for a given leaf area, and given climate, there may be limited potential to increase total fruit biomass production in kiwifruit vines.

2. Carbohydrate partitioning to kiwifruit is affected by the vine crop load. Fruit DW was enhanced at reduced crop loads, but fruit DM was not affected because fruit water contents also were increased. At high crop loads fruit DW was reduced, as were fruit water contents, so fruit DM also was not affected. Fruit turgor pressure will be lowered in response to carbohydrate accumulation in fruit cells and this may induce a pressure-driven flow of water into fruit via the phloem. Therefore, practices such as thinning that affect carbohydrate partitioning to fruit may have limited potential to manipulate fruit DM, unless they are conducted in conjunction with practices that manipulate fruit water accumulation.

3. Transpiration rates of leaves subtending fruit may influence Ca accumulation in fruit, and to a lesser extent Mg accumulation, but appear to have little effect on K and P accumulation. In fact, when rates of transpiration are low, K and P accumulation in fruit may be enhanced. This suggests that when rates of evapotranspiration are low phloem water translocation is enhanced to compensate for reductions in xylem mineral ion translocation. Alternatively, K and P may be actively partitioned towards fruit in the xylem. As physiological pitting incidence in kiwifruit is positively correlated with fruit K and P concentrations (Ferguson *et al.*, 2003), pitting incidence may be greater in low crop load vines.

4. Pruning may enhance rates of photosynthesis and stomatal conductance in kiwifruit vines by altering carbohydrate partitioning in a manner that increases overall demand for carbohydrate, thereby reducing the chance of photosynthesis being down-regulated by carbohydrate accumulation in leaves. In addition, pruning may increase rates of leaf photosynthesis by enhancing light transmission in the vine, which would explain the positive correlation between light transmission and fruit DM found in Chapter 3.

5. It is postulated that rates of cell division are affected by carbohydrate availability during flower and fruitlet development. Carbohydrate partitioning to fruit on long shoots is likely to be greater than to fruit on short shoots, because: (a) rates of photosynthesis are higher in leaves on long shoots than in leaves on short shoots, and (b) phloem differentiation is more

advanced in stalks of fruit from long shoots than in stalks of fruit from short shoots. Therefore, fruit on long shoots are likely to have more cells and greater sink strength than fruit on short shoots.

6. Calcium accumulation in fruit on long shoots is likely to be greater than in fruit from short shoots, because leaves on long shoots have greater stomatal conductance than leaves on short shoots. Moreover, xylem differentiation in the stalks of fruit from long shoots is greater than in stalks of fruit from short shoots.

7. Phloem differentiation may be regulated in response to the changing demands of sinks for carbohydrate. However, there may be a limited capacity of xylem conductance to increase in response to changes in sink demand. Consequently, the xylem capacity of the fruit stalk may restrict Ca movement into fruit.

5. Carbohydrate and Mineral Accumulation in Long, non-terminating and Short-terminating Shoots: effects of Girdling and Defoliation

5.1. Introduction

A positive correlation has been reported between kiwifruit Ca and DM concentrations that is consistent both between vines (Ferguson *et al.*, 2003), and between orchards within the same region (Smith *et al.*, 1994). Thorp *et al.* (2003b) suggested that sub-populations of fruit exist within a kiwifruit vine that have desirable physical, chemical and postharvest quality attributes. In particular, fruit from long shoots tended to have a higher DM and Ca concentration than fruit from short terminating shoots. It is possible that similar factors determine fruit Ca and DM concentrations at harvest. However, it would be surprising if there were a direct link, as Ca is largely phloem immobile and is transported predominantly in the xylem, whereas a fruit's DMC is largely dependent on carbohydrate translocation in the phloem (Clark and Smith, 1988). Furthermore, Ca is mainly accumulated during the first month of fruit growth (Clark and Smith, 1988), whereas fruit DMCs increase consistently from late December through fruit development and maturation (Richardson *et al.*, 1997).

The nature of this link between Ca and DM concentrations is unknown, yet an understanding of the factors affecting DM and Ca concentrations may enable growers to produce an increased proportion of kiwifruit with desirable fruit quality attributes. If different factors affected Ca and carbohydrate accumulation in kiwifruit, then it should be possible to produce fruit with high DMCs and low Ca concentrations or vice versa. This would help to determine to what extent factors affecting fruit DM and Ca concentrations differ.

Phloem translocation, and hence fruit DMCs, may be manipulated by girdling (Goren et al., 2004). However, girdling has no direct effect on water accumulation (Goren et al., 2004) and does not alter concentrations of K, Mg, Ca or P in kiwifruit at harvest (Currie *et al.*, unpublished data). The ability of girdling to alter a fruit's DMC depends on when the girdle is applied and the L:F ratio on the girdled shoot (Snelgar and Thorp, 1988; Lai *et al.*, 1989a; Currie, 1997). Girdling one-year-old fruiting shoots soon after flowering ('spring girdling') increased fruit cell division and expansion, and hence water and carbohydrate accumulation and FW, but reduced fruit DMCs. By contrast, when shoots were girdled in late summer ('autumn girdling'), FW only responded via cell expansion, and as starch accumulation increased, fruit DMCs tended to increase (Currie, 1997; Carreño *et al.*, 1998).

Fruit growth may be inhibited by deficits of carbohydrate in girdled shoots with a low L:F ratio (i.e. short shoots), whereas girdling will prevent carbohydrate export from shoots with a high L:F ratio (i.e. long shoots). Subsequently, carbohydrate that would normally be exported may instead be partitioned into fruit where it may promote cell division and elongation (Snelgar and Thorp, 1988; Lai *et al.*, 1989a; Currie, 1997). When shoots were isolated by girdling, a L:F ratio of two was required for a shoot to support fruit growth without having to import carbohydrate from other parts of the vine (Lai *et al.*, 1989a). However, girdling may increase shoot respiration, and thus fewer than two leaves per fruit may be required to support fruit growth on intact vines (Currie, 1997). With L:F ratios above four, the response to girdling is reduced (Seager and Hewett, 1995; Famiani *et al.*, 1997), and at very high L:F ratios carbohydrate may accumulate in leaves leading to feedback inhibition of photosynthesis (Di Vaio *et al.*, 2001). Lai *et al.*(1989a) found no effect of crop load on the photosynthetic rate or stomatal resistance of leaves on girdled kiwifruit shoots, and results in Chapter 4 indicate no effect of crop loads as low as 20 fruit m^{-2} on photosynthesis and stomatal conductance.

As Ca is transported predominantly by mass flow in the transpiration stream (Clark and Smith, 1988), its accumulation in fruit may be manipulated by defoliating shoots to reduce evaporative losses from the vine. Phloem translocation is not necessarily directly affected by defoliation, as photoassimilates can travel up to two metres to compensate for local shortages in carbohydrate supply as long as overall vine carbohydrate is not limiting (Lai *et al.*, 1989b; Buwalda and Smith, 1990b; Tombesi *et al.*, 1993).

Defoliation has been used to manipulate Ca accumulation in apple, where fruit Ca concentrations were reduced by bourse and/or primary leaf removal, especially when leaves were removed shortly after flowering when Ca was accumulating rapidly in the fruit (Ferree and Palmer, 1982; Proctor and Palmer, 1991; Volz *et al.*, 1994; Volz *et al.*, 1996). Lang and Volz (1998) suggested that defoliation reduced the movement of xylem sap containing high concentrations of Ca between leaves and fruit. In kiwifruit, a positive correlation existed between leaf area and fruit Ca concentrations in short shoots, but not in long shoots (Thorp *et al.*, 2003b). This suggests that there is a threshold leaf area below which subsequent increases in leaf numbers will increase fruit Ca concentrations, but above which fruit Ca concentrations will not be affected. As long shoots are more exposed, with more leaves and hence higher rates of transpiration than short shoots, there may be more potential to reduce shoot transpiration by defoliating long shoots than short shoots. Therefore, it could be expected that the defoliation of long shoots will have more of an effect on fruit Ca accumulation than the defoliation of short shoots.

The work presented in this chapter investigated physiological drivers of Ca (xylem), carbohydrate (phloem) and water (xylem and phloem) accumulation in fruit on long and short shoots. It is proposed that different factors affect carbohydrate and Ca accumulation in fruit, and that these factors may be independently manipulated. Thus it is possible to have a fruit with a high DM and low Ca concentration or vice versa. Furthermore, it is suggested that the different attributes of long and short shoots will cause fruit on those shoots to differ in response to girdling and defoliation treatments.

5.2. Materials and Methods

5.2.1. Experimental design and treatments

On 24 November 2001 (10 DAFB), 40 vines were selected from a commercial orchard block in Te Puna in the Bay of Plenty region, New Zealand (refer Chapter 3 for details). A completely randomised split-plot design was used, whereby eight replicate vines were (randomly) assigned to each of the following five treatments: (1) spring-defoliated (SpD), (2) spring-girdled (SpG), (3) summer-defoliated (SmD), (4) autumn-girdled (AuG), and (5) control (main plot). Within each vine, ten short shoots, (five each from the distal (short-end (SE)) and proximal (leader end: short-base (SB)) ends of the cane), and five long shoots (from the leader zone (LZ)) were tagged and treatments were applied to these shoots on: 24 November (at FB; treatments 1,2 and 5), 5 January 2002 (42 DAFB; treatment 3), and 1 February (69 DAFB; treatment 4) (sub-plot). Treatments 3 and 4 were applied after the completion of cell division, but while cells were still expanding (MacRae *et al.*, 1989). Each shoot carried 3-5 fruit.

All leaves on defoliated shoots were removed, including 'scale' leaves at the very base of the shoot and small developing leaves at the shoot tip that had not yet unfurled. The number of leaves removed varied from shoot to shoot, averaging 6.5 ± 0.18 (mean ± s.e) on short shoots and 23 ± 0.75 (mean ± s.e) on long shoots. Defoliation treatments were not maintained as shoots grew and new leaves developed. Girdling was performed using a double-bladed knife to remove a ring of bark 10 mm wide from the entire circumference of a shoot's base. After the bark was removed, the girdle was checked to ensure that all tissues external to the xylem had been removed, and that xylem tissues were not damaged during the girdling process. Two cotton wool buds were taped to either side of thin lateral shoots, which had low physical strength after girdling, to form a 'splint' to protect shoot breakage at the point of weakness at the girdle. Similarly, 'splints' were placed on 20, randomly selected, control and defoliated shoots to ensure that this practice had no unintended

effects on the experimental outcome. As no difference was found between fruit from shoots with and without splints, these shoots were not considered independently in the results (Section 5.3). Girdling treatments were not maintained, so that girdles were completely healed by 8-10 weeks after girdle application. Control shoots were left untouched

5.2.2. Harvest procedure

Fruit mineral and carbohydrate accumulation was tracked as fruit matured on dates coinciding with the beginning, middle and end points of fruit development (where end point is defined as the time until commercial maturity or harvest). On 18 December 2001, five control fruit were randomly harvested from each of the three positions (SE, SB and long) in each of the eight vines (120 fruit in total, 40 from each position). Similarly, 120 fruit (40 from each position or 5 fruit per vine) were harvested from the spring-girdled and spring-defoliated treatments on 19 and 20 December 2001, respectively. Fruit FW, DM and mineral concentrations were analysed as described in the general methods (Chapter 2) and fruit DWs and water contents (g) were deduced from DMC and FW data. Fruit stalk length and diameter were measured on three fruit stalks per vine, one from each position (48 per treatment, or 24 from each position) using digital callipers. This procedure was repeated on 22, 23, 24 and 25 January 2002 for the spring-girdled, spring-defoliated, control and summer-defoliated treatments, respectively, and on 22, 23, 24, 25 and 26 April 2002 for the spring-defoliated, spring-girdled, summer-defoliated, control and autumn-girdled treatments, respectively.

During the first harvest period fruit were growing rapidly as rates of cell division and elongation, and rates of starch, Ca, Mg, K and P accumulation were high (Hopping, 1976b; Clark and Smith, 1988). The second harvest period coincided with the start of the second phase of fruit growth, when fruit growth started to slow, coinciding with reduced rates of mineral accumulation (Hopping, 1976b; Clark and Smith, 1988). The final harvest period coincided with cessation of fruit growth and mineral accumulation, when starch conversion into soluble sugars commenced (Clark and Smith, 1988; Smith *et al.*, 1994).

5.2.3. Xylem functionality

As defoliation is known to directly affect (xylem) hydraulic conductance, xylem functionality was measured in ventromedian capillary (VMC) bundles of defoliated and control fruit. On 21 December 2001, 12 fruit were selected from long shoots and 12 fruit were selected from SE shoots (24 in total) from the control and spring defoliation treatments and their xylem functionality was

assessed as described in Section 2.7. Similarly, twelve fruit were harvested from each of the long and SE shoots for assessment of xylem functionality in fruit from the control, spring and summer defoliation treatments on 20 January 2002 and 27 April 2002.

After xylem functionality was assessed on 27 April 2002, two samples were selected from each of the three treatments (spring and summer defoliation and control) that had no dye staining in the VMC bundles. These samples, which consisted of a stalk with a small section of attached flesh, were sectioned longitudinally and analysed using a Leica FLZ III light microscope (Leica Microscopy Systems Ltd., Heerbrugg, Switzerland) to determine the site of xylem dysfunction. A more detailed assessment of the tissue surrounding the site of dysfunction was made using a compound light microscope (Olympus Vanox AHT3 (Olympus Optical Co Ltd., Tokyo, Japan)).

5.2.4. Statistical Analysis

Mean differences between fruit from the same treatment, but different lateral types, and between fruit from the same lateral type, but different treatments, were determined by ANOVA GLM as described in Chapter 2. There were insufficient differences between fruit from short-end and short-base shoots, so data were combined under the heading short (short terminating shoots). Unfortunately MINITAB® Release 14.11 software, which was the only statistical software available to me, was unable to analyse this experiment as a split-plot design with the correct degrees of freedom. This meant that analyses were restricted to main effects and interactions between variables were not able to be assessed.

5.3. Results

5.3.1. December harvest

Spring defoliation reduced fruit Ca and K concentrations, regardless of shoot length, when compared to the control (Table 5.1). Phosphorus concentrations also were reduced by spring defoliation in short shoots. Mg concentrations were never affected by spring defoliation. Spring girdling reduced fruit Ca and K concentrations in long shoots, but not in short shoots. There was no effect of spring girdling on fruit Mg or P concentrations. Fruit Ca concentrations were lower in spring-defoliated shoots than in spring-girdled shoots, regardless of shoot length. Fruit Ca concentrations also were lower in short spring-defoliated shoots than in short spring-girdled shoots, but not in long shoots. There was no difference between fruit Mg and P concentrations in

spring-girdled and defoliated shoots. Fruit stalk length and diameter also were unaffected by treatments.

Table 5.1. Several quality attributes of fruit from long, non-terminating (long) and short terminating (short) axillary kiwifruit shoots, as measured on 19 December 2001, following shoot defoliation (SpD) or girdling (SpG) on 24 November 2001 (25 days earlier).

Fruit attribute	LONG			SHORT		
	Control	SpD	SpG	Control	SpD	SpG
Ca (mg $100g^{-1}$ FW)	35_a	17_b	28_c	23_a	12_b	25_a
Mg (mg $100g^{-1}$ FW)	12_a	12_a	10_a	10_a	9.1_a	10_a
K (mg $100g^{-1}$ FW)	228_a	190_b	200_b	232_a	191_b	227_a
P (mg $100g^{-1}$ FW)	23_a	21_a	22_a	25_a	22_b	24_{ab}
Fruit stalk length (mm)	61_a	65_a	72_a	60_a	61_a	61_a
Fruit stalk diameter (mm)	3.3_a	3.5_a	3.5_a	3.1_a	3.1_a	3.1_a
Fresh weight (g)	24_a	35_b	41_c	21_a	31_b	29_b
Dry weight (g)	1.5_a	2.0_b	2.5_c	1.3_a	1.8_b	1.7_b
Water content (g)	22_a	33_b	38_c	20_a	29_b	28_b
DMC	6.5_a	5.7_b	6.0_c	6.3_a	5.7_b	5.8_b

For each shoot type (long or short), means within rows followed by the same letter are not significantly different at P <0.05. Highlighted values indicate significant differences between fruit from long and short axillary shoots (P <0.05). Fruit stalk diameters are an average of measurements taken at the proximal, mid and distal ends of the fruit stalk.

FW, DW and water content of fruit from spring-girdled or defoliated shoots was always significantly higher than that of fruit from control shoots regardless of shoot length. Fruit from spring-girdled long shoots had a greater FW, DW and water content than fruit from spring-defoliated long shoots, but there was no difference in these attributes between fruit from spring-defoliated and spring-girdled short shoots. In contrast, the DMC of fruit from control shoots was greater than the DMC of fruit from treated shoots regardless of shoot length.

Defoliation and girdling treatments often enhanced differences between fruit from long and short shoots. Fruit from spring-defoliated long shoots had higher Ca and Mg concentrations than fruit from spring-defoliated short shoots, whilst the K concentration of fruit from spring-girdled long shoots was lower than that of fruit from spring-girdled short shoots. Calcium concentrations in fruit from long control shoots were greater than in fruit from short control shoots. Fresh and dry weights, as well as fruit stalk diameter and length were greater in fruit from long shoots than in fruit from treated short shoots, when defoliation or girdling treatments were applied. In control shoots, fruit stalk diameter and fruit DW also was greater in long shoots than in short shoots.

Xylem functionality varied considerably from fruit to fruit, from zero to 100% functionality. In both long and short shoots xylem functionality was considerably greater in fruit from the control treatment than in fruit from the spring defoliation treatment (Fig 5.1). There was no difference in xylem functionality between long and short shoots (Fig 5.1).

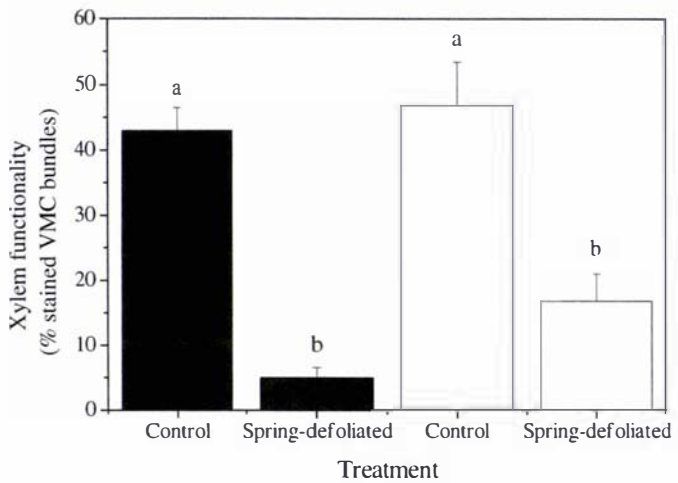


Fig 5.1. Xylem functionality of ventromedian carpellary (VMC) bundles in kiwifruit following spring defoliation of long (black) and short (white) fruiting shoots, as measured on 21 December 2001. Bars labelled with the same letter are not significantly different at P <0.001.

5.3.2. January harvest

Girdling and defoliation reduced fruit Ca concentrations regardless of shoot length (Table 5.2). Regardless of shoot length, spring girdling also reduced fruit K and P concentrations when compared to the control treatment. Defoliation did not affect fruit K and P concentrations, and none of the treatments affected fruit Mg concentrations. There also was no affect of treatments on fruit stalk length and diameter.

In contrast to the December harvest results, spring defoliation tended to reduce fruit fresh and dry weight accumulation, although differences were not always statistically significant. Summer defoliation had no effect on fresh and dry weight accumulation in fruit from long shoots, but reduced fresh weight accumulation in fruit from short shoots. Spring girdling increased fresh, but not dry weight, accumulation in fruit from long shoots. Dry weight accumulation was reduced in short spring-girdled shoots. Fruit DMCs were highest in fruit from the summer defoliation treatment and lowest in fruit from the spring girdling treatments. Spring-defoliation did not affect the DMC of fruit from long shoots, but reduced the DMC of fruit from short shoots.

In every treatment the mean measured value for each fruit attribute, except the mean fruit K and P concentrations, was greater in fruit from long shoots than in fruit from short shoots. The fruit stalk length was significantly greater in fruit from long shoots than in fruit from short shoots in fruit from control and summer-defoliated shoots.

Table 5.2. Several quality attributes of fruit from long, non-terminating (long) and short terminating (short) axillary kiwifruit shoots, as measured on 25 January 2002, following shoot defoliation on 24 November 2001 (SpD) and 5 January 2002 (SmD), or shoot girdling on 24 November 2002 (SpG).

Fruit attribute	LONG				SHORT			
	Control	SpD	SmD	SpG	Control	SpD	SmD	SpG
Ca (mg $100g^{-1}$ FW)	29_a	19_b	21_b	22_b	21_a	13_b	15_b	15_b
Mg (mg $100g^{-1}$ FW)	12_a	14_a	14_a	13_a	11_a	11_a	11_a	10_a
K (mg $100g^{-1}$ FW)	244_a	228_{ab}	239_a	210_b	249_a	226_{ab}	228_{ab}	212_b
P (mg $100g^{-1}$ FW)	34_a	31_a	36_a	20_b	37_a	34_a	38_a	22_b
Fruit stalk length (mm)	63_a	61_a	64_a	66_a	58_a	60_a	60_a	60_a
Fruit stalk diameter (mm)	3.8_a	3.8_a	3.7_a	3.8_a	3.3_a	3.4_a	3.4_a	3.4_a
Fresh weight (g)	66_a	61_b	64_{ab}	75_c	56_a	53_{ab}	51_b	54_{ab}
Dry weight (g)	6.3_a	5.8_b	6.5_a	6.8_a	5.1_a	4.7_b	5.0_{ab}	4.5_b
Water content (g)	59_a	55_b	57_{ab}	68_c	51_a	49_{ab}	46_b	50_a
DMC	9.6_a	9.5_{ac}	10.2_b	9.1_c	9.0_a	8.6_b	9.7_c	8.3_d

For each shoot type (long or short), means within rows followed by the same letter are not significantly different at P <0.05. Highlighted values indicate significant differences between fruit from long and short axillary shoots (P <0.05). Fruit stalk diameters are an average of measurements taken at the proximal, mid and distal ends of the fruit stalk.

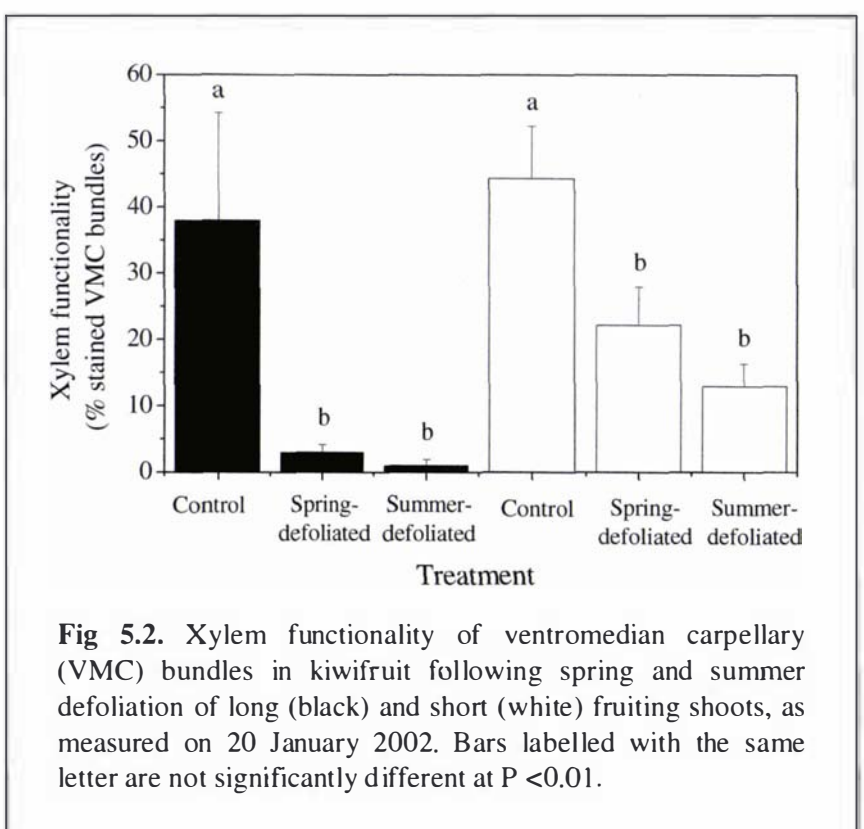


Fig 5.2. Xylem functionality of ventromedian carpellary (VMC) bundles in kiwifruit following spring and summer defoliation of long (black) and short (white) fruiting shoots, as measured on 20 January 2002. Bars labelled with the same letter are not significantly different at P <0.01.

Xylem functionality was considerably lower in fruit from defoliated shoots than in control fruit (Fig 5.2). In each treatment, xylem functionality was lower in fruit from long shoots than in fruit from short shoots, however these differences were never significant due to the large variation found between fruit (ranging from 0-100% functionality).

5.3.3. April harvest

As in previous harvests, fruit Ca concentrations were reduced by shoot defoliation (Table 5.3). Autumn girdling also reduced Ca concentrations in fruit from long shoots. Fruit P concentrations were reduced in short, summer defoliated shoots, but were not affected by treatments in long shoots. Potassium concentrations in fruit from girdled and defoliated shoots never differed from those in control fruit. However, fruit from short, autumn-girdled shoots had a higher K concentration than fruit from short, defoliated and spring-girdled shoots. As in the other harvests, fruit Mg concentrations and fruit stalk length and diameter, did not differ between treatments.

Table 5.3. Several quality attributes of fruit from long, non-terminating (long) and short terminating (short) axillary kiwifruit shoots, as measured on 25 April 2002, following shoot defoliation on 24 November 2001 (SpD) and 5 January 2002 (SmD), or shoot girdling on 24 November 2002 (SpG) and 1 February 2002 (AuG).

Fruit attribute	LONG					SHORT				
	Control	SpD	SmD	SpG	AuG	Control	SpD	SmD	SpG	AuG
Ca (mg $100g^{-1}$ FW)	32_a	19_{bc}	19_b	26_{ac}	22_{bc}	19_a	12_b	13_b	18_a	18_a
Mg (mg $100g^{-1}$ FW)	17_a	15_a	15_a	15_a	18_a	14_a	13_a	13_a	13_a	13_a
K (mg $100g^{-1}$ FW)	297_a	298_a	274_a	262_a	380_a	303_{ab}	261_a	279_a	276_a	345_b
P (mg $100g^{-1}$ FW)	39_a	37_a	33_a	38_a	38_a	41_a	37_{ab}	34_b	42_a	37_{ab}
Fruit stalk length (mm)	68_a	66_a	63_a	68_a	66_a	59_a	60_a	58_a	62_a	60_a
Fruit stalk diameter (mm)	4.1_a	3.8_a	3.9_a	4.0_a	4.1_a	3.5_a	3.5_a	3.5_a	3.6_a	3.5_a
Fresh weight (g)	105_a	91_b	99_c	115_d	111_{ad}	88_{ab}	83_a	86_{ab}	91_b	83_a
Dry weight (g)	17_a	15_b	17_a	20_c	19_c	14_a	13_a	14_a	15_a	12_b
Water content (g)	88_a	76_b	81_b	95_c	91_{ac}	74_{ab}	69_a	73_{ab}	76_b	71_{ab}
DMC	16.5_a	16.6_{ac}	17.5_b	17.5_b	17.4_{bc}	15.9_a	16.2_a	16.0_a	16.4_a	14.2_b

For each shoot type (long or short), means within rows followed by the same letter are not significantly different at $P < 0.05$. Highlighted values indicate significant differences between fruit from long and short axillary shoots ($P < 0.05$). Fruit stalk diameters are an average of measurements taken at the proximal, mid and distal ends of the fruit stalk.

As found in the January harvest, fruit from long girdled shoots generally had the highest FW and water content, while fruit from long defoliated shoots generally had the lowest FW and water content. Dry weight accumulation was reduced in fruit from long, spring-defoliated shoots and increased in fruit from long, girdled shoots. Fruit DW was reduced in short, autumn-girdled shoots. Similarly, the DMC of fruit from short autumn-girdled shoots was lower than that of fruit from the other treatments. The DMC of fruit from long shoots was increased by shoot girdling and summer defoliation treatments. Fruit DMCs were not affected by spring shoot defoliation.

Fruit fresh and dry weights, DM, Ca and Mg concentrations, and fruit stalk lengths and diameters continued to be higher in fruit from long shoots than in fruit from short shoots, regardless of treatment. There was little difference between K and P concentrations of fruit from the two different shoot types.

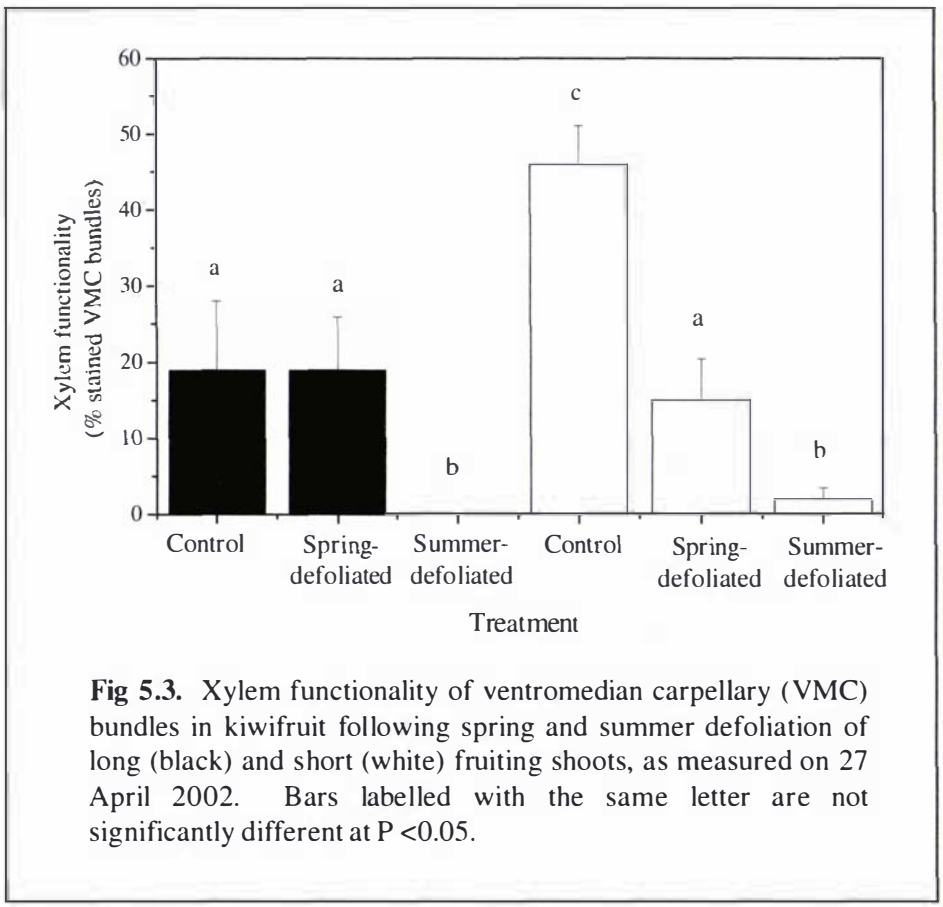


Fig 5.3. Xylem functionality of ventromedian carpellary (VMC) bundles in kiwifruit following spring and summer defoliation of long (black) and short (white) fruiting shoots, as measured on 27 April 2002. Bars labelled with the same letter are not significantly different at $P < 0.05$.

Xylem functionality was lower in fruit from the summer defoliation treatment than in fruit from the spring-defoliated and control treatments. In short shoots, xylem functionality also was lower in spring-defoliated shoots than in control fruit (Fig 5.3). Despite considerable fruit to fruit variability, xylem functionality was still lower in fruit from long-control shoots than in fruit from short-control shoots. Xylem functionality did not differ between long and short shoots from the other treatments. Dye movement into fruit was blocked in the 'brush' of the stalk, which is the

section of pedicel attached to fruit that is slightly wider in diameter than the remainder of the pedicel. There were no anatomical changes in the cells of the brush that might indicate the presence of an abscission zone in the stalk.

5.4. Discussion

5.4.1. Minerals

Calcium and defoliation

Fruit Ca concentrations were markedly reduced by defoliation in both long and short shoots. Shoot defoliation would almost entirely eliminate shoot transpiration, and this may have reduced fruit Ca concentrations by reducing xylem sap movement into the shoot. Moreover, defoliation would prevent fruit Ca concentrations being supplemented with high Ca-containing sap from the leaves as is thought to occur in apple fruit (Lang and Volz, 1998).

It is possible that defoliation also may affect mineral accumulation by upsetting the plant growth regulator (PGR) balance between the shoots and the rest of the plant. Auxins are synthesised in young developing leaves (Bangerth, 1976) that were removed in the defoliation treatments in this experiment. In addition, partial defoliation may have reduced seed numbers and/or seed weight, as has been found in *Aesculus hippocastanum* (horse chesnut) (Thalmann *et al.*, 2003), *Ligustrum vulgare* (Oleaceae) (Obeso and Grubb, 1993), and *Carya* spp. (Juglandaceae) (McCarthy and Quinn, 1992) trees when leaves were removed during early stages of seed development. As auxins are synthesised in seeds, this may reduce auxin efflux from fruit. The basipetal movement of auxin from sites of synthesis towards the roots has been directly linked to Ca accumulation in fruit (De la Fuente, 1984; Wand *et al.*, 1991a). It has been suggested that auxin concentration in fruit indirectly affects mineral accumulation by stimulating vascular differentiation, cell division and cell elongation and regulating sink development, possibly by working in conjunction with other PGRs (Gamburg, 1982; Evans, 1985; Lee *et al.*, 1997a; Aloni *et al.*, 2000, refer Section 1.6 for more information) and this may have occurred in defoliated shoots of kiwifruit. Xylem functionality data clearly showed that hydraulic conductance was reduced by defoliation despite considerable fruit to fruit variability. As auxins are involved in the regulation of vascular development (Aloni *et al.*, 2000), this may be further indirect evidence that auxin is, in some way involved in Ca accumulation in kiwifruit.

Time of defoliation

Summer defoliation reduced fruit Ca concentrations as much as spring defoliation. Within 20 days of applying the summer defoliation treatment fruit Ca concentrations had decreased by 27% compared to the control. In addition, the Ca content of fruit from the summer defoliation treatment decreased by nearly 2mg/fruit between January and April. This was unexpected because by 5 January 2002 (7 WAFB) it was anticipated that about 75% of the Ca would have accumulated in the fruit (Clark and Smith, 1988) and that fruit Ca concentrations would have been reduced only slightly by defoliation at this time. Instead it appears that only about 60% of the Ca had accumulated in fruit by 5 January 2002. This indicates that, contrary to previously published information (Clarkson, 1984; Clark and Smith, 1988; Xiloyannis *et al.*, 2001), significant amounts of Ca were accumulated late in the season despite the decrease in water accumulation in fruit as they mature. An alternative explanation for this unexpected result is that summer defoliation may have caused Ca to be remobilised out of fruit. In apple (Lang, 1990) and grape (Lang and Thorpe, 1989), sap may flow out of fruit along a water potential gradient during periods of high potential evapotranspiration. This reduces the likelihood of leaves becoming water-stressed and photosynthetic efficiency being reduced, but may affect the mineral composition in fruit. However this latter explanation, does not seem likely given that: (a) leaves were removed, so that a water potential gradient between leaves and fruit could not be established, and (b) xylem tissues in defoliated fruit were largely dysfunctional, thereby preventing xylem sap outflow from fruit.

It is likely that summer defoliation has a greater affect on late season Ca accumulation than does spring-defoliation. Xylem functionality tended to be lower in fruit from the summer-defoliated treatment than in fruit from the spring-defoliated treatment, although differences were not always significant. This means that the ratio of phloem: xylem translocation into fruit would be higher in the summer-defoliation treatment than in the spring-defoliation treatment. This imbalance would favour accumulation of phloem mobile nutrients, such as K and P over accumulation of those predominantly transported via the xylem, such as Ca. If phloem translocation were further enhanced to compensate for reductions in xylem translocation, as is suggested in Chapter 4, then this imbalance would be exaggerated. In January, when leaves had not yet started to return on summer defoliated shoots, fruit K and P concentrations and fruit DW were higher, although not always significantly so, in the summer defoliation treatment than in the spring-defoliation treatment. This may be a consequence of this imbalance. Lower xylem functionality in summer-defoliated fruit that in spring-defoliated fruit may be attributed to the larger fruit size of the former, as fruit expansion may be one of the causes of xylem dysfunction (Dichio *et al.*, 2003).

Shoot type response to defoliation

Ca accumulation was not reduced more in fruit from long shoots than in short shoots; this was in contrast to expectations, given the greater xylem dysfunction in fruit from long shoots. It is possible that Ca concentrations in xylem sap may in part be regulated independently of transpiration, and may be higher in long shoots than in short shoots (refer Chapter 9 for more detail). If fruit Ca accumulation were regulated by auxin, then it would be expected that Ca concentrations would be higher in xylem sap from long shoots than in short shoots. This is because fruit on long shoots tend to have more seeds (Lai *et al.*, 1989a) than fruit from short shoots and more leaf regrowth following defoliation, as they continue to extend. Hence more Ca could be expected to be directed towards fruit on long shoots.

It should be noted that dye infiltration methodology only provides information about the number of functioning bundles, and provides no clues as to when they became dysfunctional, or how much they retard xylem flow and therefore, the potential of fruit to accumulate Ca. In grapes, dye also stopped moving into fruit in the brush of the stalk during the later stages of fruit development, but water and Ca continue to accumulate in fruit even when their stalks are steam girdled, albeit at a lower rate (Rogiers *et al.*, 2001). It was suggested that aquaporins located in (grape) berry membranes between the brush and fruit may restrict xylem sap movement into fruit during the later stages of development (Tyerman *et al.*, 2004), but that limited amounts of Ca and water may still move into fruit by non-vascular pathways (Rogiers *et al.*, 2001). While a relationship between xylem functionality and fruit Ca concentrations cannot be assumed, it seems likely to exist given the relationships between xylem functionality and Ca accumulation found by Dichio *et al.*, (2003).

Calcium and girdling

Like defoliation, girdling tended to reduce fruit Ca concentrations. This did not appear to be due to the effects of girdling on fruit size, as large fruit did not always have a lower Ca concentration and small fruit did not always have a higher Ca concentration. This suggests that in addition to travelling in the transpiration stream, there are additional factors that may affect Ca accumulation in kiwifruit. While fruit size is negatively correlated with fruit Ca concentrations in apple (Perring and Jackson, 1975), fruit Ca concentrations also increase as seed number and fruit size increases (Tomala and Dilley, 1990; Buccheri and Di Vaio, 2004), which indicates that fruit auxin concentrations may be more important than fruit size in determining final fruit Ca concentrations.

Girdling may upset the PGR balance in a shoot by restricting PGR translocation into or out of a shoot in phloem associated tissues. Tomala and Dilley (1990) found that phloem girdling two weeks after apple fruit set reduced fruit Ca concentrations and attributed this to the effect of

girdling on auxin translocation in the phloem. Currie (1997) observed that girdling prevented export of auxin out of kiwifruit shoots, resulting in accumulation of auxin above the girdle. If girdles are applied during flowering or early fruit development seed set is reduced (by up to 40%) (Currie, 1997), possibly in response to accumulation of auxin in fruit, so as to prevent further increases in auxin production (Krishnamurthy *et al.*, 1997). These factors will reduce auxin efflux from fruit, which may directly (De la Fuente, 1984; Wand *et al.*, 1991a) or indirectly affect Ca accumulation in fruit by influencing vascular and fruit cell differentiation (Aloni *et al.*, 2000), and fruit metabolism (Lee *et al.*, 1997a).

Girdling may affect fruit Ca concentrations by affecting xylem hydraulic conductivity. Girdling restricts circulation of mineral ions, such as K, between the phloem and xylem, thereby lowering xylem sap osmotic potential (Zwieniecki *et al.*, 2004). Zwieniecki *et al.*, (2001) found that xylem sap ion concentrations influenced xylem hydraulic conductance by controlling the swelling and shrinking of pectins (hydrogels) in bordered pit membranes. When xylem sap ionic concentrations declined in response to girdling, hydrogels become swollen and xylem conductance declined by 15-24% in maple (Zwieniecki *et al.*, 2004). These reductions in hydraulic conductance may restrict movement of minerals such as Ca in the xylem and hence, their accumulation in fruit, especially if the vascular carrying capacity was already reduced in response to accumulation of auxin above the girdle.

Timing of girdling

Just as summer defoliation caused an unexpected reduction in fruit Ca accumulation, autumn girdling reduced fruit Ca accumulation also, even though about 90% of the Ca is thought to have accumulated in fruit by this time (Clark and Smith, 1988). Again, this could indicate that significant quantities of Ca accumulate in fruit during the later stages of fruit development. Alternatively, girdling may enhance Ca mobilisation out of the fruit, since leaves on girdled shoots remained intact, providing the opportunity for a negative water potential gradient to form between fruit and leaves during periods of high potential evapotranspiration.

For this theory to be correct, there must be functional xylem connections between leaves and fruit. Xylem functionality was not measured in fruit from girdled treatments in this experiment. However, data from chapter 8 suggests that girdling is more likely to restrict, rather than inhibit Ca accumulation, as dye infiltration into fruit from girdled shoots was similar to that of control fruit. This may explain why girdling reduced fruit Ca concentrations less than defoliation. Furthermore, it suggests that it is possible for Ca to be remobilised out of kiwifruit, as occurs in apple (Lang, 1990) and grape (Lang and Thorpe, 1989). In spring-girdled shoots starch may accumulate in

leaves, thereby reducing stomatal aperture and preventing the water potential of leaves becoming more negative than that in fruit.

Shoot type response to girdling

Fruit Ca concentrations were generally higher in long girdled shoots than in short girdled shoots. However, differences were not as apparent as for the defoliation treatment. Long shoots have more developing leaves than short shoots, and fruit on long shoots have more seeds. Therefore, there is more potential for auxin to accumulate in long shoots than short shoots. It is possible that girdling had more effect on Ca concentrations in fruit from long shoots, and this may explain why differences between Ca concentration of fruit on long and short girdled shoots declined. However, auxin efflux from long shoots may still be higher than that from short shoots. Consequently, vascular tissues in the stalks of long fruit may be more developed than in stalks of fruit from short shoots and this may increase the flux of Ca-containing xylem sap into the former.

Other minerals

Treatment and shoot-type effects on fruit K and P concentrations were inconsistent, whereas fruit Mg concentrations were never affected by the treatments. This suggests that the factors affecting K, P and Mg accumulation in fruit differ to those affecting fruit Ca accumulation. Smith *et al.*, (1994) investigated within and between vine variation in kiwifruit micro and macro mineral nutrients concentrations and concluded that their spatial variation within the vine could be explained based on mobility in the phloem. Some minerals, including K and P had a higher between vine variation, and a lower between shoot variation than a second group of minerals, which included Ca. The variability of a third group, which included Mg, was between these two groups. This suggests that attributes of individual shoots, such as leaf area, are more important in determining Ca accumulation than they are in accumulation of Mg, K, or P. This conclusion also was reached based on results in Chapter 4.

Fruit concentrations of K and P were reduced by defoliation, but only when it was conducted early in the season. At this stage, significant proportions of these minerals are transported in the xylem (Clark and Smith, 1988; Lang and Ryan, 1994), whereas later in the season these minerals are transported predominantly in the phloem and so would not have been affected by defoliation to the same extent. As both K and P are phloem mobile (Marschner *et al.*, 1997), their concentrations in fruit may have been reduced in response to girdling, which inhibits phloem translocation beyond the girdle and the circulation of ions between the phloem and xylem (Goren *et al.*, 2004; Zwieniecki *et al.*, 2004). The concentration of Mg in kiwifruit xylem sap is generally lower than that of K and P (Peterlunger *et al.*, 1990; Sotiropoulos *et al.*, 2002) and Mg is less mobile than both K and P in both phloem and xylem tissues (Marschner *et al.*, 1997). Therefore Mg may have

been less affected than K and P by practices that manipulate either xylem or phloem translocation. Apple fruit K and Mg concentrations at harvest were not affected by seed number at harvest (Bramlage *et al.*, 1990), which suggests that auxin may have a limited affect on accumulation of these elements in contrast to Ca. As K, P and Mg are mobile in both the xylem and the phloem (Marschner *et al.*, 1997), their accumulation would be less susceptible than Ca accumulation to factors that impact on either xylem or phloem translocation. This would explain why girdling and defoliation had less of an impact on the accumulation of these nutrients than on the accumulation of Ca. Moreover, it may explain why: (a) Mg concentrations did not differ between fruit on long and short shoots, and (b) why K and P concentrations were sometimes higher in fruit from short shoots than in fruit from long shoots, despite these shoots having a lower evapotranspiration rate (B. Dichio and C. Xiloyannis: pers. comm.) and possibly a lower auxin efflux.

5.4.2. Fruit weight and dry matter

Effects of defoliation

Defoliation (spring and summer) had variable effects on fruit DMCs in January and April, which may be related to how the timing of defoliation and fruit position in the canopy affect fresh and DW accumulation. The DW of fruit on summer-defoliated shoots was never different to that of control fruit, whereas in fruit from spring-defoliated shoots DW was often lower than that of control fruit, especially in fruit from long shoots. If carbohydrate supplies are limited during early fruit development, the potential of fruit to attract carbohydrate may be reduced, possibly by reducing phloem differentiation and cell division, so that fruit demand for carbohydrate does not exceed carbohydrate availability. Consequently, fruit from spring-defoliated shoots are probably less able to attract imported assimilate from other parts of the vine than fruit on shoots defoliated after cell division is complete (i.e. summer-defoliated shoots). This problem may be enhanced in the LZ as L:F ratios in this zone tend to be lower than in the FZ, so there may not be a large surplus of carbohydrate available for export into the fruit (Buwalda and Smith, 1990b). Several authors have stressed the importance of having high quantities of carbohydrate available during the early stages of fruit development in order to ensure good size and ripe SSC at eating ripeness (Piller and Meekings, 1997; Piller *et al.*, 1998). This may be because of the need to have high carbohydrate availability to promote seed set and hence, auxin synthesis in fruit. This in turn may affect vascular development in fruit stalks, which will determine subsequent rates of dry and fresh weight accumulation and hence the potential fruit DMC that can be attained.

As water accumulation also was reduced in fruit from spring-defoliated shoots, the DMC of these fruit tended to be the same as that in control fruit. Conversely, the DMC of fruit from summer-defoliated shoots often was higher than that in control fruit, as water accumulation was still reduced, but DW accumulation was not affected. The reduction in water accumulation caused by defoliation may be related to the effects of defoliation on shoot auxin balance and hydraulic conductance (refer Section 5.4.2 for more information).

In December, fruit from spring-defoliated shoots had considerably greater DW (25-28% greater) and FW (31-32% greater) than fruit from control shoots. However, as FW of the fruit increased more than the DW, the DMC of control fruit was 9.5-12.3% higher than that of the defoliated fruit. In December the DMC of all fruit will decrease as they go through exponential growth (Fig 1.7), so the exact fruit DMC may depend on how far through this exponential growth phase the fruit have proceeded. During early stages of fruit growth developing shoots are a stronger sink than fruit (Amano *et al.*, 1998; Piller *et al.*, 1998). Spring defoliation may reduce competition between reproductive and vegetative growth for carbohydrate, causing exponential growth to progress at a faster rate than in control fruit.

Effects of girdling

In January or April, the DW of fruit from spring and autumn-girdled long shoots was up to 15% greater than that of fruit from control shoots. Water content of these fruit was higher than that of control fruit throughout the rest of the season. However, differences between water content of fruit from spring-girdled long and control shoots decreased as the season progressed, so that by January water content of these fruit was only 3-7% greater than in control fruit. Consequently, by harvest the DMC of fruit on long, girdled shoots had shifted from being lower, to being higher than that of fruit on control shoots. The greater decline in water content of fruit from long, girdled shoots, when compared to control shoots may be related to the direct and indirect (by enhancing auxin accumulation in fruit) effects of girdling on xylem conductivity, which might restrict water accumulation in fruit (refer section 5.4.1 (Calcium and girdling) for details).

Fruit on short shoots generally have fewer seeds and may synthesise less auxin than fruit on long shoots that generally have higher seed numbers. Therefore, auxin may be less likely to accumulate in short girdling shoots than in long girdled shoots and xylem conductivity may be less affected. This would explain why the fruit water content was not reduced in fruit from short shoots when compared to in control fruit from long shoots. As DW of fruit from short, girdled shoots was lower than that of control fruit in both January and April (except in fruit from short, spring-girdled shoots whose girdles had grown over by April), and because the water content of fruit from short, girdled and control shoots did not differ, fruit DMCs were reduced in the former (except where the

girdles had healed). As girdling interrupts phloem linkages between a shoot and the rest of the vine, carbohydrates cannot be imported into the shoot if there is insufficient assimilate to support fruit growth. Consequently, fruit DW accumulation is likely to be source limited in short shoots with a low L:F ratio. Conversely, on long shoots with a high L:F ratio, excess carbohydrate can not be exported out of the shoot, so it becomes available to fruit on that shoot (Lai *et al.*, 1989a).

The timing of girdling had little effect on the DMC of fruit from long shoots, however the DMC of fruit on autumn-girdled short shoots was considerably less than in fruit on control shoots, although fruit from spring-girdled short shoots had a similar DMC to controls. In contrast, Currie (1997) found that fruit DMCs were always reduced on fruit from girdled shoots with a low L:F ratio, regardless of girdling date. In his experiments, late girdling of shoots with a high L:F ratio caused less of an increase in fruit FW than early girdling, when compared to a control treatment, but late girdling tended to have more of an impact on fruit DMCs. Fresh weight accumulation also was less when girdling was applied later in the season as in this experiment, and DMCs were increased, but not significantly. Richardson *et al.*, (1997) found that DW increased linearly as fruit matured, but that the rate of increase was higher for fruit from vines with a low crop load than for fruit from vines with a high crop load. This suggests that the potential of a fruit to accumulate carbohydrate is determined in the very early stages of fruit development, and that later in the season a fruit's DMC can only be influenced by manipulating water accumulation. As water accumulation is dependent on many factors that are not necessarily experimentally controlled, such as climate and irrigation (rain), it is not surprising that the results of these experiments differ from those of Currie (1997).

Like spring defoliation, spring girdling also enhanced fruit DW (24-40% greater) and FW (28-41% greater) accumulation in December, but reduced fruit DMCs by 7.7-7.9%. Girdling also may have advanced the exponential growth phase of fruit development by preventing carbohydrate from being exported to developing leaves on other shoots. This would reduce competition between vegetative and reproductive growth. This effect appeared to be more noticeable in fruit from long shoots than in fruit from short shoots, perhaps because there are more developing sinks in the LZ than in the FZ, so there is more competition for carbohydrate between developing vegetative and reproductive sinks.

5.5. Conclusions

In this chapter the relationship between Ca (xylem) and carbohydrate (phloem) accumulation in fruit from long and short shoots was investigated in order to explain the link between fruit Ca and

DM concentrations often found in kiwifruit. It is proposed that whilst similar factors affect Ca and carbohydrate accumulation in fruit, the ways in which these factors influence Ca and carbohydrate accumulation may differ. Consequently, fruit Ca and DM concentrations are often found to be positively correlated, but it would be possible to obtain fruit with a high Ca concentration and a low DMC or vice versa. Results have confirmed some hypotheses and provided new insights into the mechanisms affecting fruit DM and Ca concentrations in long and short shoots, including:

1. Whilst Ca is primarily translocated to fruit in the transpiration stream, evidence from this chapter suggests that Ca translocation can occur independent of water movement in the transpiration stream. It is postulated that auxin efflux from fruit may directly affect fruit Ca accumulation. It also may indirectly affect fruit Ca accumulation by regulating xylem differentiation in the fruit stalk, thereby influencing the: (a) fruit stalk hydraulic conductivity or the ease with which xylem sap can move into fruit, and (b) xylem: phloem ratio in the stalk, and therefore the balance between phloem and xylem translocation into fruit.

2. The above factors will affect DMCs in fruit by influencing fruit water content. It is possible that fruit DMCs are effected by the extent of phloem differentiation in the fruit stalk. This may determine availability of carbohydrate resources for promoting cell division, and therefore, the capacity of fruit to accumulate water, carbohydrates and other solutes.

3. The L:F ratio on a shoot may influence fruit Ca and DM concentrations by: determining the auxin source from leaves and the carbohydrate availability for seed set. Leaves also may influence: (a) Ca accumulation by determining the shoot transpiration rate and the extent of xylem sap translocation between leaves and fruit or vice versa, and (b) carbohydrate accumulation by determining the carbohydrate availability in the vicinity of the fruit for import into fruit. Management practices that alter the carbohydrate availability on a fruiting shoot, or that alter the shoot hormone balance are likely to alter fruit DM and Ca concentrations.

4. DW accumulation appears to be linked to water accumulation. Therefore, the ability to alter fruit DMCs may depend on the ability to manipulate DW accumulation without affecting water accumulation, which may be more likely later in the season when the rate of water accumulation is decreased and when sugars are being compartmentalised as starch.

5. Differences in the response of fruit on long and short shoots to girdling and defoliation may be attributed to differences in the (a) leaf number on these shoots, (b) seed number in fruit on these shoots, (c) composition of Ca and other minerals in the xylem sap of these shoots.

As long shoots tend to be more exposed to direct light and air movement and have a greater leaf area, more seeds, and possibly greater Ca concentrations in their xylem sap, they tended to produce fruit with higher Ca and DM concentrations than short shoots.

6. Substances that travel in significant amounts in both the xylem and phloem, such as K, P and Mg, are less affected by practices that directly impact on either xylem or phloem translocation. However, their accumulation may be affected by management practices that have an impact directly on xylem and phloem translocation (i.e. the combined effects of girdling and defoliation).

7. Significant amounts of Ca may be accumulated in fruit during the second and third phases of fruit development, after cell division has ceased. Similarly, significant amounts of Ca may be remobilised out of fruit during periods of high potential evapotranspiration if xylem vessels in the fruit and stalk remain functional. This accumulation could have a significant affect on the final Ca content attained in a fruit.

8. The osmotic potential of the xylem sap also may have a direct effect on (xylem) hydraulic conductivity and hence fruit DM and Ca concentrations by affecting the fruit water content.

6. Fruit Transpiration, Skin Permeance, Mineral and Carbohydrate Accumulation

6.1. Introduction

It is generally accepted that Ca travels predominantly by mass flow in the xylem transpiration stream (Clarkson, 1984; Marschner, 1995) accumulating in leaves, and other highly transpiring plant organs. Organs with low transpiration rates, such as fruit, are often deficient in Ca and may develop Ca deficiency disorders in storage, including bitter pit and blossom-end rot (Ferguson and Boyd, 2001). In contrast, K, P and to a lesser extent, Mg, are transported in both the phloem and the xylem (Marschner, 1995) and are more evenly distributed amongst the different plant organs than Ca (Buwalda and Smith, 1987).

Mango fruit Ca concentrations are positively correlated with fruit transpiration rates (Shivashankara and Mathai, 1999) and may be reduced by fruit bagging (Beasley *et al.*, 1999), which reduces the driving force for water loss and hence fruit transpiration. Results for other fruit are more equivocal. Rates of fruit transpiration have been reported to affect and have no effect on tomato (Banuelos *et al.*, 1985; Mulholland *et al.*, 2000) and grape (During and Oggionni, 1986; Boselli and Di Vaio, 1996) fruit Ca concentrations. Calcium accumulation in apple was not affected by fruit transpiration (Jones and Samuelson, 1983), yet bagging reduced apple fruit Ca concentrations (Cline and Hanson, 1992). Humidity has no or little affect on K, Mg and P accumulation, despite influencing rates of fruit transpiration (During and Oggionni, 1986; Tromp and Van Vuure, 1993; Combrink *et al.*, 1995; Fallahi *et al.*, 2001).

Discrepancies between these results may relate to the maturity of fruit at the time of assessment. Fruit transpiration, and hence the effect of fruit bagging, has less of an influence on Ca accumulation as mango fruit mature and the rate of Ca accumulation declines (Beasley *et al.*, 1999; Amarante *et al.*, 2002). Effects of bagging also may depend on its duration. It has been suggested that prolonged exposure to high humidity, as would occur in bagged fruit, may induce changes in the cuticle making it more permeable to water, or may cause a plant to utilise alternative pathways to import Ca to fruit that do not rely on rates of fruit transpiration (Hofman *et al.*, 1997). High humidity also may promote Ca accumulation by enhancing vascular differentiation (Li *et al.*, 2004). Bagging also may trap the heat of respiration, which would increase the driving force for water loss and may alter fruit cuticle structure, making it more

permeable to water (Amarante *et al.*, 2002), thereby negating any negative effects of bagging on fruit transpiration.

In kiwifruit, rates of fruit transpiration and Ca accumulation are high initially and drop as the season progresses (Smith *et al.*, 1995; Xiloyannis *et al.*, 2001), suggesting that Ca accumulation may be affected by fruit transpiration. Fruit transpiration rates appear to be linked to structural changes in the epidermal cell layers that alter fruit permeance to water vapour (Smith *et al.*, 1995; Xiloyannis *et al.*, 2001; Hallett and Sutherland, 2005). For example, kiwifruit skin permeance is high initially (100-800 nmol m^{-2} s^{-1} Pa^{-1}), drops rapidly in the first 1-2 weeks after anthesis, then declines more slowly for another eight weeks, after which time it remains relatively constant until harvest (Smith *et al.*, 1995). Around the time when permeance becomes constant, fruit transpiration rates begin to decline and there is little further increase in Ca content of the fruit (Xiloyannis *et al.*, 2001).

This decline in fruit permeance also may be caused by maturation of trichomes (hairs) on the fruit surface. As kiwifruit mature, trichome cells start to die, and may become lignified, so there are no living trichome cells when fruit are mature (Schmid, 1978; White, 1986a). Compared to young trichomes, mature trichomes are impermeable to water (Schmid, 1978), so water may not be lost from their surface. However, like young trichomes, mature trichomes may hinder water loss by trapping a boundary layer of still, humid air around the fruit, which reduces the effective driving force for water loss (D. Green: Unpublished data).

In kiwifruit, differences in trichome density between fruit on a vine may increase the variability in fruit permeance, especially while trichomes are still living and are permeable to water vapour. Water loss is negatively correlated with trichome density and its uniformity in *Wigandia urens* shrubs (Perez-Estrada *et al.*, 2000) and *Tectona grandis* (Teak) trees (Bandyopadhyay *et al.*, 2004). Many trichomes are broken off during kiwifruit development leaving areas of disrupted cells (Hopping, 1976b) that may be more permeable to water than the surrounding epidermal cells. Trichome morphology and ontogeny may be affected by the micro-climate in which a fruit develops, and hence may affect permeance of fruit from different positions within the vine.

It is possible that permeance differences between fruit from long and short axillary shoots may explain the higher transpiration rates and Ca concentrations (Xiloyannis *et al.*, 2001) found in the former. Permeance of apple (Maguire *et al.*, 1999a) and peach (Lescourret *et al.*, 2001) fruit to water vapour differs within a population of fruit from the same tree. These permeance differences may result from differences in the extent of micro-crack development in the fruit epidermis (Maguire *et al.*, 1999b; Lescourret *et al.*, 2001), and/or to physical damage to the skin surface

caused by abrasion of fruit against each other, both of which would increase water loss (Maguire *et al.*, 1999a). In apples, micro-cracks are believed to be caused by uneven rates of development of the epidermal and cuticle cell layers, which may extend and widen as fruit grow (Meyer, 1944). In addition to fruit expansion, development of micro-cracks may be exacerbated by sudden changes in soil moisture, rainfall, high relative humidity, temperature and exposure to sunlight as well as cultural factors such as rootstock, mineral nutrition, chemical sprays, pruning and thinning (Opara *et al.*, 1997).

There was no difference in permeance of kiwifruit from upper and lower canopies (Smith *et al.* (1995). Xiloyannis *et al.* (2003) found small (8%), but consistent vapour pressure deficit differences between fruit on exposed and shaded canes, despite large differences in the rate of sap flow and Ca concentrations of fruit from such canes. This suggests that transpiration rates may not differ much between fruit from different positions in the vine and that Ca accumulation may be more dependent on leaf transpiration, or other factors not related to transpiration. In apples, leaf transpiration has more of an effect than fruit transpiration on fruit Ca accumulation (Jones and Samuelson, 1983).

In this chapter, the effect of fruit transpiration on mineral accumulation in kiwifruit is tested by altering transpiration rates of fruit on the vine. This was done by bagging fruit, to reduce their transpiration, or by placing small fans in front of fruit to enhance their transpiration. Seasonal changes in permeance of fruit on long and short shoots were investigated and compared. A working hypothesis was that early season Ca accumulation is dependent, at least partially, on rate of fruit transpiration and that differences in Ca concentration of fruit on long and short shoots are related to differences in skin permeance to water vapour. Seasonal changes in trichome density of fruit from long and short axillary shoots were compared and attempts were made to determine the effect of trichomes on fruit permeability. In contrast to Ca, it is suggested that K, Mg and P concentrations would not be affected by fan and bag treatments.

6.2. Materials and Methods

6.2.1. The effect of fruit transpiration on mineral and carbohydrate accumulation in fruit from long, non-terminating and short terminating shoots

Four vines growing at the HortResearch Te Puke Orchard in the Bay of Plenty district of New Zealand were selected from the centre of two adjacent rows in the middle of an orchard block. In

each vine, nine long and nine short shoots, carrying at least three flowers, were selected and the flowers were hand-pollinated (27 November 2003). On 5 December 2003, after petal fall, the largest two fruitlets from each shoot were tagged. Tagged fruit on three long shoots and three short shoots were allocated to either the control, fan or bag treatments as described below. Consequently, for each treatment there were 48 fruit, 24 of which came from long shoots and 24 from short shoots. Half of the fruit (1 fruit per shoot; 72 fruit in total or 24 from each treatment) were harvested on 12 January 2003, 46 days after full bloom (DAFB), and the other half were harvested on 20 April 2003, 144 DAFB. The fresh weight (FW), DMC and concentration of K, Mg, Ca and P was determined for each fruit. On one third of the fruit stalks (16 in total per treatment, eight from fruit on long shoots and eight from fruit on short shoots), external and vascular dimensions and estimated secondary xylem conductance were measured as described in the general methods (Chapter 2). Fruit dry weight (DW) and water contents (g) were deduced from FW and DMC measurements.

Treatments

Fig 6.1. Fan treatment design

Fan treatment

On 5 December 2002, immediately after petal fall when fruit Ca concentrations start to rapidly increase (Clark and Smith, 1988), 48 fans (55 mm diameter, 12V DC) were suspended in the canopy, 10 cm away from 24 fruitlets on long shoots and 24 fruitlets on short shoots, using 1.6 mm diameter pieces of wire so that they pointed directly at the fruitlet (Fig 6.1). Fans ran for 24 h a day and were regularly checked (to ensure that they remained functional for the time that they were suspended in the canopy, and that they remained in their set positions). Fans were removed from the canopy on 22 January 2003, shortly after the initial fruit harvest. It was decided to remove the fans at this time (exactly eight weeks after anthesis), as it coincided with the completion of cell division after which time very little Ca is thought to accumulate in the fruit (Ferguson, 1980; Clark and Smith, 1988; Xiloyannis *et al.*, 2001) and because the power supply was unavailable from the end of January until harvest. As a preliminary test to evaluate this technique, detached fruitlets (approximately 10g in weight) were hung by their fruit stalks for 2 h in front of these fans, in a laboratory at approximately 17°C and

in front of these fans, in a laboratory at approximately 17°C and 75% RH. On average they lost 8% more weight than detached fruitlets held in the laboratory away from the fans.

Bag treatment

On the same day that fans were suspended in the canopy, 24 fruitlets (12 each from 12 different long and 12 different short shoots) were bagged using 140 x 180 mm light grey paper bags with a red inner surface (Kobayashi Bag Mfg. Co., Ltd, Japan), and 24 fruitlets (12 each from 12 different long and 12 different short shoots) were bagged using very fine gauze bags (200 x 200 mm in diameter), secured over the fruit with a twist tie. A small section was chopped off the corner of the paper bags to allow water drainage. These bags were used as it was thought that the paper bags might reduce gas exchange in the fruit, causing them to abscise, but would be more effective at reducing fruit transpiration, whilst the gauze bags might have been too porous to have any significant effect on fruit transpiration, especially in windy conditions. Bags were kept on the fruit until they were harvested. When the bags were evaluated on young, detached fruitlets in the laboratory (conditions as for the fan trial), the RH in the bags increased by 3-8%, compared to ambient air, after the fruitlets had been bagged for 2 h.

Control treatment

No fans or bags were used.

Statistical analysis

This experiment was analysed as a 3 (treatments) x2 (shoot type) factorial design using ANOVA GLM as described in the general methods (Chapter 2).

6.2.2. Effect of shoot type on fruit permeance

Mature kiwifruit vines growing at the HortResearch Te Puke Research Orchard in the Bay of Plenty district of New Zealand were used in this experiment. Average daily temperature during the experiment was 16.3°C, and ranged from 8.3-24.7°C. Vines were growing on open-pollinated 'Bruno' seedling rootstocks and were managed for commercial production on a pergola-trained system. Fruit were harvested from long and short axillary shoots at intervals from 8 December 2003 (14 days after full bloom) until harvest on 10 May 2004; the time between sampling periods ranging from 2-7 weeks depending on stage of fruit development. At each harvest 18 fruit were randomly selected, from each shoot type, from within a bay taking care not to select fruit from vines on the bay ends. Fruit were harvested with pedicels attached and immediately placed on cotton-wool pads in single layer trays within polyliners awaiting assessment later that day. For

each shoot type, ten fruit were used to assess fruit permeance, five were used to calculate surface area, and three to determine trichome density.

Fruit permeance

Weight changes due to water loss, and respiration (carbon loss) were determined and permeance values calculated for individual fruit. Fruit respiration rates were determined before and after measurements of total weight loss and an average of these values was used to determine the respiratory weight loss.

Respiratory loss

Pedicels of each fruit were removed and fruit were weighed before covering the picking scar (where the pedicel attaches to the fruit) with a thin layer of petroleum jelly to prevent water loss, and the fruit were re-weighed. Fruit were placed in sealed plastic containers, of known volume, that had been flushed with pure oxygen and left at 20°C for 1-4 h before their respiration rates were determined by taking a 1 ml sample from the headspace, and injecting into an Infrared CO_2 Analyser (Servomex, UK). Output was recorded on a Hewlett Packard 3395 Intergrator (Agilent Technologies, Palo Alto, California) calibrated against standard CO_2. The volume of CO_2 in the headspace was calculated based on the assumption that 1 g fruit tissue was equivalent to 1 cm^3. Respiratory weight loss was calculated using standard formulae (Banks *et al.*, 1995; Yearsley *et al.*, 1996):

$$m(CO_2) = \left(\frac{V \times P}{R \times T}\right) \times mw \qquad (6.1)$$

$$C = \left(\frac{m(CO_2)}{M \times t}\right) \times \frac{3}{8} \qquad (6.2)$$

Where:

$m(CO_2)$ = Mass CO_2 produced per fruit (kg)

V = Volume headspace (m^3)

P = Atmospheric pressure (Pa)

R = Universal gas constant (J mol^{-1} K^{-1})

T = Temperature (K)

mw = Molecular weight CO_2

C = Carbon loss (kg kg^{-1} h^{-1})

M = Mass of fruit (kg)

t = Time (h)

Water loss and calculation of permeance

After fruit respiration rates had been calculated for the first time, fruit were re-weighed and immediately arranged on two trays that fitted inside a 20 L plastic bucket around PVC tubing (65mm inside diameter, 200mm height) held in the bucket centre. This extended from the bucket's base beyond the height of the second tray, leaving a 70 mm margin between the top of the tube and the bucket lid. A 12V DC fan, 55 mm in diameter placed in the bottom of the tube ensured air was circulated around the bucket. This fan was connected to a power supply via a small hole drilled in the side of the bucket at its base. The bucket was sealed with an air-tight lid, and air was passed through a saturated $Mg(NO_3)_2$ solution and into the bucket via an inlet valve in the lid, and out again via an outlet tube in the lid. In addition to bubbling air through a $Mg(NO_3)_2$ solution and into the bucket, two small containers (45 mm high x 65 mm wide) were half-filled with this solution and placed on each tray. Humidity inside the bucket was monitored with a humidity sensor inserted in a small hole in the centre of the bucket lid. Two buckets were used for the experiment and they were placed in a temperature-controlled room at 20°C ± 0.5°C. Using this system, humidity in the bucket was maintained at around 60% RH (± 6% RH at 20°C). Each bucket contained fruit from both shoot types, five fruit from one shoot type being placed on the top tray in the first bucket and on the bottom tray in the second bucket.

After 5-24 h, depending on stage of fruit maturity, fruit were re-weighed and respiration rates measured again as described above. Rate of water loss (r'_{H_2O}) was calculated as the difference between the total weight loss whilst the fruit were in the bucket, and the average respiratory weight loss, and fruit permeance values were determined for each fruit using the steady state solution of Fick's first law of diffusion (Equation 6.3: Nobel, 1991):

$$P'_{H_2O} = \frac{r'_{H_2O}}{\Delta p_{H_2O} SA} \qquad (6.3)$$

Where:

P'_{H_2O} = Ease with which water can escape from the fruit or the effective permeance of the fruit surface to movement of water vapour under prevailing conditions (nmol m^{-2} s^{-1} Pa^{-1}).

r'_{H_2O} = Rate of water loss (mol s^{-1})

Δp_{H_2O} = Difference in partial pressure of water vapour between the environment and inside the fruit, (Pa)

SA = Surface area (m^2)

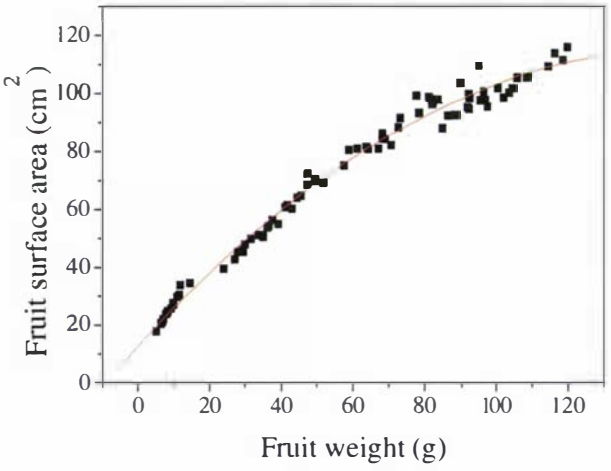


Fig 6.2. Relationship between fruit weight and surface area, calculated using insulation tape, as an estimate of surface area. R^2 = 0.988. $Y = 12.745+1.354X-0.0045X^2$.

Calculation of fruit surface area

Ten fruit (five from each shoot type) were selected to represent the range in size of fruit used for permeance assessment. Fruit stalks were removed and fruit were weighed before being covered in adhesive tape. Tape was then peeled from the fruit and the area of tape required to cover the fruit, which corresponded to the fruit surface area (Banks, 1985), was estimated using Photoshop® 7.0 software (Adobe®, California). Fruit surface area was plotted against weight and the equation of the line calculated to determine accurately (Fig 6.2) the surface area of the remaining 20 fruit used for permeance assessments. This method was accurate regardless of fruit size and shape, which changed from spherical to ellipsoidal shape as fruit matured. Mathematical approaches to calculating surface area also were evaluated based on the assumption that the fruit resembled a shape or shapes (spheres, cylinders, ellipses and/or hemispheres, Baten and Marshall, 1943; Galbreath, 1976; Clayton *et al.*, 1995) from which surface areas could be calculated mathematically. However, a function could not be found that accurately determined surface area regardless of fruit size and/or shape. Depending on stage of fruit development these estimates were 3-74% higher than those estimated using the equation presented in Fig 6.2.

Trichome density

Following permeance assessment, the number of trichomes was determined for three fruit each from the two different shoot types (six fruit in total). For each of these fruit, three 1 mm^2 areas were selected from the proximal, distal and mid-sections of the fruit and the number of hairs in these areas was calculated (18 areas in total) using a Leica MZ FLIII stereomicroscope (Leica Microscopy Systems Ltd., Heerbrugg, Switzerland). Within each of these areas a trichome was randomly selected (18 trichomes in total) and its surface area was calculated, on the assumption that it resembled a cone shape, using the following equation:

$$SA = \pi r\left(\frac{1}{2}r + \left(r^2 + h^2\right)\right) \qquad (6.4)$$

Where:

SA = surface area (of a cone)

r = trichome diameter at the base (widest part)

h = trichome length

The trichome density (SA mm^{-2}) was determined by multiplying the number of trichomes per square millimetre by the average trichome area. This value was then multiplied by the fruit SA to calculate an 'adjusted' fruit SA.

Trichomes and fruit permeance

To evaluate the effect trichomes have on fruit permeability, unsuccessful attempts were made to remove trichomes from the fruit surface by shaving or cutting trichomes at their bases with a small pair of nail scissors. Following trichome removal it was planned to repeat the above procedure with hairless or partially hairy fruit, and compare permeance values with fruit that had not been shaved or clipped. However, trichomes could not be removed completely without damaging the epidermal surface of the fruit (as shown by the appearance of discolouration on the epidermal cell surfaces). This meant that any comparisons undertaken would have been between damaged and non-damaged fruit, rather than between fruit with and without trichomes, so this idea was abandoned. Therefore, instead of direct permeance measurements, the surface area was adjusted by allowing for the additional surface area of the trichomes (see previous), and re-calculating permeance values.

Statistical analysis

Mean differences in permeance, SA, trichome density or respiration rate measurements between shoot types or assessment times were determined using one-way ANOVA (Chapter 2). Individual curves (exponential for the permeance data and asymptotic for the SA data) were fitted to the data for each shoot type and a total error sums of squares ($error_{SSC}$) was calculated. In addition, data for fruit from long and short shoots were combined and a single (common) curve was fitted to the data and a total common $error_{SSC}$ was calculated. In order to test the null hypothesis (H_o) that the individual curves (fitted to data for long and short shoots) differed significantly, and therefore, that the variation in data could not adequately be described by one curve alone, the following equation was used and its significance tested using the f-stat:

$$H_o = \frac{\text{Total error}_{SS}\ (\text{common}) - \text{Total error}_{SS}\ (\text{two curves})}{\Delta\ \text{parameter number}} \times \frac{df_{error}}{\text{Total error}_{SS}\ (\text{two curves})} \qquad (6.5)$$

Where:

Δ parameter number = reduction in the number of parameters achieved by using one curve, instead of two curves to describe the data.

df_{error} = Error degrees of freedom.

6.3. Results

6.3.1. The effect of fruit transpiration on mineral and carbohydrate accumulation in fruit from long, non-terminating and short terminating shoots

Fruit bagged in paper bags were pale green in colour, indicating that chlorophyll synthesis had been adversely affected by bagging. However, other fruit and fruit stalk attributes did not differ significantly between fruit in the two different kinds of bags, and therefore results for these attributes were combined.

January-harvested fruit

Fruit attributes

Fans reduced the FW and water content of fruit on short shoots when compared to fruit from the bag and control treatments (Table 6.1). In short shoots, fruit from the fan treatment also had a lower DMC than fruit from the control treatment. Treatments did not affect the fresh or dry weight, and hence DMC, of fruit from long shoots. Fruit mineral composition was more affected by treatments in fruit from long than in fruit from short axillary shoots. In fruit from long shoots, Ca and Mg concentrations were approximately 30 and 13% higher, respectively in fruit from the fan treatment than the bag treatment (on both a FW and a DW basis). Fruit Ca contents also were 5-6 mg (29-33%) higher per fruit in fruit from the fan and control treatments than in bagged fruit. Fruit P concentrations and content showed the opposite response to Ca, and was higher in bagged fruit than in fruit from the fan and control treatments. This was especially noticeable in fruit from short shoots. Treatment effects on fruit K concentrations were inconsistent between shoot types.

Fans tended to enhance fruit attribute differences between fruit from long and short shoots, whereas fruit bagging tended to reduce differences, when compared to the control treatment (Table 6.1). Fan treatments increased both fresh and dry weights of fruit from long shoots when compared to fruit from short shoots, and as a consequence there was no difference in the DMC of fruit from these two shoot types. The fan treatment also increased fruit Ca concentrations and the

content of all minerals, except P, in fruit from long shoots relative to that of fruit from short shoots. Fruit Ca content also was greater in control fruit from long shoots than in control fruit from short shoots. Control treatments increased fruit K and P concentrations, and the content of P, in short shoots compared to that in long shoots. Bagged fruit from short shoots also had a higher P concentration than fruit from long shoots.

Table 6.1. Attributes of kiwifruit from long and short axillary shoots harvested on 12 January 2003 (46 DAFB) following control, fan and bag treatments applied after petal fall.

Fruit attribute	Long shoots			Short shoots		
	Control	Fan	Bag	Control	Fan	Bag
Fresh weight (g)	43 $_{a}$	41 $_{a}$	40 $_{a}$	40 $_{a}$	34 $_{b}$	40 $_{a}$
Dry weight (g)	2.8 $_{a}$	2.7 $_{a}$	2.6 $_{a}$	2.5 $_{a}$	2.2 $_{a}$	2.6 $_{a}$
Water content (g)	40 $_{a}$	38 $_{a}$	37 $_{a}$	37 $_{a}$	31 $_{b}$	37 $_{a}$
DMC	6.5 $_{a}$	6.7 $_{a}$	6.6 $_{a}$	6.4 $_{a}$	6.7 $_{b}$	6.6 $_{ab}$
Ca (mg 100g^{-1} FW)	40 $_{ab}$	43 $_{a}$	30 $_{b}$	33 $_{a}$	31 $_{a}$	28 $_{a}$
Mg (mg 100g^{-1} FW)	8.4 $_{ab}$	9.0 $_{a}$	7.9 $_{b}$	8.7 $_{a}$	8.4 $_{a}$	8.0 $_{a}$
K (mg 100g^{-1} FW)	183 $_{a}$	200 $_{b}$	188 $_{ab}$	199 $_{a}$	196 $_{a}$	187 $_{a}$
P (mg 100g^{-1} FW)	19 $_{a}$	21 $_{ab}$	24 $_{b}$	25 $_{ab}$	24 $_{a}$	28 $_{b}$
Ca (mg 1g^{-1} DW)	6.2 $_{a}$	6.5 $_{a}$	4.5 $_{b}$	5.2 $_{a}$	4.6 $_{a}$	4.2 $_{a}$
Mg (mg 1g^{-1} DW)	1.3 $_{ab}$	1.4 $_{a}$	1.2 $_{b}$	1.4 $_{a}$	1.3 $_{a}$	1.2 $_{a}$
K (mg 1g^{-1} DW)	28 $_{a}$	30 $_{a}$	29 $_{a}$	31 $_{a}$	29 $_{b}$	28 $_{ab}$
P (mg 1g^{-1} DW)	2.8 $_{a}$	3.2 $_{b}$	3.7 $_{b}$	3.9 $_{ab}$	3.5 $_{a}$	4.2 $_{b}$
Ca content (mg)	17 $_{a}$	18 $_{a}$	12 $_{b}$	13 $_{a}$	11 $_{a}$	11 $_{a}$
Mg content (mg)	3.6 $_{a}$	3.7 $_{a}$	3.1 $_{a}$	3.5 $_{a}$	2.9 $_{a}$	3.2 $_{a}$
K content (mg)	79 $_{a}$	81 $_{a}$	74 $_{a}$	79 $_{a}$	66 $_{a}$	74 $_{a}$
P content (mg)	8.0 $_{a}$	8.7 $_{a}$	9.6 $_{a}$	9.8 $_{a}$	7.8 $_{b}$	10.9 $_{a}$

In each row, for each shoot type, means followed by the same letter are not significantly different at $P < 0.05$. Within a treatment, highlighted values indicate a significant difference between fruit from long and short axillary shoots ($P < 0.05$).

Fruit stalk attributes

Treatments had no effect on the vascular characteristics of fruit on long shoots (Table 6.2). Phloem and xylem areas and estimated secondary xylem conductance were reduced by the fan treatment in fruit stalks of fruit from short shoots.

The fan treatment enhanced fruit stalk differences between fruit from long and short shoots, whereas fruit bagging reduced differences between fruit from these shoot types relative to the control treatment (Table 6.2). Fans increased the xylem and phloem areas and estimated secondary xylem conductance and vessel number in fruit from long shoots relative to that in fruit from short shoots. In control fruit only the pith and xylem areas differed between fruit from these two shoot types.

Table 6.2. Attributes of fruit stalks from fruit on long and short axillary shoots harvested on 12 January 2003 (46 DAFB) following the application of control, fan and bag treatments at petal fall.

Fruit attribute	Long			Short		
	Control	Fan	Bag	Control	Fan	Bag
Fruit stalk length (mm)	66 $_a$	66 $_a$	68 $_a$	58 $_a$	61 $_a$	62 $_a$
Fruit stalk diameter (mm)	3.34 $_a$	3.36 $_a$	3.18 $_a$	3.20 $_a$	3.02 $_a$	3.27 $_a$
Pith area (mm^2)	0.32 $_a$	0.28 $_a$	0.27 $_a$	0.21 $_a$	0.23 $_a$	0.21 $_a$
Xylem area (mm^2)	1.35 $_a$	1.39 $_a$	1.20 $_a$	1.08 $_a$	0.80 $_b$	0.98 $_{ab}$
Phloem area (mm^2)	1.20 $_a$	1.12 $_a$	1.14 $_a$	1.14 $_a$	0.85 $_b$	1.08 $_{ab}$
Secondary xylem vessel number	41 $_a$	40 $_a$	35 $_a$	35 $_a$	24 $_a$	29 $_a$
Estimated secondary xylem conductance (arbitary units)	47 $_a$	56 $_a$	33 $_a$	35 $_a$	6 $_b$	24 $_a$

In each row, for each shoot type, means followed by the same letter are not significantly different at $P < 0.05$. Within a treatment, highlighted values indicate a significant difference between fruit from long and short axillary shoots ($P < 0.05$). Fruit stalk diameter values are an average of measurements taken at the fruit, mid and shoot ends of the stalk.

April-harvested fruit

Fruit attributes

No effect of the fan treatment on fruit quality attributes was sustained until the April harvest following fan removal from the canopy in January (Table 6.3), and many effects of bagging observed in January were not noticeable in April. In fruit from long shoots, fruit Mg concentrations (g^{-1} DW) were greater in the fan treatment than the bag treatment, but Mg concentrations were not effected when considered on a FW basis. In fruit from short shoots, the DMC was greater in fruit from the control treatment than in bagged fruit, as DW accumulation was reduced by bagging. In these shoots bagging also reduced the concentration and content of Ca relative to that in fruit from control and fan treatments. Between January and April Ca

concentrations were reduced by up to 47% and Ca contents were increased by up to 32%, in fruit on long shoots from the control and fan treatments. This compares to a 23% reduction in the Ca concentration of bagged fruit on long shoots and a 48% increase in fruit Ca content.

In contrast to January, the bag treatment enhanced fruit attribute differences between fruit from long and short shoots, whereas the fan treatment had little effect, when compared to a control treatment (Table 6.3). The DMC was greater in fruit from long shoots than in fruit from short shoots in both the control and bag treatments. The bag treatment also enhanced Ca concentration and content, FW and DW of fruit from long shoots when compared to those from short shoots.

Table 6.3. Attributes of kiwifruit from long and short axillary shoots harvested on 20 April 2003 (144 DAFB) following the application of control, fan and bag treatments at petal fall.

Fruit attribute	Long			Short		
	Control	Fan	Bag	Control	Fan	Bag
Fresh weight (g)	94 $_{a}$	93 $_{a}$	98 $_{a}$	89 $_{a}$	85 $_{a}$	80 $_{a}$
Dry weight (g)	14 $_{a}$	14 $_{a}$	15 $_{a}$	13 $_{a}$	12 $_{a}$	11 $_{a}$
Water content (g)	80 $_{a}$	79 $_{a}$	84 $_{a}$	76 $_{a}$	72 $_{a}$	69 $_{a}$
DMC	15.4 $_{a}$	14.7 $_{a}$	14.9 $_{a}$	14.8 $_{a}$	14.3 $_{ab}$	13.9 $_{b}$
Ca (mg $100g^{-1}$ FW)	27 $_{a}$	23 $_{a}$	23 $_{a}$	24 $_{a}$	24 $_{a}$	14 $_{b}$
Mg (mg $100g^{-1}$ FW)	11 $_{a}$	12 $_{a}$	10 $_{a}$	11 $_{a}$	12 $_{a}$	10 $_{a}$
K (mg $100g^{-1}$ FW)	260 $_{a}$	277 $_{a}$	233 $_{a}$	289 $_{a}$	287 $_{a}$	302 $_{a}$
P (mg $100g^{-1}$ FW)	31 $_{a}$	34 $_{a}$	33 $_{a}$	35 $_{a}$	36 $_{a}$	34 $_{a}$
Ca (mg $1g^{-1}$ DW)	1.8 $_{a}$	1.6 $_{a}$	1.6 $_{a}$	1.6 $_{a}$	1.6 $_{a}$	1.0 $_{b}$
Mg (mg $1g^{-1}$ DW)	0.71 $_{ab}$	0.82 $_{a}$	0.67 $_{b}$	0.77 $_{a}$	0.84 $_{a}$	0.73 $_{a}$
K (mg $1g^{-1}$ DW)	17 $_{a}$	19 $_{a}$	16 $_{a}$	19 $_{a}$	20 $_{a}$	22 $_{a}$
P (mg $1g^{-1}$ DW)	2.0 $_{a}$	2.3 $_{a}$	2.2 $_{a}$	2.3 $_{a}$	2.5 $_{a}$	2.5 $_{a}$
Ca content (mg)	25 $_{a}$	21 $_{a}$	23 $_{a}$	22 $_{a}$	20 $_{a}$	12 $_{b}$
Mg content (mg)	9.9 $_{a}$	11 $_{a}$	9.4 $_{a}$	10 $_{a}$	9.9 $_{a}$	8.4 $_{a}$
K content (mg)	236 $_{a}$	259 $_{a}$	225 $_{a}$	266 $_{a}$	241 $_{a}$	250 $_{a}$
P content (mg)	28 $_{a}$	32 $_{a}$	32 $_{a}$	32 $_{a}$	30 $_{a}$	28 $_{a}$

In each row, for each shoot type, means followed by the same letter are not significantly different at $P < 0.05$. Within a treatment, highlighted values indicate a significant difference between fruit from long and short axillary shoots ($P < 0.05$). Fans were removed from vines on 22 January 2003.

Fruit stalk attributes

In fruit from long shoots, fruit bagging increased average fruit stalk diameters relative to those observed in control fruit (Table 6.4). In fruit from short shoots, fruit stalk length was enhanced, but xylem area, secondary xylem vessel number and estimated xylem conductance were reduced, by bagging compared to in control fruit. Fruit stalk diameters were greater in fruit from long shoots than in fruit from short shoots from the fan and bag treatments (Table 6.4). The bag treatment also increased the xylem area and secondary xylem vessel number in long fruit when compared to that in short fruit. Fruit stalk length was greater in control and fan fruit from long shoots than in fruit from short shoots.

Table 6.4. Attributes of fruit stalks from fruit on long and short axillary shoots harvested on 20 April 2003 (144 DAFB) following the application of control, fan and bag treatments at petal fall.

Fruit attribute	Long			Short		
	Control	Fan	Bag	Control	Fan	Bag
Fruit stalk length (mm)	64 $_{a}$	68 $_{a}$	63 $_{a}$	55 $_{a}$	59 $_{a}$	66 $_{b}$
Fruit stalk diameter (mm)	3.45 $_{a}$	3.56 $_{ab}$	3.77 $_{b}$	3.61 $_{a}$	3.22 $_{a}$	3.25 $_{a}$
Pith area (mm^2)	0.30 $_{a}$	0.31 $_{a}$	0.32 $_{a}$	0.25 $_{a}$	0.28 $_{a}$	0.23 $_{a}$
Xylem area (mm^2)	1.44 $_{a}$	1.29 $_{a}$	1.43 $_{a}$	1.26 $_{a}$	1.31 $_{a}$	0.97 $_{b}$
Phloem area (mm^2)	1.52 $_{a}$	1.48 $_{a}$	1.52 $_{a}$	1.55 $_{a}$	1.44 $_{a}$	1.33 $_{a}$
Secondary xylem vessel number	36 $_{a}$	30 $_{a}$	37 $_{a}$	43 $_{a}$	37 $_{ab}$	21 $_{b}$
Estimated secondary xylem conductance (arbitary units)	23 $_{a}$	22 $_{a}$	20 $_{a}$	32 $_{a}$	30 $_{a}$	10 $_{b}$

In each row, for each shoot type, means followed by the same letter are not significantly different at $P < 0.05$. Within a treatment, highlighted values indicate a significant difference between fruit from long and short axillary shoots ($P < 0.05$). Fruit stalk diameter values are an average of measurements taken at the fruit, mid and shoot ends of the stalk. Fans were removed from vines on 22 January 2003.

6.3.2. Effect of shoot type on fruit permeance

Fruit permeance and surface area

Fruit surface area (SA) increased asymptotically from 8-89 DAFB, after which time it increased only slightly (Fig 6.3). Two curves were better able to explain variation in the data than one curve ($P \leq 0.05$), indicating that fruit from long shoots consistently had a greater SA than fruit from short shoots. However, when specific comparisons were made at each sampling period, differences

between fruit from the two different shoot types were only significant from 63 DAFB. From this time until harvest, SA of fruit from long axillary shoots was, on average, 9 cm^2 greater than that of fruit from short shoots ($P \leq 0.05$).

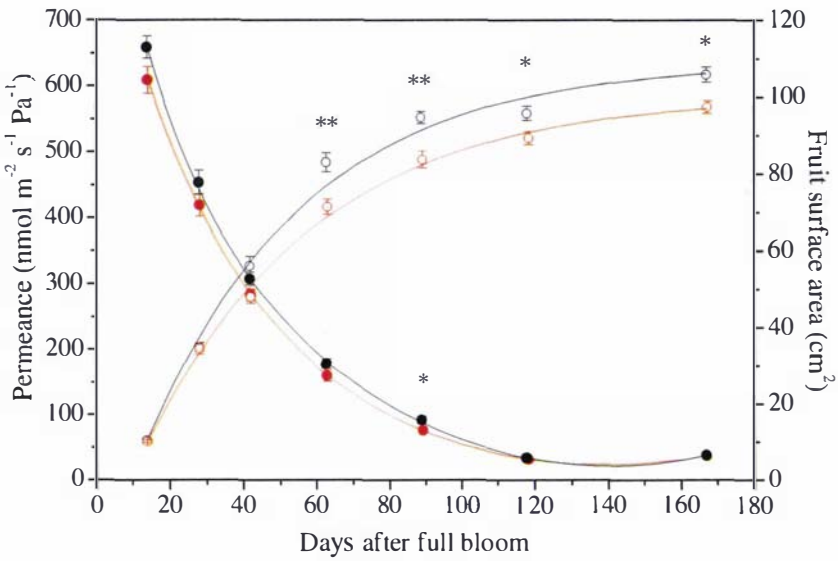


Fig 6.3. Seasonal changes in permeance (closed symbols) and surface area (open symbols) of fruit from long (black) and short (red) axillary shoots. Data points are mean values (± s.e.). Stars indicate significant differences between shoot types at * $P < 0.05$, ** $P < 0.01$. Values are adjusted to account for respiratory weight loss.

In contrast to SA, fruit permeance decreased exponentially from early December (eight days after full bloom (DAFB)) until late March (118 DAFB) and remained steady thereafter (Fig 6.3). Two curves fitted to the data for fruit from long and short shoots were better able to explain seasonal changes in permeance than one curve ($P \leq 0.01$). Over the entire season permence of fruit from long shoots was significantly greater than that of fruit from short shoots. However, when permeance values for fruit from these two shoot types were compared at each sampling period, differences were only significant at 89 DAFB. Permeance differences between fruit from the two shoot types, and variation in permeance of individual fruit, tended to decrease as the season progressed, until harvest when the permeance of all fruit was virtually identical. When surface area data were plotted against permeance data for each shoot type, two significantly different lines were obtained (Fig 6.4: $P \leq 0.01$). For each 10 cm^2 increase in SA, there was a decrease of 68 and 65 nmol m^{-2} s^{-1} Pa^{-1} in permeance for fruit from short and long shoots, respectively.

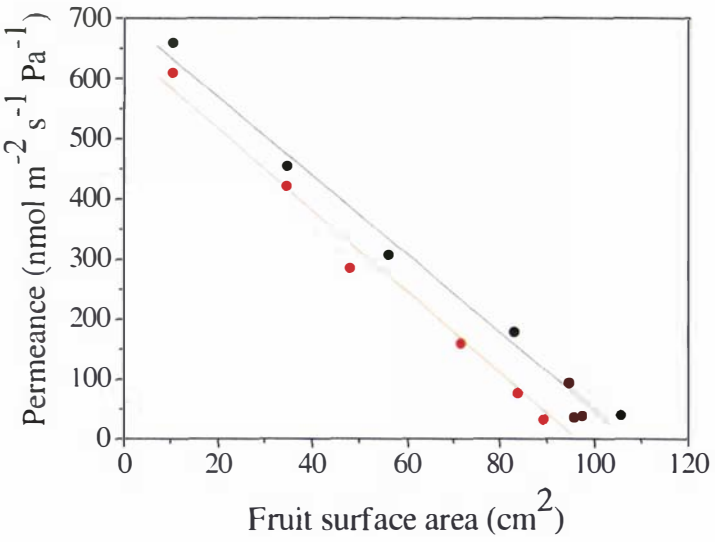


Fig 6.4. Relationship between permeance and surface area of fruit from long (black) and short (red) shoots. Data points are average values.

Trichome density decreased rapidly as fruit surface area increased (Fig 6.5). This was due to a decrease in the number of trichomes per square millimetre as the fruit expanded (i.e. increased distance between trichomes) rather than to any change in the surface area of each hair. There was no difference in the number of trichomes or surface area of individual trichomes, and hence trichome density between fruit from long and short axillary shoots, and therefore data for these two shoot types were combined.

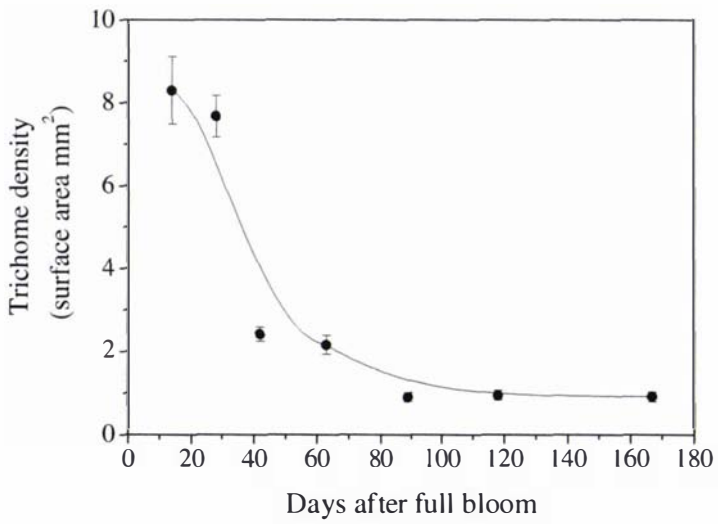


Fig 6.5. Trichome density in 'Hayward' kiwifruit as measured at intervals from flowering. Data points are mean values (± s.e.) for the combined data of fruit from long and short shoots.

When permeance values were re-calculated after 'adjusting' fruit SA to account for the increased area added by the trichomes, permeance values were reduced by more than 90% in fruit from the first few sampling times, but there was almost no effect on permeance of fruit from the later sampling times, as trichome surface area per square millimetre decreased and became insignificant compared to the increase in fruit SA. Since skin resistance to water loss increased as the season progressed, as indicated by the decline

in fruit permeance, it is clear that simple calculations adjusting permeance according to trichome attributes are not valid and are probably based on an incorrect assumption as to the permeance of trichomes *per se*.

Fruit respiration

The proportion of total weight loss due to respiration increased slightly as the season progressed in both fruit from long and short shoots, but never accounted for more than 4% of the total weight loss (data not shown).

6.4. Discussion

6.4.1. Mineral accumulation and fruit transpiration

Fruit Ca contents and concentrations were affected by treatments in both January- and April-harvested fruit, whereas treatment effects on K, Mg and P concentrations were inconsistent between harvests. As all of these minerals, except Ca, are phloem mobile it is likely that the treatments influenced fruit transpiration rates, and hence xylem translocation of mineral nutrients towards the fruit. If it is assumed that fans enhanced fruit transpiration and bags reduced fruit transpiration in both the laboratory and the orchard, then the finding that Ca concentrations, and to a lesser extent Mg concentrations, were highest in fruit from the fan treatment and lowest in the bag treatment, reinforces this theory. Fruit DW was never affected by treatments, which suggests that phloem transport was little affected by treatments. Therefore, K, Mg and P could have still been transported to fruit via the phloem, to compensate for any decline in translocation in the xylem sap, especially later in the season when water is thought to travel predominantly via the phloem (Ferguson, 1980).

At the January harvest, treatments affected fruit K, Mg and P accumulation, but there was no effect of treatments on these minerals at the April harvest. In January, patterns of Mg accumulation in fruit from long shoots were similar to those for Ca, in that concentrations were highest in fruit from the fan treatment and lowest in fruit from the bag treatment, which suggests that fans enhanced fruit transpiration and hence Ca and Mg movement into fruit in the transpiration stream. However, fruit Ca concentrations were 30% greater in the fan treatment than the bag treatment, whereas this difference was only 12% for Mg, which probably reflects its greater phloem mobility (Clark and Smith, 1988). Early in the season significant amounts of K are still transported in the xylem (Clark and Smith, 1988; Lang and Ryan, 1994), and as K is more mobile than both Mg and

Ca in the xylem (Marschner, 1995), this may explain why fruit K concentrations were significantly increased in fanned fruit from long shoots when compared to fruit from long, control shoots. Fruit P concentrations were increased by bagging. As P is transported predominantly in the phloem (Clark and Smith, 1988), this suggests that phloem water translocation may have been increased in response to reduced xylem water translocation caused by bagging. This would explain why treatments affected fruit transpiration, but generally had no effect on fruit water contents or FW, and why bagging did not reduce K concentrations.

At the January harvest, treatment effects were more noticeable in fruit from long shoots than in fruit from short shoots. This may be because the skin of fruit from short shoots was less permeable to water than the skin of fruit on long shoots and fruit on short shoots tend to be located in shaded parts of the vine, so their transpiration rates may already be lower when compared to fruit on long shoots. There also is evidence that mineral concentrations were affected by the xylem transport capacity in January-harvested fruit from short shoots. In short shoots, not only was the water content and FW of fruit from the fan treatment reduced compared to fruit from the other treatments, but estimated fruit xylem conductance was also. This may have restricted water flow through the xylem, thereby negating any positive effects that this treatment may have had on Ca accumulation by enhancing fruit transpiration rates. This would explain why in long shoots, differences were observed in the Ca content and concentration of fruit from the fan and bag treatments, whereas no differences were observed between these treatments in fruit from short shoots from the January harvest.

It is not known why estimated xylem conductance was reduced in fruit from short shoots that had been exposed to the fan treatment. In citrus, pear and persimmon fruit the extent of vascular development in the fruit stalk has been found to be positively correlated with fruit size (Nii, 1980b; Nii, 1980c; Nii, 1980a; Bustan *et al.*, 1995). This correlation also was found in the present experiments, because the size of fruit from the fan treatment was reduced (34g) when compared to that of fruit from the control and bag treatments (40g). However, it is not known whether fruit size was reduced because vascular tissues were poorly developed or vice versa.

In experiments by Li *et al.* (2004), humidity enrichment to 60% RH increased vascular development in capsicum fruit when compared to control fruit where the air RH could be as low as 34%. As capsicum plants grown in a high RH environment (>70% RH) had a greater stomatal conductance and transpiration rate than plants grown in a low RH environment (<50% RH: Zabri and Burrage, 1998), it was suggested that this improved the plant water status, which favoured vascular development (Li *et al.*, 2004). Other studies also suggest that vascular development is dependent on plant water status (Lovisolo and Schubert, 1998; Gribble *et al.*, 2003). As fans

lowered the water content of the fruit it is possible that this also hindered development of the vascular tissues and restricted water, and hence Ca movement into the fruit. In addition, the reduction in water accumulation would explain why DMCs were increased in fanned fruit from short shoots.

It was interesting that mineral accumulation was not more affected in fruit bagged in paper bags than in fruit bagged in gauze bags, as one might expect that fruit transpiration and therefore, the fruit water status, would be more affected by the former. Perhaps the small hole cut in the corner of the paper bags to allow water drainage allowed an equivalent amount of moisture to escape from the bag as the many small holes in the gauze bags. Alternatively, the fact that paper bags did not affect mineral accumulation any more than gauze bags may indicate that fruit transpiration has less of an affect on mineral accumulation than other factors.

6.4.2. Shoot type effects

Permeance

Permeance of fruit on short shoots declined more than that of fruit on long shoots, as the fruit SA increased. Consequently, permeance of fruit on long shoots was consistently higher than that of fruit on short shoots. As fruit transpiration rates are positively correlated with permeance (Xiloyannis *et al.*, 2001), and as Ca travels primarily in the transpiration stream of the plant (Clarkson, 1984), then permeance differences may contribute to differences in Ca concentration of fruit from long and short shoots. However, the effect of permeance was small compared to the effect of leaf area on fruit Ca (refer Chapter 5 for more information), as was found in apples (Jones and Samuelson, 1983).

In other studies a negative relationship was found between decreasing trichome density and plant permeance to water vapour (Perez-Estrada *et al.*, 2000; Lenssen *et al.*, 2001). D. Green (unpublished data) suggested that kiwifruit permeance increased as trichome density declined after finding that permeance of hairy 'Hayward' fruit was 7-8 nmol m^{-2} s^{-1} Pa^{-1} lower than that of the less hairy 'Hort16A' fruit. They suggested that trichomes on 'Hayward' fruit trapped a layer of still, humid air around the fruit, the boundary layer, which decreased fruit permeance by reducing the effective driving force for water loss. However, in this study trichome density did not differ between fruit from the two shoot types, therefore it is unlikely that this was a factor influencing these permeance differences. Differences between trichome densities of the individual fruit used

in this study would be small compared to differences between 'Hayward' and 'Hort16A' fruit, and may explain why no relationship was found between trichome density and permeance.

Permeance differences may, however, be related to differences in the ontogeny and morphology of the trichomes (timing of initiation and death and structure). Kiwifruit trichomes may be of two types, large multiseriate and small, two-celled uniseriate trichomes (White, 1986a); multi-celled and branched trichomes are more effective at preventing water loss than trichomes that are less elongated and less complex in structure, such as uniseriate trichomes (Bandyopadhyay *et al.*, 2004). 'Hort16A' fruit have more uniseriate trichomes than 'Hayward' fruit (I Hallet; pers. comm.), which may explain why 'Hort16A' skin is more permeable to water vapour. Light exposure has been shown to influence trichome development (Perez-Estrada *et al.*, 2000), and could possibly increase the ratio of multiseriate to uniseriate trichomes on fruit on long exposed shoots when compared to fruit on short, shaded shoots.

Permeance also may be influenced by skin structural features, such as the development of micro-cracks as seen in apple fruit (Maguire *et al.*, 1999b). Hallet and Sutherland (2005) noted that a thick layer of dead, radially compressed cells with suberised cell walls began to develop over the hypodermis of 'Hayward' fruit approximately seven weeks after petal fall. As fruit expanded this suberised cell layer developed cracks that eventually turned into lenticel-like structures. In mature fruit these cracks or lenticels may be the only route for water loss as suberised cells are largely impermeable to water vapour; fruit covered with oil sprays for insect control after the formation of this suberised cell layer often abscise as they experience an oxygen deficit (McKenna *et al.*, 1995; Allison and McKenna, 2002). In this experiment fruit from long shoots were larger than fruit from short shoots, as shown by their greater SA. The greater expansion of these fruit may have put considerable strain on the suberised cell layer, thereby enhancing crack development in these fruit compared to fruit on short shoots. Once fruit reach a certain size (90-110g: Fig 6.4) and fruit growth rates start to slow, permeance stops declining and may even slightly increase, as happens in apples (Maguire and Banks, 2000). Consequently, differences between fruit from long and short shoots decrease as the season progresses (Fig 6.3).

Mineral concentrations

By April, treatments had no effect on the Ca content and concentration of fruit from long shoots, despite there being differences earlier in the season. This suggests that in fruit from short shoots the effects of bagging may have been cumulative as the fruit matured, whereas fruit from long shoots managed to overcome the negative effects of bagging on Ca accumulation. This shows that fruit have the potential to accumulate significant amounts of Ca beyond eight weeks after anthesis, which is the time when most (≈ 80%) Ca is thought to have accumulated in the fruit under normal

circumstances (Clark and Smith, 1988; Xiloyannis *et al.*, 2001). As fruit permeance and fruit transpiration are very low during the later half of fruit development (Smith *et al.*, 1995; Xiloyannis *et al.*, 2001), some other factor(s) must have been driving Ca accumulation in the fruit.

Ability of fruit from long shoots to accumulate Ca later in the season may be attributed to the greater leaf area on long shoots, when compared to short shoots, which could possibly increase the volume of high Ca-containing xylem sap from the leaves towards fruit (Lang and Volz, 1998: refer to Chapter 5 for more information). Furthermore, more auxin may be synthesised in long shoots as there are more developing leaves on long shoots than on short shoots and fruit on long shoots tend to have more seeds than fruit from short shoots (refer Section 8.3.3 for details). This did not appear to affect vascular differentiation in long and short shoots (see Table 6.4), but may have enhanced cell division and elongation and fruit metabolism, thereby promoting Ca and carbohydrate accumulation in fruit from long shoots (Gamburg, 1982; Evans, 1985; Lee *et al.*, 1997a). In addition, auxin may directly affect Ca accumulation in the fruit (De la Fuente, 1984; Wand *et al.*, 1991a: refer Section 1.2.3 for more information).

The inability of fruit from short-bagged fruit to overcome the negative effects of bagging on Ca accumulation appear to relate to the effects of this treatment on vascular differentiation, as xylem conductivity is determined by the structure and size of xylem vessels (Tyree and Ewers, 1991; Schultz and Matthews, 1993). Bagging reduced estimated secondary xylem conductance in fruit from short shoots when compared to fruit from the other treatments, and negatively affected Ca accumulation. Phloem area also was reduced by bagging, as was the fruit DMC. It is not known why vascular differentiation was reduced in bagged fruit from short shoots, but it is doubtful that it is related to bagging effects on plant water accumulation, as there was not a similar response in stalks of bagged fruit from long shoots.

Interestingly, Ca content and concentration, FW, DW and DMCs were nearly always greater in fruit from long shoots than in fruit from short shoots (although differences were not always significant) despite the treatment applied to the fruit and/or time of fruit harvest. This implies that either the rate of Ca and carbohydrate accumulation in fruit on long shoots is greater or that Ca and carbohydrates are being accumulated at the same rate, but in greater quantities, or a combination of the above. Rates and quantities in which water, carbohydrates and Ca arrive in the fruit may be affected by a combination of the above factors including, increased (a) skin permeance and hence transpiration of fruit from long shoots, (b) sink strength in fruit from long shoots, (c) leaf area on long shoots increasing the pull of Ca towards these shoots and potentially increasing the production and, hence availability of carbohydrate, (d) increased vascular development in fruit from long shoots (bag treatment only).

6.4.3. Mineral accumulation and fruit permeance

Both in this study and in studies by Smith *et al.* (1995), fruit permeance dropped rapidly around the same time that fruit Ca concentrations are reported to plateau in kiwifruit (Clark and Smith, 1988). This decline in permeance appears to be related to structural changes in the epidermal cell layers (Xiloyannis *et al.*, 2001), but may also relate to the cessation of trichome initiation, which occurs around six weeks after flowering (White, 1986b) and coincides with the time of very rapid fruit growth and hence SA; as fruit continued to increase in size then this lead to an automatic decrease in trichome density in this experiment. From this time on the proportion of living to dead trichomes decreases and hence the surface area for water loss is reduced (White, 1986b). Reductions in the SA for water loss may be partially off-set by reductions in the boundary layer resistance to water loss that would occur as trichome density increased. Although differences in permeance between fruit from long and short shoots were small, in the vine they may be exaggerated, as fruit on long shoots are more exposed than fruit on short shoots, the boundary layer might be broken down and permeance increased, compared with the deeper boundary layer and reduced permeance of fruit on shaded shoots.

6.5. Conclusions

Effects of fruit transpiration on Ca accumulation in kiwifruit vines were small compared to the effects of leaf area on Ca accumulation (refer Chapter 5 for more information). Fruit transpiration is likely to have the greatest effect on Ca accumulation during the early stages of fruit development when fruit permeance is high.

1. Calcium, but not carbohydrate accumulation, was affected by changes in water accumulation. Reductions in water accumulation reduce fruit Ca concentrations, but increase the DW: FW ratio in the fruit and hence, fruit DMCs. Therefore, fruit Ca and DM concentrations are both influenced by water accumulation in kiwifruit, but in different ways.

2. Changes in fruit transpiration appear to have a greater effect on Ca accumulation in fruit from long shoots than in fruit from short shoots, particularly during the early stages of fruit development. This may be because fruit on long shoots have a higher permeance and are more exposed than fruit on short shoots, so Ca accumulation is naturally greater in the former, and there is more potential to influence Ca accumulation by manipulating fruit

transpiration. This may be the basis for long/short differences in fruit Ca concentrations within a vine.

3. In addition to being influenced by water accumulation, fruit mineral and carbohydrate concentrations may be affected by other factors. These factors may relate to the effects of leaf area on sap movement between leaves and fruit, and shoot auxin synthesis on cell division and elongation, fruit metabolism and vascular development (refer Chapters 5 and 8 for more information). It is suggested that when the estimated xylem conductance is reduced below a threshold level, Ca accumulation is restricted and fruit Ca concentrations decline. Therefore, factors that affect vascular differentiation, possibly by altering fruit auxin levels and/or the plant water status, also may affect Ca accumulation.

4. The affect of fruit transpiration on mineral accumulation during the early stages of fruit development is dependent on mineral ion mobility in the xylem. Nutrients such as K and P that are more mobile in the phloem and whose distribution in the plant is less dependent upon xylem hydraulic conductance, are less affected by fruit transpiration.

5. Calcium concentrations were consistently higher in fruit from long shoots than in fruit from short shoots, probably because long shoots have more leaves than short shoots and fruit on long shoots have more seeds (sites of auxin biosynthesis).

6. Fruit permeance was not related to trichome density, but may be related to micro-crack development in the epidermal cell layers as fruit expand or to trichome ontogeny and morphology. Reductions in the SA for water loss caused by decreasing trichome density as fruit expand may be partially off-set by reductions in the boundary layer for water loss.

7. Seasonal Trends in Vascular Development of Fruit Stalks From Long, Non-terminating and Short, Terminating Shoots

7.1. Introduction

Knowledge of the development and organisation of the vascular system associated with fruit is necessary to clearly appreciate the structure-function relationship between vascular development and fruit quality. This is particularly true for vascular development within the fruit stalk, as this provides the final pathway between a root or shoot (source) and a fruit (sink), through which all minerals and photosynthates will travel. This pathway represents a 'bottle-neck' in the water transport pathway, as vessels in the fruit stalk are considerably smaller than those in subtending shoots (Cordon, 1993). Therefore, average vessel diameter in the fruit stalk may be important in determining the rate of solute transfer to a fruit.

Fruit carbohydrate accumulation does not appear to be restricted by the phloem transport capacity of the fruit stalk (Darnell and Martin, 1987; Guardiola *et al.*, 1993; Salvador *et al.*, 1994; García-Luis *et al.*, 2002). Information on the effect of xylem transport capacity on fruit mineral accumulation is more equivocal. Calcium deficiency symptoms were more prominent in grape varieties with constriction zones in their pedicels than in those without (Lee, 1989; During and Lang, 1993). Biasi and Altamura (1996) found that vascular differentiation was enhanced, and fruit Ca and Mg concentrations also were higher, in fruit stalks of light-grown kiwifruit plants compared with shade-grown plants. These results suggest that there may be a causative link between extent of vascular differentiation in kiwifruit fruit stalks and fruit Ca and Mg concentrations.

Anatomical investigations of phloem and xylem tissue in apple pedicels have been used to successfully predict sap flows to fruit in relation to mineral nutrition (Lang and Ryan, 1994), and may offer some clues on the extent to which Ca and/or carbohydrate translocation is restricted by vascular capacity of kiwifruit (fruit) stalks. There are no published reports on vascular development in stalks of kiwifruit. The aims of this study were to: (a) investigate seasonal development of vascular tissues in kiwifruit stalks, (b) to compare vascular development in fruit stalks from fruit with different abilities to accumulate carbohydrates and/ or minerals, namely long and short axillary shoots, and (c) to determine whether any constriction zones exist in the fruit stalk by analysing the spatial distribution of vessels along the stalk's length.

7.2. Materials and methods

7.2.1. Experimental design

Mature kiwifruit vines growing at HortResearch's Te Puke Research Centre, Bay of Plenty, New Zealand were used. Flowers or fruit, with stalks attached, were harvested from long and short axillary shoots 13 different times between 6 November 2002 (21 days prior to full bloom) and 20 April 2003 (just prior to harvest); the time between sampling periods ranging from 6 to 55 days depending on the stage of fruit development. At each harvest, eight fruit/flowers, four from each shoot type (long and short), were randomly harvested from within a single bay taking care not to select fruit/flowers from vines on the bay ends (52 samples (fruit or flowers with attached stalks) from each shoot type in total from 13 different sampling times). Fruit were sampled from any one of 15 vines from within the same bay at each harvest. Fruit stalk lengths and diameters, vascular dimensions and estimated secondary xylem conductance were measured as described in Section 2.4. An additional six fruit (three from each shoot type) were harvested, with fruit stalks attached, on 2 April 2003 for analysis of the spatial distribution of vessels along the stalk length. This was assessed by measuring the vascular dimensions and estimated secondary xylem conductance each tenth of the way along the stalk, as measured from the fruit/flower end. Fresh weights were recorded for each fruit.

7.2.2. Statistical analysis

At each sampling time, differences between means for fruit from the two shoot types were determined using two-way ANOVA. Where the F-stat indicated a significant difference between treatments the Fisher's protected least significant difference (LSD) procedure was used to establish significance between treatment means. Time constraints prevented more than four stalks from each shoot type being assessed at each harvest date. The low number of samples was taken into consideration when analysing trends in vascular development and differences between shoot types.

7.3. Results and discussion

7.3.1. Flower and fruit stalk growth

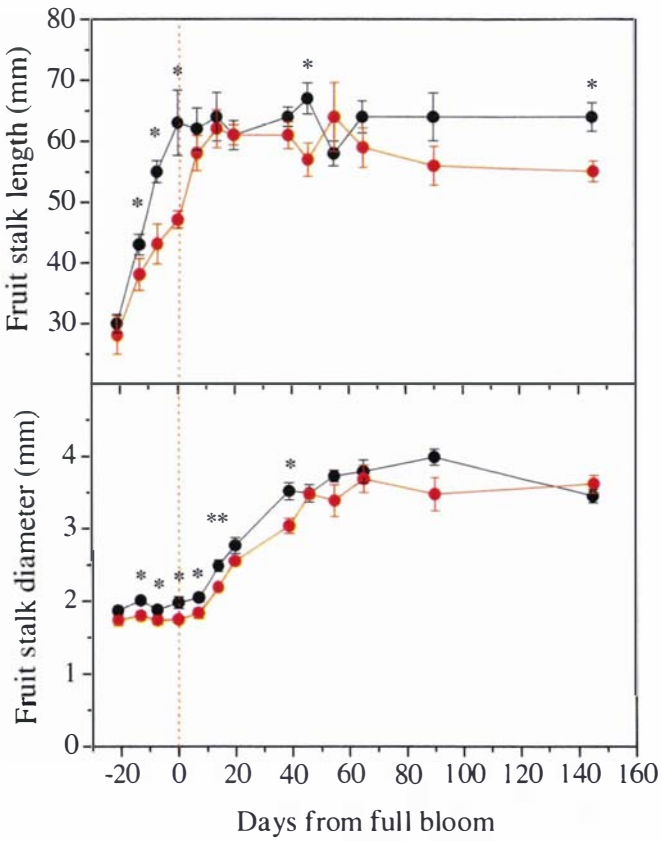


Fig 7.1. Flower and fruit stalk length and diameter development in stalks from long (black) and short (red) shoots. Stalk diameter was measured at the mid-point, stem and fruit/flower ends of the stalk, and an average diameter calculated. Data are means of four observations (± s.e.). Stars indicate significant differences between shoot types at * P <0.05 and ** P <0.01.

Flower stalk length increased rapidly until anthesis and then remained constant until the final harvest in April. In contrast, the fruit stalk diameter did not increase until after anthesis, reaching maximum values 42-46 days later (Fig 7.1), similar to that found in apples (Privé *et al.*, 1988; Dražeta *et al.*, 2004a). During early stages of development, flower stalks from long shoots were wider and longer than stalks from short shoots, but once maximum lengths or diameters were obtained there was little difference between stalks of fruit from the two different positions. Stalk length was greater in fruit sampled from long shoots than in fruit sampled from short shoots at the final sampling date. However, as this difference was not obtained in the three prior sampling dates, and as fruit stalk length did not change significantly from 3.5 DPFB (stalks from long shoots) to 7 DAFB (stalks from short shoots), it is likely that this can be attributed to chance variation in the samples collected.

7.3.2. Vascular development in the fruit stalk

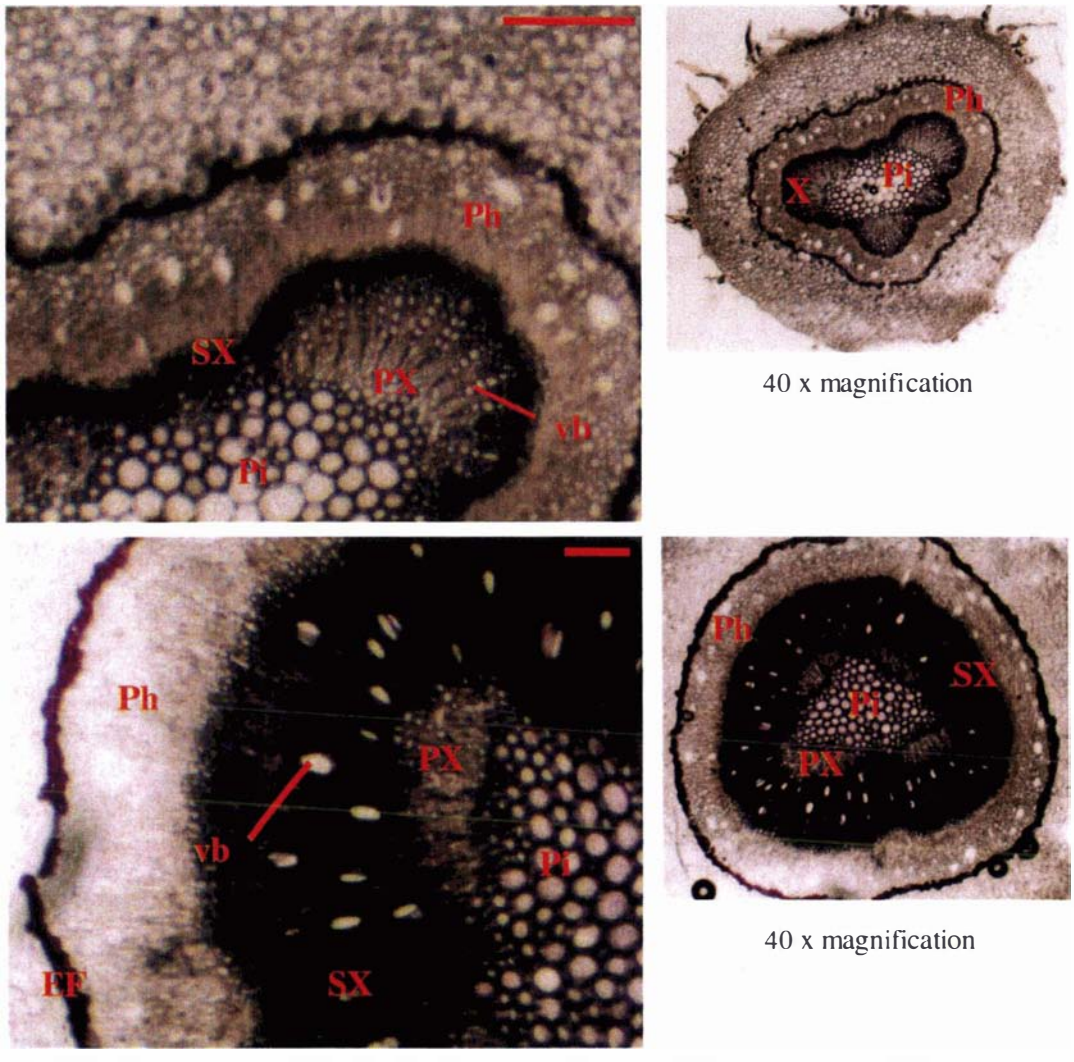


Fig 7.2. Cross-sections of kiwifruit pedicels two (top) and 21 weeks after anthesis. Images on the left-hand side are a close up of those on the right-hand side. Ph = phloem, SX = secondary xylem, PX = primary xylem, Pi = pith, vb = vascular bundle, EF = extraxylary fibre. Bar = 0.2 mm.

Vascular tissues in kiwifruit fruit stalks are illustrated in Figure 7.2. Phloem areas began to increase at anthesis around one week prior to the increase in secondary xylem area (Fig 7.3). Phloem area did not increase significantly beyond 21 DAFB in fruit stalks from short shoots and 46 DAFB in fruit stalks from long shoots. At 40 DAFB there was a sudden rise in the phloem area in fruit stalks from short shoots. This may be due to chance variation in fruit selection as, due to the time taken to assess each sample, it was only feasible to assess four fruit/flower stalks from each shoot type at each sampling time. Therefore, the likelihood of these stalks having a higher than average phloem area at this sampling time due to chance variation is relatively high. Maximum xylem area was reached around three weeks after maximum phloem areas were

obtained (≈ 40 days and 65 days after flowering for fruit stalks from fruit on short and long shoots, respectively). Pith area remained low (≈ 0.25 mm^2) and constant from 21 days prior to anthesis until harvest in late April.

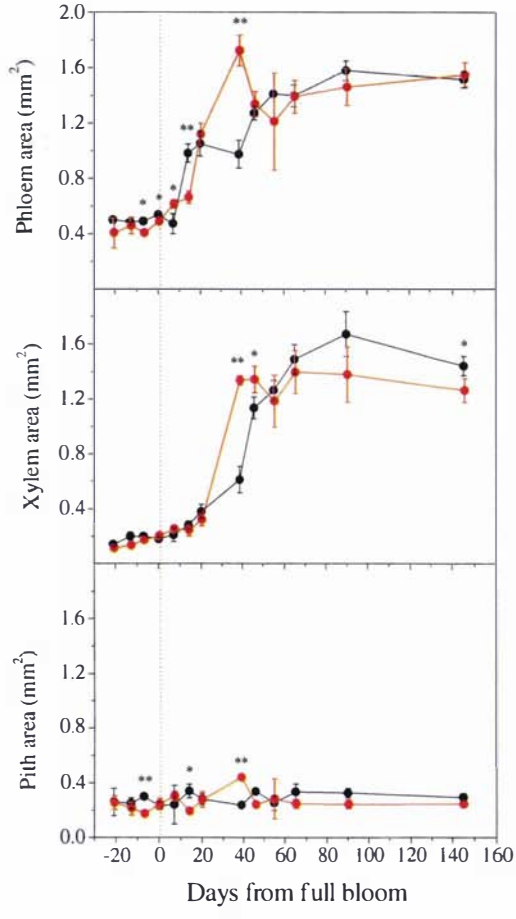


Fig 7.3. Secondary phloem, xylem and pith development in flower and fruit stalks from long (black) and short (red) shoots. Data are means of four observations (± s.e.). Stars indicate significant differences between shoot types at *, P <0.05 and ** P <0.01.

At anthesis the xylem comprised about 6.5% of the total flower stalk cross-sectional area (assuming the flower stalk is cylindrical in shape) in both short and long shoots, but by harvest this value was closer to 15%. The proportion of total fruit stalk area occupied by phloem remained relatively constant throughout the sampling period, averaging 15 and 18% of the total flower/fruit stalk area in stalks from short and long shoots, respectively. Subsequently, the phloem: xylem ratio was high (3.6) at the start of the season until maximum xylem areas were attained. After this time, the ratio of phloem area: xylem area was roughly equal and there were no significant increases in the total vascular area. In contrast, the area of phloem exceeded that of xylem by a ratio of 2.65 ± 1.0 (± s.e) in apple (Dražeta *et al.*, 2004a). As Ca is phloem immobile this may provide an explanation for the low Ca status of apple fruit when compared to that of kiwifruit (Kirkby and Philbeam, 1984).

Initial phloem areas were slightly greater in fruit stalks from long shoots than in stalks from short shoots. This may relate to the seed number in the fruit, as Antognozzi *et al.*, (1991) found that auxin synthesis was positively correlated with fruit seed numbers in kiwifruit. Vascular tissues were shown to be initiated earlier and their development was more advanced in fruit with many seeds than in fruit with few seeds. Piller *et al.* (1998) suggested that early fruit abscission, which coincided with the initiation of secondary phloem differentiation, was caused by shortages of assimilate, and that fruit on short shoots, with a low leaf: fruit ratio, were more likely to abscise than fruit on long shoots. The

greater early initiation of secondary phloem development in stalks of fruit from long shoots may ensure that there are adequate supplies of carbohydrate to maintain steady rates of cell division and elongation during the initial phase of fruit growth, thereby ameliorating fruit abscission. Adequate supplies of carbohydrate also may ensure development of a large number of cells into which subsequent minerals and carbohydrates can be loaded, and could help to explain why final DM and Ca concentrations are higher in fruit from long shoots than in those from short shoots. Lai *et al.* (1990) found that fruit on long shoots were on average 12% larger than fruit on short shoots. It is likely that this is because these fruit have more cells, as treatments that increase fruit size, such as CPPU application (Woolley *et al.*, 1991; Cruz-Castillo *et al.*, 2002) and girdling at anthesis (Currie, 1997) also increase fruit cell number in kiwifruit. Moreover, early-initiated flowers have a greater cell number in their ovaries at anthesis and develop into larger fruit than late-initiated flowers (Cruz-Castillo *et al.*, 2002).

At about 40 DAFB xylem areas were slightly higher in fruit stalks from short shoots than in stalks from long shoots, but after this time xylem area was always numerically higher in fruit from long shoots than in fruit from short shoot and was significantly higher at the final sampling date in April. Pith area was affected by shoot type differences, but effects were not consistent between sampling times and varied from date to date.

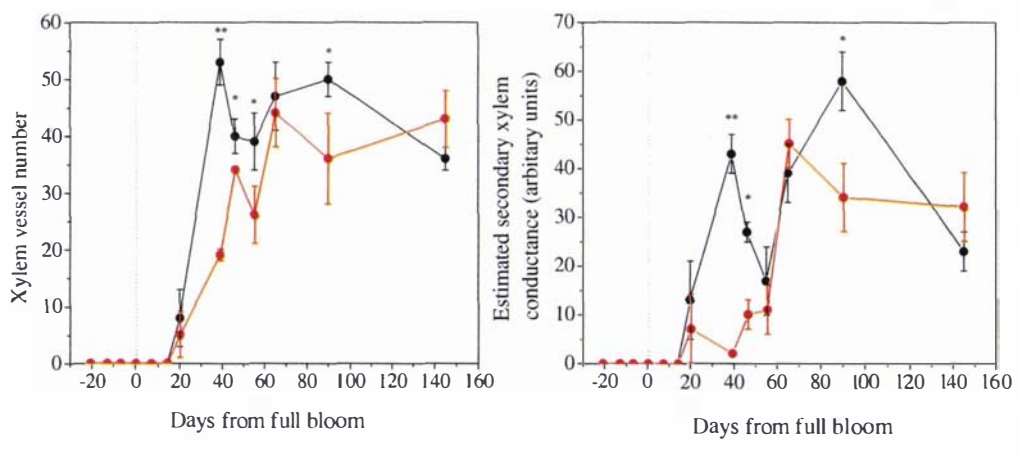


Fig 7.4. Seasonal changes in secondary xylem vessel number and estimated conductance in stalks from flowers and fruit on long (black) and short (red) shoots. Data are means of four observations (± s.e.). Stars indicate significant differences between shoot types at * P <0.05 and ** P <0.01.

At about one week after secondary xylem areas began to increase (17 DAFB), vessels began to form in the secondary xylem and subsequently estimated secondary xylem conductance began to increase (Fig 7.4). Secondary xylem vessel numbers increased until 40-46 DAFB, coincident with

the attainment of maximum fruit stalk diameter. Estimated xylem conductance varied considerably throughout the growing period, especially in stalks from fruit and flowers on long shoots. These variations may relate to the small number of samples (four) collected from each shoot type at each date, or they may be ascribed to cycles of vessel breakage (resulting from stretching caused by fruit stalk growth) and repair (resulting from early season cambial activity) similar to those reported to occur in kiwifruit (Dichio *et al.*, 2003). Maximum estimated secondary xylem conductance was attained 65 DAFB in fruit stalks from short shoots and 90 DAFB in stalks from long shoots.

These results suggest that the shift from procambial to cambial development occurs at, or shortly after anthesis, as in apple (Dražeta *et al.*, 2004a) and citrus (García-Luis *et al.*, 2002) fruit. Between 40-65 DAFB cambium activity appears to slow in kiwifruit, as new xylem vessels are no longer developed and the xylem area ceases to increase. The cambium becomes inactive 65-90 DAFB, around the same time the cambium becomes inactive in citrus (García-Luis *et al.*, 2002), but around 32 days after the time when the cambium becomes inactive in apple fruit (Barden and Thompson, 1963; Privé *et al.*, 1988; Dražeta *et al.*, 2004a). Kiwifruit Ca concentrations start to decline (Ferguson, 1980; Clark and Smith, 1988; Xiloyannis *et al.*, 2001) about the time that cambium activity slows. This may result from damage caused to the xylem by fruit stalk elongation and expansion not being "repairable" once the cambium was inactive. Consequently, the xylem would become dysfunctional, Ca movement would cease and Ca accumulation into fruit would be restricted. Cambium activity starts to slow and ceases in fruit stalks from long shoots roughly 25 days later than in stalks from short shoots. Subsequently, carbohydrates and minerals may be able to enter the fruit over a longer period of time in the former and this could explain why fruit from long shoots often have higher DM and Ca concentrations than fruit from short shoots.

Table 7.1. The percentage of small (< 8 μm), medium (8-14 μm) and large (> 14 μm) secondary xylem vessels and their contribution to the total estimated secondary xylem conductance in fruit stalks from long and short shoots.

% of total...	Long	Short
..vessels < 8 μm in diameter	60 (2.0)	55 (3.0)
..vessels 8-14 μm in diameter	28 (1.2)	30 (1.2)
..vessels >14 μm in diameter	12 (0.3)	15 (0.8)
..conductance contributed by vessels < 7 μm in diameter	3 (0.1)	2 (0.1)
..conductance contributed by vessels 8-14 μm in diameter	28 (1.5)	31 (2.1)
..conductance contributed by vessels > 14 μm in diameter	69 (6.9)	67 (5.2)

Data are mean values (± s.e.) of measurements made between 6 November 2002 and 20 April 2003.

Estimated xylem conductance also was higher in fruit stalks from long

shoots than in fruit stalks from short shoots during the first few weeks of secondary xylem development when rates of Ca accumulation are highest (Clark and Smith, 1988). In fruit stalks from both shoot types, small diameter (< 8 μm) vessels were the most abundant, but contributed the least to the total conductance, while large diameter vessels (> 14 μm) were the least abundant, but contributed the most to the total conductance (Table 7.1) since hydraulic conductance is related to the fourth power of the vessel diameter (Tyree and Ewers, 1991). The average vessel diameter in fruit stalks from both shoots types was 18 μm, compared to 16 μm in 'Cox's Orange Pippin' (Cox) and 'Gala' apples (Lang and Ryan, 1994) and 14 μm in 'Granny Smith' apples (Dražeta *et al.*, 2004a). Corresponding estimated conductance values, for a given number of vessels, were calculated to be 64, 80 and 98% of those of kiwifruit, for 'Granny Smith', 'Gala' and 'Cox' apples, respectively. This larger estimated conductance value in kiwifruit might partially explain why these fruit have a higher Ca concentration than apple fruit at harvest (Kirkby and Philbeam, 1984).

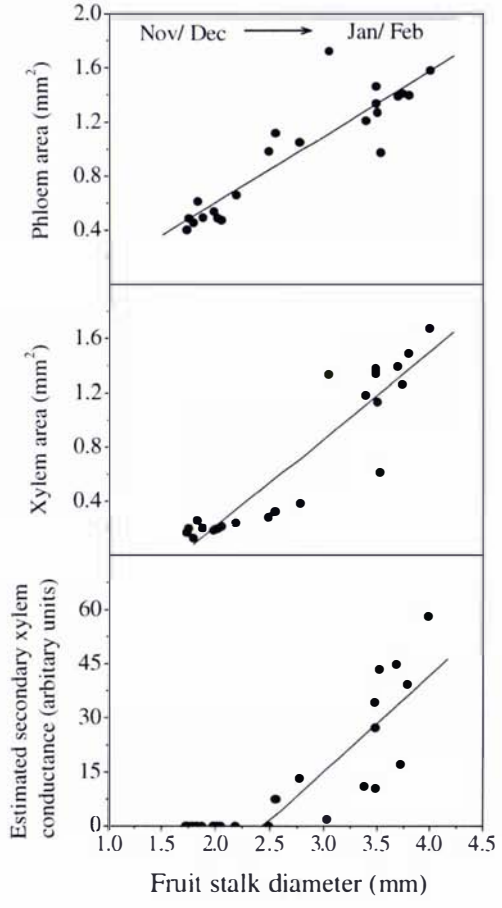


Fig 7.5. Relationship between flower/fruit stalk diameter, phloem (R^2 = 0.82) and xylem (R^2 = 0.69) areas and estimated secondary xylem conductance (R^2 = 0.53 for diameters > 2.25 mm). Data points are averages from each sampling period.

The increase in xylem and phloem area closely matched the increase in fruit/flower stalk diameter (Fig 7.5: $P < 0.001$). Pith area was not correlated with fruit or flower stalk diameter (data not shown). Phloem area increased linearly with increasing fruit/flower stalk diameter. Linear regression also was used to describe the relationship between fruit/flower stalk diameter and xylem area. However, data indicates that this relationship may in fact be non-linear. More data points are required to confirm this, and to justify the use of non-linear regression to describe the relationship between these two variables. Increases in fruit/flower stalk diameter preceded increases in estimated secondary xylem conductance, but once xylem conductance began to increase it was linearly correlated with fruit stalk diameter (Fig 7.5: $P < 0.003$). As carbohydrate and mineral accumulation is likely to be enhanced as

vascular capacity in the fruit stalk increases, this may explain why fruit size was closely correlated (R^2 = 0.72) with fruit stalk diameter in studies by Lai *et al.* (1990). Fruit stalks had completed elongation before xylem and phloem areas began to increase, and final fruit stalk lengths were not correlated with phloem or xylem areas or estimated secondary xylem conductance (data not shown).

7.3.3. Spatial distribution of phloem and xylem tissues within the fruit stalk

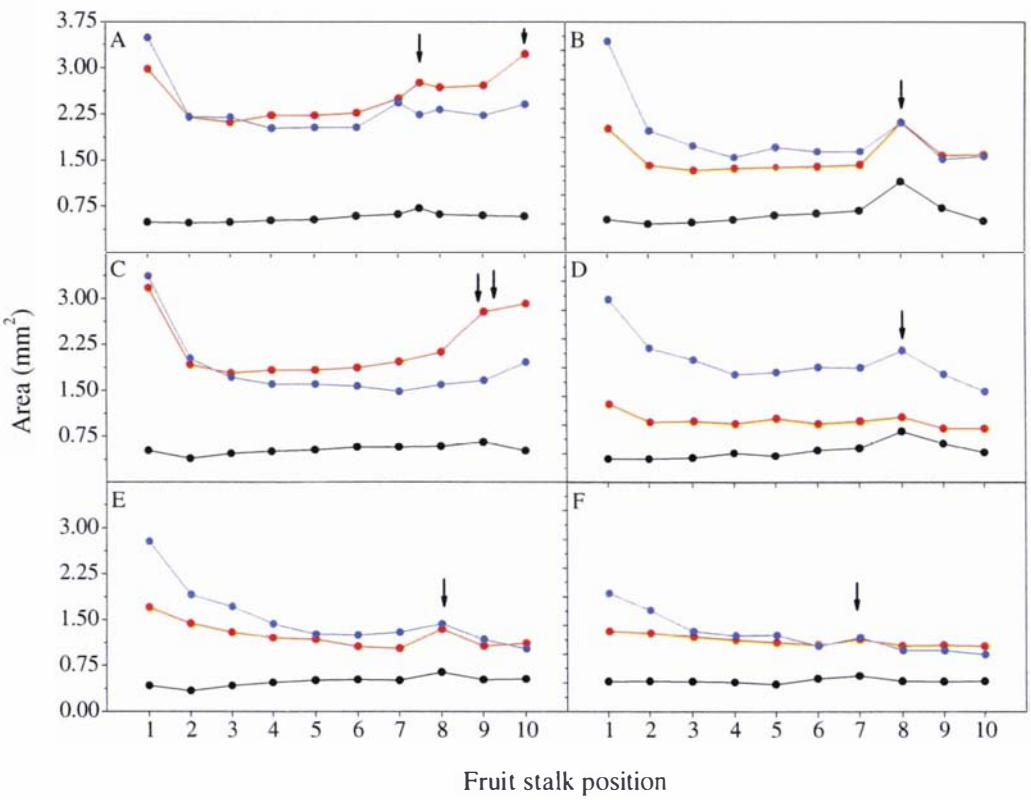


Fig 7.6. Spatial distribution in pith (black), xylem (red) and phloem (blue) areas down a fruit stalk. Each plot represents data from one fruit stalk. Measurements were taken each tenth of the way along the stalk length, as measured from the fruit (position 1) to the shoot (position 10) ends of the stalk. Arrows indicate the position of bracts where side flowers may have arisen (the pedicel-peduncle junction). A, C, and E illustrate data from fruit stalks attached to long shoots and B, D, and F illustrate data from fruit attached to short shoots.

Xylem and phloem cross-sectional area decreased sharply in the initial few centimetres of the pedicel (as measured from the fruit end) and then remained relatively constant except across the pedicel-peduncle junction where their areas tended to increase. In most fruit the ratio of phloem: xylem area was greater than one at the fruit end of the stalk and decreased to one or less at the

shoot end of the stalk. Pith area remained relatively constant along the entire length of the stalk, increasing only at the pedicel-peduncle junction (Fig 7.6).

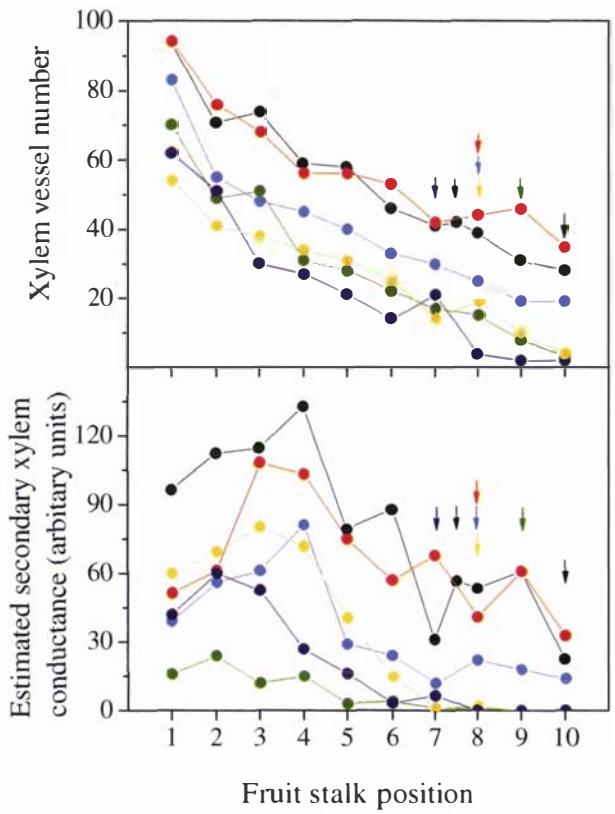


Fig 7.7. Spatial distribution in xylem vessel number (top) and estimated conductance (bottom) down a fruit stalk. Each curve represents data from one fruit stalk. Measurements were taken each tenth of the way along the stalk length, as measured from the fruit (position 1) to the shoot (position 10) ends of the stalk. Arrows, corresponding to the colour of each curve, indicate the position of the pedicel-peduncle junction. Black, red and yellow curves illustrate data collected from fruit stalks attached to long shoots and dark blue, light blue and green curves illustrate data collected from fruit attached to short shoots.

Xylem vessel number decreased almost linearly from the fruit to the shoot ends of the stalk. Xylem conductance increased in the initial few centimetres of the pedicel (as measured from the fruit end), despite a decrease in xylem and phloem areas and xylem vessel number, and then decreased towards the shoot end of the stalk. Dražeta *et al.* (2004a) also found that estimated xylem conductance decreased towards the fruit and shoot (spur) ends of the fruit stalk. However, in their experiments xylem vessel number remained constant along the length of the pedicel, which suggests that estimated xylem conductance decreased because of a reduction in the average vessel size, rather than vessel number. Such hydraulic constriction zones have been found in branch junctions between small and large branches in white cedar (*Thuja occidentalis*), balsam fir (*Abies balsamaea*) and eastern hemlock (*Tsuga Canadensis*) trees (Tyree *et al.*, 1983; Ewers and Zimmerman, 1984a; Ewers and Zimmerman, 1984b), and may be similar to those found between the fruit and the pedicel and between the pedicel/ peduncle and the spur/ shoot (Lee, 1989; During and Lang, 1993).

Xylem vessel number and conductance did not show any consistent patterns across the pedicel-peduncle junction. The estimated xylem conductance and vessel number tended to be greater in fruit stalks attached to long shoots than in fruit stalks attached to short shoots (Fig 7.7). This may affect the ease with which solutes can be accumulated in fruit, and could explain the trend for higher Ca concentrations in fruit from long shoots when compared with fruit from short shoots. There was no relationship between fruit size, vessel number or estimated secondary xylem conductance (data not shown). This might be a further indication that the relationship between fruit stalk diameter, which is linearly related to fruit size (Lai *et al.*, 1990), and xylem area is not linear.

Vascular development is enhanced by light (Biasi and Altamura, 1996) and upwards shoot orientation (Schubert *et al.*, 1999; Lovisolo *et al.*, 2002). Therefore vascular differentiation may differ in fruit stalks from long and short shoots, as long shoots tend to grow up towards the sun, whereas short shoots tend to be shaded and often grow with a downward orientation. The ability of these factors to influence vascular differentiation may relate to their effects on auxin metabolism and/ or distribution (Biasi and Altamura, 1996; Schubert *et al.*, 1999; Lovisolo *et al.*, 2002), as auxins are known to regulate vascular differentiation (Aloni *et al.*, 2000). The seed number in a fruit also could influence vascular development, because auxins are synthesised in seeds (Nitsch, 1950). As fruit on long shoots tend to be larger than those on short shoots, they may have more seeds and this also could explain why vascular development was greater in fruit from the former. Moreover, fruit on long shoots usually develop from early-opening flowers, whereas fruit on short shoots usually develop from late-opening flowers and may be slower to develop than fruit on long shoots (Lai *et al.*, 1990). The timing of flower evocation may influence the extent of auxin efflux from developing fruit and this may affect a fruit's ability to compete for carbohydrates (Bangerth, 1989). In the following chapter the possibility that auxin export from the seeds influences vascular differentiation and subsequent mineral and carbohydrate accumulation is explored.

7.4. Conclusions

Anatomical investigations of phloem and xylem tissue in kiwifruit fruit stalks provided an indication of the extent to which carbohydrate and mineral accumulation may be affected by the vascular capacity in stalks from long and short shoots. Findings from this chapter confirm findings from chapters 5 and 8, in relation to the vascular capacity of fruit from long and short shoots. The following conclusions also may be confirmed by repeating this study with a larger number of samples.

1. During the first few weeks of flower/fruitlet development, there were indications that phloem differentiation was more advanced in fruit stalks from fruit on long shoots than in stalks from fruit on short shoots. This may facilitate enhanced cell division in fruit from long shoots when compared to fruit from short shoots by increasing the availability of carbohydrates and minerals for structural growth. Subsequently, fruit from long shoots may have a greater sink potential or ability to attract carbohydrates and minerals throughout the season than fruit from long shoots, causing fruit Ca concentrations and DW to be generally greater in the former (Thorp *et al.*, 2003b).

2. Cambial activity ceased later in the season in the stalks of fruit on long shoots than in the stalks of fruit on short shoots. Therefore, carbohydrates and minerals may be able to accumulate over an extended period of time in fruit on long shoots when compared to those on short shoots.

3. Estimated secondary xylem conductance appeared to be slightly greater in fruit stalks from fruit on long shoots than in stalks from fruit on short shoots; this could account for the tendency for Ca accumulation to be greater in fruit from long shoots than in fruit from short shoots.

4. At the same time as the vascular cambium became inactive, fruit Ca concentrations began to decline, suggesting that once the cambium is inactive xylem vessels become dysfunctional as they are stretched and broken during fruit expansion (Dichio *et al.*, 2003) and are not able to be repaired. This means that hydraulic conductivity would be reduced, thereby causing a decline in water and calcium accumulation into fruit later in the season (Smith *et al.*, 1995; Xiloyannis *et al.*, 2001).

5. Increases in fruit stalk diameter closely matched increases in xylem and phloem tissue areas and estimated secondary xylem conductance, which suggests that pedicel diameter could be used to provide an indication of the vascular capacity in the fruit stalk and therefore of a fruit's potential to obtain minerals and carbohydrates. In the case of the phloem, this increase was linear. However, data indicated that the increases in xylem area might not be directly proportional to increases in fruit stalk diameter. This suggests that phloem differentiation may increase in response to increases in fruit size, or that fruit may develop in response to increases in phloem area. However, xylem area may not increase in response to increases in fruit size, hence the reason why fruit Ca concentrations are often negatively correlated with fruit size. It is proposed that the extent of phloem development in the fruit stalk during the first few weeks after anthesis sets the potential for subsequent DW

accumulation, but does not directly influence carbohydrate accumulation during the later stages of fruit development. However, the extent of xylem differentiation and functionality may have a considerable impact on the accumulation of phloem immobile nutrients, such as Ca, during both the early and late stages of fruit development (as proposed in conclusions 3 and 4).

6. Seasonal trends in vascular development were very similar to those in apple fruit. However the final phloem: xylem ratios were higher in apple fruit than in kiwifruit and the frequency of vessels in the larger size classes was lower, which may partially explain the high Ca status of kiwifruit fruit when compared to apple.

8. TIBA and Fruit Seed Number Influences Mineral and Carbohydrate Accumulation in Kiwifruit: A Potential Role For Auxins

8.1. Introduction

Results from the previous chapters suggest that Ca accumulation is, at least partially, influenced by the conductance of fruit stalk xylem vessels. Evidence for such a proposal includes the: (a) positive correlation between Ca accumulation and estimated secondary xylem conductance, (b) reduction in Ca accumulation (Clark and Smith, 1988; Xiloyannis *et al.*, 2001) from the time when the cambium becomes inactive and new xylem tissues are presumably no longer synthesised, (c) greater estimated vascular conductance and higher Ca concentration in stalks of fruit from long shoots than in stalks of fruit from short shoots, (d) greater estimated vascular conductance and higher Ca concentration in kiwifruit than in apples, and (e) inability of reductions in estimated secondary xylem conductance to affect the transport of minerals such as K, Mg and P that are transported in both the xylem and phloem. Other studies also have suggested a link between xylem conductivity and Ca accumulation in kiwifruit (Antognozzi *et al.*, 1991; Biasi and Altamura, 1996; Dichio *et al.*, 2003).

A link between carbohydrate accumulation and phloem conductivity is more difficult to establish because phloem conductance is not easily measured and it cannot be assumed that phloem area is directly correlated with phloem conductance, as not all phloem cells are conductive. There are suggestions that carbohydrate accumulation is not affected by phloem conductance (Barden and Thompson, 1963; Darnell and Martin, 1987; Guardiola *et al.*, 1993; Salvador *et al.*, 1994; García-Luis *et al.*, 2002). Results from previous chapters indicate that phloem capacity may increase in conjunction with increases in sink demand for carbohydrate and so never becomes limiting.

There is substantial evidence to suggest that vascular differentiation is regulated by auxins, most likely in conjunction with other plant growth regulators (PGRs) or plant substances (Aloni *et al.*, 2000; Uggla *et al.*, 2001; Ye, 2002: refer Section 1.6.1 for more information). In addition to regulating vascular cell differentiation and controlling vascular patterning, auxins also may influence carbohydrate and mineral accumulation indirectly by regulating the: (a) proliferation (division and elongation) of other cell types (Gamburg, 1982; Evans, 1985; Ljung *et al.*, 2001; Uggla *et al.*, 2001), (b) processes controlling phloem loading and unloading (Lee *et al.*, 1997a; Cole and Patrick, 1998), and (c) competition between competing sinks within a plant (Bangerth

and Ho, 1984; Bangerth, 1989; Cutting and Bower, 1989). There also is widespread evidence that fruit Ca accumulation is directly affected by the basipetal movement of auxin away from the fruit (De la Fuente, 1984; Wand *et al.*, 1991a: refer Section 1.2.3 for more information).

Auxins are synthesised in developing leaves and seeds and are transported basipetally to 'target tissues', such as the vascular cambium, where they take effect (Kaufman *et al.*, 1995; Bennett *et al.*, 1998). Auxin transport inhibitors, such as 2,3,5-triiodobenzoic acid (TIBA), 1-N-naphthylphthalamic acid (NPA) and chloroflurenolmethylester (CME) have been shown to reduce Ca accumulation when applied to tomato (Hamamoto *et al.*, 1998), pome (Raese *et al.*, 1995), avocado (Cutting and Bower, 1989) and stone (Wand *et al.*, 1991b) fruit or their stalks. Auxin transport inhibitor effects are specific to Ca and are independent of any treatment affect on evapotranspiration and/or Ca movement between cation exchange sites (Banuelos *et al.*, 1987).

The above studies all concluded that auxin transport inhibitors affected Ca accumulation directly by inhibiting basipetal indolyl-3-acetic acid (IAA) movement. This however, does not explain why fruit abscission was enhanced by auxin transport inhibitor application to stalks of peach (Ramina *et al.*, 1986), citrus (Yuan *et al.*, 2003) and apple (Dražeta *et al.*, 2004a) fruit, why TIBA altered the SSC and titratable acidity of grapes (Yakushiji *et al.*, 2001), and why NPA application to the stalks of kiwifruit (Currie, 1997) and apple (Dražeta *et al.*, 2004a) fruit greatly reduced auxin efflux from the fruit, fruit size and total seed weight. Given the wide range of processes thought to be regulated by auxin (described above), it is likely that in addition to directly affecting Ca accumulation, auxin transport inhibitors may indirectly affect the accumulation of carbohydrates and minerals by influencing vascular differentiation, cell division and elongation and by altering fruit metabolism.

Dražeta *et al.*, (2004a) found that NPA application to stalks of apple fruit shortly after full bloom (FB), reduced vessel diameters and vessel numbers proximal to the point of application. Conversely, a mixture of auxins and gibberellins applied to tomato trusses shortly after FB, stimulated peduncle and pedicel growth, xylem differentiation, and to a lesser extent phloem differentiation, and increased uptake of carbohydrates, water and mineral ions (Starck *et al.*, 1988). In kiwifruit it has been suggested that PGR efflux from fruit increases as seed number increases, as does vascular differentiation in the fruit stalk (Antognozzi *et al.*, 1991). Application of TIBA and NPA to kiwifruit in late December reduced fruit fresh weight (FW), fruit stalk diameters, total seed weight and Ca concentrations at harvest, but had no affect on K and Mg concentrations (D. Woolley and S. Lawes, pers. comm.). The decrease in pedicel diameter suggests that vascular differentiation may have been inhibited, as pedicel diameter is positively correlated with both phloem and xylem area (refer Chapter 7), and this may have restricted carbohydrate and Ca

accumulation. It also is possible that IAA export from young fruit favours import of not just Ca, but also substrates for growth of fruit and seeds (D. Woolley and S. Lawes: pers. comm.), and that vascular capacity is increased to cope with the greater demands of rapidly growing fruit.

Numerous studies suggest that the early stages of fruit growth (carbohydrate and/or water uptake) are dependent on early auxin accumulation, but that at later stages of fruit development growth can occur independent of auxin (Guardiola *et al.*, 1993; Tao *et al.*, 1994; Currie, 1997; Lee *et al.*, 1997a; Lee *et al.*, 1997b; Ofosu-Anim *et al.*, 1998). In kiwifruit, positive correlations between fruit seed number (auxin supply) and fruit growth are noticeable shortly after FB (Hopping and Hacking, 1983). Fruit size is a function of cell number, cell size and cell density and it has been suggested that the number of cells in a fruit determines its capacity to increase in volume and import photoassimilates (Ho, 1992). However, when kiwifruit seed numbers were reduced by style excision, FW and cell size were greatly reduced, but not cell number (Currie, 1997). Other studies found a positive correlation between kiwifruit fruit size and cell number (Kurosaki and Mochizuki, 1990; Cruz-Castillo *et al.*, 2002) and suggested that auxins, most likely in conjunction with cytokinins and gibberellins, enhanced cell division during the early stages of fruit development (Tao *et al.*, 1994; Fang *et al.*, 2000).

The effects of seed number on mineral accumulation are equivocal. Seedless tomato fruit had lower Ca concentrations and a higher incidence of blossom end rot (BER), a Ca-related deficiency disorder (Banuelos *et al.*, 1987), and a lower rate of basipetal IAA efflux (Sjut and Bangerth, 1984) than seeded fruit. Low seed numbers were associated with reduced fruit Ca and increased bitter-pit in 'Braeburn' (Brookfield *et al.*, 1996; Broom *et al.*, 1998), 'Red Delicious', 'Golden Delicious' (Di Vaio, 2004), and 'McIntosh' apples, but not in 'Spartan' apples (Tomala and Dilley, 1990). Mineral contents, but not concentrations, were affected by seed numbers in grape fruit (Boselli *et al.*, 1995). The duration over which Ca, and to a lesser extent Mg, could accumulate in grape berries increased with increasing seed number, which suggests that the xylem dysfunction that occurs in the later stages of fruit development (During and Lang, 1993; Greenspan *et al.*, 1994), occurs later in fruit with more seeds. Durability of the xylem also has been associated with increased seed numbers in pistachio nuts (Polito, 1999).

In kiwifruit, the growth response to increasing seed number declines as seed numbers are increased above about 800 seeds (Pyke and Alspach, 1986; Lawes *et al.*, 1990; Currie, 1997). This may be because as seed numbers are increased the weight of each consecutive seed is reduced, and smaller seeds are poorer sources of auxin than larger seeds (Currie, 1997). Alternatively, a threshold level of auxin may be required to stimulate vascular differentiation, fruit growth and possibly Ca accumulation, and further increases in auxin, above this threshold level, will have little additional

impact on fruit size. There was little difference in the response of kiwifruit to NPA concentrations over the range of 1.7-3.4 mM (Currie, 1997).

As fruit on long shoots tend to have more seeds than fruit on short shoots (Lai *et al.*, 1990), it is likely that vascular differentiation and hence Ca accumulation will be more affected by auxin transport inhibitor application in fruit from long shoots than in fruit on short shoots. Bangerth (1976) found that auxin sprays did not reliably increase Ca concentrations in well pollinated apple fruit, but that they could increase fruit Ca concentrations in growth regulator induced parthenocarpic apple fruit that produce little of their own auxin. Conversely, auxin transport inhibitors were not able to further reduce fruit Ca concentrations in parthenocarpic fruit, whereas Ca concentrations were considerably reduced in well-pollinated fruit.

This chapter includes three experiments. The first two investigated the effects of TIBA on vascular development in fruit stalks of kiwifruit and subsequent effects on mineral and carbohydrate accumulation. In the first experiment, TIBA was applied during the early stages of fruit and flower development, when IAA efflux from fruit is believed to be crucial for fruit development, when vascular tissues are differentiating, and when Ca is still rapidly accumulating in fruit, and during the latter stages of fruit growth when fruit growth is believed to occur independent of IAA. TIBA also was applied later in the season when Ca accumulation rates are very low, and when cambium activity declines. It was thought that early applications of TIBA (prior to 21 DAFB) would affect vascular differentiation, particularly xylem differentiation and that this might affect Ca, FW and DW accumulation, but would have little affect on the accumulation of K, Mg or P or fruit DMCs, and that effects of TIBA would decline at later application dates.

In the second experiment TIBA was applied to fruit on shoots that had been girdled or defoliated in order to determine whether TIBA has any additional effects on mineral and carbohydrate accumulation other than those reported in Chapter 5 (where shoots were girdled or defoliated, but fruit were not treated with TIBA). This should help determine to what extent leaf area may be affecting carbohydrate and mineral accumulation and to what extent carbohydrate and mineral accumulation may be affected by other factors, such as treatment effects on vascular development, cell division and elongation and fruit metabolism.

Finally, the third experiment investigated the relationship between fruit seed number, vascular development and subsequent mineral and carbohydrate accumulation. This experiment was used to assess whether any effects of TIBA on vascular differentiation, fruit growth and mineral accumulation could be replicated by reducing auxin concentrations in a more 'natural' way.

Information gained should help establish whether mineral and carbohydrate accumulation was restricted by the vascular capacity in the fruit stalk and whether there is a possibility that auxin is involved in mineral and carbohydrate accumulation in kiwifruit.

8.2. Materials and methods

8.2.1. TIBA and fruit quality

Experimental design for 2001/2002: preliminary trial

On 6 December 2001 (21 DAFB), during the cell division phase of fruit growth when Ca is still rapidly accumulating in fruit (Clark and Smith, 1988), 50 vines were selected from a block of vines growing in an orchard on Parish Line Rd, Ardmore, South Auckland, New Zealand. In each of these vines, four short shoots (200 in total) were selected, each with around 3 fruit (roughly 600 fruit in total). Fruit on half of these shoots were suspended, for ten seconds, in a 50 mg L^{-1} TIBA (2,3,5-triiodobenzoic acid) solution made by dissolving 50 mg of TIBA in 2.5ml 1 N NaOH and 20 ml ethanol before adding 0.05 ml of Pulse® penetrant (Monsanto Company, USA) and making the solution up to 1 L with distilled water. Fruit on the remaining 100 shoots were treated with a control solution made as above, but without the TIBA. As there were no data available on TIBA use in kiwifruit it was decided to use a 50 mg L^{-1} TIBA solution, as this was the most commonly used concentration reported in the literature (Bangerth, 1976; Cutting and Bower, 1989; Wand *et al.*, 1991b; Hamamoto *et al.*, 1998).

A further four shoots were selected from the same vines on 11 January 2002 (57 DAFB), after the main period of Ca accumulation and cell division (Clark and Smith, 1988), and TIBA and control solutions were applied as on 6 December 2001. On 2 May 2002, 50 fruit were harvested from each treatment (a) TIBA 1: TIBA applied on 6 December 2001, (b) Control 1: control solution applied on 6 December 2001, (c) TIBA 2: TIBA applied on 11 January 2002, and (d) Control 2: control solution applied on 11 January 2002). Fruit FW, DM and SS concentrations and K, Mg, Ca and P contents and concentrations were measured in addition to fruit stalk diameter and length, as described in the general methods (Chapter 2). The remaining fruit (between 150-250 fruit per treatment) were harvested into modular bulk packs with plastic liners and put into cool storage for postharvest assessment of firmness, SSC, rot and storage disorder incidence as described in the general methods (Chapter 2). Differences between treatments were analysed using a one-way ANOVA model.

Experimental design for 2002/2003

TIBA concentration

On 5 December 2002 (8 DAFB), ten vines were selected from within a bay of vines growing in a block at the HortResearch Te Puke Research Centre in the Bay of Plenty, New Zealand. In each of these vines, ten short shoots were tagged and two shoots were allocated to each of the following treatments (20 shoot replicates per treatment): (a) fruit on the shoots were treated with a 0 mg L^{-1} (control), (b) 50 mg L^{-1}, (c) 100 mg L^{-1}, (d) 150 mg L^{-1} or (e) 200 mg L^{-1} TIBA solution (application as described in the preliminary trial above). One fruit per shoot was harvested on 7 January 2003 (41 DAFB), and one fruit per shoot was harvested on 5 May 2003 (159 DAFB) for FW, DMC and mineral ion content and concentration measurements as described in Chapter 2 (general methods). In addition, fruit stalk external and vascular tissue dimensions and estimated secondary xylem conductance were assessed on half of the fruit from each treatment as described in Chapter 2. Total DW and water content (in grams) of each fruit were deduced from DMC and FW data. This equated to 20 fruit and 10 fruit stalks being assessed for each treatment at each of the mentioned harvest dates.

TIBA application timing

On 31 October 2002, 28 days prior to full bloom (DPFB) twelve vines were selected from the same bay as used (above) in the TIBA concentration trial. Two shoots were selected in each of these vines 28 DPFB, and flower buds on one of these shoots were treated with a 100 mg L^{-1} TIBA solution, whilst buds on the remaining shoot were treated with a control solution as described above. This procedure was repeated 21, 14 and 7 DPFB and 0, 7, 14 and 21 DAFB. Consequently, in each vine flowers/fruit on eight shoots were treated with a TIBA solution and flowers/fruit on eight shoots were treated with a control solution. This meant that flowers/fruit on 24 shoots were treated at each TIBA application time. Half of these flowers/fruit were treated with a TIBA solution (average ≈ 25 flowers/fruit per treatment) while the other half were treated with a control solution (average ≈ 25 flowers/fruit per vine). A 100 mg L^{-1} solution was chosen in order to try and increase the response to TIBA obtained in the preliminary trial (refer to the results for more information). In ten of the vines, one fruit per shoot was harvested on 10 January 2003 and one fruit per shoot was harvested on 21 April 2003 for assessment as described above. Subsequently, at each harvest date, 10 control and 10 treated fruit were assessed for each application date and fruit stalks from half of these fruit were assessed as described above.

Xylem Functionality

On 10 January 2003 (44 DAFB), four TIBA-treated fruit and four untreated fruit, from each of the eight TIBA application times, were randomly selected from the 12 experimental vines and xylem

functionality of the ventromedian carpellary (VMC) bundles was assessed using dye infiltration as described in Section 2.7. This procedure was repeated on 11 January 2003, so in total 128 fruit were assessed, 64 from each TIBA application time, 32 of which had been treated with TIBA and 32 of which had been left as a control. These dates were selected for assessing xylem functionality as they were just prior to the time when the cambium becomes inactive (refer Chapter 7). Therefore, it was expected that dye would still be accumulating in the fruit, but that experimental results would not be greatly affected by cycles of xylem dysfunction and repair that occur during the early stages of fruit development (Dichio *et al.*, 2003).

Storage

After the April harvest, any remaining fruit were harvested into single layer trays with polyliners and kept in cool storage for 24 weeks at 0°C. After this time, eight fruit were selected from each treatment (TIBA or control), for each application time (128 fruit in total) and their firmness and SSC contents were assessed as described in Chapter 2.

Statistical analysis

Mean differences between fruit suspended in different concentrations of TIBA were determined as described in the general methods (Chapter 2). Data from the different application times were grouped into the following four categories: TIBA applied 21-28 DPFB, TIBA applied 7-14 DPFB, TIBA applied 0-7 DAFB and TIBA applied 14-21 DAFB, based on the fact that fruit in each of these categories had very similar averages for each of the assessed variables. For each of the four categories, mean differences between TIBA and untreated fruit (80 treated and untreated fruit, 40 from each category) were determined by one-way analysis of variance. Where the F-stat indicated a significant difference between treatments the Fisher's protected least significant difference (LSD) procedure was used to establish significance between treatment means. Results for each category were plotted using bar graphs.

8.2.2. TIBA plus girdling or defoliation of long and short shoots and fruit quality

Experimental design

Mature kiwifruit vines, cv 'Hayward' growing at a Te Puke orchard (on Number 3 Rd) in the Bay of Plenty district of New Zealand were used in this experiment. Fifteen vines were selected from within an orchard block, taking care to avoid vines from outside rows. In each vine, six long and six short shoots, carrying at least two flowers, were selected, as illustrated in Figure 8.1, and the flowers were hand-pollinated (28 November 2002). At petal fall (7 December 2002), four shoots

were selected from each vine, two of each type (long and short), and were defoliated. On the same date, two long shoots and two short shoots also were selected from each vine to be girdled, and two long and two short shoots were selected as controls (left untouched). On 9 December 2002, all of the fruit on one girdled, one defoliated and one control shoot, of each shoot type, were suspended in a 100 mg L^{-1} TIBA solution (as described in Section 8.2.1) for 10 seconds. Fruit on the remaining shoots were treated with a control solution containing no TIBA (Fig 8.1). Consequently, there were a total of 180 shoots treated, 60 of which were girdled, 60 defoliated and 60 left as controls. For each shoot treatment (girdled, defoliated or control) 30 of the 60 shoots were long and 30 were short. Fifteen of these 30 shoots were treated with TIBA and 30 were left as controls.

Fruit and fruit stalk attributes

On 14 January 2003 (48 DAFB), ten vines were selected, and one fruit was harvested from each shoot in these vines. One fruit also was harvested from each shoot in the same ten vines on 30 April 2003 (154 DAFB) after cambium activity had ceased. Fruit FW, DMCs, and K, Mg, Ca and P concentrations and contents were assessed as described in the general methods (Chapter 2). In addition, fruit stalk lengths and diameters were measured on one third of the fruit from each shoot treatment. Consequently, for each shoot treatment 120 fruit and 40 fruit stalks were assessed, half of which were from long shoots (60 and 20, respectively) and half of which were from short shoots. Of these 60 fruit, 30 were treated with TIBA and 30 were left as controls. Similarly, ten of the 20 fruit stalks were from fruit treated with TIBA and ten were from untreated fruit.

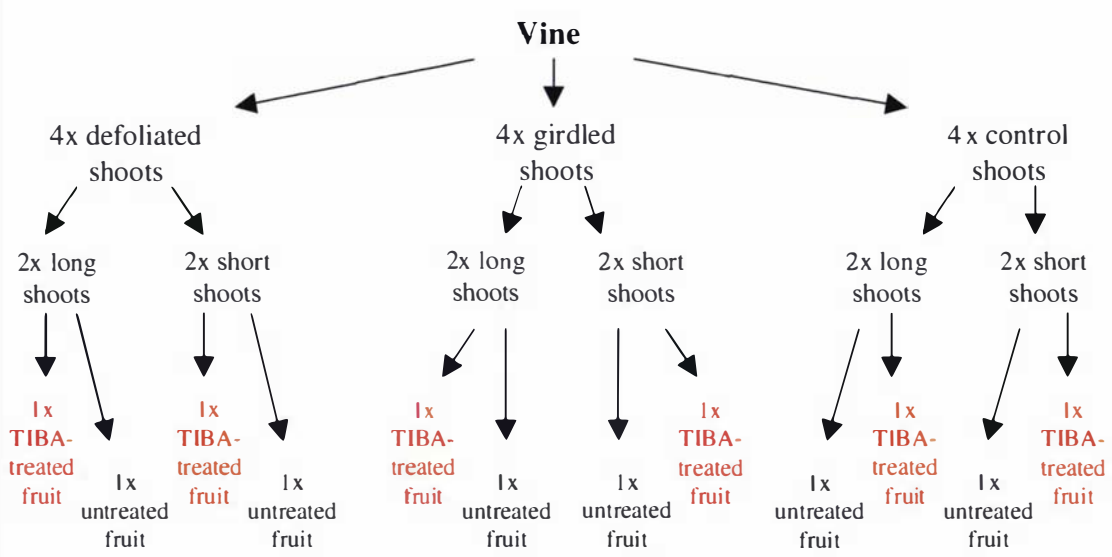


Fig 8.1. Diagrammatic representation of one vine replicate. TIBA was applied to all the fruit on half of the experimental shoots, as indicated by the red text. Ten vine replicates were used in the experiment.

Xylem functionality

On 15 and 16 January 2003, 6 fruit were harvested from girdled long shoots and 6 fruit were harvested from girdled short shoots from the same ten vines as used above. Of these six fruit, three had been treated with TIBA and three had not. This procedure was repeated for the defoliation and control treated shoots and VMC bundles were assessed for their xylem functionality as described in Section 2.7. These harvest dates were selected for the reasons described in Section 8.2.1.

Storage trials

Any remaining fruit on the ten harvested vines, in addition to fruit on the five un-harvested vines, were packed into modular bulk boxes with polyliners and put in cool storage for assessment 24 weeks after harvest as described in the general methods (Section 2.9). For every fruit treatment (TIBA or control) x shoot type (long or short) x shoot treatment interaction (control, girdled or defoliated) 15 fruit were assessed (60 per shoot treatment (girdled, defoliated and control) of which 30 were from long shoots and 30 were from short shoots).

Statistical analysis

This experiment was analysed as a 3 (shoot treatments) x2 (shoot type) x2 (with and without TIBA) factorial design. As time restraints restricted the number of fruit that could be sampled, it was only possible to assess one shoot per (shoot treatment x shoot type x TIBA) treatment (i.e.; there was no replication for the combination of variables). Consequently, it was not possible to assess interactions between TIBA treatment and shoot-type or between TIBA treatment and shoot treatments. Therefore, statistical analyses only investigated main (treatment) effects. Within each shoot treatment (girdled, defoliated or control), one-way ANOVA was used to identify where significant differences existed between fruit from different shoot types (long or short) or between TIBA-treated and control (or untreated) fruit, as described in Section 2.10. This method also was used to identify where significant differences existed between fruit from the different shoot treatments (girdled, defoliated or control) and data were plotted as bar graphs.

8.2.3. Seeds and fruit quality

Experimental design

Mature 'Hayward' kiwifruit vines growing at the HortResearch Te Puke Orchard in the Bay of Plenty district of New Zealand, were used in this experiment. Six vines were selected for the study, three each from two adjacent rows. Within each vine, 18 short shoots were selected and were thinned to two flowers per shoot (216 fruit in total). These 18 shoots were divided in six

groups of three, each with 36 fruit, and each group was marked with a different colour tag representing the six different treatments to be applied. Flowers on tagged laterals were enclosed in terylene sleeves to exclude both insect and wind-carried pollen from staminate vines. Within 2 days of flower opening each group of shoots was randomly assigned one of the following treatments: 20 ml suspension media, 1 L distilled water and 5 ml marker dye with 4 g (control: made according to manufacture's recommendations), 0.8, 0.6, 0.4, 0.2, or 0.04 g pollen, as described below. Each treatment was prepared and applied before proceeding with the next treatment. Treatments were applied in a random order and flowers re-bagged until petal fall when the stigmas were no longer receptive.

Formation of Pollen Suspensions and Pollen Spraying

Staminate pollen that had been collected during flowering in 2003 and stored at –18°C over aqueous H_2SO_4 mixtures was thawed and mixed in PollenAid® suspension media, made up with distilled water, and a non-toxic red marker dye. This suspension was agitated for 10min in a plastic (PollenAid®) mixer, and applied to newly opened flowers (within 2 days of opening) using a KiwiPollen Cambrian sprayer® (hand-held 1.5L pressure sprayer with a hand-operated pump) within one hour of being agitated. Each flower was sprayed for around 0.25 seconds, from 10 cm away, to provide a light coat of pollen. Treated flowers were distinguishable by the pink colour of the marker dye.

Harvest

On 21 January and 26 April 2004 (52 and 154 days after DAFB, respectively), 15 fruit were harvested from each of the six treatments, except the 0.04 g pollen suspension solution treatment, because most of the fruit from this treatment had abscised so it was only possible to harvest six fruit in January and April. Fruit stalk lengths and diameters, xylem and phloem areas and estimated secondary xylem conductance were measured in stalks from ten of the fifteen fruit from each treatment, or all of the stalks from the 0.04 g pollen suspension solution treatment as described in the general methods (Chapter 2). Each fruit was weighed and its dimensions were determined as described in Section 2.3, before cutting the fruit in half. One of these halves was weighed and bagged for seed number determination as described below. From the remaining half an equatorial slice (5-6 mm) was taken for assessment of the fruit DMC. A thin slice (2-3 mm) also was taken from either side of this slice to determine fruit Ca, Mg, K and P concentrations as described in the general methods (Section 2.5).

Seeds from the April harvest were dark in colour and were easy to separate from the flesh and count. However, seeds from the January harvest were pale in colour and were difficult to separate from the flesh. Moreover, once separated from the flesh they shrank to the size of a speck of dust

and tended to stick together making it a near impossible task to count them. As the objective of this experiment was to determine the effect of fruit seed number on carbohydrate and mineral accumulation and vascular development in fruit stalks, seeds counts had to be highly accurate. Therefore, it was decided not to analyse the January data (vascular, mineral and carbohydrate data) until data from the April harvest had been assessed. Results from the April harvest were unambiguous (Section 8.3.3), so it was decided that it was not necessary to analyse January data.

Seed Number

After removing skin from each fruit half, the flesh was cut into small pieces and left to digest for 2-4 days in 5 ml L^{-1} pectinase (Rohapect D5l, Carter & Associates, NZ). Digested flesh was forced through a fine sieve with high-pressure water to free seeds from the pulp. Extracted seeds were dried at 50°C for 4-6 h prior to counting. Total seed number per fruit was estimated by dividing the number of seeds by the percentage of the total FW of the fruit half. In order to assess the accuracy of this method for estimating seed number, a further 20 fruit, of varying sizes, were randomly selected from the experimental vines and cut in half. Both halves of the fruit were weighed and the seed number in each half was determined. Using this method total fruit seed number could be determined with 99% accuracy (P=0.001: Fig 8.2). This meant that the maximum difference between the actual and estimated seed number was 18 seeds, no more than 3% of the total seed number. Estimates of fruit seed numbers clearly showed that the different dilutions of the PollenAid® solution were successful in producing fruit with different seed numbers (Fig 8.2).

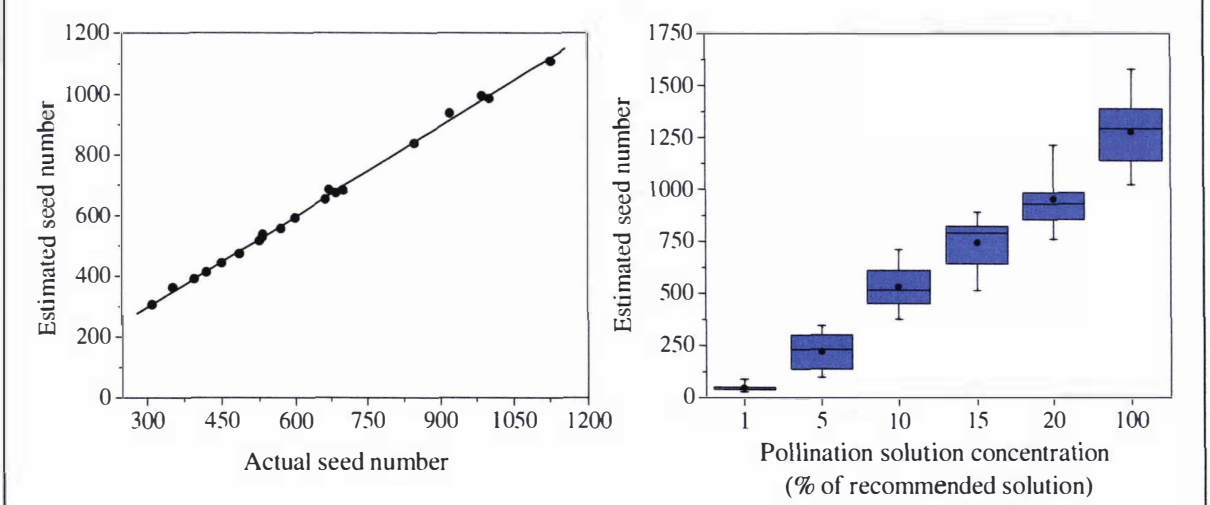


Fig 8.2. Relationship between actual and estimated seed numbers (Left: see Section 8.2.3 for details) and between the pollen solution concentration and the estimated seed number (right). Boxes illustrate the upper and lower quartile values, with a line to show the median value and a dot to indicate means for each treatment. Whiskars show the data range, and crosses indicate outliers for each treatment. Treatments are all significantly different at $P < 0.001$.

Statistical analysis

Two-way ANOVA was used to identify significant treatment effects, and where F-stat values were significant Fisher's LSD procedure was used to identify mean differences between treatments. Relationships between fruit seed numbers and fruit and fruit stalk characteristics were plotted and curves were fitted to data using Origin 7.5 (Massachusetts, USA). Where relationships between variables were linear, simple regression was used to establish R^2 values and significance values for curves fitted to the data. Where non-linear curves were fitted to the data, an F-stat was calculated for each curve based on R^2 values given by Origin 7.5 and the degrees of freedom for each curve (based on the number of parameters used to fit each curve and the total number of observations).

8.3. Results

8.3.1. TIBA and fruit quality

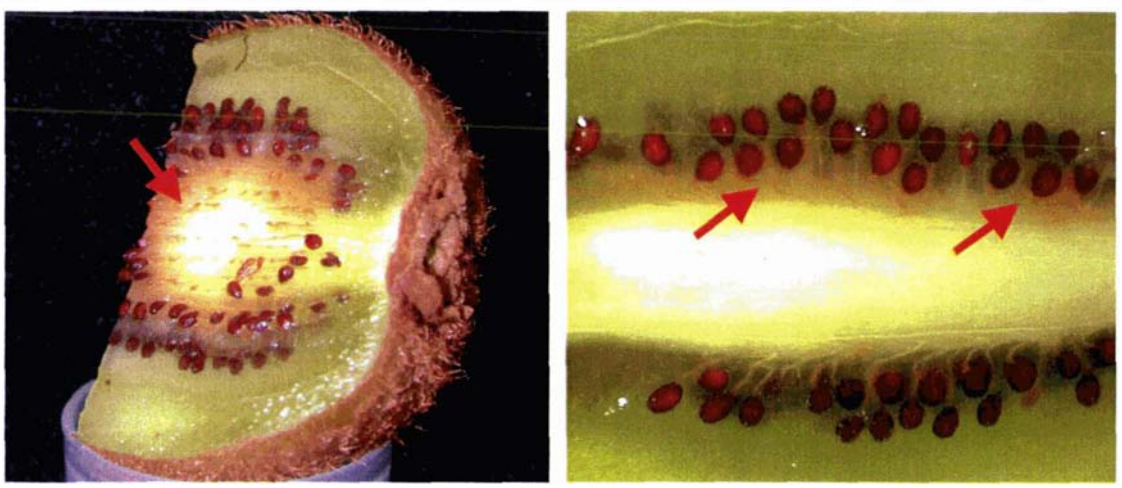


Fig 8.3. Vascular discolouration in TIBA-treated fruit. Arrows point to the discolouration

Preliminary trial

When TIBA was applied during cell division, only the pedicel diameter was reduced ($P < 0.05$). While fresh and dry weights, DMCs, SSC and contents of Ca, Mg and P were all numerically lower in TIBA-treated fruit than in untreated fruit, no statistically significant differences occurred (data not shown). The SSC of TIBA 1 treated fruit was, on average, 12% lower than in fruit from the other treatments ($P < 0.05$) between 12-16 weeks at $0^{O}C$, but after 24 weeks at $0^{O}C$ there was no treatment effect on fruit SSC. In contrast, fruit firmness did not differ between treatments after

12 or 16 weeks at 0°C, but after 24 weeks firmness of TIBA 1 treated fruit was 40-60% lower than that of fruit from the other treatments ($P < 0.001$). Fruit DMCs were 5-10% lower ($P < 0.05$) in TIBA 1 treated fruit than in fruit from other treatments after 16 weeks at 0°C. There was no effect of treatment on storage rots. After 24 weeks at 0°C, approximately 5% of fruit were pitted, 25% had slight LTB and 10% severe LTB, however pitting and LTB incidence did not differ between treatments. Many of the softer fruit (< 0.8 kgf (7.8 N), that did not develop LTB, developed a brown discolouration in the vascular tissue. This discolouration appeared to develop from the seeds into the surrounding cortex, usually towards the core, and was more apparent in TIBA-treated fruit than in untreated fruit (Fig 8.3).

On the basis of these results it was decided to repeat the TIBA experiments in the 2002/2003 season using different concentrations of TIBA, and applying TIBA at different times closer to anthesis.

TIBA concentration

January-harvested fruit

TIBA at concentrations of 50 mg L^{-1} or greater affected all fruit quality attributes measured, except fruit stalk length, DMCs and concentrations of K and P (Table 8.1). Xylem area was more reduced by TIBA application than phloem area. Estimated secondary xylem conductance was reduced by nearly 100% in TIBA-treated fruit when compared to untreated fruit, due to a large drop in both the number of vessels and the average vessel diameter. In control and TIBA-treated fruit, large diameter vessels (>15 μm) accounted for 62 and 0% of the total estimated conductance, respectively. Fruit quality attributes were not enhanced further (Fig. 8.4) by TIBA concentrations greater than 50 mg L^{-1} (see also lack of significance between the 50 and 100 mg L^{-1} TIBA treatments (Table 8.1)).

May-harvested fruit

In May-harvested fruit, TIBA at concentrations > 50 mg L^{-1} reduced fruit stalk diameter, FW and DW accumulation, the fruit content of all minerals, xylem and phloem area and estimated secondary xylem conductance (due to a decline in both the number of vessels and the average vessel diameter: Table 8.2). DMCs were reduced in fruit treated with 50 mg L^{-1}, but not 100 mg L^{-1} TIBA, when compared to the control treatment. In contrast to the January results, Ca and Mg concentrations were not significantly reduced by TIBA application in May-harvested fruit. This was because fruit Ca and Mg concentrations decreased approximately 38% less in TIBA-treated fruit than in untreated fruit during the period between 7 January 2003 and 5 May 2003 (from 41

DAFB), while the Ca and Mg contents of TIBA-treated fruit increased by 20-29% and 8-9% more, respectively, than in untreated fruit.

Table 8.1. Several fruit quality attributes, measured on 7 January 2003, following application of TIBA, at 50 and 100 mg L^{-1} on 5 December 2002, 8 DAFB.

Fruit attribute	Control	TIBA 50 mg L^{-1}	% difference from the control	TIBA 100 mg L^{-1}	% difference from the control
Fruit stalk length (mm)	59 a	59 a		67 a	
Fruit stalk diameter (mm)	3.6 a	2.2 b	-38	2.4 b	-32
Fresh weight (g)	35 a	22 b	-37	19 b	-46
Dry weight (g)	2.3 a	1.4 b	-39	1.3 b	-45
Water content (g)	33 a	21 b	-37	18 b	-46
DMC	6.6 a	6.2 a		6.7 a	
Ca (mg $100g^{-1}$ FW)	40 a	24 b	-40	21 b	-48
Mg (mg $100g^{-1}$ FW)	8.3 a	6.5 b	-22	5.8 b	-30
K (mg $100g^{-1}$ FW)	192 a	196 a		210 a	
P (mg $100g^{-1}$ FW)	22 a	22 a		23 a	
Ca content (mg)	13.8 a	5.6 b	-59	4.0 b	-71
Mg content (mg)	2.96 a	1.43 b	-52	1.12 b	-62
K content (mg)	68 a	43 b	-37	40 b	-41
P content (mg)	7.8 a	4.9 b	-37	4.4 b	-44
Phloem area (mm^2)	1.47 a	0.78 b	-47	0.73 b	-50
Xylem area (mm^2)	1.17 a	0.38 b	-68	0.30 b	-74
Secondary xylem vessel number	49 a	7.5 b	-85	1.30 b	-97
Estimated secondary xylem conductance (arbitary units)	40 a	0.14 b	-100	0.02 b	-100

In each row means followed by the same letter are not significantly different at $P < 0.001$. Fruit stalk diameter values are an average of measurements taken at the fruit, mid and shoot ends of the stalk.

Table 8.2. Several fruit quality attributes, measured on 5 May 2003, following application of TIBA, at 50 and 100 mg L^{-1} on 5 December 2002, 8 DAFB.

Fruit attribute	Control	TIBA 50 mg L^{-1}	% difference from the control	TIBA 100 mg L^{-1}	% difference from the control
Fruit stalk length (mm)	59 a	64 a		64 a	
Fruit stalk diameter (mm)	3.45 a	2.96 b	-14	2.59 b	-25
Fresh weight (g)	92 a	77 b	-16	60 c	-35
Dry weight (g)	14.3 a	11.4 b	-20	9.2 c	-36
Water content (g)	78 a	66 b	-16	51 b	-35
DMC	15.5 a	14.7 b	-5	14.9 ab	
Ca (mg $100g^{-1}$ FW)	24 a	19 a		20 a	
Mg (mg $100g^{-1}$ FW)	12 a	11 a		11 a	
K (mg $100g^{-1}$ FW)	254 a	253 a		266 a	
P (mg $100g^{-1}$ FW)	36 a	36 a		35 a	
Ca content (mg)	23 a	14 b	-39	13 b	-43
Mg content (mg)	11.4 a	8.1 b	-29	6.5 b	-43
K content (mg)	239 a	193 ab		158 b	-34
P content (mg)	33 a	28 ab		21 bc	-36
Phloem area (mm^2)	1.55 a	1.22 b	-21	0.86 c	-45
Xylem area (mm^2)	1.42 a	0.82 b	-42	0.55 b	-61
Secondary xylem vessel number	47 a	1.25 b	-97	1.25 b	-97
Estimated secondary xylem conductance (arbitary units)	29.00 a	0.05 b	-100	0.01 b	-100

In each row means followed by the same letter are not significantly different at $P < 0.001$. Fruit stalk diameter values are an average of measurements taken at the fruit, mid and shoot ends of the stalk.

In addition to Ca and Mg, the effect of TIBA on other fruit quality attributes also was less noticeable in May than in January. In May-harvested fruit, 100 mg L^{-1} TIBA was required to obtain an equivalent response to 50 mg L^{-1} TIBA in January-harvested fruit (refer Tables 8.1 and 8.2 and Figure 8.4). The response to TIBA was not increased by applying more than 100 mg L^{-1} TIBA, except for fruit Ca concentration, where Ca concentration of fruit treated with 200 mg L^{-1} TIBA (16 mg $100g^{-1}$ FW) was significantly less than in untreated fruit (24 mg $100g^{-1}$ FW).

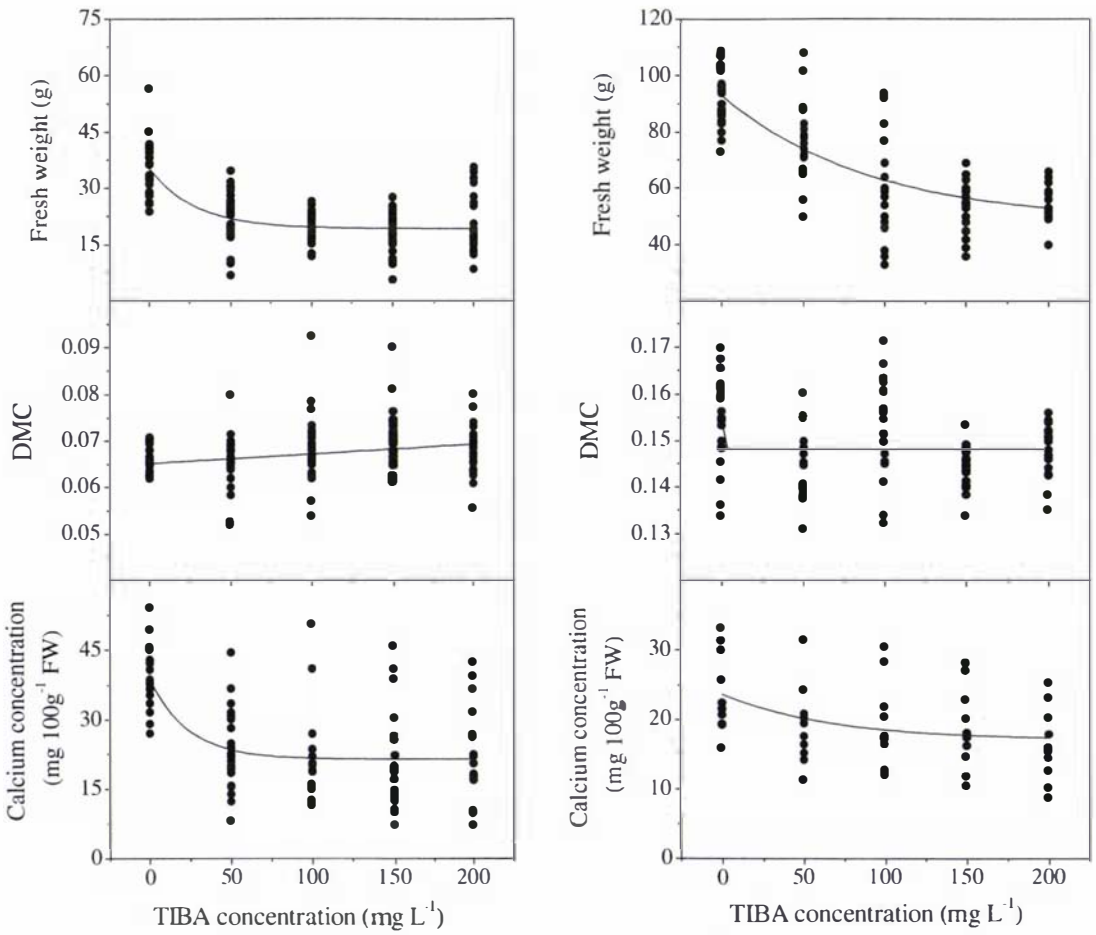


Fig 8.4. Fruit FW, DM and Ca concentrations, as measured on 7 January 2003 (left-hand side) and 5 May 2003 (right-hand side), following TIBA application on 5 December 2002, 8 DAFB. Each point represents data from one fruit.

Timing of TIBA application

January-harvested fruit

TIBA affected xylem area more than phloem area, especially when applied in the first few weeks after anthesis. Estimated secondary xylem conductance was reduced to negligible levels when TIBA was applied 0-7 DAFB (Fig 8.5), due to a large decrease in both number of vessels and average vessel diameter. In untreated fruit, vessels < 8 μm in diameter and vessels < 14 μm in diameter contributed 10% and 51%, respectively, to the total estimated conductance, compared with 75% and 0%, respectively, for fruit treated with TIBA 0-7 DAFB. Estimated secondary xylem conductance also was reduced when TIBA was applied 21-28 DPFB due to a 60% reduction in the average vessel diameter. TIBA application between 28 DPFB to seven DAFB

resulted in a 14-24% reduction in the FW, DW and water content of the fruit when compared to the control treatment, however, DMCs were not affected by TIBA application at any of the times tested (data not shown).

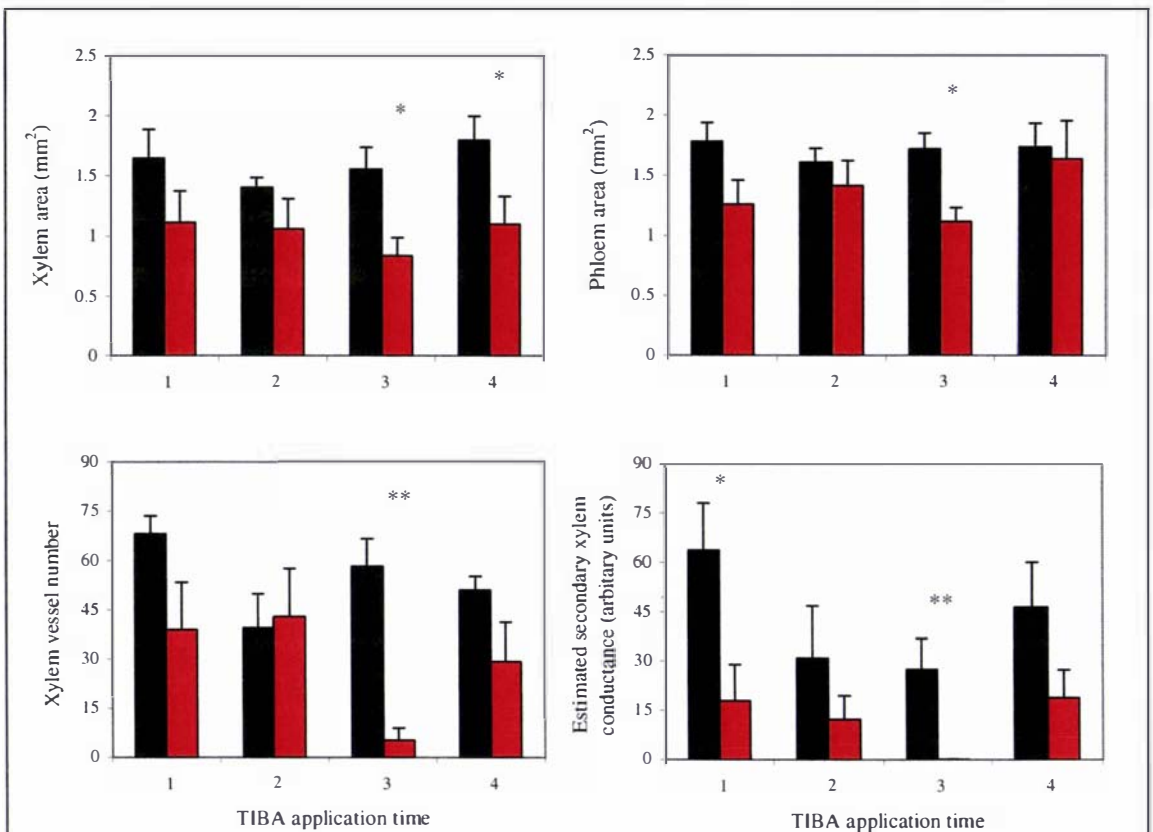


Fig 8.5. Xylem and phloem area, secondary xylem vessel number and estimated conductance, as measured on 10 January 2003, following TIBA application 21-28 DPFB (time 1), 7-14 DPFB (time 2), 0-7 DAFB (time 3) or 14-21 DAFB (time 4). Black bars represent control fruit and red bars TIBA-treated fruit, and are mean values (± s.e.). Stars are used to show significance at * P <0.05, ** P <0.01, *** P <0.001.

Calcium was the only nutrient consistently reduced by TIBA application, although a significant difference between TIBA-treated fruit and untreated fruit was only found when TIBA was applied 14-21 DAFB. Fruit Mg concentrations were increased when TIBA was applied 14-21 DAFB, whilst they were reduced when TIBA was applied 21-28 DPFB. TIBA had no effect on the K and P concentrations in the fruit (Fig 8.6). Fruit Ca content was significantly reduced when TIBA was applied 7-14 DPFB (P <0.05), whilst the Mg content was reduced when TIBA was applied 21-28 DPFB and 0-7 DAFB, as was the P content (P <0.05). The fruit K content was only reduced when TIBA was applied 21-28 DPFB (P <0.05, data not shown).

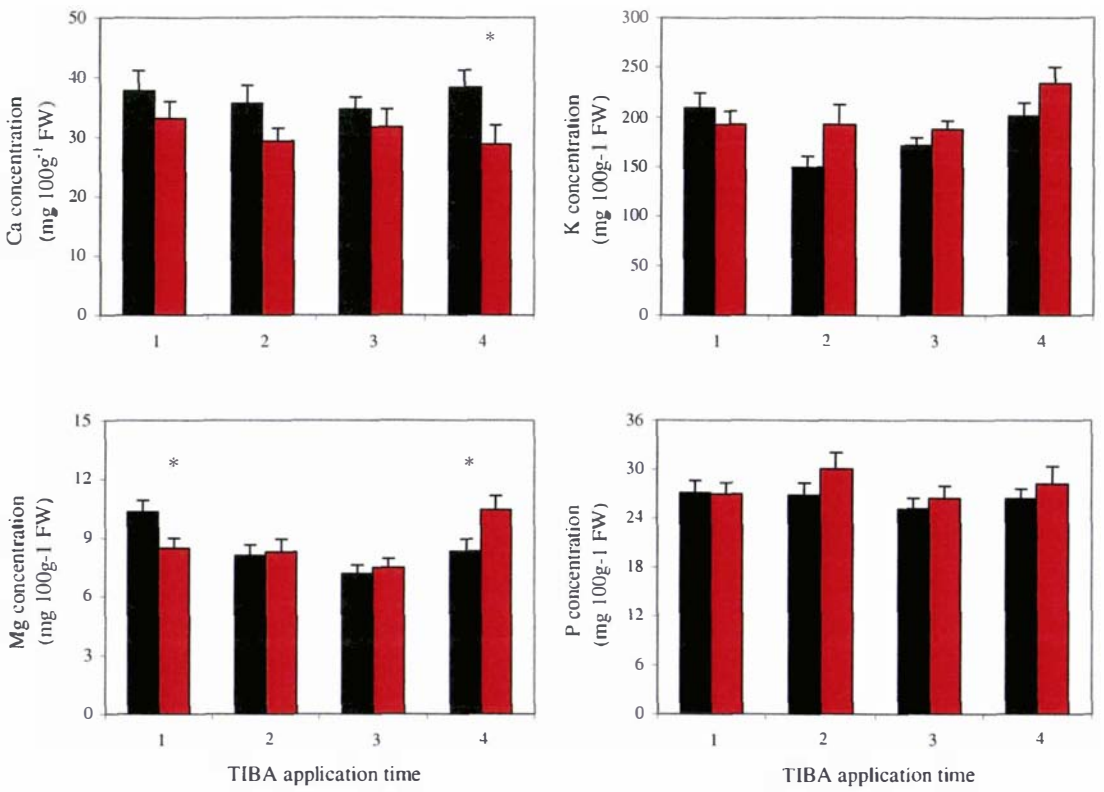


Fig 8.6. Fruit Ca, K, Mg and P concentrations, as measured on 10 January 2003, following TIBA application 21-28 DPFB (time 1), 7-14 DPFB (time 2), 0-7 DAFB (time 3) or 14-21 DAFB (time 4). Black bars represent control fruit and red bars TIBA-treated fruit, and are mean values (± s.e.). Stars are used to show significance at * P <0.05, ** P <0.01, *** P <0.001.

April-harvested fruit

In April-harvested fruit, the xylem area was reduced when TIBA was applied between 14 DPFB to seven DAFB, however phloem area was only reduced when TIBA was applied 7-14 DPFB. Estimated secondary xylem conductance was reduced to negligible levels when TIBA was applied from 14 DPFB to seven DAFB (Fig 8.7). This was due to an 80% reduction in xylem vessel number and a 66% reduction in the average vessel diameter. FW, DW and fruit water content responded similarly to TIBA application and were all reduced by 21-25% when TIBA was applied between 14 DPFB and seven DAFB. Dry weight was reduced more than the fruit water content when TIBA was applied 0-7 DAFB, and as a result fruit DMCs were 4% lower in fruit treated with TIBA at this time than in untreated fruit (Fig 8.8).

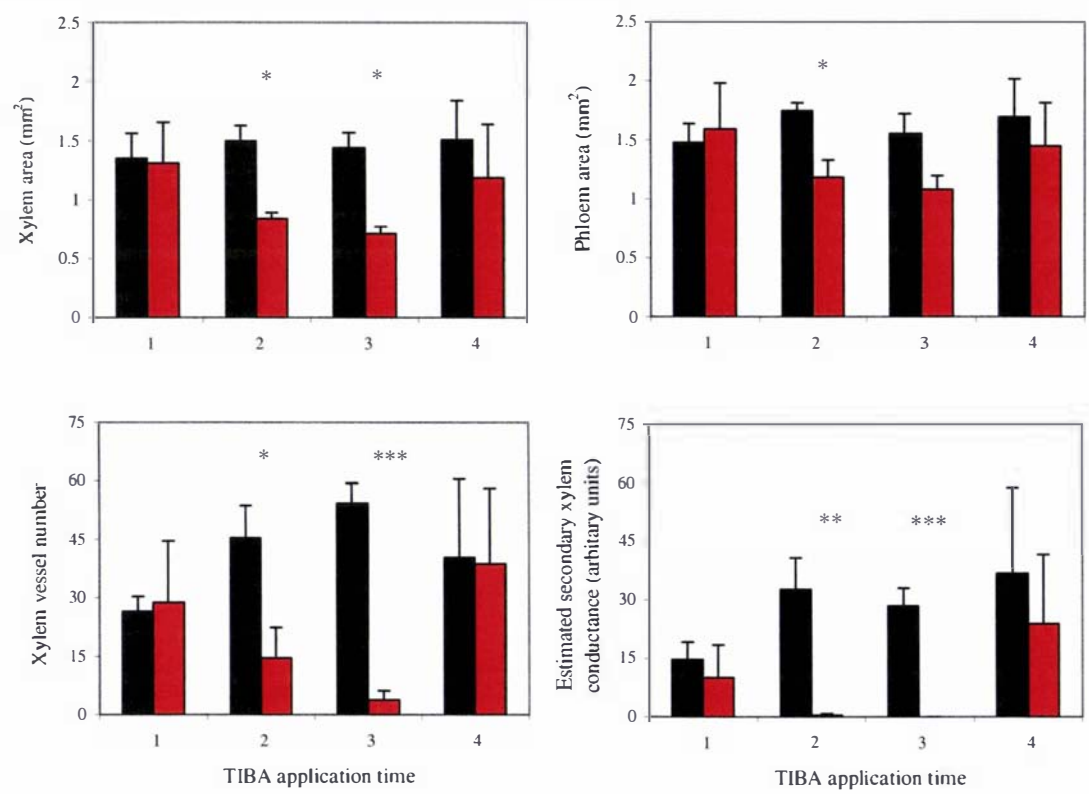


Fig 8.7. Xylem and phloem area, secondary xylem vessel number and estimated conductance, as measured on 21 April 2003, following TIBA application 21-28 DPFB (time 1), 7-14 DPFB (time 2), 0-7 DAFB (time 3) or 14-21 DAFB (time 4). Black bars represent control fruit and red bars TIBA-treated fruit, and are mean values (± s.e.). Stars are used to show significance at * P <0.05, ** P <0.01, *** P <0.001.

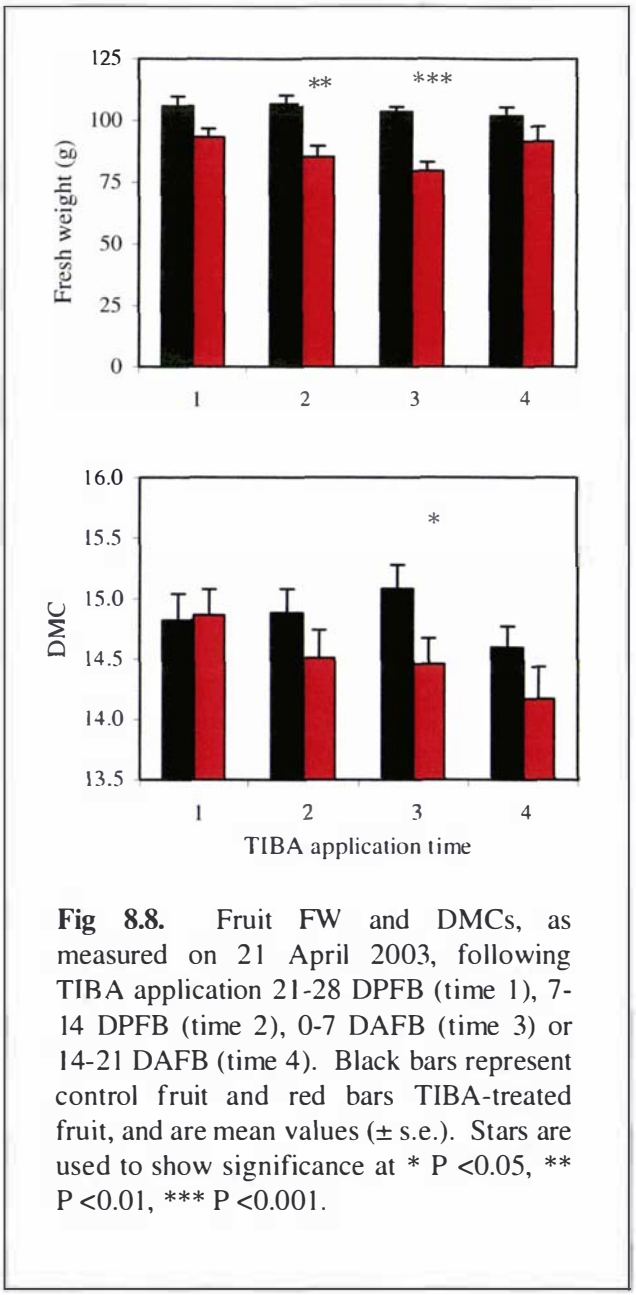


Fig 8.8. Fruit FW and DMCs, as measured on 21 April 2003, following TIBA application 21-28 DPFB (time 1), 7-14 DPFB (time 2), 0-7 DAFB (time 3) or 14-21 DAFB (time 4). Black bars represent control fruit and red bars TIBA-treated fruit, and are mean values (± s.e.). Stars are used to show significance at * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

In April-harvested fruit TIBA application, at any time, did not affect fruit mineral concentrations despite the fact that Mg and Ca concentrations were significantly affected in January-harvested fruit (Fig 8.9). The FW, DW and water content of TIBA-treated fruit increased by a similar amount to that in the untreated fruit between the January and April harvests. However, during the days between these harvests the Ca concentration was reduced by 13% more in untreated fruit than in fruit that had been treated with TIBA 14-21 DAFB. Consequently, the Ca concentration of the TIBA-treated and untreated fruit was about equal at harvest. The contents of K, Mg and P were significantly reduced when TIBA was applied 7-14 DPFB ($P < 0.05$). Fruit Ca content was never affected by TIBA application at the times investigated.

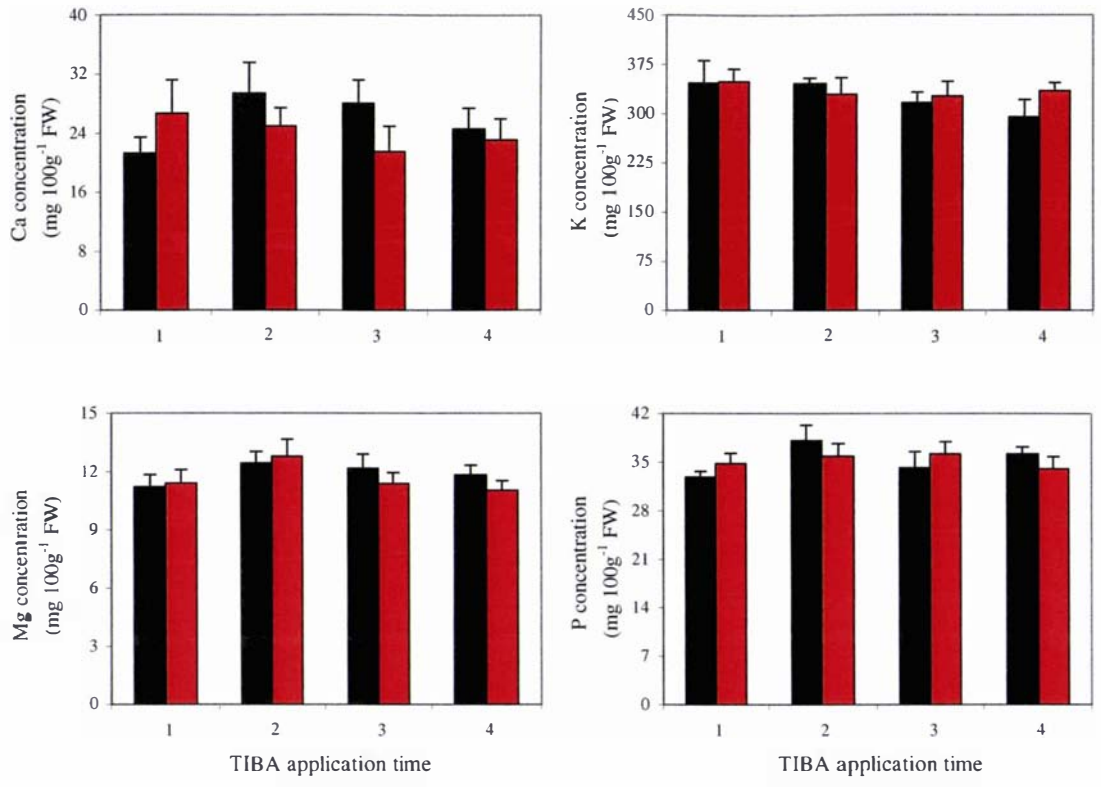


Fig 8.9. Fruit Ca, K, Mg and P concentrations, as measured on 21 April 2003, following TIBA application 21-28 DPFB (time 1), 7-14 DPFB (time 2), 0-7 DAFB (time 3) or 14-21 DAFB (time 4). Black bars represent control fruit and red bars TIBA-treated fruit, and are mean values (± s.e.).

Xylem functionality

TIBA reduced xylem functionality of the VMC bundles by 70 and 88% when applied 0-7 and 14-21 DAFB, but had no affect when applied prior to FB (Fig 8.10).

Storage trial results

After 24 weeks in storage fruit firmness ranged from 6.7-8.4 N and SSC ranged from 11-12.2%. TIBA application time did not significantly affect fruit firmness. However, TIBA application between 0-7 DAFB reduced fruit SSC by 8% when compared to fruit from the control treatment ($P < 0.05$). There was no evidence of the vascular discolouration observed in the preliminary TIBA trial.

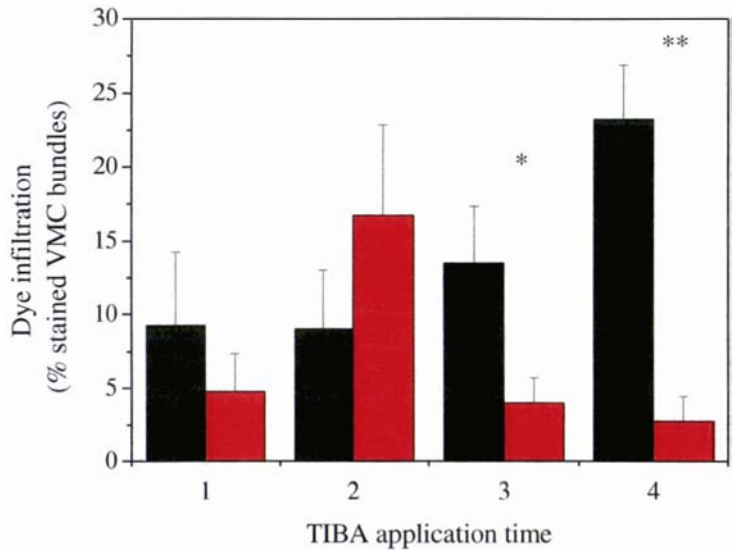


Fig 8.10. Functionality of xylem vessels in the ventromedian carpellary (VMC) bundles, as measured on 10-11 January 2003, following TIBA application 21-28 DPFB (time 1), 7-14 DPFB (time 2), 0-7 DAFB (time 3) or 14-21 DAFB (time 4). Black bars represent control fruit and red bars TIBA-treated fruit, and are mean values (± s.e.). Stars are used to show significance at * P <0.05, ** P <0.01, *** P <0.001.

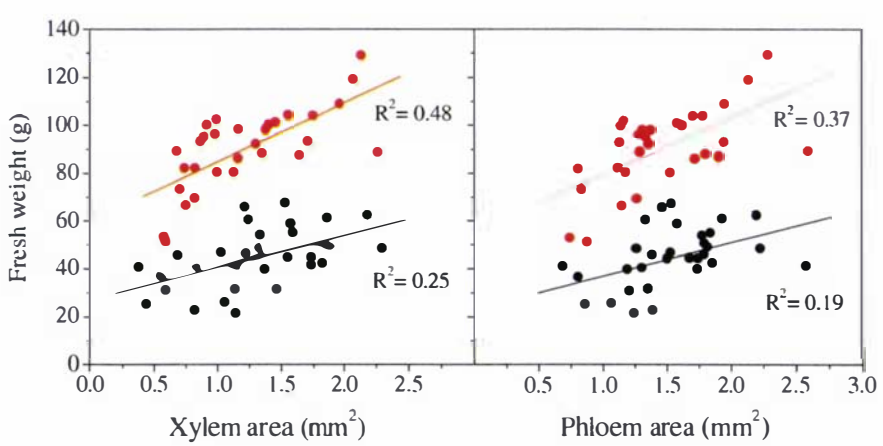


Fig 8.11. Relationship between xylem (left) and phloem (right) area and fruit FW, as measured on 10 January 2003 (black data points) and 21 April 2003 (red data points). TIBA was applied to all fruit. Data include points from each TIBA application time (between 28 DPFB and 21 DAFB). Each point represents data for one fruit.

Correlations between vascular development and fruit quality

Fruit FW was linearly correlated with xylem and phloem area in January and April (Fig 8.11). Similarly, fruit DW was linearly correlated with xylem and phloem area, whilst fruit DMCs were never significantly correlated with either the xylem or phloem area (data not shown). Calcium concentrations of fruit in January ($P < 0.001$) and May ($P < 0.05$) were positively correlated with estimated secondary xylem conductance (Fig 8.12). Magnesium concentrations of fruit in January ($P < 0.01$), but in May, were positively correlated also with estimated secondary xylem conductance, although the correlations were not as strong as for Ca. Potassium and P concentrations were never significantly correlated with estimated secondary xylem conductance (Fig 8.12).

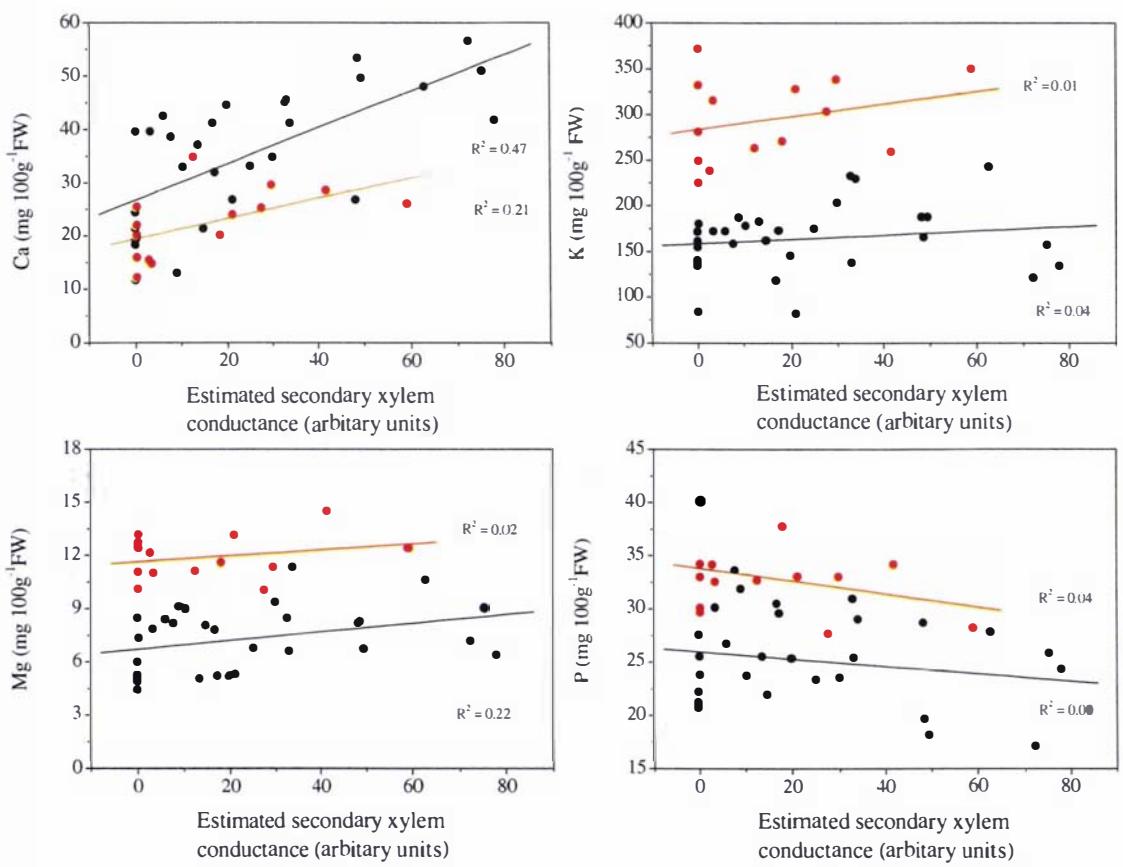


Fig 8.12. The relationship between estimated secondary xylem conductance and fruit Ca, Mg, K and P concentrations in January (black data points) and May (red data points). Data include points from each TIBA application time (between 28 DPFB and 21 DAFB). Each point represents data for one fruit.

In fruit from the January harvest, but not the May harvest, K ($P < 0.05$, $R^2 = 0.10$), Mg ($P < 0.05$, $R^2 = 0.13$) and Ca ($P < 0.01$, $R^2 = 0.21$) concentrations were positively correlated with xylem area.

Fruit Mg concentrations also were positively correlated with phloem area in January ($P < 0.01$, $R^2 = 0.20$), but not in May. Phloem area was not significantly correlated with fruit K, Ca or P concentrations (data not shown).

8.3.2. Effects of TIBA plus girdling or defoliation on fruit quality

January-harvested fruit

Control shoot treatment

TIBA reduced fruit stalk diameter, FW, DW and water content of fruit regardless of shoot type (Table 8.3). Fruit stalk length and fruit DMCs were unaffected by TIBA application. Calcium concentrations were reduced by TIBA application in fruit from long, but not short shoots. Fruit Mg, K and P concentrations were not affected by TIBA regardless of shoot type. Calcium, Mg and K contents were reduced by TIBA application in fruit from both long and short shoots, but fruit P contents were not affected by TIBA application. Fruit stalk diameters of TIBA-treated fruit from long shoots were greater than those of TIBA-treated fruit from short shoots. There were no differences between the attributes of untreated fruit from long and short shoots.

Defoliation shoot treatment

TIBA reduced fruit water and K contents regardless of shoot type (Table 8.3). Fruit stalk diameter and fruit Ca concentrations and contents were reduced in fruit from long shoots, whilst TIBA application to fruit on short shoots reduced fruit stalk length, fruit FW, DW and Mg content. Fruit DM, Mg, K and P concentrations and P contents were not affected by TIBA in long or short shoots. Calcium concentrations and contents were greater in untreated fruit from long shoots than in untreated fruit from short shoots. TIBA-treated fruit from long shoots had longer fruit stalks, and greater fresh and dry weights, water contents and Mg contents than TIBA-treated fruit from short shoots. Fruit stalk diameter, fruit DMCs, fruit Mg, K and P concentrations and fruit K and P contents never differed between shoot types.

Girdling plus TIBA treatment

TIBA treatment had a smaller effect on fruit from girdled shoots than on fruit from defoliated and control shoots (Table 8.3); only the DW of fruit from long shoots was reduced by TIBA application. Fruit from long shoots always had a greater fruit stalk length than fruit from short shoots. The DW of untreated fruit from long shoots was greater than the DW of untreated fruit from short shoots. None of the other fruit attributes differed significantly between fruit on long and short shoots, regardless of the fruit treatment.

Table 8.3. Several fruit quality attributes, as measured on 14 January 2003, following TIBA application to fruit on girdled or defoliated long and short axillary shoots on 9 December 2002.

Fruit or fruit stalk attribute	Fruit treatment	Shoot type	Axillary shoot treatment			Treatment differences
			Control	Defoliated	Girdled	
Fruit stalk length (mm)	Control	Long	71_{a}	72_{a}	71_{a}	
	TIBA	Long	62_{a}	74_{a}	73_{a}	
	Control	Short	61_{a}	76_{a}	62_{b}	
	TIBA	Short	66_{a}	58_{b}	63_{b}	
Fruit stalk diameter (mm)	Control	Long	3.4_{a}	4.0_{ab}	3.2_{a}	* C_{a}, D_{b}, G_{a}
	TIBA	Long	3.0_{b}	3.1_{c}	3.2_{a}	
	Control	Short	3.7_{a}	3.5_{bc}	3.1_{a}	
	TIBA	Short	2.7_{c}	2.8_{c}	2.7_{a}	
Fresh weight (g)	Control	Long	49_{a}	48_{a}	49_{a}	
	TIBA	Long	32_{b}	41_{a}	39_{a}	
	Control	Short	55_{a}	43_{a}	38_{a}	
	TIBA	Short	35_{b}	33_{b}	34_{a}	
Dry weight (g)	Control	Long	3.4_{a}	3.4_{a}	3.8_{a}	
	TIBA	Long	2.1_{b}	2.9_{a}	2.7_{b}	* C_{a}, D_{b}, G_{ab}
	Control	Short	3.8_{a}	3.0_{a}	2.6_{b}	
	TIBA	Short	2.4_{b}	2.2_{b}	2.3_{b}	
Water content (g)	Control	Long	45_{a}	45_{a}	45_{a}	
	TIBA	Long	30_{b}	38_{b}	37_{ab}	
	Control	Short	51_{a}	40_{a}	36_{ab}	
	TIBA	Short	33_{b}	30_{c}	31_{b}	
DMC	Control	Long	6.9_{a}	7.1_{a}	7.5_{a}	
	TIBA	Long	6.7_{a}	7.2_{a}	6.9_{a}	* C_{a}, D_{b}, G_{ab}
	Control	Short	7.0_{a}	6.8_{a}	6.7_{a}	
	TIBA	Short	6.9_{a}	6.9_{a}	6.9_{a}	

Continued on next page...

Fruit or fruit stalk attribute	Fruit treatment	Shoot type	Axillary shoot treatment			Treatment differences
			Control	Defoliated	Girdled	
Ca (mg $100g^{-1}$ FW)	Control	Long	36_a	30_a	35_a	
	TIBA	Long	23_b	19_b	29_a	
	Control	Short	30_{ab}	16_b	28_a	* C_a, D_b, G_a
	TIBA	Short	22_b	10_b	19_a	* C_a, D_b, G_a
Mg (mg $100g^{-1}$ FW)	Control	Long	8.3_a	9.2_a	8.8_a	
	TIBA	Long	6.7_a	9.0_a	7.5_a	
	Control	Short	8.0_a	8.2_a	8.5_a	
	TIBA	Short	7.3_a	6.7_a	6.9_a	
K (mg $100g^{-1}$ FW)	Control	Long	184_a	188_a	187_a	
	TIBA	Long	197_a	180_a	178_a	
	Control	Short	203_a	204_a	196_a	
	TIBA	Short	205_a	206_a	204_a	
P (mg $100g^{-1}$ FW)	Control	Long	16_a	19_a	18_a	
	TIBA	Long	18_a	15_a	17_a	
	Control	Short	21_a	18_a	21_a	
	TIBA	Short	23_a	18_a	16_a	
Ca content (mg)	Control	Long	15_a	12_a	14_a	
	TIBA	Long	6.1_b	6.6_b	9.4_{ab}	
	Control	Short	14_a	5.6_b	8.7_{ab}	
	TIBA	Short	6.3_b	2.8_b	5.4_b	
Mg content (mg)	Control	Long	4.1_a	4.4_a	4.3_a	
	TIBA	Long	2.1_b	3.7_a	2.9_a	
	Control	Short	4.4_a	3.5_a	3.2_a	
	TIBA	Short	2.6_b	2.2_b	2.3_a	
K content (mg)	Control	Long	90_a	90_a	92_a	
	TIBA	Long	63_b	74_{bc}	69_a	
	Control	Short	112_a	88_{ac}	74_a	* C_a, D_b, G_{ab}
	TIBA	Short	72_b	68_b	69_a	
P content (mg)	Control	Long	7.8_a	9.1_a	8.8_a	
	TIBA	Long	5.8_a	6.2_a	6.6_a	
	Control	Short	12_a	7.7_a	8.0_a	
	TIBA	Short	8.1_a	5.9_a	5.4_a	

Data are mean values. For each shoot treatment (column), values followed by the same letter are not significantly different at $P < 0.05$. Within a row values are significantly different at * $P < 0.05$, as indicated in the treatment differences column for control (C), defoliated (D) and girdled (G) shoots.

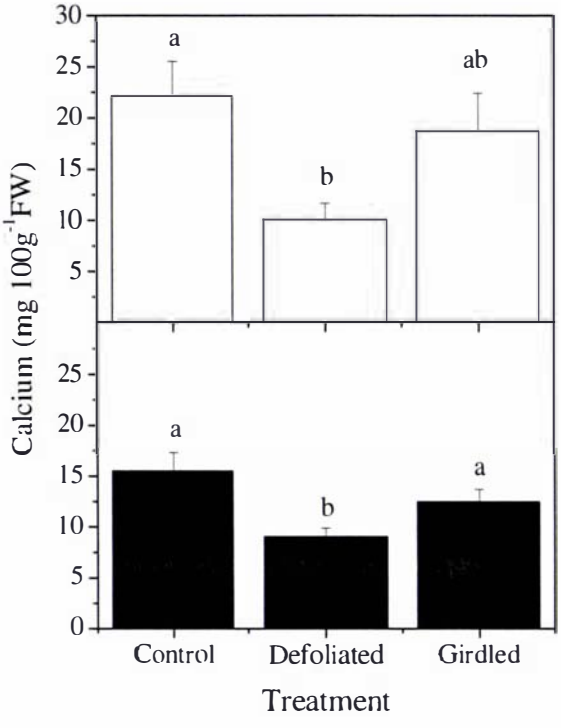


Fig 8.13. Calcium concentrations in TIBA-treated fruit from short control, defoliated and girdled shoots, as measured on 14 January 2003 (white bars) and 30 April 2003 (black bars). Bars represent mean values (± s.e.). Treatments with the same letters above the bars are not significantly different at P <0.05. TIBA was applied on 9 December 2002, 11 DAFB.

Comparison between treatments

The Ca concentration of fruit from short defoliated shoots was lower than that of fruit from short control and girdled shoots (Table 8.3). This was especially noticeable in TIBA-treated fruit, where Ca concentrations of fruit from short defoliated shoots were 55% lower than those in fruit from control shoots (Fig 8.13). TIBA-treated fruit from long-defoliated shoots had a 28% greater DW content than TIBA-treated fruit from long (control) shoots, and hence a 7% greater DMC (Fig 8.14). Untreated fruit from long-defoliated shoots had a 20% wider fruit stalk diameter than long (control) shoots and a 22% wider diameter than long-girdled shoots (P <0.05, data not shown). Fruit K contents were lower in short untreated fruit from defoliated and girdled shoots than in untreated fruit from control shoots.

In January, Ca (content): K (content) ratios in TIBA-treated and untreated fruit averaged 0.09 and 0.15, respectively, whilst Ca: P ratios averaged 0.91 and 1.54, respectively.

April-harvested fruit

Control treatment

Effects of TIBA on fruit from long shoots did not persist until April for any of the fruit quality attributes measured in fruit from control shoots (Table 8.4). This was because the DW and water content of TIBA-treated fruit increased more between the January and April harvests than in untreated fruit. Calcium concentration decreased more in untreated fruit than in TIBA-treated fruit, maybe as a result of greater fresh and dry weight gains in these fruit. Fruit stalk diameter and fruit FWs, DWs and water contents remained lower in TIBA-treated fruit from short shoots than in untreated fruit from short shoots. Fruit DMCs were higher in long shoots than short

shoots, regardless of the fruit treatment. TIBA-treated fruit from long shoots also had a higher fruit stalk diameter and fruit FW, DW and water content than TIBA-treated fruit from short shoots.

Table 8.4. Several fruit quality attributes, measured on 30 April 2003, following TIBA application to fruit on girdled or defoliated long and short axillary shoots on 9 December 2002.

Fruit or fruit stalk attribute	Fruit treatment	Shoot type	Axillary shoot treatment			Treatment differences
			Control	Defoliated	Girdled	
Fruit stalk length (mm)	Control	Long	67_{a}	65_{a}	65_{a}	
	TIBA	Long	64_{a}	66_{a}	59_{a}	
	Control	Short	68_{a}	57_{a}	60_{a}	
	TIBA	Short	61_{a}	59_{a}	60_{a}	
Fruit stalk diameter (mm)	Control	Long	3.6_{a}	4.3_{a}	3.9_{a}	
	TIBA	Long	3.5_{a}	3.8_{b}	3.4_{b}	* C_{a}, D_{b}, G_{a}
	Control	Short	3.6_{a}	3.6_{c}	3.9_{a}	
	TIBA	Short	3.0_{b}	3.3_{c}	3.1_{b}	
Fresh weight (g)	Control	Long	116_{a}	129_{a}	126_{a}	
	TIBA	Long	106_{a}	105_{b}	98_{ab}	
	Control	Short	106_{a}	108_{b}	93_{ab}	
	TIBA	Short	81_{b}	87_{c}	74_{b}	
Dry weight (g)	Control	Long	19_{a}	22_{a}	21_{a}	
	TIBA	Long	17_{a}	18_{b}	16_{b}	
	Control	Short	17_{a}	17_{bc}	15_{bc}	
	TIBA	Short	12_{b}	14_{c}	11_{c}	
Water content (g)	Control	Long	97_{a}	107_{a}	105_{a}	
	TIBA	Long	88_{a}	87_{bc}	82_{ab}	
	Control	Short	89_{a}	91_{ac}	78_{ab}	
	TIBA	Short	68_{b}	73_{b}	63_{b}	
DMC	Control	Long	16.8_{a}	17.3_{a}	16.9_{a}	
	TIBA	Long	16.5_{ab}	16.8_{a}	15.9_{ab}	* C_{ab}, D_{a}, G_{b}
	Control	Short	15.8_{bc}	15.6_{b}	16.0_{ab}	
	TIBA	Short	15.0_{c}	15.4_{b}	14.6_{c}	

Continued on next page...

Fruit or fruit stalk attribute	Fruit treatment	Shoot type	Axillary shoot treatment			Treatment differences
			Control	Defoliated	Girdled	
Ca (mg $100g^{-1}$ FW)	Control	Long	30_a	17_a	22_a	
	TIBA	Long	23_{ab}	18_a	22_a	
	Control	Short	20_{ab}	12_{ab}	19_a	
	TIBA	Short	16_b	9.1_b	12_a	* C_a, D_b, G_{ab}
Mg (mg $100g^{-1}$ FW)	Control	Long	11_a	12_a	12_a	
	TIBA	Long	11_a	12_a	13_a	
	Control	Short	11_a	11_a	13_a	
	TIBA	Short	11_a	11_a	11_a	
K (mg $100g^{-1}$ FW)	Control	Long	249_a	282_a	279_a	
	TIBA	Long	254_a	238_a	277_a	
	Control	Short	252_a	260_a	311_a	
	TIBA	Short	268_a	293_a	319_a	
P (mg $100g^{-1}$ FW)	Control	Long	23_a	24_a	28_a	
	TIBA	Long	23_a	22_a	27_a	
	Control	Short	21_a	25_a	29_a	
	TIBA	Short	28_a	25_a	27_a	
Ca content (mg)	Control	Long	34_a	22_a	28_a	
	TIBA	Long	24_{ab}	19_a	22_a	
	Control	Short	21_{ab}	13_{ab}	18_a	
	TIBA	Short	13_b	7.9_b	8.9_b	
Mg content (mg)	Control	Long	13_a	15_a	15_a	
	TIBA	Long	12_a	13_{ab}	13_{ab}	
	Control	Short	12_a	12_{ab}	12_{ab}	
	TIBA	Short	8.9_a	10_b	8.1_b	
K content (mg)	Control	Long	289_a	364_a	352_a	
	TIBA	Long	269_{ab}	250_b	271_b	
	Control	Short	267_{ab}	281_b	289_b	
	TIBA	Short	217_b	255_b	236_b	
P content (mg)	Control	Long	27_a	31_a	35_a	
	TIBA	Long	24_a	23_a	26_{ab}	
	Control	Short	22_a	27_a	27_{ab}	
	TIBA	Short	23_a	22_a	20_{ab}	

Data are mean values. For each shoot treatment (column), values followed by the same letter are not significantly different at $P < 0.05$. Within a row values are significantly different at * $P < 0.05$, as indicated in the treatment differences column for control (C), defoliated (D) and girdled (G) shoots.

Defoliation plus TIBA

Fruit FWs, DWs and water contents were reduced by TIBA application in fruit from defoliated shoots, regardless of shoot type (Table 8.4). In fruit from long shoots TIBA also reduced fruit stalk diameter and fruit K contents. Effects of TIBA on fruit Ca concentrations, and the content of Ca and K, did not persist until the April harvest. In the case of Ca, this was because Ca concentrations were reduced by 38% more between the January and April harvests in untreated fruit from long shoots than in TIBA-treated fruit from long shoots. Fruit from long shoots always had a greater fruit stalk diameter, FW, DW and DMC than fruit from short shoots. TIBA-treated fruit from long shoots continued to have a higher Ca concentration and content than TIBA-treated fruit from short shoots. K contents were higher in untreated fruit from long shoots than in untreated fruit from short shoots.

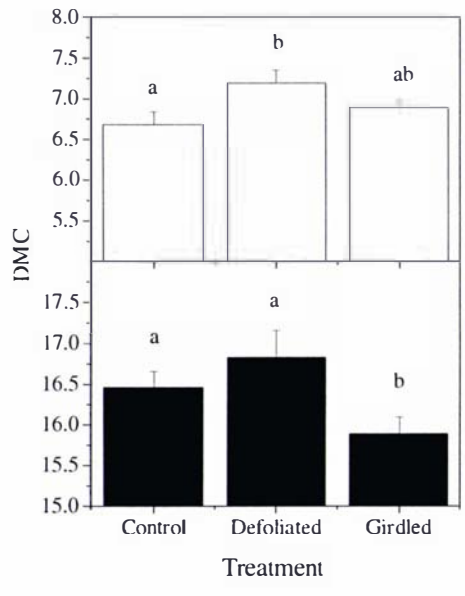


Fig 8.14. DMC in TIBA-treated fruit from long control, defoliated and girdled shoots, as measured on 14 January 2003 (white bars) and 30 April 2003 (black bars). Bars represent mean values (± s.e.). Treatments with the same letters above the bars are not significantly different at $P < 0.05$. TIBA was applied on 9 December 2002, 11 DAFB.

Girdling plus TIBA

TIBA application always reduced fruit stalk diameter in fruit from girdled shoots (Table 8.4). In fruit from long shoots TIBA also reduced fruit DW and fruit K contents, whilst in fruit from short shoots TIBA application reduced fruit DMCs and fruit Ca contents. Fruit DW was always greater in fruit from long shoots than in fruit from short shoots. Fruit DM and Ca concentrations also were higher in TIBA-treated fruit from long shoots than in TIBA-treated fruit from short shoots. K contents were higher in untreated fruit from long shoots than in untreated fruit from short shoots.

Comparison between treatments

The Ca concentration of TIBA-treated fruit from short-defoliated shoots was 43% lower than that of TIBA-treated fruit from short (control) shoots and 24% lower than that of TIBA-treated fruit from short-girdled shoots (Fig 8.13). The DMC of TIBA-treated fruit from long-girdled shoots was 4% lower than that of TIBA-treated fruit from long (control) shoots, and 5% lower than that of TIBA-treated fruit from long-defoliated shoots (Fig 8.14). The fruit stalk diameter of TIBA-

treated fruit from long-defoliated shoots was 11% greater than that of TIBA-treated fruit from long-girdled shoots ($P < 0.05$, data not shown).

Xylem functionality

TIBA reduced xylem functionality by 42% in fruit from control shoots and by 71% in fruit from defoliated shoots. Xylem functionality was not reduced in fruit from girdled shoots or in fruit from short control and defoliated shoots. In control shoots, xylem functionality was greater in untreated fruit from long shoots than in untreated fruit from short shoots, although fruit size did not differ between untreated fruit from these shoots. In defoliated shoots, xylem functionality was 65% lower and fruit size 30% greater in TIBA-treated fruit from long shoots than in TIBA-treated fruit from short shoots. Xylem functionality tended to be lower in fruit from defoliated shoots than in fruit from control or girdled shoots. Shoot treatment effects on xylem functionality were most apparent in fruit from long shoots.

Table 8.5. Xylem functionality and corresponding fruit fresh weights in control and TIBA-treated fruit from long and short control, defoliated or girdled shoots, as measured on 15-16 January 2003.

		Xylem functionality (%)				Fruit fresh weight (g)			
		Axillary shoot treatment				Axillary shoot treatment			
Fruit treatment	Shoot type	Control	Defoliated	Girdled	Treatment differences	Control	Defoliated	Girdled	Treatment differences
Control	Long	1.00_a	0.63_a	0.56_a	**	52_a	56_a	47_a	
TIBA	Long	0.58_b	0.18_b	0.64_{ab}	**	41_b	43_b	42_a	
Control	Short	0.53_b	0.53_a	0.71_{ab}		49_a	53_a	36_b	**
TIBA	Short	0.71_b	0.51_a	0.79_b		40_b	30_c	37_a	*
		0.71	0.46	0.68	*	46	46	41	

Data are mean values. For each shoot treatment (column), values followed by the same letter are not significantly different at $P < 0.05$. Within a row, highlighted values differ significantly from un-highlighted values at * $P < 0.05$ and ** $P < 0.01$.

Storage trial results

The only difference between TIBA and untreated fruit after storage was found in fruit from long, defoliated shoots where untreated fruit were 18% softer than TIBA-treated fruit (Table 8.6). Firmness of TIBA-treated fruit from long, girdled shoots was greater than that of TIBA-treated fruit from short, girdled shoots. Defoliation resulted in higher DMCs in fruit from long shoots than in fruit from short shoots. The rSSC of untreated fruit from long, defoliated shoots was higher in untreated fruit from short, defoliated shoots. The DMC of TIBA-treated fruit was greater from long, control shoots than from short, control shoots, whilst DMCs were greater for untreated fruit from long girdled shoots than for untreated fruit from short girdled shoots.

Table 8.6. Postharvest attributes of control and TIBA-treated fruit from long and short control, defoliated and girdled axillary shoots, as measured after 24 weeks in storage at 0°C from 30 April 2003.

Fruit or fruit stalk attribute	Fruit treatment	Shoot type	Axillary shoot treatment			Treatment differences	
			Control	Defoliated	Girdled		
Firmness (N)	Control	Long	7.7_{a}	5.9_{a}	8.1_{ab}	***	C(a), D(b), G(a)
	TIBA	Long	8.1_{a}	7.2_{b}	8.9_{a}	*	C(ab), D(a), G(b)
	Control	Short	7.2_{a}	6.4_{ab}	7.2_{b}		
	TIBA	Short	8.0_{a}	7.0_{b}	7.6_{b}		
rSSC (%)	Control	Long	12.7_{a}	13.6_{a}	12.4_{a}	*	C(ab), D(a), G(b)
	TIBA	Long	12.4_{a}	12.9_{ab}	12.2_{a}		
	Control	Short	13.2_{a}	12.4_{b}	11.9_{a}		
	TIBA	Short	12.2_{a}	12.1_{b}	12.3_{a}		
DMC	Control	Long	16.4_{ab}	16.1_{a}	16.3_{a}		
	TIBA	Long	17.0_{a}	15.8_{a}	15.5_{ab}	*	C(a), D(ab), G(b)
	Control	Short	15.7_{b}	14.4_{b}	15.2_{b}	*	C(a), D(b), G(ab)
	TIBA	Short	14.8_{b}	14.6_{b}	15.2_{b}		
Rots (% incidence)	Control	Long	1.8_{a}	1.8_{a}	4.5_{a}		
	TIBA	Long	0.0_{a}	0.0_{a}	1.3_{a}		
	Control	Short	0.0_{a}	4.8_{a}	0.0_{a}		
	TIBA	Short	3.6_{a}	2.7_{a}	0.0_{a}		
LTB (% incidence)	Control	Long	27_{a}	62_{a}	38_{a}	***	C(a), D(b), G(a)
	TIBA	Long	34_{a}	53_{a}	25_{a}	***	C(a), D(b), G(a)
	Control	Short	36_{a}	63_{a}	28_{a}	***	C(a), D(b), G(a)
	TIBA	Short	39_{a}	56_{a}	38_{a}	**	C(a), D(b), G(a)

Data are mean values. For each shoot treatment (column), values followed by the same letter are not significantly different at $P < 0.05$. Within a row values are significantly different at * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, as indicated for control (C), defoliated (D) and girdled (G) shoots.

Comparison between treatments

More than half of the fruit from defoliated shoots showed signs of LTB, whereas less than 40% of the fruit from control and girdled shoots showed signs of LTB (Table 8.6). Fruit from defoliated, long shoots (TIBA-treated and untreated fruit) were softer than fruit from girdled and control, long shoots. The DMC of TIBA-treated fruit from girdled, long shoots was lower than that of TIBA-treated fruit from long, control shoots and untreated fruit from long, girdled shoots had lower rSSC than untreated fruit from defoliated, long shoots. Untreated fruit from short, defoliated shoots had a lower DMC than untreated fruit from short, control shoots. Rot incidence was very low (< 5%) and did not differ between treatments. There was no evidence of physiological pitting or vascular discolouration after 24 weeks in storage.

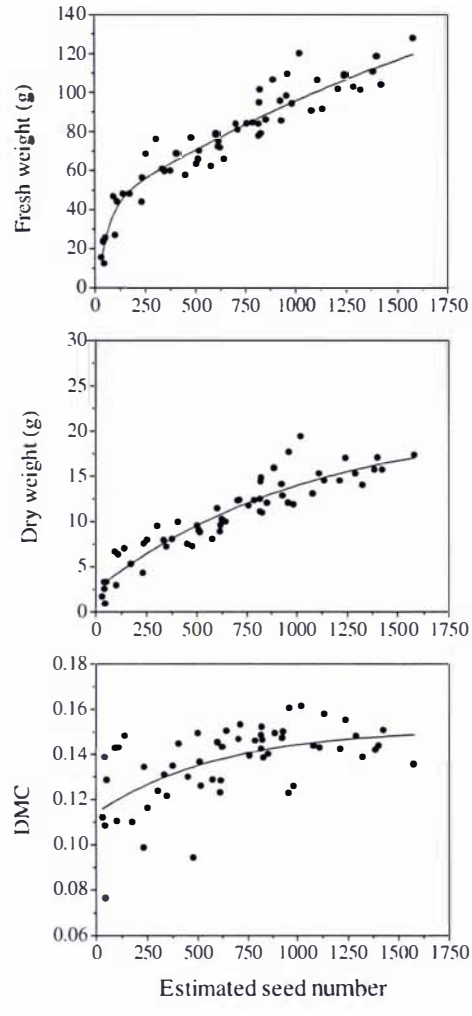


Fig 8.15. Relationship between estimated seed number, FW (top; R^2 = 0.91, P <0.001), DW (centre; R^2 = 0.86, P <0.001) and fruit DMC (bottom; R^2 = 0.34, P <0.05), as measured on 26 April 2004.

8.3.3. Seeds and fruit quality

Fruit FW, DW and DMCs increased asymptotically with increasing seed number (Fig 8.15). In January fruit fresh and dry weights increased as the concentration of pollen in the suspension medium increased from 0.04-0.6 g and from 0.04-0.8 g, respectively (P <0.001). Fruit DMCs were highest in fruit treated with the full strength PollenAid® solution and lowest in fruit treated with a 1 or 5% (0.04-0.2 g pollen) PollenAid® solution (P <0.001).

Fruit fresh and dry weights continued to increase over the seed range found in this experiment, although fruit DMCs increased little as seed numbers increased above 750 seeds. For example, average fruit DMCs increased by 17%, on average, as the seed number increased from 50 to 750 seeds, an increase of 700. However, as the seed number increased by 700 from 750-1450 seeds, fruit DMCs only increased by 5%. There was a positive linear relationship between fruit Ca and Mg concentrations over the seed number range found in this experiment. However, fruit K and P concentrations were not affected by fruit seed number (Fig 8.16). The content of all minerals was positively correlated with both seed number, and fruit FW (P = 0.001: data not shown). Fruit Ca: K ratios and Ca: P ratios for fruit with more than 800 seeds were 0.09 and 0.97, respectively; whilst for fruit with less than 800 seeds they were 0.06 and 0.63, respectively.

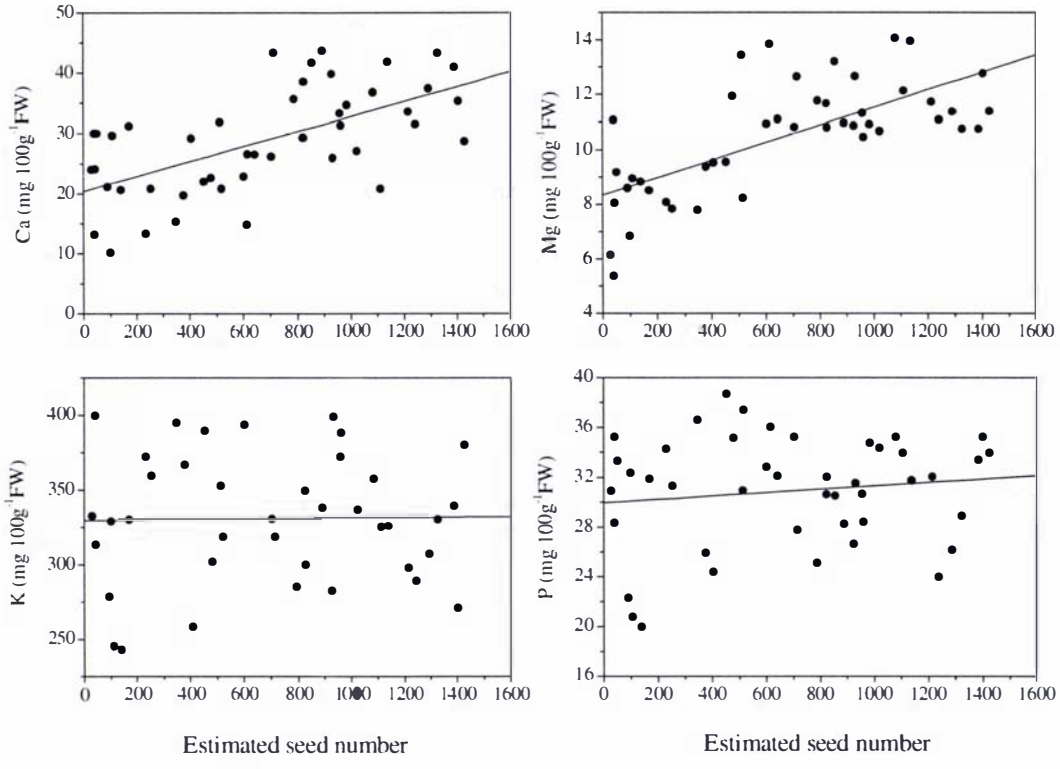


Fig 8.16. Relationship between the estimated fruit seed number and fruit Ca (R^2 = 0.36, P <0.001), Mg (R^2 = 0.46, P <0.001), K (not significant), P (not significant) concentrations, as measured on 26 April 2004.

Fruit stalk length was not related to estimated seed number in April. Similarly, in January fruit stalk length did not change as the concentration of pollen was increased in the suspension solution (data not shown). An asymptotic relationship was found between estimated fruit seed number and fruit stalk diameter in April (R^2 = 0.76, P <0.001), and in January, fruit stalk diameters increased as the concentration of pollen in the suspension solution increased (P <0.001). Fruit stalk diameter was linearly related to fruit fresh and dry weights (R^2 = 0.80, P <0.001) and DMCs (R^2 = 0.39, P <0.001: data not shown).

Fruit stalk phloem and xylem areas increased linearly with increasing estimated fruit seed number and the phloem to xylem ratio decreased linearly as the estimated fruit seed number increased (Fig 8.17). Pith area was not related to the estimated seed number (data not shown). Secondary xylem vessel number also increased linearly with increasing seed number and estimated secondary xylem conductance increased exponentially with increasing fruit seed number (Fig 8.18). Estimated secondary xylem conductance was negligible in fruit with less than 500 seeds, but increased rapidly in fruit with more than 750 seeds. The contribution made by large diameter vessels (>14

μm) to estimated secondary xylem conductance increased as the fruit seed number increased, whereas the contribution made by small diameter vessels (<8 μm) declined (P= 0.001; Fig 8.19). Consequently, fruit with fewer than 400 seeds had more small diameter vessels than large or medium (8-14 μm) diameter vessels and fruit with more than 800 seeds had more large diameter vessels than small diameter vessels (P <0.001).

There also were more large diameter vessels than medium diameter vessels in fruit with more than 800 seeds (P <0.001), whilst fruit with between 400-800 seeds had more medium diameter vessels than small or large diameter vessels (P <0.001; Fig 8.19). There was a positive linear correlation between fruit Ca and Mg concentrations and estimated secondary xylem conductance. However, fruit K and P concentrations were not significantly correlated with estimated secondary xylem conductance (Fig 8.20).

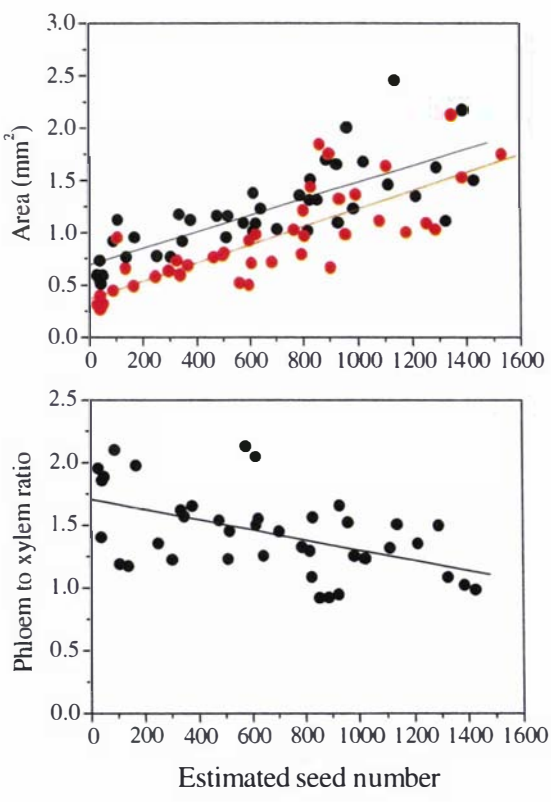


Figure 8.17. Relationship between the estimated fruit seed number and phloem (black; R^2 = 0.60, P <0.001) and xylem (red; R^2 = 0.63, P <0.001) areas (top) and the phloem: xylem ratio (bottom; R^2 = 0.24, P <0.001), as measured on 26 April 2004.

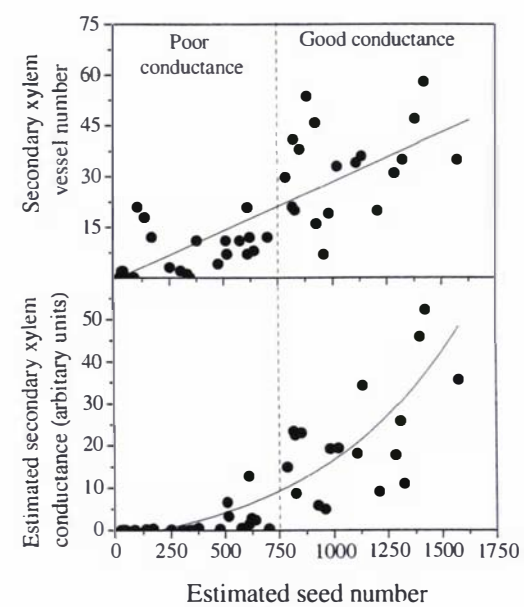


Figure 8.18. Relationship between the estimated fruit seed number and secondary xylem vessel number (top; R^2 = 0.59, P < 0.01) and estimated conductance (bottom; R^2 = 0.68, P < 0.01).

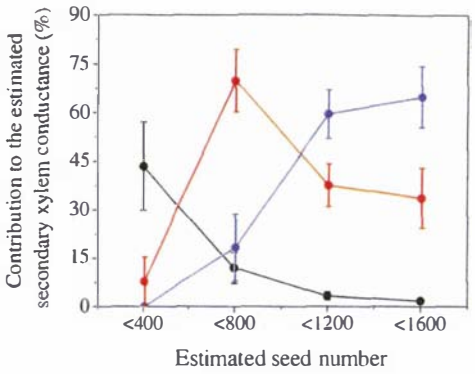


Fig 8.19. Percentage of small (<8 μm: black), medium (8-14 μm: red), and large (>14 μm: blue) diameter vessels contributing to the estimated secondary xylem conductance in fruit with low (<400), low-medium (<800), medium-high (<1200), and high (<1600) seed numbers. Data points are means (± s.e.) and were determined from fruit harvested on 26 April 2004.

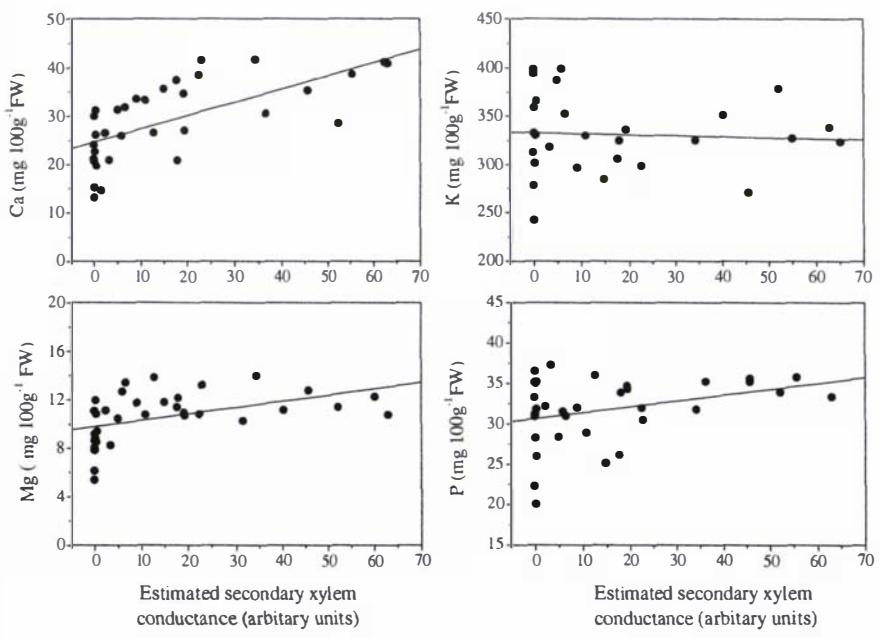


Figure 8.20. Concentrations of Ca ($R^2 = 0.33$, $P < 0.01$), Mg ($R^2 = 0.19$, $P < 0.05$), K (not significant) and P (not significant) in kiwifruit in relation to estimated secondary xylem conductance of fruit stalks.

8.4. Discussion

8.4.1. Mineral ion accumulation

Calcium and Mg accumulation in kiwifruit seems to be dependent on the extent of auxin synthesis in, and efflux from, developing fruit. Both TIBA application and seed number influenced Ca and Mg concentrations, and both are well known to affect auxin synthesis in, and efflux from, kiwifruit (Lai *et al.*, 1990 and D. Woolley and S. Lawes: pers. comm.) and other fruit (Nitsch, 1950; Banuelos *et al.*, 1987; Wand *et al.*, 1991a; Hamamoto *et al.*, 1998). In kiwifruit, vascular differentiation also was reduced by TIBA application during the initial period of fruit stalk and fruit development, and vascular differentiation was reduced in fruit with low seed numbers. Phloem differentiation was less affected by TIBA application and low seed numbers than xylem differentiation, which is in keeping with the finding that phloem differentiation can proceed at lower auxin concentrations than xylem differentiation (Aloni, 1987). This meant that the phloem: xylem ratio was increased at low seed numbers and following TIBA application to fruit between 0-21 DAFB.

It is proposed that the effect of auxin on Ca and Mg accumulation resides in the regulation of vascular development, as auxins are believed to provide the stimulus for vascular differentiation (Aloni and Zimmerman, 1983; Aloni *et al.*, 2000). TIBA application and declining seed numbers may indirectly affect Ca and Mg accumulation in fruit by reducing xylem differentiation in the fruit stalk and fruit, thereby restricting the movement of phloem immobile solutes into the fruit (Fig 8.21). The increased phloem: xylem ratio also would favour the accumulation of phloem mobile ions over phloem immobile ions. Evidence for this proposal includes: (a) the mutual decline in xylem differentiation and in fruit Ca and Mg concentrations in response to these treatments, and hence the positive correlation between fruit Ca, and to a lesser extent, Mg concentrations and estimated secondary xylem conductance, and (b) the finding that the effects of TIBA and pollination treatments on fruit Ca, Mg, K and P concentrations were related to mineral ion mobility in the phloem; K and P accumulation was not affected by these treatments and Mg accumulation was less affected than Ca accumulation. This may be evidence that xylem translocated minerals are more affected than phloem translocated minerals by reductions in auxin synthesis in, and/or efflux from, fruit, and supports the finding that xylem differentiation is more affected than phloem differentiation.

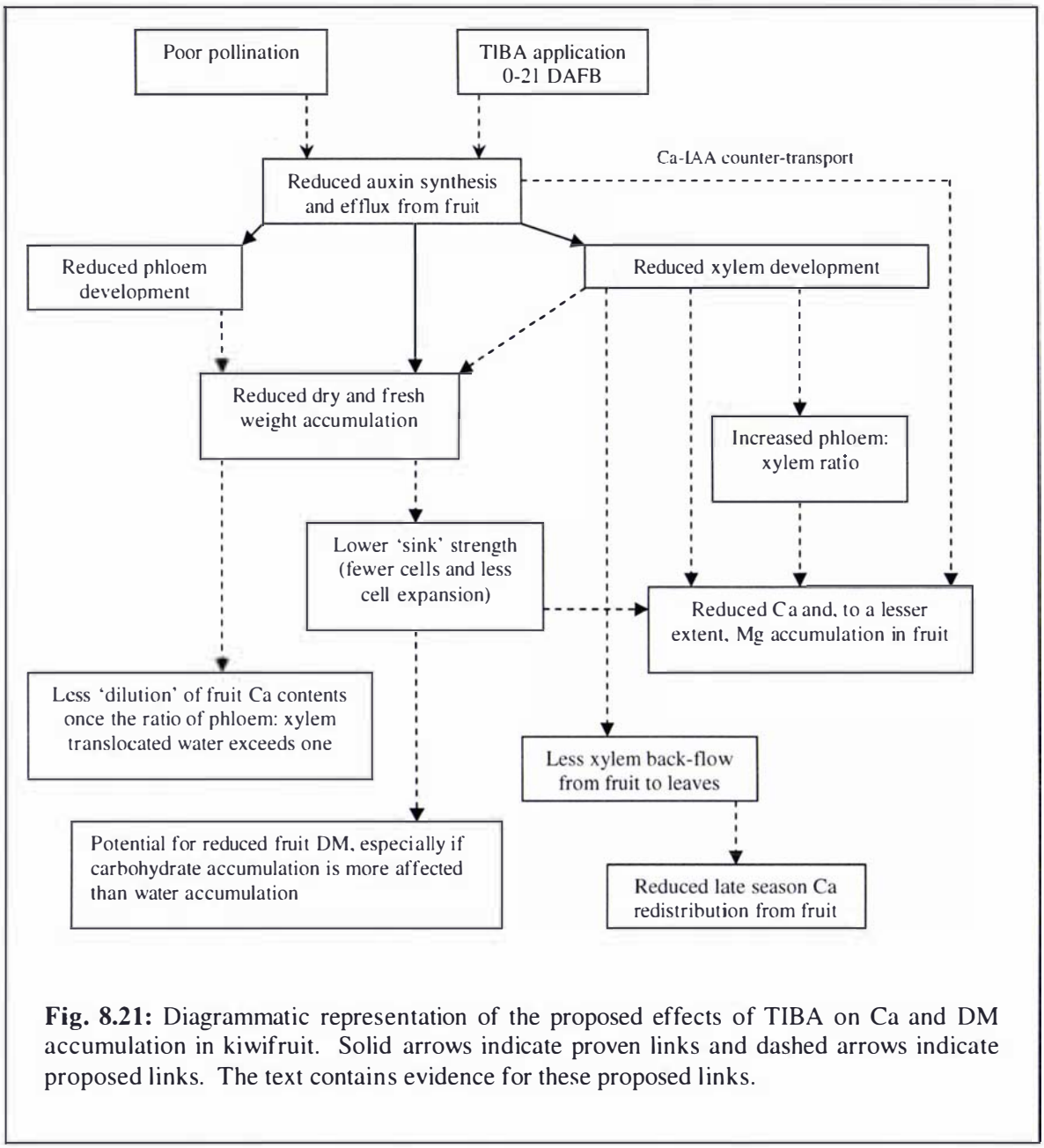


Fig. 8.21: Diagrammatic representation of the proposed effects of TIBA on Ca and DM accumulation in kiwifruit. Solid arrows indicate proven links and dashed arrows indicate proposed links. The text contains evidence for these proposed links.

In addition to affecting vascular cell differentiation, TIBA may affect differentiation and elongation of other cell types within the fruit, which could indirectly affect Ca and Mg accumulation by altering the driving force for xylem flow into fruit. Cell numbers and cell size were not measured in this experiment, but should be investigated in the future. Acropetal Ca ion transport into fruit also may be directly affected by the basipetal counter transport of IAA from shoots to roots (Cutting and Bower, 1989; Wand *et al.*, 1991b; Raese *et al.*, 1995; Hamamoto *et al.*, 1998). However, this would not affect Mg accumulation and could not explain the effects of TIBA on fresh and dry weight accumulation (described in Section 8.4.2), as the ability of basipetal

auxin translocation to influence acropetal nutrient translocation is very specific to Ca (Banuelos *et al.*, 1987).

The effect of TIBA on Ca accumulation was not sustained until April. The reason why Ca accumulated in TIBA-treated fruit to the same concentration as in untreated fruit by the April harvest is unclear, as under normal circumstances only small amounts of Ca (less than 20% of the total Ca content) are thought to accumulate in fruit from eight WAFB (Clark and Smith, 1988; Xiloyannis *et al.*, 2001). Furthermore, estimated vascular conductance remained lower in TIBA-treated fruit stalks than in control stalks until harvest, so Ca accumulation in TIBA-treated fruit would presumably also be restricted until harvest. Therefore, it is unlikely that differences between these treatments declined because more Ca accumulated in TIBA-treated fruit than in untreated fruit during the later stages of fruit development.

Declining Ca accumulation later in the season may be attributed to xylem dysfunction within the fruit, as vessels are stretched and broken during fruit growth (Dichio *et al.*, 2003) and to declining rates of fruit transpiration due to a reduction in fruit permeance to water vapour as fruit mature (Xiloyannis *et al.*, 2001). However, fruit FW continued to increase until later in the season, suggesting that water still accumulated via the phloem. In apples (Lang and Thorpe, 1989) and grapes (During *et al.*, 1987) there may be a shift from xylem- to phloem-supplied water during later stages of fruit development. Assuming that xylem flow into fruit is reduced later in the season, then fruit Ca concentrations may decline more in untreated than in TIBA-treated fruit, because the FW of the former is greater than the FW of the latter. Consequently, Ca concentrations would be more diluted in untreated fruit than in TIBA-treated fruit (Fig 8.21).

Fruit FW increased by a similar amount between January and April in TIBA-treated and untreated fruit from defoliated and girdled shoots. However, on average fruit FW increased by 16% more between January and April in TIBA-treated fruit than in untreated fruit. Therefore, it is surprising that fruit Ca concentrations would be less diluted in this treatment than in untreated fruit. Between January and April, average fruit Ca contents increased by 30% more and fruit Ca concentrations declined by 41% less, in TIBA-treated fruit from control shoots than in untreated fruit from these shoots. These values were 21 and 76% for fruit from defoliated shoots. In fruit from girdled shoots Ca contents increased by 9% more, between January and April, in untreated fruit than in TIBA-treated fruit, but fruit Ca concentrations decreased by 10% less in TIBA-treated fruit than in untreated fruit. These data suggest that the decline in Ca concentration differences between TIBA-treated and untreated fruit were not solely related to differences in FW of fruit from these treatments.

Substantial quantities of xylem sap may flow out of apple (Lang, 1990) and grape (Lang and Thorpe, 1989) fruit towards leaves during periods of peak potential evapotranspiration. It is possible that Ca also was redistributed from kiwifruit during the later stages of fruit development when average daily temperatures and hence, potential rates of leaf evapotranspiration were higher. As xylem dysfunction was greater in TIBA-treated fruit than in untreated fruit, redistribution of Ca from fruit to leaves may have been less in TIBA-treated fruit than in untreated fruit (Fig 8.21). Consequently, late season Ca concentrations may decline less in TIBA-treated than in untreated fruit. These findings would explain why differences between the Ca: K and Ca: P ratios of TIBA-treated and untreated fruit declined by 17% in fruit from control shoots between January and April.

Fruit Ca accumulation was more affected by TIBA in fruit from long shoots than in fruit from short shoots. This may be because fruit on long shoots usually have more seeds (Lai *et al.*, 1990), and accumulate more Ca (Thorp *et al.*, 2003b), than fruit from short shoots. This means that TIBA treatment might have more potential to reduce Ca accumulation in fruit from long shoots than in fruit from short shoots. Moreover, TIBA might have more potential to reduce IAA efflux from the former, as PGR efflux from seeds is positively correlated with the fruit seed number (Trustrum, 1983; Lawes *et al.*, 1990; Currie, 1997). It also suggests that Ca concentrations may be higher in fruit from long shoots than in fruit from short shoots because auxin efflux from fruit on long shoots is likely to be greater than from fruit on short shoots with a low seed number. This proposal is supported by evidence from Chapter 7 that xylem differentiation occurs earlier in stalks of fruit from long shoots than in stalks of fruit from short shoots.

Accumulation of phloem mobile ions, such as K and P, and to a lesser extent Mg, may have been less affected by TIBA-treatment than Ca, because: (a) phloem transport was less affected than xylem transport, (b) unlike Ca, accumulation of phloem mobile mineral ions in fruit is not solely dependent on the maintenance of xylem function, and (c) phloem mineral translocation is less likely to be affected by the narrowing of sieve elements than xylem mineral translocation would be by the narrowing of vessel cells, because solutes are actively transported in the phloem, whereas they are predominantly passively transported in the xylem (Marschner, 1995).

8.4.2. Fresh and dry weight accumulation

TIBA consistently reduced both fresh and dry weight accumulation in fruit from long and short shoots, although reductions were not always significant. Fresh and dry weight accumulation also was positively correlated with fruit seed number. This suggests that the ability of fruit to accumulate carbohydrate and water is, like Ca and Mg, affected by rates of auxin synthesis in fruit

and/or the extent of auxin efflux from fruit. Auxin may affect carbohydrate and water accumulation in fruit directly, by regulating the active loading of carbohydrates into storage organelles, or indirectly by stimulating the conversion of accumulated sucrose into insoluble carbohydrates such as starch and other cellular constituents (Fig 8.21). In melon and eggplant fruit it has been proposed that auxin stimulates invertase activity, and that this stimulates phloem unloading by triggering the conversion of sucrose to fructose and glucose (Lee *et al.*, 1997a; Lee *et al.*, 1997b). A role for invertase has been implicated in carbohydrate metabolism of developing kiwifruit (E. MacRae: pers. comm.). However, the effect of auxin on invertase activity and carbohydrate metabolism in immature kiwifruit is not known and could be investigated in the future.

Auxin may affect carbohydrate accumulation indirectly by influencing differentiation of vascular and fruit cells (Gamburg, 1982; Evans, 1985; Aloni *et al.*, 2000; Ljung *et al.*, 2001). In this study xylem and phloem areas were reduced by TIBA application and poor pollination, and fruit fresh and dry weight accumulation was positively correlated with phloem and xylem area. The potential of kiwifruit to accumulate carbohydrate is thought to be related to fruit cell number (Currie, 1997). Cell division in kiwifruit is completed six weeks after anthesis (Hopping, 1976b). In kiwifruit, fruit DW tends to increase linearly from the time when cell division ceases despite climatic variations that would be expected to alter rates of carbohydrate accumulation (Richardson *et al.*, 1997). This suggests that rates of DW accumulation are predetermined in some way during the initial stages of fruit development. It is proposed that the extent of phloem development in young flowers and fruit somehow determines the amount carbohydrate that can be transported into fruit for incorporation into cell walls and other cellular structures, and therefore, the extent of fruit cell division in fruit (Fig 8.21). Subsequent cell elongation will set the potential for further carbohydrate and water accumulation. In fruit with few cells, rates of DW accumulation will be lower than in fruit with many cells (Fig 8.22).

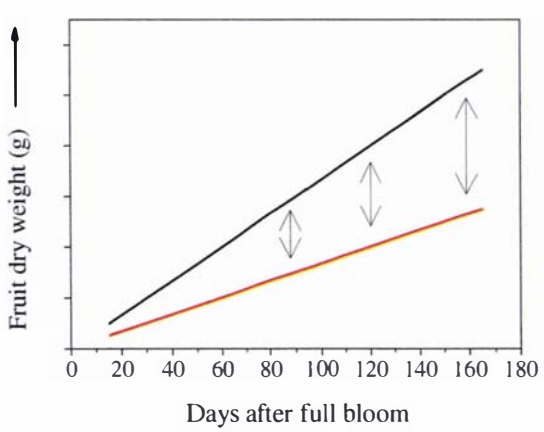


Fig 8.22. Proposed rate of dry weight increase in fruit with low (red) and high (black) cell numbers. Figure adapted from Richardson *et al.* (1997).

Unlike Ca, the effects of TIBA on fruit DMC were often more apparent as fruit matured. This may be because differences in the DW of fruit with low and high cell numbers and hence, different rates of DW accumulation,

increase as fruit mature, as is shown in Figure 8.22. This theory would only be plausible if the ratio of fresh: dry weight accumulation was not affected by TIBA application (Fig 8.21). Generally fruit fresh weights increase in conjunction with increases in DW, perhaps because of the effects of DW accumulation in fruit on fruit turgor pressure (see Section 5.4 for more information). If fruit metabolic activity was reduced by TIBA treatment, or TIBA reduced rates of sugar loading into storage organelles (see above), then the relationship between fresh and dry weight accumulation may be disrupted, and fruit DMCs may be reduced also. Alternatively, TIBA may restrict water accumulation in fruit by influencing xylem and phloem development, and this could increase fruit DMCs, thereby masking the negative effects of TIBA on fruit DW accumulation. Effects of TIBA on fruit DMCs will therefore depend on the effects of TIBA on the ratio between fresh and dry weight accumulation. If carbohydrate supplies are limited, or conditions are not conducive for carbohydrate accumulation in fruit, then differences between TIBA-treated and untreated fruit may be marginal.

Effects of TIBA on fruit fresh and dry weight accumulation did not increase as the TIBA concentration increased above 50-100 mg L^{-1}. However, as fruit seed numbers increased, fruit fresh and dry weights continued to increase, albeit at a slower rate once the seed number exceeded 250 seeds. In apple, fruit DW also increases as the fruit seed number increases (Broom *et al.*, 1998; Di Vaio, 2004), as does the auxin efflux from the fruit (Callejas and Bangerth, 1998). Currie (1997) found that cell division was stimulated as seed numbers in 'Hayward' fruit increased from 0-270 seeds, but that cell division was not enhanced by further increases in seed number above 270 seeds. He suggested that a threshold level of auxin is required to stimulate cell division and expansion in kiwifruit. Once this seed number is exceeded, other factors, such as the availability of carbohydrate and the metabolic activity of fruit may limit fruit development.

There was little further increase in fruit DMCs after the seed number exceeded 750. An asymptotic curve fitted to FW data showed that, on average, fruit with 750 seeds weigh 84 g. This suggests that fruit with a count size of less than 36, which are small because they have a low seed count, are more likely to have unacceptable DMCs (i.e. below the low threshold limit of 14.5 set by ZESPRI™). Fruit DMCs may decline as seed numbers in the fruit increase, because as seed number increases the weight of each additional seed is reduced and lighter seeds synthesise less PGRs than heavier seeds (Trustrum, 1983; Lawes *et al.*, 1990; Currie, 1997). Therefore, lighter seeds would have less of an affect on fruit development than heavier seeds. Furthermore, as seed number is increased, the phloem: xylem ratio declines, so more water may be accumulated than carbohydrate, which would lower the rate at which fruit DMCs increased.

8.4.3. Girdling and defoliation in relation to TIBA effects on fruit quality

Girdling tended to reduce the effects of TIBA on fruit quality and xylem functionality in VMC bundles. This may be because both TIBA (Muday and De Long, 2001) and girdling (Currie, 1997) block IAA movement beyond the site of treatment application. Therefore, IAA efflux from fruit on girdled shoots may be reduced regardless of whether TIBA is applied to fruit or not. TIBA also may reduce the transport capacity of phloem tissues, as indicated by the reduction in phloem area. However, this appeared to have little additional affect on carbohydrate, K and P accumulation in fruit. This supports the hypotheses of others that carbohydrate translocation is little affected by the transport capacity of the phloem (García-Luis *et al.*, 2002).

Unlike girdling, the effects of defoliation on fruit Ca persisted regardless of whether TIBA was applied to fruit or not, indicating that the effects of defoliation on Ca accumulation were independent of any effects of TIBA on Ca accumulation. Defoliation may reduce fruit Ca concentrations by reducing the flow of xylem sap towards a shoot, and by reducing Ca redistribution from leaves to fruit (refer Chapter 5 for more information). As proposed in Figure 8.21, TIBA may affect Ca accumulation by reducing xylem transport capacity and hence, passive flow of xylem sap into fruit, or by reducing the IAA-Ca counter-transport of Ca ions into fruit. At any one time both of these mechanisms may be operating to regulate xylem sap flow, and hence Ca accumulation in fruit.

8.4.4. TIBA and fruit quality in storage

Treatment effects on fruit composition at harvest were often reflected in the postharvest quality of fruit. For example, fruit from defoliated shoots had a higher incidence of LTB and were softer than fruit from the other treatments. This may be attributed to reduced Ca concentrations in these fruit when compared to fruit from the other treatments, as others also have reported that fruit firmness and incidence of other storage disorders are positively correlated with fruit Ca concentrations (Hopkirk *et al.*, 1990; Prasad *et al.*, 1990). Fruit Ca: K and Ca: P ratios also were 15-36% lower in fruit from the defoliation treatment than in fruit from the other treatments, which probably contributed to their poor storage quality. Fruit firmness and LTB incidence may not have been affected by TIBA treatment alone, as fruit Ca concentrations and fruit Ca: K and Ca: P ratios did not differ between TIBA-treated and untreated fruit by harvest.

In the preliminary trial, vascular tissues in TIBA-treated fruit developed a brown discolouration after 24 weeks in storage. Avocado fruit with low Ca concentrations showed increased vascular

browning when compared to fruit with high Ca concentrations (Thorp *et al.*, 1997). Fruit Ca concentrations were lower in TIBA-treated than in untreated fruit, and this may have increased fruit susceptibility to vascular browning. However, vascular browning was not observed in TIBA-treated or untreated fruit in the other experiments. As experiments were not all conducted on the same orchard, the browning effect may have been due to differences in edaphic or climatic factors between orchards. Fruit from the South Auckland orchard in which the preliminary trial was conducted had a lower DMC than fruit from the Te Puke orchards, and TIBA-treated fruit from this orchard were also softer than untreated fruit, despite there being no significant effect of TIBA on fruit Ca and DMCs. Perhaps then, some other factor was weakening the resistance of fruit from this orchard to the adverse effects of TIBA. Others have suggested that environmental influences can affect disorder incidence and fruit quality in storage (Ferguson and Boyd, 2001).

8.5. Conclusions

TIBA application and fruit seed number had a significant effect on carbohydrate, mineral and water accumulation in kiwifruit, most likely due to the effect of these treatments on auxin synthesis in, and efflux from, developing fruit.

1. Calcium accumulation appears to be affected by both the transport capacity of the xylem (this chapter), and the rate of xylem sap movement into fruit (refer Chapters 5 and 6). It is suggested that the synthesis of IAA in fruit and the efflux of IAA from fruit was reduced by TIBA treatment and poor pollination. This negatively affected xylem differentiation. Subsequently, fruit concentrations of phloem immobile ions (e.g. Ca), or ions that are relatively immobile in the phloem (e.g. Mg), were reduced. Evidence supporting this proposal includes the:

    a. finding that the greatest affects of TIBA on Ca and Mg accumulation coincided with the period when secondary xylem tissues were developing in fruit (Chapter 7) and the time when IAA concentrations reportedly peaked in kiwifruit;
    b. positive linear relationship between fruit Ca and Mg concentrations and estimated secondary xylem conductance;
    c. reduction in xylem functionality in TIBA-treated fruit and in estimated secondary xylem conductance in the stalks of TIBA-treated fruit;
    d. reduction in IAA efflux caused by TIBA application in other fruit crops.

2. Calcium may be redistributed from fruit to leaves during periods of high potential evapotranspiration. It is suggested that loss of Ca will be greater in fruit with a large number of functioning vessels and high conductance in the fruit stalk than in fruit where xylem vessels are largely dysfunctional and xylem conductance in the fruit stalk is low.

3. Once phloem and fruit cell differentiation are complete, 3-6 weeks after full bloom, the transport capacity of the phloem does not appear to restrict carbohydrate accumulation. However, it is proposed that the rate of phloem differentiation during the initial stages of fruit development determines the amount of carbohydrate that can be translocated into flowers and fruitlets for incorporation into cell walls and other cellular structures, and hence the extent of fruit cell division. This then sets the capacity of fruit to accumulate carbohydrates, water and minerals.

4. When auxin efflux from fruit is reduced, the accumulation of phloem immobile minerals, such as Ca, in fruit may be more reduced than accumulation of phloem mobile minerals, because: (a) reductions in vessel diameter are likely to have a greater effect on xylem mineral transport than reductions in sieve element diameter will have on phloem transport, as solutes are passively transported in the xylem, whereas they are actively transported in the phloem, (b) phloem differentiation is less affected by low auxin concentrations than xylem differentiation, and (c) they are entirely dependent on the transport capacity of the xylem for translocation into fruit. As K and P are very mobile in both phloem and xylem, their concentrations were less affected than fruit Ca and Mg concentrations by TIBA application and reductions in fruit seed number.

5. During the initial stages of fruit and flower development (14 DPFB to 21 DAFB), it is possible that a threshold level of auxin is required to trigger vascular and fruit cell differentiation. Carbohydrate, mineral and water accumulation will be reduced when auxin levels fall below this threshold, but will not be enhanced when auxin concentrations exceed this threshold level. Auxins may stimulate vascular and fruit cell division during the initial stages of fruit and flower development (14 DPFB to 21 DAFB), but will have little affect on fruit development after this time.

6. Auxin synthesis in, and efflux from, fruit on long shoots may be greater than in/from fruit on short shoots, as fruit on long shoots generally have more seeds. This may explain why vascular and fruit cell differentiation is more advanced during the initial stages of fruit development in fruit from long shoots than in fruit from short shoots (Chapter 7), and why fruit Ca concentrations and dry weights are generally higher in fruit from long shoots than in

fruit from short shoots. In fruit from long shoots, high auxin concentrations also may enhance the activity of sugar metabolising enzymes and active loading of sugars into storage organelles. Thus fruit carbohydrate concentrations could increase without altering the turgor pressure in fruit and this might increase fruit DMCs.

9. Non-destructive Measurement of Mineral Concentrations in Xylem Sap of Different Kiwifruit Shoot Types Using Spittlebugs

9.1. Introduction

Variation in fruit mineral concentrations may be attributed to differences in the flux of sap flowing to fruit from different positions within the vine, or to differences in the xylem sap mineral ion composition accumulating in fruit. In previous chapters experiments were designed to manipulate xylem flux into fruit by reducing rates of leaf and/or fruit transpiration (e.g. by inducing stomatal closure at low crop loads (Chapter 4), defoliating shoots (Chapter 5), and by using fans and bags to alter rates of fruit transpiration (Chapter 6)), or by reducing the capacity of the xylem and phloem tissues (Chapter 8). These treatments had a considerable affect on fruit Ca concentrations, although the concentration of the more phloem mobile ions, Mg, K and P was not always affected. In this chapter *in situ* Ca, Mg, K and P concentrations in xylem sap collected from pedicels of fruit from long and short shoots were analysed. The objective was to determine whether xylem mineral transport to different parts of the vine, such as to fruit on long and short shoots, may be differentially restricted or regulated, and whether this might partially account for the variation in mineral concentration of fruit located at different positions in the vine.

In situ measurements of xylem mineral transport in kiwifruit are limited, including measurement of sap constituents associated with different shoot types and temporal changes. This is due to difficulties in: (a) extracting xylem sap without contamination from neighbouring tissues and cut cell surfaces (Schurr, 1998), (b) collecting sap from transpiring plants, where sap is held under strong negative pressure during transpiration, and (c) penetrating xylem vessels without causing embolisms that disrupt sap flow (Schurr, 1998; Malone et al., 2002a). Previous studies have used indirect methods to collect xylem sap, such as vacuum-extraction (Bollard, 1953) and collection of bleeding sap (Ferguson *et al.*, 1983), which are not associated with transpiring conditions and may not reflect the composition within the transpiration stream of an intact plant (Ferguson *et al.*, 1983; Schurr, 1998). Xylem flow rates tend to be higher, and driving forces different, under transpiring compared to non-transpiring conditions (Watson *et al.*, 2001). Moreover, sap has usually been collected from trunks or large canes and this sap may be quite different in composition to that in the axillary shoots and/or fruit stalks. It would be preferable to analyse sap flowing into the fruit stalks themselves, as these are the final conduit to the fruit.

Recently a technique for collecting xylem sap has been developed that uses the xylem-feeding meadow spittlebug (*Philaenus spumarius*). This enables continuous, non-destructive collection of xylem sap that can be analysed subsequently for xylem mineral content at high temporal resolution (Malone et al., 1999; Malone et al., 2002a). As this insect feeds it secretes droplets of watery excreta in which the concentration of all inorganic ions, except ammonium ions, was very close to that of the xylem sap of tomato (*Lycopersicon esculentum*) and bean (*Vicia faba*) plants on which insects were feeding (Malone et al., 2002a; Ponder et al., 2002). This relationship between excreta and sap composition is remarkably robust, holding true for different plant species and sap compositions (Malone *et al.*, 2002b; Ponder *et al.*, 2002). In ten minutes enough sap can be collected to detect changes in sap composition (Ponder et al., 2002).

In this experiment spittlebugs were used as a tool to investigate xylem sap mineral concentrations in fruit stalks from long and short terminating shoots of 'Hayward' kiwifruit. Excreta was analysed to confirm that higher Ca concentrations occurred in fruit from long axillary shoots that in fruit from short shoots, and to determine whether differences in the mineral composition of fruit from these shoots were associated with specific differences in xylem concentration and flow of Ca and other mineral nutrients into the fruit. Relationships between xylem sap composition and attributes of the shoot from which sap was collected, such as the shoot L:F ratio, rate of leaf transpiration, and distance from the main leader were investigated. These relationships are discussed in relation to the hypothesis that differences in the attributes of specific shoots can explain much of the within-vine variation in kiwifruit mineral concentrations.

9.2. Materials and methods

9.2.1. Plant material

Two adjacent mature 31-year-old 'Hayward' kiwifruit vines growing at the HortResearch Te Puke Research Centre in the Bay of Plenty district of New Zealand were used in this experiment. Vines were growing on open-pollinated 'Bruno' seedling rootstocks and were managed for commercial production on a pergola-trained system.

9.2.2. Bug collection and xylem sap feeding

Spittlebugs (*Philaenus spumarius* L. [Homoptera: Cercopidae]) from uncultivated grassland adjacent to the research orchard were collected by sweeping a butterfly net over the grass and then sucking the spittlebugs into a container with an aspirator. Once collected, spittlebugs were kept in the laboratory in a plastic cage containing potted weeds from the collection area. An aspirator was then used to transfer spittlebugs from this plastic cage into cages mounted on vines.

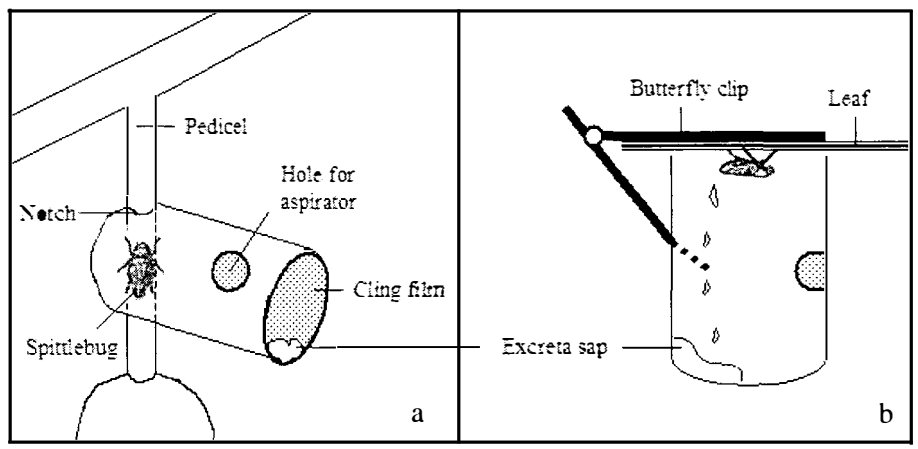


Fig 9.1. Cages used to contain the spittlebugs whilst feeding on kiwifruit (a) fruit stalks and (b) leaves.

Fruit stalk cages (Fig 9.1a) consisted of a piece of clear plastic tubing, approximately 25 mm in length and 20 mm in diameter, with one end cut on a slight angle. Food grade polyethylene film was glued over the flat end of the tubing, and a pair of diametrically opposed 'U'-shaped notches were cut in the angled end of the tubing. These notches fitted around the fruit stalk in such a way that the cage sloped downwards to ensure that spittle accumulated at a common collection site. In one side of the cage a hole was cut to fit the aspirator tubing so that insects could be transferred into the cage. Once insects were inside the cage the hole was blocked with putty. Each cage was secured to the fruit stalk using Parafilm®. This design allowed accumulated spittle to be viewed and collected by piercing the film at the bottom of the cage with a hypodermic needle on a plastic syringe, without disrupting the feeding insects. A clip cage, made from a hair clip and plastic tubing, was used when insects were caged on the abaxial surface of a leaf lamina (Fig 9.1b). Spittle accumulated at the covered bottom end of the tubing and was collected with a needle and syringe as described above.

9.2.3. Collection of excreta sap

The insect spittle, hereafter called excreta sap, was collected over several days at four different stages of fruit development, from December to mid-February during the summer of 2003/04. In December and January excreta sap was collected from fruit stalks of 20 fruit on long and 20 fruit on short axillary shoots. Long shoots tended to be closer to the leader towards the proximal end of the parent canes, and short shoots further from the leader towards the distal end of the parent cane (Thorp *et al.* (2003b). Any effect of the spatial separation of these two shoot types on mineral concentrations in the xylem sap was tested by destructive sampling described below. As there was no detectable effect of vine or vine side (east or west) on excreta sap mineral concentrations, samples from different vines or different sides of the vine were considered as individual random samples.

One cage was placed on one fruit per shoot, and each cage contained two or three insects. In February insects no longer fed consistently on fruit stalks, and therefore cages were placed on prominent lateral veins of leaves arising from the same nodes as the fruit. Ten leaves were used for each shoot type, with cages containing one or two insects. Excreta sap was collected at approximately 2 h intervals between 08:00 and 17:00. The exact collection times were dependent on insect feeding rates and subsequent excreta sap accumulation. Variation in rate and timing of insect feeding meant that excreta sap was not collected from every cage at every sampling time. Collected excreta sap was transferred from the syringe to a micro-centrifuge tube and frozen at –20°C pending analysis. Ion concentrations were not correlated with size of excreta sap samples (data not shown), indicating that results were not affected by sample evaporation between collection times.

9.2.4. Excreta sap analysis

The composition of K, Ca and Mg in excreta sap was determined using atomic absorption spectrophotometry as described in Chapter 2. Samples were thawed and shaken before removal of a sub-sample and addition of 0.01 mM $LaCl_3$. Sub-sample size ranged between 50-150 µl, depending on the original sample size, and this was diluted in proportion with 2-6 ml of $LaCl_3$ solution. Sub-samples were collected from tubes containing small (<70 µl), medium (70-150 µl), and large (150 µl+) volumes of excreta sap in order to eliminate any affect of sample size and feeding frequency on results. If the diluted sample size was more than 5 ml, Ca, Mg, and K concentrations could all be determined. In contrast, only one or two of the above ions could be analysed in samples of less than 5 ml.

Phosphorus concentrations were measured colormetrically by the molybdate blue reaction (Turner *et al.*, 1977), in most cases using different samples to those that were analysed for Ca, Mg, and K. This was because sample volumes were usually too small to allow analysis of all four minerals. Sub-sample sizes for P were no smaller than 100 µl, and other than adding 4 ml of colour reagent no further dilutions were made, as P levels were otherwise undetectable.

Once all analyses were completed, tubes were weighed and the remaining excreta sap evaporated in an oven at 60°C. Tubes were re-weighed and the volume of excreta sap calculated, after adding the amount used for analysis.

9.2.5. Spittlebug excreta sap and xylem sap composition

Artificial sap solutions, of known mineral composition, were used to test that composition of excreta sap did not differ significantly from that of xylem sap (Ponder *et al.*, 2002). Two different mineral ion solutions were used; high concentration (8 mM KH_2PO_4, $CaCl_2$, $Mg(NO_3)_2$ and 3 mM sucrose) and low concentration (2 mM concentrations of the above compounds with 3 mM sucrose). Mineral concentrations used encompassed the range of concentrations measured previously in destructively harvested *Actinidia* sap (Ferguson *et al.*, 1983). Fruit were picked from the vine with fruit stalks still attached, and the fruit stalk re-cut under water to remove emboli. The cut fruit stalk ends were then immediately placed into micro-centrifuge tubes containing artificial sap solutions and positioned in front of a fan to promote fruit transpiration. After 2 h insects were caged onto the fruit stalks and left to feed, and excreta sap was collected at approximately 2 h intervals. Thirty-five fruit were used, with two insects per fruit. However, not all the insects fed, so only 24 excreta sap samples were collected for comparison with the artificial sap solutions. Excreta sap and samples of the artificial sap were analysed for Ca, Mg, K and P concentrations by inductively-coupled plasma mass spectrometry at a commercial laboratory (Hill Laboratories, Cambridge, NZ).

9.2.6. Vacuum-extraction of xylem sap

Sap was destructively harvested from current season's fruiting canes at the end of February to determine if there was a gradient in sap mineral composition between the central leader and the periphery of the crown. All axillary shoots were pruned from the canes in the early afternoon and the cane immediately cut into three sections; proximal (closest to the leader), mid, and distal. Xylem sap was extracted from these sections, in random order, using a hand-pumped vacuum-

extraction apparatus (Pate *et al.*, 1994), with the cane section connected to the apparatus using rubber tubing and hose clamps. To limit contamination, the cut end of the cane was washed with distilled water and wiped with blotting paper, and the first few drops of xylem fluid discarded. Between 0.25-10 ml of xylem sap was extracted per cane section and stored in screw-cap micro-tubes at –20°C pending analysis. Samples were analysed for four nutrients (P, K, Mg and Ca) using the methods described in Chapter 2.

9.2.7. Axillary shoot attributes

Measurements were made of fruit and leaf water potentials on 18 December 2003 and on 14 January 2004 using a pressure bomb (Soil Moisture Equipment Corp., Santa Barbara, California). Both non-exposed and exposed leaves were used on the assumption that the former had much lower transpiration rates. Twenty-eight measurements were recorded each day between 10:30 and 15:00 h, comprising eight fruit measurements, and 10 exposed and 10 non-exposed leaves. On the 19 and 27 December 2003 and 14 January 2004, stomatal conductance and rates of leaf transpiration were measured using a portable photosynthesis system (Li 6400, Licor, Nebraska). On short axillary shoots these measurements were made on the most distal leaf on the shoot. On long shoots measurements were made on the first leaf past the fruit, and on the last, or penultimate fully expanded leaf. Measurements were recorded in ambient light on fine days with blue skies and the occasional cloud between 11:00 and 14:00 h. Twenty measurements were recorded each day, ten for each shoot type. Temperatures in the leaf chamber ranged from 27-31°C, relative humidity 36-56%.

The following data were recorded in December for each axillary shoot that had been used for excreta sap extraction: (1) distance from the central leader, (2) axillary shoot number (shoots numbered down the cane from central leader to cane tip), (3) leaf: fruit ratio (L: F ratio), (4) leaf area (measured with a Li 3100 area meter. Licor, Nebraska), (5) axillary shoot length, and (6) which side of the vine the axillary shoot was situated on.

9.2.8. Statistical analysis

Mean differences in xylem sap composition between shoot types or sap collection times were determined by one-way ANOVA. Where the F-stat indicated a significant difference between treatments the Fishers protected least significant difference (LSD) procedure was used to establish significance between treatment means. This method also was used to identify significant

differences between attributes of the two different shoot types. One-sample t-tests were used to determine whether the ratio of mineral concentration in artificial sap to that of insect excreta differed from unity.

Daily trends in xylem sap composition were modelled using a polynomial function. Relationships between Ca, Mg, K and P concentrations in the excreta sap and shoot attributes (shoot leaf area, L: F ratio, length and distance from the central leader) were investigated by grouping the shoot attribute variables into nominal categories (for example: leaf area ≤1000 cm^2, ≤2000 cm^2, and ≤3000 cm^2) and calculating average ion concentrations (± SE) for each of these categories. This information was plotted as a bar graph so that significant trends between categories, and between collection times could be identified.

9.3. Results

9.3.1. Bug feeding patterns

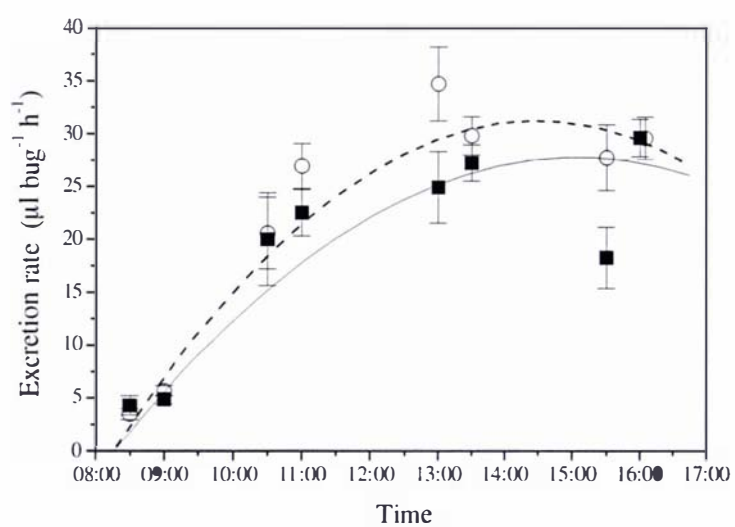


Fig 9.2. Rate of excreta sap production throughout the day (2 December 2002 – 30 January 2004). Open circles: excreta sap collected from insects caged on short shoots; solid squares: excreta sap collected from long shoots. Data points are means values (± SE) calculated using fruit stalk data only. Between 32-214 samples were analysed at each of the times indicated

Individual insects tended to feed sporadically rather than continuously throughout the day, although there was a general trend for excretion rates to increase and reach a maximum towards early afternoon (Fig 9.2). This trend was little affected by date (data not shown) or axillary shoot type. Excreta sap was excreted at an average rate of 23 μl $insect^{-1}$ h^{-1}, although there was much variation in the rate at which insects fed and in the amount of excreta sap they released.

As the season progressed the volume of excreta sap produced declined (Fig 9.3), until February when insects ceased to feed on fruit stalks. At this stage cages were transferred from lignified fruit stalks to softer leaf midrib tissue where feeding did resume although only small quantities of excreta sap were produced (Fig 9.3). The seasonal decline in excreta sap volume was particularly noticeable in insects caged on long axillary shoots; they produced less excreta sap than insects caged on short shoots, except in late December when the highest volumes of excreta sap were produced (Fig 9.3).

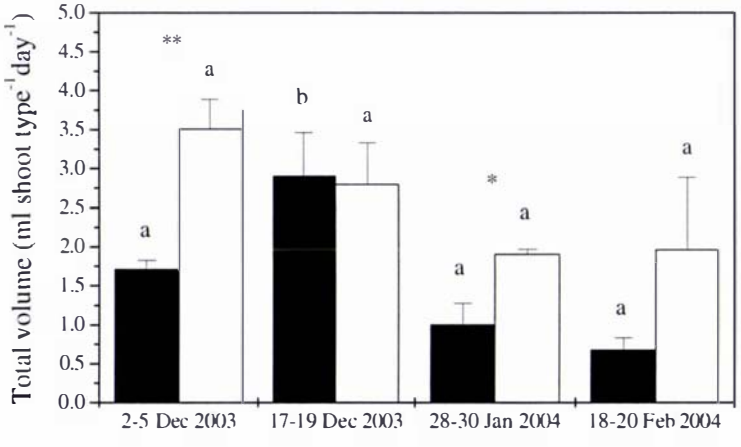


Fig 9.3. Seasonal changes in the volume of excreta sap produced per insect, per day. Solid bars represent excreta sap from insects caged on long axillary shoots, whilst open bars represent excreta sap from insects caged on short shoots. Excreta sap collected during 18-20 February 2004 was taken from leaves. All other excreta sap was collected from fruit stalks. Bars are mean values (± SE) for each time period. Letters are used to indicate significant differences between time periods for each shoot type ($P < 0.05$), whilst stars indicate significant differences between shoot types (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Between 27-202 samples were assessed at each of the dates indicated.

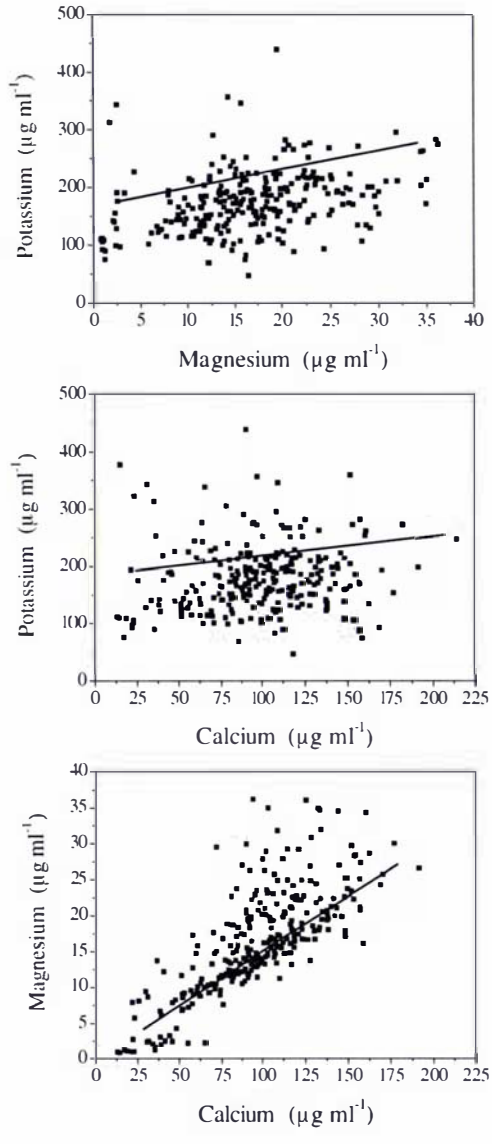


Fig. 9.4. Relationship between Ca, Mg, and K concentrations in the xylem sap. Data points represent data collected in December 2003 and January 2004. P <0.001, r^2 = 0.15, 0.04 and 0.64 for the top, middle and bottom graphs, respectively.

9.3.2. Excreta sap ion composition

Within a vine there was considerable variation in excreta sap concentration. Inter-quartile ranges for Ca, Mg, K and P were 68.7-116 µg ml^{-1}, 12.2-22.5 µg ml^{-1}, 143-217 µg ml^{-1}, and 6.1-9 µg ml^{-1}, respectively, for data collected from the December to January sampling period. Potassium was always the most abundant of the four nutrients measured, followed by Ca, Mg and P in decreasing order. Excreta sap Ca concentrations correlated with Mg concentrations, but not with K concentrations (Fig 9.4). Excreta sap Mg concentrations did not correlate with K concentrations (Fig 9.4). Correlations with P could not be made due to limited sample volumes.

There was a seasonal pattern in mineral concentration in excreta sap, but no daily patterns were evident. Magnesium and Ca fruit stalk excreta sap concentrations followed a similar pattern, peaking mid-late December, and declining towards the end of January (Fig 9.5). This decline was accompanied by a rise in K and P concentrations in late January. Excreta sap mineral concentrations decreased considerably from January to February for all minerals except P (Fig 9.5). This may be a tissue type effect, rather than a time effect, as excreta sap was collected from leaves of axillary shoots in February and from fruit stalks in December and January. There was little change in the proportions of these minerals, relative to one another, from January to February despite a change in tissue type from which excreta sap was collected. Except for K, there was greater seasonal variation in mineral concentrations of excreta sap collected from long axillary shoots, than in excreta sap collected from short shoots (Fig 9.5).

9.3.3. The effect of axillary shoot-type on xylem sap composition

When data from each sampling date was combined, average Ca and Mg concentrations were higher, (17 and 23%, respectively), in excreta sap from fruit stalks on long shoots than in fruit stalks from short shoots. There was no difference in K excreta sap concentrations in fruit stalks from the two different shoot types, and P concentrations were, on average, 28% higher in fruit stalks on short shoots, than on long shoots.

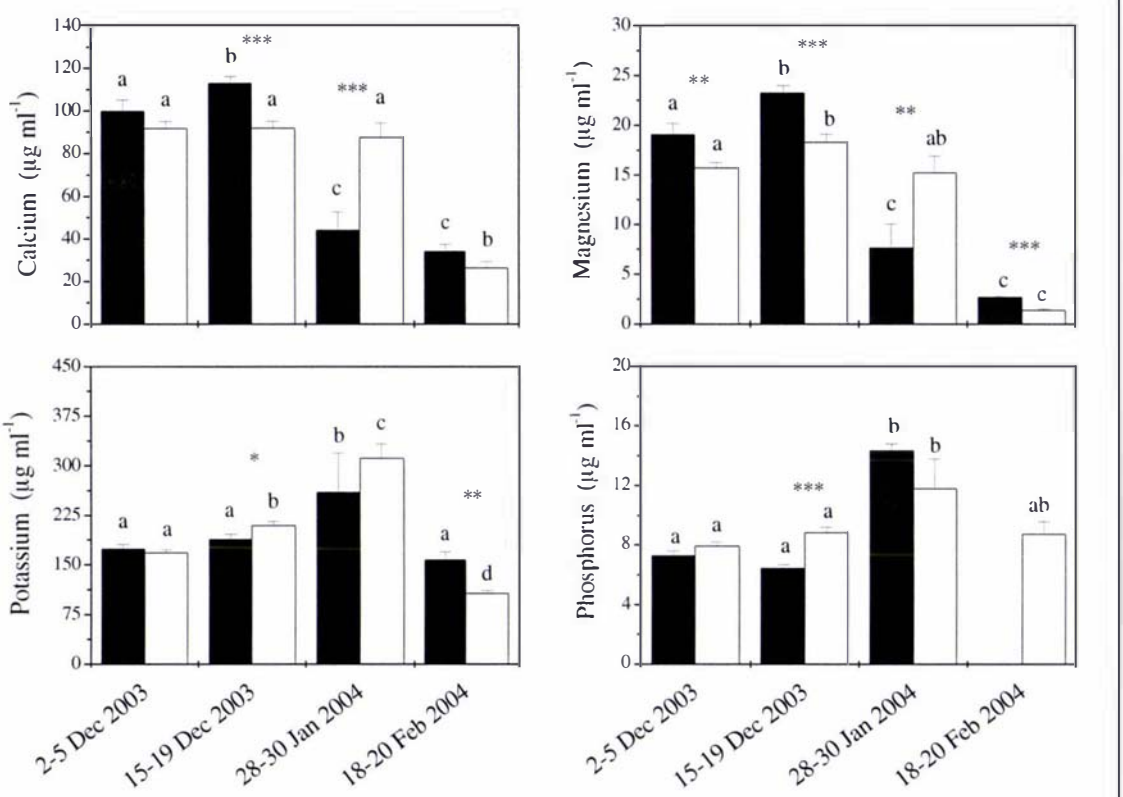


Fig 9.5. Seasonal changes in excreta sap mineral ion concentrations. Solid bars represent excreta sap from insects caged on long axillary shoots, whilst open bars represent excreta sap from insects caged on short shoots. Excreta sap collected during 18-20 February 2004 was taken from leaves. All other excreta sap was collected from fruit stalks. Bars are mean values (± SE) for each time period. Letters are used to indicate significant differences between time periods for each shoot type ($P < 0.05$), whilst stars indicate significant differences between shoot types (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Between 22-111 samples were assessed at each of the indicated dates for each mineral.

At the start of December, Mg concentrations were greater in excreta sap from fruit stalks on long shoots than in sap collected from fruit stalks on short shoots, but no differences existed in the concentration of other minerals between the two shoot types (Fig 9.5). By mid to late December Mg and Ca concentrations were higher in fruit stalks on long shoots than in fruit stalks on short

shoots, whereas the opposite pattern was noted at the end of January. In contrast to Ca and Mg, K and P sap concentrations were initially higher in fruit stalks from short shoots, than in fruit stalks from long shoots, however these differences did not persist through January. Potassium and Mg concentrations were greater in excreta sap collected in mid-February from the mid-ribs of leaves on long shoots, than in sap collected from leaves on short shoots (Fig 9.5).

Attributes of the shoot (distance from the central leader, length, leaf area and L:F ratio), from which excreta sap was collected, were not related to Ca, Mg, K or P concentrations in the excreta sap (data not shown). Long shoots tended to have leaves with higher transpiration rates and stomatal conductance values, were more exposed (in terms of light incident on the shoots) and had leaves with lower leaf water potential values than short shoots (Table 9.1).

Table 9.1. Attributes of long and short shoots.

Shoot Attribute	Short terminating axillary shoots	Long, non-terminating axillary shoots
Distance from the leader (m)	1.56 ± 0.017	0.56 ± 0.008
Leaf area (m^2)	0.05 ± 0.001	0.29 ± 0.003
Leaf: fruit ratio	2.31 ± 0.056	5.79 ± 0.251
Axillary shoot length (m)	0.15 ± 0.003	1.01 ± 0.015
Leaf xylem water potential (mPa)	-0.26 ± 0.014	-0.31 ± 0.014
Leaf water potential (mPa)	-0.41 ± 0.025	-0.51 ± 0.023
Fruit water potential (mPa)	-0.31 ± 0.007	-0.28 ± 0.010
Rate of transpiration (mol m^{-2} s^{-1})	1.24 ± 0.180	3.47 ± 0.347
Total conductance (mol H_20 m^{-2} s^{-1})	0.06 ± 0.010	0.21 ± 0.027
Photosynthetic photon flux (μmol m^{-2} s^{-1})	521 ± 95	1536 ± 373

Data are means of 20 axillary shoots (± SE). For long shoots average transpiration rates, and total conductance to water vapour (boundary and stomatal) values, represent means of leaves from both the proximal and distal ends of the axillary shoot. Mean values of all variables are significantly different between long and short shoots ($P < 0.05$), except fruit water potential values, which were not significantly different.

9.3.4. Ion concentration gradients down a cane

There was no ion concentration gradient from the proximal to distal regions of the canes for any of the minerals tested in vacuum-extracted sap samples (Table 9.2).

Table 9.2. Mineral concentration gradient in sap down a cane.

Cane section	Concentration (μg ml^{-1})			
	Calcium	Magnesium	Potassium	Phosphorus
Leader (proximal)	28.73	5.49	101.89	9.15
Mid	30.26	3.86	90.70	7.95
End (distal)	28.00	4.64	111.45	8.82

Data are mean values of 30 samples (n =10 per cane section) collected by vacuum-extraction. Data from the different cane sections were not significantly different at P <0.05.

9.3.5. Calibration solution results

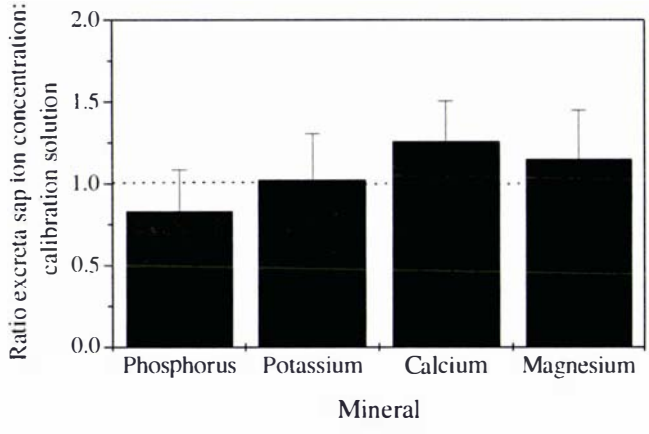


Fig 9.6. Mean ratio (± SE) of excreta sap ion concentration verses that in the corresponding calibration solution. For each mineral 34 samples were assessed.

Comparisons were made between pairs of excreta sap and calibration solution samples (collected at the same time) by dividing the concentration of each ion in the excreta sap by its concentration in the corresponding calibration solution (Fig 9.6). For each of the minerals assessed, the resulting ratio never differed significantly from one, which indicates that ion concentrations in the excreta sap and calibration solution samples were not significantly different.

9.4. Discussion

9.4.1. Xylem sap mineral composition

Inorganic ion concentrations in kiwifruit xylem sap were virtually identical to those in sap ingested and excreted by spittlebugs. Therefore, spittlebugs can be used to accurately estimate kiwifruit xylem sap composition. Similar results were reported for spittlebugs feeding on tomato

(*Lycopersicon esculentum*) (Ponder *et al.*, 2002). This also provides evidence that the species of plant on which the insect is feeding, or composition of the xylem sap, may not necessarily affect ion concentration in the excreta relative to that of the ingested material.

Concentrations of Ca, Mg, K and P found in kiwifruit xylem sap in this study were comparable to those reported previously (Ferguson *et al.*, 1983; Peterlunger *et al.*, 1990; Sotiropoulos *et al.*, 2002). Moreover, proportions of minerals in the xylem, relative to one another, were similar to those found in the excreta of spittlebugs (Ponder *et al.*, 2002) and cicadas (Cheung and Marshall, 1973) feeding on xylem sap of species other than kiwifruit.

9.4.2. Insect feeding patterns

Feeding rates increased towards the early afternoon for spittlebugs feeding on fruit stalks attached to both long and short shoots. Brodbeck *et al.* (1993) found that leafhoppers could adjust their feeding rates to match diurnal fluctuations in xylem fluid chemistry. It is possible that spittlebugs also are capable of this, although feeding patterns did not appear to relate specifically to concentrations of any of the minerals measured in this experiment. No relationship was found between the (diurnal) time of sap collection and xylem sap mineral concentrations.

Feeding rates declined as the season progressed due to a drop in the number of insects feeding and increases in the duration of breaks between feeding. As a consequence of this reduction in feeding it was not possible to collect excreta sap for analysis after 20 February 2004, nearly 13 weeks after anthesis. By this stage more than 70% of the total Mg and nearly 80% of the total Ca may have accumulated in the fruit (Clark and Smith, 1988), and concentrations of these mineral in the xylem sap were more than 65% lower than at the start of the season. Furthermore, more than 60% of the total K and P may have accumulated in the fruit (Clark and Smith, 1988) and there was no evidence to suggest that concentrations of either of these minerals in the xylem sap increased towards the end of the season, or that concentration differences between fruit on short and long shoots were consistently increasing. Consequently, data collected after this time would be unlikely to provide valuable information regarding the effect of xylem sap composition on Ca, Mg, K and P concentrations in fruit from short and long shoots at harvest.

9.4.3. Spatial variation in xylem sap mineral ion concentrations: evidence that minerals are partitioned on a demand basis

In kiwifruit Ca and Mg appeared to be preferentially available to fruit on long axillary shoots rather than to fruit on short shoots, while the opposite trend was noted for P and K. Thorp *et al.* (2003a; 2003b) noted that fruit on long axillary shoots had higher Ca and Mg concentrations, and lower P and K concentrations, than fruit on short shoots. As there was no inorganic ion concentration gradient down a cane, it is doubtful that these concentration differences were due to the different position of the two shoot types on the cane. Thorp *et al.* (2003b) compared fruit from short shoots at three positions along a cane and found no significant differences in fruit Ca and Mg concentrations among fruit from the different positions, although K and P concentrations were higher in fruit from short axillary shoots nearer to the distal ends of the canes.

The high correlation between Ca and Mg xylem sap concentrations, and the decline in Ca and, to a lesser extent, Mg sap concentrations with increased concentrations of the more phloem-mobile nutrients, such as K and P, suggests that the mechanisms controlling Ca and Mg translocation within the plant may be regulated by similar factors. As K and P are both highly mobile and travel in both the xylem and phloem (Marschner, 1995), their sap concentrations may be less affected by internal regulation systems and shoot attributes. By contrast, Ca transport is restricted to the xylem, where it travels via mass flow in the transpiration stream and is influenced by cation exchange processes (Ferguson and Bollard, 1976). The mobility of Mg is intermediate between that of Ca and K or P (Marschner, 1995).

Declining concentrations of Ca and Mg in the xylem sap, reduced fruit transpiration (Chapter 6, Xiloyannis *et al.*, 2001) and vascular dysfunction (Dichio *et al.*, 2003) may individually or together explain the decline in accumulation of these minerals in fruit as it nears maturity (Clark and Smith, 1988), as well as explaining why the Ca concentration of fruit from short shoots does not 'catch up' with that in fruit on long shoots (Thorp *et al.*, 2003b), despite the existence of higher sap Ca concentrations in fruit stalks from short shoots towards the end of January.

Fruit mineral concentrations will depend on both flow rates of sap flowing into a fruit and the mineral ion concentration differences in sap being transported to the fruit. Thorp *et al.* (2003a; 2003b) and Smith *et al.* (1994) concluded that final fruit mineral concentrations were related to their position on the vine during development after finding correlations between leaf transpiration rates and fruit Ca concentrations (Smith *et al.*, 1994) and leaf area and fruit Ca concentrations on short axillary shoots (Thorp *et al.*, 2003b). When soil water availability was not limiting, sap flow

rates were positively correlated with whole-plant transpiration in banana (Lu *et al.*, 2002), mango (Lu and Chacko, 1997), and grape (Lu *et al.*, 2003). It is not surprising then that leaf area and fruit Ca concentrations are correlated, given that sap flow towards fruit is likely to be enhanced on shoots that are more exposed or have a high leaf to fruit ratio (Table 9.1). This study provides evidence that in addition to differences in sap flow rate, variations in the xylem sap composition may be responsible for the high fruit to fruit variation in mineral concentration found within a kiwifruit vine, such as that between fruit on long and fruit on short axillary shoots (Ferguson *et al.*, 2003).

It is possible that xylem loading with mineral ions can occur independent of water transport (Smith, 1991), as can mineral ion translocation (Siebrecht *et al.*, 2003). This would explain the lack of correlation between xylem sap mineral concentrations, leaf areas and leaf transpiration rates found in this study. Rates of Ca translocation, and xylem sap Ca concentrations also were unaffected by rates of transpiration in experiments by Malone (2002b) and Atkinson *et al.* (1992). Differences between the excreta sap concentrations in fruit stalks from long and short shoots and the high levels of within-vine variation in (xylem) bleeding sap concentrations found in other studies (Ferguson *et al.*, 1983; Peterlunger *et al.*, 1990; Sotiropoulos *et al.*, 2002) suggest that mechanisms may be operating to control flow of, or partitioning of, ions towards specific plant parts.

Two explanations are suggested to explain the differential partitioning of mineral ions towards specific parts of the plant that results in differences in composition between long and short shoots. First, certain minerals are preferentially diverted towards long or short axillary shoots. Xylem loading of mineral nutrients can occur independent of water transport (Smith, 1991; Atkinson *et al.*, 1992) and may be controlled by anion- and K- channel activity (Marschner, 1995; Siebrecht *et al.*, 2003). Siebrecht *et al.* (2003) indicated that mineral translocation rates in poplar were unrelated to sap flow rates and suggested that active xylem loading enabled plants to regulate mineral nutrient translocation in response to shoot demand. They also stated that demand for nutrients was correlated with leaf age, with newer leaves, such as those growing on long shoots, acting as stronger sinks than old leaves. Ferguson *et al.* (1983) also suggested that a preferential loading/ unloading system was responsible for within-vine variation in the xylem sap mineral concentration in kiwifruit. Some legume species can regulate Ca translocation via the transpiration stream by sequestering xylem sap Ca in their roots and/or shoots (Atkinson *et al.*, 1992). Calcium may be preferentially diverted towards fruit on long shoots, during periods of rapid fruit growth, for incorporation into the cell wall structure. Conversely, K and P may be preferentially diverted towards fruit on short shoots. Auxins have been shown to regulate anion-

(Barbier-Brygoo *et al.*, 1999) and K- channel (Philippar *et al.*, 2004) activity and therefore, may regulate mineral ion distribution within the plant.

Second, because mineral ions are being pulled passively rather than actively at a greater rate towards long shoots than short shoots, they may accumulate at cation exchange sites in the vascular tissues of long shoots. This accumulation could result in reserve pools forming at these sites from which minerals may be released into the xylem, adding to those already mobilised from the roots. By contrast in short shoots, where the transpiration rates are lower relative to total leaf area, there is less potential to accumulate such reserve pools. This explanation would fit the relationship found by Thorp *et al.* (2003b) between leaf area on short axillary shoots and mineral ion concentration in the fruit. As Ca and, to a lesser extent, Mg (Clark and Smith, 1988) are phloem immobile and are dependent on passive transport in the transpiration stream, these minerals would be more likely to accumulate in fruit from long shoots. Minerals such as K and P that are very mobile in the phloem would be less likely to accumulate at cation exchange sites in long shoots.

Hydraulic constrictions at branch junctions may affect the movement of ions within plants (Tyree *et al.*, 1983; Ewers and Zimmerman, 1984a; Ewers and Zimmerman, 1984b). These constrictions are particularly noticeable where a small branch arises from a much larger one (Ewers and Zimmerman, 1984a), such as might be found in kiwifruit at the junction between a short axillary shoot and its parent cane. Although not considered in this study, these hydraulic constrictions could reduce the capability of a shoot to supply water (and nutrients) to its leaves and fruit and might help to explain the higher Ca, and to a lesser extent, Mg levels in long shoots than in short shoots.

9.5. Conclusions

Spittlebugs were used to detect differences in the composition of xylem sap collected from fruit stalks on long and short axillary shoots of kiwifruit and the following hypotheses were made:

1. Differences in the mineral concentration of fruit on long and short shoots may be due to variations in the mineral concentration of xylem sap reaching fruit, in addition to differences in sap flow rate. Fruit Ca and Mg concentrations were higher, and P concentrations lower, in sap collected from fruit stalks on long shoots than on short shoots. Xylem sap K concentrations in long and short shoots did not differ.

2. Xylem sap concentration differences between these two shoot types may occur because some minerals are preferentially diverted towards specific parts of the plant, a process that may be regulated by plant hormones such as auxin. Alternatively, Ca and Mg may accumulate at cation exchange sites in long, non-terminating shoots where they can be released into the xylem, adding to those already mobilised from the roots. These differences may explain why fruit from long shoots have higher Ca and Mg concentrations and lower P and K concentrations, and are less likely to develop physiological pitting in storage than fruit from short shoots.

3. Xylem loading with mineral nutrients may occur independent of water transport.

4. Spittlebugs provide a useful tool for the study of xylem transport dynamics in kiwifruit. However, their seasonal availability, feeding habits and preferences need to be considered when using them as a tool to collect xylem sap.

10. General Discussion

10.1. Thesis objectives

In 1997 the New Zealand Kiwifruit Marketing board created the ZESPRI™ brand in an attempt to position New Zealand kiwifruit internationally as an upmarket fruit category and to relieve price competition (Beverland, 2001). It was recognised that the reputation and ultimate success of the ZESPRI™ brand would depend on the ability of the New Zealand kiwifruit industry to satisfy consumer demands for product quality. Consequently, a considerable amount of time and money was allocated towards identifying attributes that consumers desired in kiwifruit products. Research showed that consumers placed more value on internal quality attributes of kiwifruit, such as taste, nutrition and postharvest quality, than on external attributes such as fruit size and freedom from defects (Ferguson, 2004; Harker, 2004). Therefore, production and marketing strategies were targeted towards delivering fruit with sought after internal quality attributes to consumers (e.g., the Taste ZESPRI™ initiative).

Fruit DM and mineral concentrations are among the determinants of internal quality (Feng *et al.*, 2003; Ferguson *et al.*, 2003; Maguire and Mowat, 2003; Harker, 2004), yet there is a poor understanding of the factors affecting fruit mineral and DM concentrations in kiwifruit vines. In practice there is considerable variation in the DM and mineral composition of fruit arriving at the packhouse. This makes it difficult for industry to segregate and extract value from fruit that have high inherent internal quality and increases the risk of consumers receiving fruit that are 'unacceptable'. This lack of knowledge in what causes this quality variation makes it difficult for industry to identify and implement production practices that both reduce variation in fruit internal quality and raise fruit internal quality. This is a major concern for industry as the success of many of their marketing strategies, such as the Taste ZESPRI™ initiative, is dependent on the ability of growers to consistently produce fruit with high and guaranteed internal quality.

Therefore, the overall objective of this research was to determine what factors influence carbohydrate, water and mineral accumulation in kiwifruit (Ca, K, Mg and P). In each experiment, fruit were selected from long and short shoots, from whence fruit are known to differ in their DM and mineral composition. Experiments were designed to investigate how specific practices could affect carbohydrate, water and mineral accumulation in fruit from these shoots and the implications of these practices to fruit quality. Results of these experiments are used to identify management technologies with potential to reduce variation in fruit internal quality and raise the

internal quality standard of ZESPRI™ fruit. It is suggested that fruit Ca and DM concentrations will both be influenced by the timing and extent of vascular development in the fruit and fruit stalk and by leaf area development on the fruiting shoot. These factors are discussed in detail.

10.2. Factors affecting Ca and carbohydrate accumulation in kiwifruit

Similar factors appear to influence Ca and carbohydrate accumulation in kiwifruit, although the way in which they affect Ca and carbohydrate partitioning to fruit differs (Fig 10.1). Fruit Ca accumulation is driven, at least partially, by the gradient in water potential that exists between the soil and air (Marschner, 1995). This gradient is maintained by evaporation of water from leaves, which controls the flow-rate or flux of water through xylem in the transpiration stream. In contrast to Ca, carbohydrate partitioning to fruit is driven by turgor pressure gradients that are generated by active or passive loading (in sources)/unloading (into sinks) (Patrick, 1997; Lalonde *et al.*, 1999).

Defoliation and crop load experiments demonstrated that Ca accumulation in kiwifruit is influenced by the transpiration rate of leaves subtending fruit (Fig 10.1). There is little effect of the transpiration rate of leaves not on the fruiting shoot on fruit Ca accumulation (Chapters 4-5). In apples, leaves closest to fruit have the biggest effect on apple fruit Ca concentrations (Proctor and Palmer, 1991; Volz *et al.*, 1994). It was suggested that, in apples, high concentrations of Ca accumulate in leaves during the day whilst they are transpiring, and that this Ca is transported from leaves to fruit in the xylem sap at night when the water potential of fruit becomes more negative than that of the leaves (Lang and Volz, 1998). A similar thing may occur in kiwifruit. In addition, Ca ions may be pulled towards transpiring leaves, and could accumulate at cation exchange sites within axillary shoots (Chapter 9). This accumulation could lead to the formation of reserve pools, and Ca ions from these pools may be released into the xylem sap adding to those already mobilised from the roots. Therefore, factors that reduce rates of leaf transpiration also may reduce kiwifruit Ca concentrations. Rates of fruit transpiration also may have a small effect on Ca accumulation in fruit, particularly during the early stages of fruit development when the fruit skin is highly permeable to water vapour and fruit have a high surface area to volume ratio (Chapter 6, Fig 10.1).

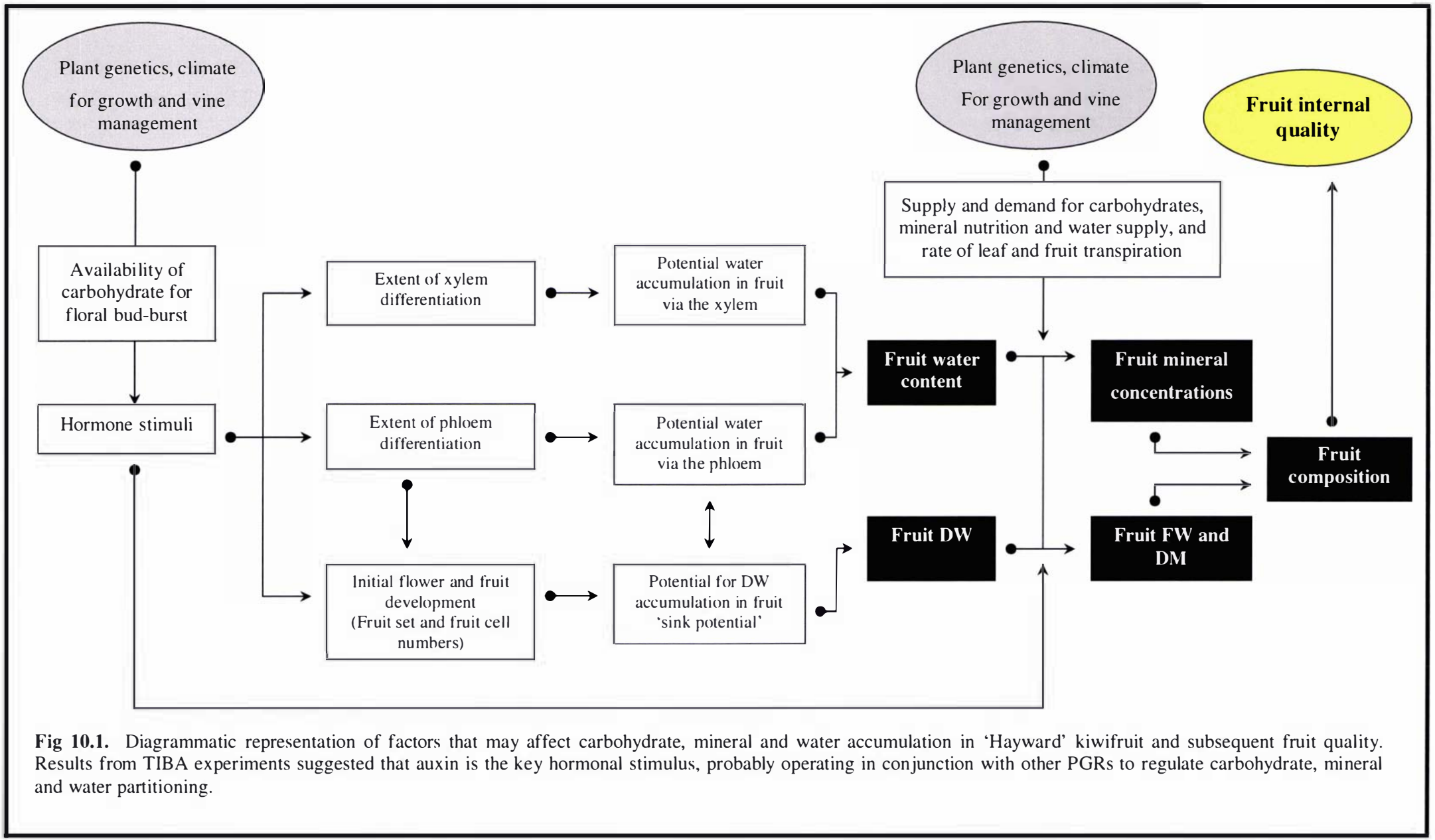


Fig 10.1. Diagrammatic representation of factors that may affect carbohydrate, mineral and water accumulation in 'Hayward' kiwifruit and subsequent fruit quality. Results from TIBA experiments suggested that auxin is the key hormonal stimulus, probably operating in conjunction with other PGRs to regulate carbohydrate, mineral and water partitioning.

Fruit on long shoots generally have higher fruit Ca concentrations than fruit on short shoots as long shoots: (a) have a greater leaf area and hence, a greater surface area for water loss than short shoots (Chapter 5), (b) are generally more exposed than short shoots, so the water potential difference (vapour pressure difference) between the leaves/fruit and the environment is greater, (c) have a greater skin permeance and hence greater rates of fruit transpiration, (d) leaves on long shoots have a higher stomatal conductance than leaves on short shoots, so would be expected to have higher rates of transpiration.

Fruit carbohydrate accumulation also is influenced by vine/shoot leaf areas, or more specifically by the L:F ratio within a vine/shoot, which determines the supply of, and demand for, carbohydrate (Chapters 3, 4 and 5, Fig 10.1). Photosynthesis is little affected by crop load in kiwifruit, as at low crop loads 'surplus' carbohydrate is utilised in the development of new shoots or is partitioned into fruit (fruit size increases) (Chapter 4). Similar findings were observed in grapes (Chaumont *et al.*, 1994; Rubio *et al.*, 2004). Shoot growth may enhance the demand for carbohydrate in kiwifruit, thereby increasing the photosynthetic efficiency of vines (Chapter 4); rates of photosynthesis were higher on long, non-terminating shoots that have a high L:F ratio and continue to extend until late in the season than on short-terminated shoots that have a low L:F ratio and extend very little after flowering. This suggests that vegetative growth is a stronger sink for carbohydrate in vine crops than in tree crops such as apple. In apple, fruit is the predominant sink for carbohydrate, and carbohydrate partitioning to fruit may be reduced at low crop loads (high L:F ratio), as photosynthesis is down-regulated (Palmer, 1992; Fallahi and Simons, 1993; Wünsche *et al.*, 2000). This occurs due to an imbalance between carbohydrate metabolism and absorbed excitation energy (Wünsche *et al.*, 2000).

In addition to being affected by leaf area, there is evidence that plant growth regulators (PGRs) might regulate Ca and carbohydrate partitioning to fruit. In Table 10.1 evidence is provided, that auxin, which is thought to be synthesised in the nucellus (Nitsch, 1970), developing leaves (Ljung *et al.*, 2001) and seeds (Nitsch, 1950), regulates vascular differentiation in the fruit stalk and fruit and that this determines the potential for water and carbohydrate (DW) accumulation via the xylem and/or phloem, thereby indirectly affecting fruit Ca and carbohydrate accumulation (Chapters 5-9, Fig 10.1). Evidence in Table 10.1 was derived from an assessment of the vasculature of 675 pedicels (Chapters 3, 4 and 6-8), xylem functionality of 236 pedicels (Chapters 5 and 8) and from auxin transport inhibitor (TIBA) and pollination experiments (Chapter 8). Other PGRs also may be involved, either alone or in conjunction with auxin, in regulating carbohydrate and Ca partitioning in kiwifruit.

Table 10.1. Evidence that auxin may indirectly affect carbohydrate and Ca accumulation in kiwifruit by regulating vascular differentiation in flower/fruit stalks and/or fruit cell division

PHLOEM	XYLEM
• TIBA application during the initial period of secondary phloem differentiation has the greatest effect on fruit DW (C8). • Kiwifruit DW and volume (related to DW) increases at a constant rate from the early stages of fruit development (Hall *et al.*, 1996; Richardson *et al.*, 1997) when phloem (C7) and fruit (Hopping, 1976) tissues are rapidly dividing. After phloem differentiation and cell division have ceased the effects of girdling (C5, Currie, 1997) defoliation (C5) and thinning (Currie *et al.* 2005: unpublished data) on carbohydrate accumulation in fruit are reduced when compared to the effects of these treatments applied during the period of phloem differentiation and cell division in kiwifruit. This suggests that factors that determine the rate of carbohydrate accumulation in kiwifruit are determined during the initial period of fruit development and are likely to be related to the extent of phloem differentiation and cell division in fruit. • During the initial stages of flower/fruit development secondary phloem areas are greater in flowers/fruit from long shoots (high seed number) than in flowers/fruit from short shoots (low seed number; C7). Subsequently, fruit on long shoots tend to be larger and have a greater DW than fruit on short shoots. Larger fruit size may be related to an increase in cell numbers, as fruit from early-opening flowers have a higher cell number in the ovary at flowering and develop in to larger fruit than fruit from late-opening flowers (Cruz-Castillo *et al.*, 2002). • The number of seeds in a fruit is positively correlated with the cell number in a fruit (Currie, 1997) and the fruit DW at harvest (C8). • Carbohydrate accumulation is inhibited in fruit with a low seed number that are growing in close proximity to fruit with a high seed number, even when carbohydrate is readily available (Lai *et al.*, 1990). • IAA concentrations peak when rates of cell division and phloem differentiation peak in kiwifruit (Tao *et al.*, 1994).	• Fruit Ca concentrations are positively correlated with secondary xylem conductance (C4&8). TIBA, an auxin transport inhibitor, reduces secondary xylem conductance and fruit Ca concentrations in kiwifruit when applied during the period of peak IAA efflux from fruit (Tao *et al.*, 1994) when secondary vascular tissues are rapidly dividing (C8). In addition, TIBA reduces IAA efflux from, and Ca accumulation in, kiwifruit (D. Woolley and S. Lawes: unpublished data), apple (Benson and Stahly, 1972), tomato (Hamamoto *et al.*, 1998), avocado (Cutting and Bower, 1989) and nectarine (Wand *et al.*, 1991) fruit when applied during the early stages of fruit development. • Xylem conductance and fruit Ca concentrations increase as the seed number in a fruit is increased. • Seeds are a known site of auxin synthesis and girdling, which has been shown to reduce fruit seed numbers in 'Hayward' kiwifruit (Currie, 1997), also reduces fruit Ca concentrations (C5). • Auxins have been shown to regulate vascular differentiation (Mattsson *et al.*, 1999; Aloni *et al.*, 2000) and: - Fruit Ca concentrations increase very little after the cambium becomes inactive (C7), despite rates of leaf transpiration remaining unchanged. - Vascular tissues in fruit become dysfunctional as they are broken during fruit expansion (Dichio *et al.*, 2003) and this inhibits the flow of (Ca-containing) xylem sap into fruit (C5&8). - The cambium remains active until later in the growing season in stalks from fruit on long shoots (high seed number) than in stalks from fruit on short shoots. • Long shoots have more developing leaves than short shoots and fruit on long shoots have more seeds than fruit on short shoots, therefore the auxin efflux from long shoots is likely to be greater than that from short shoots. This may explain why secondary xylem conductance tends to be greater (C7&8) and fruit Ca concentrations are generally higher (C3,4,5,6,8&9) in fruit from long shoots than in fruit from short shoots.

Where evidence was derived from this thesis, chapter numbers (C3-C9) are specified.

As passive forces regulate the flux of sap flow through xylem vessels, Ca accumulation has potential to be directly affected by factors that alter fruit stalk xylem conductance (xylem vessel numbers, size and/or continuity) at all stages of flower/fruit development (Fig 10.1). The phloem is the first 'link' to be established between a developing flower bud and the vine (Chapter 7). Therefore, the timing and extent of phloem differentiation in flower/fruitlet stalks may indirectly affect carbohydrate accumulation in kiwifruit by determining the carbohydrate availability for cell division (Chapters 7 and 8). The extent of cell division also may be determined by the carbohydrate availability at flowering, and may influence the subsequent capacity of a fruit to accumulate carbohydrate (fruit sink strength) during the latter stages of fruit development (Fig 10.1). Others have also found that kiwifruit development is influenced by the carbohydrate availability during flowering and the early stages of fruit development (Piller *et al.*, 1998). It has been suggested that the carbohydrate availability at flowering determines a kiwifruit's sink strength by influencing vascular development and/or seed set (Lai *et al.*, 1990; McPherson *et al.*, 2001).

In mandarins, the cross-sectional area of dorsal vascular bundles was measured on completion of cell division (9 weeks after anthesis) and was correlated with final fruit volume at harvest (Marsh *et al.*, 1999). In apricot (Jackson and Coombe, 1966), Japanese pear (Zhang et al., 2005) and apple (Goffinet et al., 1995) fruit, carbohydrate availability at anthesis determined the extent of cell division in flowers/fruitlets and subsequently, the final fruit size at harvest. Correlations between fruit cell number and fruit size at anthesis also have been found in apple (Denne, 1963) and olive fruit (Rapoport et al., 2004). Cultivar fruit size differences in species as diverse as kiwifruit (Currie, 1997), strawberry (Cheng and Breen, 1992), peach (Scorza et al., 1991), apple (Harada et al., 2005), melon (Higashi et al., 1999) and cherry (Yamaguchi et al., 2004) were related to differences in cell number. Taken together with the evidence from this thesis, it is most likely that carbohydrate and mineral accumulation in fruit is affected by the extent of vascular and cell differentiation in the fruit and fruit stalk during the early stages of fruit development.

During the later stages of fruit development phloem capacity in the fruit stalk does not appear to restrict carbohydrate partitioning to kiwifruit (Chapters 4 and 7), citrus (Guardiola *et al.*, 1993; García-Luis *et al.*, 2002) or strawberry (Darnell and Martin, 1987) fruit, because as fruit enlarge (sink demand is enhanced) there is a simultaneous increase in the fruit stalk phloem capacity. Conversely, xylem conductance does not necessarily increase as fruit size increases, and there is only a weak correlation between fruit FW and xylem area (Chapters 4 and 7). Because of this Ca concentrations are often diluted as fruit size increases, particularly if the phloem: xylem ratio in the fruit stalk is increased.

The effects of fruit stalk xylem and phloem differentiation on carbohydrate and mineral accumulation in kiwifruit may relate to the way in which solutes are transported in the xylem and phloem. Xylem transport is not thought to depend on the transfer of physical (turgor) signals between sources and sinks. This differs from the situation in the phloem, where physical signals may relay information between sources and sinks so that fruit development (i.e. the development of vascular tissues) can be coordinated to match assimilate production. Cowan *et al.* (2001) proposed that hormone metabolism was affected by the carbohydrate status of developing fruit and that this directly or indirectly affected fruit cell proliferation by affecting gene expression in a fruit.

Plant growth regulators may directly regulate carbohydrate partitioning to fruit by: (a) controlling the enzymatic conversion of sugars to starch and other compounds once unloaded from the phloem, (b) controlling the active loading of carbohydrates into vacuoles and other cell organelles, or by (c) switching assimilate movement between symplastic and apoplastic pathways to maintaining an appropriate pressure gradient between leaves and fruit (Daie, 1989; Morris, 1996; Cole and Patrick, 1998; Lalonde *et al.*, 1999). Figure 10.1 highlights where hormonal stimuli, produced during flowering and/or early fruit development, may be able to influence carbohydrate accumulation in fruit by regulating the above processes.

Even though PGRs may have the ability to coordinate fruit development according to changes in carbohydrate availability, fruit size is not always correlated with fruit cell numbers in a range of fruits including kiwifruit (Currie, 1997), as well as tomato (Bertin, 2005), apple (Stanley *et al.*, 2000; Atkinson *et al.*, 2001) blueberry (Cano-Medrano and Darnell, 1997) and cucumber (Marcelis, 1993). When carbohydrate supplies are limited later in the season, carbohydrate accumulation in fruit, and hence cell enlargement, may be restricted (Currie, 1997: Fig 10.1). Therefore, despite having a large number of cells, a fruit may not reach its potential DW (as determined by the number of cells it contains). Conversely, when carbohydrate is readily available (i.e. there is surplus carbohydrate available after the demands of all sinks have been met) cell expansion may be maximised as may the fruit DW (Stanley *et al.*, 2000). A situation may occur whereby a small fruit has more cells than a large fruit, but the cells are smaller than those in the large fruit. Moreover, two fruit could be found with identical cell numbers, but one may have larger cells, and therefore a greater FW than the other. In this way, cells within a sink may compete with each other for a supply of carbohydrate (Bertin, 2005).

Although signals are not thought to be transferred between sources and sinks in the xylem, based upon evidence presented in Chapter 9, it is possible that Ca is actively partitioned towards fruit from specific positions within the vine, such as fruit on long shoots. Basipetal auxin transport has

been found to have a direct effect on acropetal Ca transport to fruit (De la Fuente, 1984) and other plant organs (Cutting and Bower, 1989), which may be mediated through auxin affects on vascular development. This effect is very specific to Ca, and is independent of any effects on Ca movement in the transpiration stream or by cation exchange (Banuelos *et al.*, 1987). Perhaps PGRs transfer signals between the roots, shoots and fruit to regulate calcium and carbohydrate partitioning within the vine. Cytokinins are transported from roots to rapidly transpiring organs in the transpiration stream (Aloni *et al.*, 2005), and therefore they may work in conjunction with auxins to regulate carbohydrate and mineral partitioning. Buer and Muday (2004) recently suggested that flavonoids, which are synthesised in most plant organs, may act as negative regulators of auxin transport, thereby controlling the movement of auxin signals between source and sink, and hence with the potential to regulate carbohydrate and Ca partitioning to fruit.

10.3. Factors affecting fruit DM, Mg, K and P concentrations

Fruit DMCs are primarily dependent on the rate of carbohydrate translocation to fruit via the phloem and on the rate of water translocation to fruit via the xylem and phloem (Fig 10.1). Other solutes also are transported in the xylem and phloem tissues and make a small contribution to the total fruit DW. DMCs may be increased by either increasing carbohydrate partitioning to fruit without increasing fruit water accumulation or by reducing fruit water accumulation without reducing the fruit DW. Whilst vine management practices, such as girdling (Lai *et al.*, 1989a) and thinning (Richardson *et al.*, 1997) can influence rates of carbohydrate partitioning to fruit, few practices have been found to have a major impact on fruit DMCs. In Chapters 3-6 and 8, FW and DW accumulation usually increased simultaneously, which suggests that in kiwifruit the potential for water and carbohydrate accumulation is linked (Fig 10.1). Unless sugars are converted to starch or other insoluble compounds, or are stored within organelles, once unloaded from the phloem, they will lower the water potential in fruit (sink) cells, thereby inducing water movement into fruit and subsequent cell enlargement. Therefore, fruit DMCs may depend on the utilisation and/or storage (in vacuoles and other organelles) of unloaded carbohydrates.

Like DMCs, fruit K, Mg and P concentrations also depend on transport processes occurring in both the phloem and xylem (Fig 10.1). Consequently, factors affecting accumulation of these minerals in fruit often differed from those affecting Ca (Chapters 3-6, 8 and 9), which is largely immobile in the phloem (Clark and Smith, 1988). Accumulation of these minerals in fruit may depend on the stage of fruit development (Chapters 5, 6 and 8) and leaf transpiration rates (Chapter 4). It is likely that reductions in K, P and, to a lesser extent, Mg translocation in the xylem can be compensated for by increases in phloem translocation of these minerals (Chapters 4 and 6). In

comparison to Ca, these minerals were often little affected by the vine manipulations investigated in this research (Chapters 5, 6, 8). As long as soils are not deficient in these minerals, and root health is maintained, then uptake and distribution of these minerals within the vine is unlikely to be limiting enough to affect fruit quality.

10.4. Potential to manipulate fruit DM and Ca concentrations to benefit fruit quality

As similar factors affect carbohydrate and Ca accumulation, theoretically it should be possible to maximise both fruit DM and Ca concentrations by manipulating specific aspects of vine growth to alter the L:F ratio within the fruiting shoot or to alter vascular and fruit cell differentiation (Fig 10.1). Management practices to increase DM and Ca concentrations in fruit should be targeted at: (a) enhancing vascular differentiation and cell division in developing flowers and fruitlets, and (b) maximising carbohydrate partitioning to fruit and minimising fruit water accumulation once cell division is complete (from 30-50 DAFB (Hopping, 1976b): Fig 10.1).

During the period from bud-burst through to flowering it is critical to ensure that local carbohydrate availability is maximised, as although carbohydrates can be redistributed from reserve pools, this is not enough to support the current season's growth (Buwalda and Smith, 1990a; Piller and Meekings, 1997). Maximising carbohydrate availability at this time will ensure that there are sufficient resources available to promote vascular and cell differentiation in flowers and fruit and their associated tissues, thereby determining the potential of a fruit to accumulate carbohydrates and Ca during later stages of development. Local carbohydrate availability at this time will depend on leaf area development within the canopy, as the photosynthetic capacity of a vine increases as the LAI increases (Buwalda, 1994), so long as leaves are not shaded (Grant and Ryugo, 1984b; Lionakis *et al.*, 1997).

Early initiated leaves attain maximum photosynthetic capacity earlier than later initiated leaves, and larger leaves, such as those found on long shoots have a greater photosynthetic capacity than smaller leaves, such as those found on short terminated shoots (Chapter 4, and Piller and Meekings, 1997; Greer and Halligan, 2001). Therefore, carbohydrate availability may be improved by selecting larger, more vigorous canes, than thin weak canes. However, in leader-pruned (LP) vines the photosynthetic efficiency of leaves on thin canes may be higher than in conventionally-pruned (CP) vines, because these canes are as productive as large diameter canes on CP vines, and the postharvest quality of fruit from thin canes in LP vines is as good as that of fruit on thin canes on CP vines (Thorp *et al.*, 2003a). Nonetheless, large diameter canes are still more productive than small diameter canes in LP vines, although vigorous replacement canes that

arise from the leader zone of the vine (bull canes) should be removed as they are initiated, as they may shade shoots in the fruiting zone of the vine and compete with fruit for a source of carbohydrate (Chapter 3). The only times that it may be beneficial to leave bull canes in the canopy until later in the season may be when (a) there is a very cool spring and canopy development is very slow and/or there is a risk of frost, and (b) when HiCane® was not applied at the correct time and resulting bud-break was poor (low vine vigour). Under these circumstances bull canes may provide a valuable source of carbohydrate when carbohydrate supplies would otherwise be limited and they may protect the inner canopy from frost damage.

Thinning may increase carbohydrate partitioning to fruit, and therefore has the potential to increase fruit DMCs if water movement into fruit is not also increased. If thinning is to be conducted, it should be performed as close to flowering as possible, so as to maximise carbohydrate partitioning to the remaining fruit on the vine. Fertiliser applications should be timed to maximise leaf growth in spring, and the application of HiCane® or other practices that stimulate early bud-burst and rapid canopy development is likely to increase carbohydrate availability for vascular and fruit cell differentiation. Pollination also is very important, as seeds appear to have a strong influence on DW and Ca partitioning to fruit (Chapter 8).

Once phloem differentiation and cell division are complete and leaves are fully developed (around 50 days after bud-burst), carbohydrate availability should be maximised to ensure that (a) there is enough carbohydrate to satisfy requirements of the fruit and (b) that there is carbohydrate available for partitioning to roots and reserve pools, as this may affect bud-break and vine growth in the following season (Chapter 3). Pruning should be undertaken to ensure that leaves are not shaded and to remove vigorous shoot growth from the leader zone (Chapter 3). Pruning also may improve leaf photosynthetic efficiency by increasing the demand for carbohydrate (Chapter 4). In Chapters 3 and 4 it is suggested that an LAI of between 3 and 4 will maximise photosynthetic efficiency and carbohydrate partitioning to fruit. The use of reflective ground covers to improve light interception in the canopy also may be beneficial. Fertilisers should be applied to ensure that adequate nutrients are taken up to support shoot and fruit growth, and to prolong the time until leaf fall.

Later in the season, during the final phase of fruit growth when rates of water accumulation starts to decline (from about 10 WAFB), it also may be possible to restrict water accumulation in fruit, thereby increasing fruit DMCs at harvest, by:

1. **Girdling shoots:** to restrict water movement into fruit via the phloem. Girdling also may cause ions, such as K to be redirected into the xylem, thereby reducing (xylem) hydraulic

conductance (Zwieniecki *et al.*, 2004: refer Section 5.4.1) and further reducing the flow of water into fruit. Autumn cane girdling increased fruit DMCs in the experiments of Thorp *et al.* (unpublished data) and autumn shoot girdling increased DMCs in long shoots that had a high L:F ratio, but not in short shoots that had a low L:F ratio (Chapter 5).

2. **With holding irrigation:** to restrict water movement into fruit in both the xylem and phloem. Late-season deficit irrigation increased total soluble solids concentrations and/or DMCs of grape (Salon *et al.*, 2004), peach (Gelly *et al.*, 2003), and apple (Mills *et al.*, 1996; Mpelasoka *et al.*, 2000) fruits. In apple, cell expansion was reduced when irrigation was withheld from fruit, and fruit were smaller, but cell division was not affected (Atkinson *et al.*, 1998). In kiwifruit, rainfall prior to harvest reduced fruit soluble solids concentrations (Salinger *et al.*, 1993) and fruit DMCs (W. Snelgar pers. comm.), which suggests that deficit irrigation in the later stages of fruit development may have potential to increase fruit DMCs. However, severe water stress (irrigating to less than 65% of the soil water holding capacity) may reduce photosynthesis, and hence photosynthate availability (Chartzoulakis *et al.*, 1993a). Currently the kiwifruit industry is evaluating the use of reflective ground covers to increase whole vine photosynthesis and fruit DMCs (M. Currie: pers. comm.). In addition to increasing light interception in the vine, these covers also may prevent rain from being adsorbed by the soil, thereby lowering the soil water content.

3. **Complete defoliation of selected shoots:** to reduce the flow of water towards fruit in the transpiration stream. Under normal circumstances very little Ca accumulates in fruit after this time (Clark and Smith, 1988; Xiloyannis *et al.*, 2003) and fruit Ca accumulation may occur independent of water movement in the transpiration stream (Chapters 6 and 8). Therefore, it is unlikely that fruit Ca accumulation will be adversely affected by such a treatment. Furthermore, unless whole vine carbohydrate supplies are limited, carbohydrate can be transported to shoots where there is a shortage of carbohydrate (Lai *et al.*, 1988; Lai *et al.*, 1989a) and this may explain why fruit DMCs were increased by 0.5-1% units in fruit from summer defoliation long and short shoots. In order to avoid whole vine carbohydrate shortages, defoliation should be aimed at increasing the DMC of fruit on short shoots with a low L:F ratio, which typically produce fruit with a low DMC.

Seasonal variations in fruit Ca and DM concentrations could be reduced if growers paid careful attention to both the short- and long-term effects of specific management practices on Ca and carbohydrate accumulation (Chapter 3). When fruit DMCs were compared on high and low DMC orchards over a two-year period (Fig 1.1), fruit DMCs were reduced in the second season in 25% of the high DMC orchards and 0% of the low DMC orchards. This suggests that the decline was

not due to unfavourable climatic conditions, but because the increase in fruit DMCs attained in the first season was not sustainable. Carbohydrate partitioning to fruit may have occurred to the detriment of the root and/or reserve pools and this may have affected vascular differentiation and cell division in the following season (Fig 10.1). In apple, cell division in the current season may be negatively affected by heavy cropping in the previous season (Bergh, 1985).

It also is possible that certain practices may only be effective in certain environmental conditions. In Chapter 3 data is presented which indicates that the advantageous effects of LP might be reduced in unfavourable environmental conditions, such as in spring frosts and in high winds. Moreover, although LP tended to increase fruit DMCs when compared to CP, over the three-year period of this experiment fruit DMCs actually decreased, suggesting that other factors (not related to the pruning system used) had an over-riding influence over the maximum potential DMC (Fig 10.1). In contrast to results from other studies (M. Currie: unpublished data), DMCs were not significantly reduced at high crop loads (Chapter 4), which suggests that the crop load that can be carried by a vine before DMCs are reduced may be dictated by the climate and conditions in which a vine develops. Figure 1.1 also show that DMCs were only increased significantly (by more than 0.5% units) in 33% of the high DMC orchards. This suggests that in the best-performing orchards the potential for further increases in fruit DMC may be minimal or that for a given climate there is a limit to how much 'Hayward' kiwifruit DMCs can be increased. Further increases in fruit DMC (above the 17-20% attained on high DMC orchards) may be limited by a lack of starch storing cells in fruit or to some other genetic characteristic of the fruit/vine (Fig 10.1).

10.5. Directions for future research

Three broad areas of future research are proposed to improve internal quality in kiwifruit; to investigate the role of PGRs in carbohydrate and mineral nutrition in kiwifruit, to investigate the affects of different management practices and climatic conditions on specific physiological processes that may influence carbohydrate and mineral accumulation in kiwifruit, and to evaluate the potential of different fruit components to be used as alternative indicators of kiwifruit internal quality. The recommendations proposed here could be applied to any of the *Actinidia* species.

1. The role of PGRs

There is a strong indication that endogenous PGRs influence fruit carbohydrate and mineral partitioning to fruit by influencing the vascular differentiation and cell division. However, PGR concentrations were not measured in this thesis. It was assumed that because auxin transport inhibitors, such as TIBA, greatly reduced auxin efflux from kiwifruit (Currie, 1997

and D. Woolley and S. Lawes (pers. comm.)) and other fruit (Benson and Stahly, 1972; Cutting and Bower, 1989; Wand *et al.*, 1991a; Raese *et al.*, 1995; Hamamoto *et al.*, 1998), that kiwifruit auxin efflux also was reduced by TIBA application in this study. Therefore, opportunities exist to examine the role that PGRs play in carbohydrate and mineral partitioning in kiwifruit. In particular, one could investigate the affects of specific PGRs and/or combinations of PGRs on vascular and fruit cell differentiation and subsequent mineral, water and carbohydrate accumulation. The role of PGRs in phloem unloading also should be investigated. Once PGR roles in carbohydrate and mineral partitioning have been ascertained, an investigation could proceed into how exogenous PGRs may be used to increase carbohydrate and Ca partitioning into kiwifruit. A greater understanding of the role of PGR in influencing carbohydrate and mineral accumulation in kiwifruit also may help to identify and/or breed for cultivars with high fruit DM and/or Ca concentrations.

2. Canopy management and fruit quality

A greater understanding is required of how different management and environmental influences affect specific physiological processes occurring within the fruit and other plant organs, such as cell division and photosynthesis, and how this in turn affects the kiwifruit internal quality. This may be achieved by exposing plants to different management practices and/or climatic conditions at different stages of the growing season and examining the effects of treatments on fruit and vine physiology. In order to simulate commercial practice, multifaceted experiments could be designed to examine the effects of different combinations of management practices and/or different management practices in different climatic conditions on vine structure and function. Experiments should be continued over several seasons to quantify any long-term or carry over affects on fruit quality and fruit should be sampled from different positions in the vine known to differ in composition and internal quality. Specific management practices that could be investigated include: late season shoot girdling and defoliation, deficit irrigation, and plant growth regulator treatments.

4. Evaluation of alternative internal quality indicators

Despite the range in DM and Ca concentrations in fruit from these experiments, there was little affect of treatments on fruit sensory or postharvest quality. This may be attributed to the sampling methods used to collect fruit, the number of fruit sampled, or to treatment effects on fruit DM and Ca concentrations being too small to cause perceivable differences in fruit quality. It also is possible that fruit DM and Ca concentrations are poor indicators of kiwifruit internal quality and that better indicators are available. In order to evaluate the

efficacy of alternative internal quality indicators, a greater understanding is required of how specific fruit components and combinations of these components contribute to sensory taste and postharvest quality in kiwifruit. Precise upper and lower acceptance limits could then be established for each compound identified as having a significant affect on kiwifruit internal quality. The effects of specific management practices and climatic conditions on these fruit components could then be evaluated as described above. It is suggested that more information is required on the effects of specific mineral nutrients (i.e. mineral nutrition), soluble sugars and organic acids on kiwifruit internal quality. The effects of these fruit components on volatile production in storage and subsequent consumer acceptance should also be examined.

10.6. Conclusions

The factors affecting carbohydrate, Ca and, to a lesser extent, Mg accumulation are similar, although they do not necessarily affect carbohydrate, Ca and Mg accumulation in the same way. Factors affecting K and P accumulation in fruit often differed to those affecting Ca accumulation in fruit. Theoretically then, it should be possible to consistently increase and reduce variability in fruit DM, Ca and Mg concentrations and to reduce fruit K and P concentrations, in order to optimise kiwifruit quality. It is suggested that growers have the potential to increase, and reduce variability in fruit DM and Ca concentrations at harvest, by applying vine management technologies at very precise times to target specific physiological events, and by considering both the short- (<1 year) and long- (>1 year) term affects of vine management technologies on fruit quality. For this reason, future research should focus more on the influence of specific physiological events on fruit quality and on the physiological implications of specific management practices rather than on the changes in fruit composition induced by these management practices (i.e. the increase or decrease in fruit DM and Ca concentrations). A greater understanding of how specific practices impact on fruit and vine physiology will enable growers to better manipulate kiwifruit internal quality in order to produce fruit that are more desirable to consumers. This will help the New Zealand kiwifruit industry to attain its goal of positioning ZESPRI™ kiwifruit in an upmarket fruit category for which they can demand a price premium over kiwifruit from other countries.

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APPENDIX I: International Conference and Industry Presentations

Oral presentations were made at the following conferences:

1. The 5th International Symposium on Kiwifruit, Wuhan, China, 15-20 September 2002
2. The 5th International Postharvest Symposium, Verona, Italy, 6-11 June 2004

In addition, I presented work at the September 2004 ZESPRI™ DM field days and data from my work was presented at the November 2004 ZESPRI™ Innovation Forum. I also will be presenting data at the November/December 2005 canopy management field days.

APPENDIX II

Table A1. Chemicals utilised in fruit assessment

Chemical name	Formula/ Abbreviation	Source
Ethanol	C_2H_5OH	BDH AnalaR®, Poole, England
Paraformaldehyde	$CH_3CH_2CO[CH_2]_3CH_3$	BDH AnalaR®, Poole, England
Phloroglucinol	$C_6H_6O_3$	SIGMA-Aldrich®, St Louis, USA
Glycerine	$C_3H_8O_3$	Andrew Industries, Auckland, New Zealand
Hydrochloric acid (95%w/w)	HCl	BDH AnalaR®, Poole, England
Nitric acid (70% w/w)	HNO_3	BDH AnalaR®, Poole, England
Perchloric acid (70% w/w)	$HClO_4$	BDH AnalaR®, Poole, England
Lanthanum chloride	$LaCl_3$	SIGMA-Aldrich®, St Louis, USA
Octylphenol ethoxylate (Triton™X)	$C_{24}H_{42}O_6$	Dow Chemical Company, Michigan, USA
Sulphuric acid (95-97% w/w)	H_2SO_4	BDH AnalaR®, Poole, England
L-Ascorbic Acid (Vitamin C)	$C_6H_8O_6$	SIGMA-Aldrich®, St Louis, USA
K Antimonyl tartrate	$K(SbO)C_4H_4O_6.3H_2O$	May & Baker Ltd, Dagenham, England (Now Merck,
Ammonium Molybdate	$(NH_4)_6Mo_7O_{24}.4\ H_2O$	BDH AnalaR®, Poole, England
Adenosine-5'-triphosphate	ATP	SIGMA-Aldrich®, St Louis, USA
Nicotinamide adenine dinucleotide phosphate	NADP	SIGMA-Aldrich®, St Louis, USA
Glucose-6-phosphate dehydrogenase-hexokinase	G_6PDH-HK	ROCHE, Basel, Switzerland
Phosphoglucoisomerase	PGI	ROCHE, Basel, Switzerland
B-fructosidase (Invertase)	-	SIGMA-Aldrich®, St Louis, USA
Amyloglucosidase	AMG	ROCHE, Basel, Switzerland
A-amylase	-	SIGMA-Aldrich®, St Louis, USA
Toluidine blue O	$C_{15}H_{16}N_3SCl$	Merck, Darmstadt, Germany