RESOURCE ALLOCATION IN KIWIFRUIT (*Actinidia chinensis*)

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ABSTRACT

Kiwifruit growers in New Zealand receive financial incentives to produce high yields of fruit with high individual dry matter concentrations (DMCs). Several vine management techniques are available to growers to enable them to direct more resources into production of fruit rather than into other sinks such as root growth and shoot extension. The long term consequences of these management techniques are not well understood. The overall objective of the work described in this thesis was to investigate how manipulating whole vine source-sink relationships affects fruit quality, long-term vine health and productivity in 'Hort16A' kiwifruit vines.

A compensatory reduction in flower numbers occurred as a result of whole vine carbohydrate depletion (famine treatment) and producing high crop loads of high DMC fruit with reduced leaf area (minimal pruning, standard nitrogen). Keeping crop loads low did not result in increased productivity, instead additional resources were allocated to root growth (feast treatment). Isolating the canopy from the roots by extended trunk girdling was the technique that enabled high flower numbers to be maintained across seasons.

Increasing individual fruit DMC generally enabled fruit to be harvested earlier than fruit with lower DMC. This was because flesh colour change, the main harvest criterion, occurred earlier in fruit from treatments where DMC was increased. Fruit softening behaviour was less affected by changes in DMC than flesh colour change, meaning that low DMC fruit could be softer at commercial harvest that more mature high DM fruit. The implications of this finding for storage performance were discussed.

Vines showed few of the common responses to carbohydrate depletion. There was no evidence of increased individual leaf area, reduced specific leaf weight, upregulated leaf photosynthesis or increased shoot growth. Uptake and allocation of some mineral nutrients within the vines was affected, but few visible signs of leaf nutrient deficiencies were seen. The results suggest that vines respond to carbon depletion primarily be altering resource allocation between flowering and root growth, rather than by altering its ability to capture carbon.

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LIST OF ABBREVIATIONS AND TERMS USED

A	Net photosynthesis
BB	Bud break
C_i	Internal leaf CO_2 concentration
CP	Conventional pruning
CPPU	N-(2-chloro-4-pyridyl)-N´-phenylurea
DAMB	Days after mid-bloom
DMC	Dry matter concentration
DW	Dry weight
ETG	Extended trunk girdling
FBB	Floral bud break
F/FS	King flowers per floral shoot
FW	Fresh weight
GA	Gibberellic acid
g_s	Stomatal conductance
ha	hectare
IAA	indole-3-acetic acid
KF/Bud	King flowers per winter bud
LA	Leaf area
LAI	Leaf area index
LSD	Least significant difference (between two means)
LTB	Low temperature breakdown
MP	Minimal pruning
N	Newton
NCER	Net CO_2 exchange rate
NPA	1-N-naphthylphthalamic acid
NSC	Non-structural carbohydrates
PGRs	Plant growth regulators
SE	Standard error (of the mean)
SSC	Soluble solids concentration
SLW	Specific leaf weight
VBB	Vegetative bud break
Year 1	Spring 2007 to winter 2008 where spring = Sept. to Dec., winter = May to Aug.
Year 2	Spring 2008 to winter 2009
Year 3	Spring 2009 to winter 2010

1 INTRODUCTION

The fruit and vegetable market is highly competitive and demand for greater variety and higher quality is increasing (Ecklund-Axelson and Axelson, 2000). The New Zealand kiwifruit industry has responded by ranking fruit by fruit dry matter concentration (DMC) as an indicator of consumer acceptability of ripe fruit (Harker et al., 2009) and by developing new cultivars. The yellow-fleshed 'Hort16A' kiwifruit (*Actinidia chinensis* Planch. var. *chinensis*) has been the most successful new cultivar to date and is marketed as ZESPRI® GOLD Kiwifruit (Ferguson, 2011). Commercial planting of 'Hort16A' began in the late 1990s and 'Hort16A' fruit now comprise around one quarter of New Zealand's kiwifruit exports (Anon., 2012). The green-fleshed 'Hayward' cultivar (*Actinidia deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson) still makes up the majority of all plantings in New Zealand. Unfortunately 'Hort16A' vines are particularly susceptible to the bacterial disease *Pseudomonas syringae* pv. *actinidiae* (Psa), and the future of the cultivar is uncertain (Anon., 2012). Nevertheless the work described in this thesis may also be of value to growers of new cultivars that are more tolerant to Psa.

A) B)

Figure 1.1. Canopy of A) 'Hort16A' and B) 'Hayward' kiwifruit vines. Photographs courtesy of Martin Heffer.

The challenge for New Zealand kiwifruit growers is to maintain a competitive edge by consistently improving fruit quality whilst still increasing yields. In 2003, for example, yields of 30 to 36 tons per hectare (t ha^{-1}) were reported for 'Hort16A' vines (Patterson et al., 2003), whereas today the best 'Hort16A' orchards can produce 60 t ha^{-1} (Patterson and Currie, 2011). The challenge for kiwifruit researchers in New Zealand is to develop and maintain an understanding of consumer acceptability and to

work with kiwifruit growers to consistently produce high yields of fruit that meet consumer expectations, of, for example size and taste. The majority of the research on kiwifruit productivity published in the past 30 years was carried out on 'Hayward' vines, which are less productive than 'Hort16A' vines, and before individual fruit DMC became an important quality parameter.

There are some concerns that recent increases in vine productivity, coupled with the focus on increased individual fruit DMC, could deplete vine reserves and ultimately damage the vines. This is particularly of concern to 'Hort16A' growers because their vines carry on average higher crop loads than 'Hayward' vines, and individual 'Hort16A' fruit have higher fresh weight (FW) and DMC than 'Hayward' vines (Table 1). Results from a large-scale survey carried out across 'Hayward' and 'Hort16A' orchards in New Zealand indicated that 'Hort16A' kiwifruit DMC was on average 1.6%-units higher than that of 'Hayward' (Mowat and Kay, 2007).

Table 1.1 Terminology commonly used to calculate kiwifruit productivity, and examples from high-producing 'Hort16A' and 'Hayward' orchards (adapted from Patterson and Currie, 2011).

	Per fruit		Per canopy m^2		Per canopy hectare	
Cultivar	FW (g)	DMC (%)	fruit	DW (kg)	~t.e.	t
'Hort16A'	125	18.5	65	1.50	18,000[a]	60
'Hayward'	110	17.0	40	0.74	12,000[b]	43

FW = fresh weight; DMC = dry matter concentration; DW = FW x DMC x no. fruit; t.e.= tray equivalents; [a]tray contains 30 fruit; [b]tray contains 33 fruit; t = tonnes.

Vine management systems used by kiwifruit growers to increase productivity often involve source-sink manipulations that result in carbohydrates generated by leaves (sources) being allocated to fruit, rather than competing sinks such as shoots and roots. Girdling, for example, involves severing the phloem so that carbohydrates are unable to travel from the canopy to the roots. Summer pruning and fruitlet thinning reduce the amount of competition between fruit and shoots, and among fruit for carbohydrates. If fruit increasingly become the primary sink for carbohydrates, then it is necessary to understand how other sinks, including the roots, will be affected. Both root function and root reserves status could be adversely affected if high yields of high DM fruit are produced year after year. In addition, the source-sink

manipulations are likely to affect within-plant allocation of other resources, such as mineral nutrients and plant hormones, not just carbohydrates. While plant hormones were not measured in the work described in this thesis, their role in balancing source-sink relationships cannot be ignored and will be discussed where particularly relevant.

The focus of the work described in this thesis is to determine how source-sink manipulations, designed to alter allocation of carbohydrates to fruit, and applied over several consecutive seasons, affect vine productivity. Key measures of vine productivity include the fruit quality attributes size, DMC, maturity, harvest criteria and storage performance and indicators of vines health measured by return bloom and canopy growth. In the remainder of this Introduction the key measures of vine health will be discussed, along with the treatments that will be applied to vines to alter source-sink relationships.

1.1 VINE PRODUCTIVITY

1.1.1 Fruit dry matter concentration

Consumer acceptance of kiwifruit is strongly influenced by sweetness and acid perception (Rossiter et al., 2000; McMath et al., 1992). Kiwifruit are harvested before they are ripe, and fruit DMC at harvest correlates well with ripe fruit soluble solids content (Jordan et al., 2000; Burdon et al., 2004) meaning that DMC is a fruit property that can be measured at, or before, harvest that will give an indication of consumer acceptability. The relationship between fruit DMC and consumer acceptability of ripe fruit has been confirmed in large-scale sensory experiments (Harker et al., 2009). To maintain the competitive edge for the ZESPRI brand, growers receive a financial premium for growing fruit with higher DMC (Patterson and Currie, 2011).

There is substantial scientific literature on the effects of different orchard management techniques on fruit quality and productivity across a range of perennial fruit crops, but very little of this literature includes measurement of fruit DMC as a quality attribute. Consequently a large amount of research has recently been carried out on developing kiwifruit vine management techniques that can consistently increase fruit DMC (Patterson and Currie, 2011). However, it is not known if vines

are able to consistently maintain high levels of productivity without eventual depletion of reserves.

To understand how resources are allocated within the vine, it is necessary to quantify allocation among different parts of the vine including among leaves and fruit and perennial reserves. It is also necessary to make sure that fruit composition is not being altered by long-term source-sink manipulation. The standard method for measuring kiwifruit DMC involves taking an equatorial slice from the fruit, typically 3-5 mm-thick and oven-drying to constant weight at 65 ºC (Snelgar et al., 2005; Feng et al., 2006). An alternative non-destructive method of measuring DMC uses visible-near infra red measurements made after the equipment has been calibrated and validated against DMC taken from equatorial slices (McGlone et al., 2007). Orchard productivity estimates using dry weight (DW) per m^2 are based on whole fruit FW multiplied by the DMC sampled from an equatorial slice (Patterson and Currie, 2011; Thorp et al., 2011). It is not known if DMC measured from an equatorial slice is a good estimator of whole fruit DM contents. The work carried out in this thesis is based on determining how whole vine resource allocation is affected by different vine management techniques. Any sub-samples taken from the vines need to accurately reflect vine composition. Results also need to be comparable to those made using standard industry sub-sampling methods. In Chapter 3, various methods of sampling fruit will be compared.

1.1.2 Harvest criteria for 'Hort16A' kiwifruit

Maturity indices are attributes that are used to determine if fruit are suitable for harvest and will be of acceptable eating quality to the consumer (Kader, 1999). The main commercial harvest for 'Hayward' kiwifruit occurs when the mean SSC (soluble solids concentration) reaches 6.2 % (Hopkirk et al., 1986). At this time fruit are still firm and they can be handled during the packing process without risk of damage (Donald, 1990; Mitchell et al., 1991). The main commercial harvest of 'Hort16A' occurs when flesh colour changes from green to yellow, when the mean flesh hue angle reaches 103^o. An alternative measurement system involves ranking 90 fruit, lowest to highest, according to their hue angle and main commercial harvest can occur if the 87th fruit has a hue angle of $\leq 107.5^o$ (Anon., 2009).

Sometimes 'Hort16A' fruit start to soften on the vine as they undergo colour change (Snelgar et al., 2005). If the fruit are softening rapidly as the flesh is degreening, they are vulnerable to damage during harvest and grading (Patterson et al., 2003). There is some evidence that 'Hort16A' fruit with higher DMC can have advanced maturity. Non-destructive testing of large batches of individual fruit after harvest using visible near infrared analysis could characterise a population of fruit with lower DMC, lower SSC and greener flesh colour (Clark et al., 2004). Fruit from vines treated to increase DMC had higher SSC, were softer and had lower hue angles than fruit from vines treated to reduce DMC (Boyd and Barnett, 2011). Treatments applied to girdled 'Hayward' laterals that increased fruit DMC, also increased SSC, and therefore harvest date, but did not affect firmness (Seager et al., 1995). It is not known how increased DMC will affect maturation and harvest date in 'Hort16A' and whether all maturity attributes (flesh colour, firmness and SSC) are affected to the same degree.

1.1.3 Fruit storage performance

To meet ZESPRI's requirement for year-round supply, fruit must be stored for several months until fruit grown in the northern hemisphere comes into production. Storage losses can occur because fruit soften rapidly, making them susceptible to damage during handling, fruit can also develop rots and disorders such as low temperature breakdown (LTB; Figure 1.2). Internal symptoms of LTB include a ring of granular, water-soaked tissue at the stylar (or beak) end of the fruit (Figure 1.2B). Small circular rots are often seen on the skin of 'Hort16A' fruit affected by LTB (Boyd and Barnett, 2011). In both cultivars less mature fruit are more susceptible to LTB during storage than more mature fruit (Lallu, 1997; Clark et al., 2004; Maguire et al., 2005; Stafiotakis et al., 2005; Boyd and Barnett, 2011). Preharvest Ca sprays increased 'Hayward' flesh Ca concentrations and reduced LTB incidence (Gerasopoulos and Drogoudi, 2005). Little information is available on Ca status and LTB in 'Hort16A' fruit, although Boyd and Barnett (2011) found no consistent relationship between LTB incidence and fruit Ca concentration.

The pattern of 'Hayward' fruit softening in coolstore involves an initial period of little or no softening (depending on firmness at harvest), followed by a period of rapid softening, then softening slows and sometimes increases again at the end of storage (MacRae et al., 1989). Similar patterns are observed for other kiwifruit species

including 'Hort16A' (White et al., 2005). Softening behaviour can be quantified by 1) measuring firmness after a set period in coolstore, 2) measuring days in coolstore to reach a firmness threshold, or 3) calculating the rate of softening during the most rapid phase.

A) B)

Figure 1.2 'Hort16A' kiwifruit stored at 1.5ºC for 20 weeks and affected by low temperature breakdown showing A) external rots and B) water-soaked tissue at the beak end of the fruit.

Maturity at harvest and fruit composition affect softening in 'Hayward' kiwifruit. Generally, advanced maturity, high Ca and low N (nitrogen) were most often associated with firmer fruit, although results were not consistent. The incidence of unacceptably soft 'Hayward' fruit after a set time in storage tended to be higher in lines of fruit with low DMC, Ca and P (phosphorus), and high N concentrations (Maguire and Mowat, 2003). Softening rate in coolstore was slower in batches of fruit harvested at advanced maturity (high ratio of SSC to DMC) and in those with high Ca/N ratios and high Mg (magnesium) concentrations (Feng et al., 2006). Sprays and dips that increased fruit Ca concentrations reduced the rate of fruit softening early in storage (Moras and Nicolas, 1987; Hopkirk et al., 1990; Gerasopoulos et al., 1996; Basiouny and Basiouny, 2000), although an increase in fruit Ca concentration as result of preharvest Ca sprays or postharvest Ca dips was not accompanied by a change in firmness after storage (Boyd et al., 2006), and the time to reach a set firmness (18 N) was increased by postharvest Ca dips despite fruit Ca concentrations being unaffected (Cooper et al., 2007).

Little work has been carried out on the factors that affect postharvest softening of 'Hort16A' fruit, although Boyd et al. (2006) found both preharvest Ca sprays and

postharvest Ca dips increased fruit Ca concentrations without affecting firmness after a set time in coolstore. There is evidence that 'Hort16A' fruit with higher DMC have advanced maturity (Clark et al., 2004; Boyd and Barnett, 2011), but the effect of fruit maturity on softening behaviour in storage has not been fully explored. In 'Hayward' fruit, advanced maturity was associated with reduced softening in storage, but this may not be the case with 'Hort16A' fruit where the fruit softening and SSC accumulation are more advanced at commercial harvest than 'Hayward' fruit. There are concerns from some kiwifruit growers that fruit from girdled vines stores less well than fruit from intact vines (Currie et al., 2007), and this may be a consequence of the advanced maturity of girdled fruit. It is not known if practices that increase fruit DMC do so at the expense of carbohydrate allocation to the roots, and whether this could adversely affect mineral nutrient uptake and allocation to fruit, with possible adverse consequences for fruit storage performance.

1.1.4 Flowering and yield

Most of the research on bud break and kiwifruit has been carried out on 'Hayward' vines, but similar patterns have been observed for 'Hort16A' vines (Richardson and Walton, 2007). 'Hort16A' vines have higher natural bud break than 'Hayward' vines. For example 'Hayward' and 'Hort16A' growing at the same site had final bud break values of 46.3 ± 2.8 % and 56.0 ± 1.9 %, respectively (Snowball, 1997). Conventional 'Hayward' and 'Hort16A' orchards are usually treated with Hi-Cane® (hydrogen cyanamide, H_2CN_2) to help overcome a lack of winter chilling. Research has shown that vines that experience colder winters usually break bud earlier and over a shorter time frame than vines in regions where winter temperatures are warmer. Shoots that develop from buds that break first are more likely to produce flowers than buds that open later (Grant and Ryugo, 1982). The shorter the spread in bud-break the more flowers produced per winter bud (McPherson et al., 1994).

Manipulations carried out on 'Hayward' vines in one season can reduce flower numbers (return bloom) in the following season. Reduced return bloom has been reported as a result of defoliation (Buwalda and Smith, 1990; Cooper and Marshall, 1991; Cruz-Castillo et al., 2010), high crop loads (Burge et al., 1987), or excessive shading (Grant and Ryugo, 1984; Buwalda and Meekings, 1993).

Most female kiwifruit flowers develop into fruit. Linsley-Noakes (1989) reported that all pollinated 'Hayward' flowers set fruit. Similar data are not available for 'Hort16A' flowers. In most seasons, 'Hort16A' flowers need to be thinned to approximately 60 to 70 flowers per m^2 to maintain a fruit size profile that meets market requirements (Patterson and Currie, 2011). Quantification of bud break and flower numbers is not a measure of fruit yield, more an indicator of potential yield. Yield and crop load are usually set by the grower each season, by thinning flowers or fruitlets, except in seasons when flower numbers are lower than the required density.

'Hort16A' vines are able to carry higher crop loads of fruit with higher FW than 'Hayward' vines. In addition to producing more flowers than 'Hayward' vines, 'Hort16A' show a marked size response to the biostimulant Benefit®, which has enabled FW of 'Hort16A' fruit to increase from around 95 g to 125 g whilst still maintaining high DMC. Benefit® application has not been successful in increasing FW in 'Hayward' fruit (Patterson and Currie, 2011).

The combination of higher crop loads, larger FW and higher DMC of 'Hort16A' kiwifruit means that around 50 % more dry weight per m^2 of canopy is removed from 'Hort16A' vines than 'Hayward' vines as fruit each year (Table 1.1). This may mean that 'Hort16A' vines are more susceptible to reserve depletion if, on top of their naturally higher productivity, techniques such as Benefit® application and girdling are regularly applied to increase vine productivity.

The orchard management techniques used in this thesis to influence within-vine source-sink relationships, and potentially fruit DMC, will be discussed in the following section. Relatively little information has been published on 'Hort16A' vine productivity, therefore the research described below relates to 'Hayward' vines, unless specifically stated. The primary aim of these orchard management techniques is to alter allocation of non-structural carbohydrates (NSC) to fruit relative to other sinks, however other plant resources such as water and mineral nutrients are also likely to be affected, hence the generic term 'resource' is sometimes used.

1.2 SOURCE-SINK MANIPULATIONS

1.2.1 Summer pruning and crop load adjustment

The kiwifruit vine is an aggressively-growing plant that requires careful canopy management (Ferguson, 1990; Figure 1.3). The balance between vegetative growth and fruit production can be seen in terms of competition; as vigorous shoot growth competes with growing fruit for resources (Greer et al., 2003), as does regrowth generated from pruning cuts (Minchin et al., 2010). If the canopy is too dense, fruit quality and return bloom can be adversely affected (Grant and Ryugo, 1984; Davison 1990; Snelgar et al., 1998). Fruit grown in more shaded positions within the canopy or in denser canopies are generally smaller (Grant and Ryugo, 1984) with lower DMC (Snelgar et al., 1998), and soften in coolstore more rapidly than those grown in less shaded environments (Snelgar et al., 1998; Cooper et al., 2007). Increased summer pruning and a more open canopy improves the microclimate around fruit and can reduce the incidence of 'Hayward' storage rots caused by *Botrytis cinerea* (Michailides and Elmer, 2000; Miller et al., 2001). 'Hayward' replacement canes (shoots that grow in spring and are tied down in winter to form part of the canopy framework) that grew in shaded environments tended to have smaller basal diameters, lower DMC, higher winter bud mortality and less flowers per inflorescence than replacement canes that grew in more exposed environments (Grant and Ryugo, 1984).

Figure 1.3 'Hort16A' canopy photographed in December 2007, about 2 months after bud break showing the vigorous growth of long shoots.

During summer pruning it is important to optimise the balance between excessive vigour and maintaining sufficient leaf area to support fruit production. In 'Hayward' vines, excessive pruning limited fruit FW and reduced return bloom in the subsequent season, presumably because reserves were utilised for current season's fruit growth (Buwalda and Smith, 1990). A leaf-to-fruit ratio of 3:1 on fruiting canes was required to produce acceptably-sized kiwifruit at a cropload of 40 fruit per m^2 (Cooper and Marshall, 1991). Snelgar and Thorp (1988) reported that 'Hayward' kiwifruit FW increased at a rate of 5 to 6 g for every additional 100 cm^2 of leaf area, although very high leaf areas could lead to a reduction in FW (Snelgar and Martin, 1997). With a crop load of 30 fruit per m^2, altering the leaf-to-fruit ratio from 3:1 to 2:1 did not affect return bloom, however a further reduction in the ratio to 1:1 reduced return bloom from over 70 % floral shoots to 21 % in the following year (Cooper and Marshall, 1991).

'Hort16A' vines produce more fruit and are more vigorous than 'Hayward' vines, and extension of long shoots continues later into summer than is typical of 'Hayward' vines (Patterson et al., 2003). Although there are clear differences between 'Hayward' and 'Hort16A' canopies, little is known about the effect of canopy density on fruit quality in 'Hort16A'.

Altered leaf-to-fruit ratios. The effects of induced carbon starvation have been studied in a range of long-lived perennial plants. In forest ecosystems, carbon starvation is usually induced by repeated defoliation (Valladares et al., 2007; Anderegg and Callaway, 2012), and plants typically respond by reallocating resources toward carbohydrate production. For example, shoot growth increases, at the expense of root or reproductive growth (Karlsson and Weih, 2003; Siham et al., 2005; Stevens et al., 2008). In addition, leaves from defoliated trees often have increased specific leaf area (SLA; area/weight ratio; Meyer 1998), higher photosynthetic rates (Nykänen and Koricheva, 2004), higher N concentrations (Médiène et al., 2002) and delayed senescence (Meyer, 1998) relative to leaves from intact trees (Siham et al., 2005; Valladares et al., 2007).

It is not known if repeatedly carrying high crop loads of high DMC fruit in 'Hort16A' vines will eventually lead to C starvation, and if it does, then how the vines would respond. Defoliation reduced the growth of new roots and advanced leaf senescence in partially defoliated 'Hayward' vines (Buwalda and Smith, 1990). In single shoot experiments, leaf removal did not affect the photosynthesis rates of the remaining leaves (Lai et al., 1989). If leaf photosynthesis does not increase and leaf senescence is advanced in defoliated kiwifruit vines, then resource allocation to reserves and reproduction will be adversely affected by C starvation. Defoliation and high crop loads have adversely affected return bloom in 'Hayward' vines (Burge et al., 1987; Buwalda and Smith, 1990; Cooper and Marshall, 1991; Cruz-Castillo et al., 2010), but the effects on reserve status and mineral nutrient uptake and allocation are not well understood. Apart from reduced crop loads, deficiencies of some mineral nutrients might occur if vines are repeatedly over-cropped. It is important to learn how growers can mitigate against poor root function and nutrient deficiencies, if indeed these do occur as a result of carbohydrate depletion.

1.2.2 Trunk girdling

The practice of severing the phloem, by removing of a strip of bark from the trunk or branches without damaging the underlying tissues, is known as girdling. Related techniques involve severing the phloem with a knife cut and are known as scoring, ringing or cincturing (Noel, 1970). These techniques are used in horticulture to improve fruit set, increase fruit size and advance fruit maturity (Goren et al., 2004). Girdling disrupts the transport of assimilates from the canopy to the roots, resulting in NSC accumulation above the girdle and decreased NSC in the roots when the girdle remains open (Roper and Williams, 1989). Most plant responses to girdling are believed to be caused by increased availability of carbohydrates to developing fruit above the girdle, although other changes brought about by girdling include reduced vigour and hence reduction in competition from vegetative sinks, changes in endogenous hormone levels (Cutting and Lyne, 1993) and changes in gene-expression (Li et al., 2003).

Kiwifruit berries have two periods of rapid growth (Figure 1.4), the first occurs during the period of rapid cell division and the second whilst cell enlargement occurs (Hopping, 1976). The time of girdling relative to fruit growth affects the response.

For example girdling whilst cell division is still occurring is used to increase FW in kiwifruit (Patterson and Currie, 2011) and other fruit crops such grapes (Harrell and Williams 1987a). It is thought that the NSC that accumulates above the girdle provides extra energy for cell division, but it is also possible that girdling affects plant hormone levels by interrupting the supply of sugar or auxin to the roots and affecting the production of root-derived hormones (Goren et al., 2004). Trunk girdling and gibberellic acid (GA) application both increased fruit size in grape vines, although it is not clear if the size response to girdling was caused by an increase in endogenous GA concentration in the canopy (Harrell and Williams 1987a). An increase of GA-like substances in tissues above the girdle has been reported (Wallerstein et al., 1973).

Trunk-girdling in late summer during cell enlargement is used to increase fruit DMC in both 'Hort16A' and 'Hayward' vines (Boyd and Barnett, 2011; Patterson and Currie, 2011). The increase in DMC is caused by an increase in DW accumulation relative to FW accumulation. This increase in DW with little associated increase in FW is believed to occur because assimilates are still being produced but are unable to travel to the roots, therefore accumulate in the fruit. Girdling at this time in apple (Wargo et al., 2004), peaches and nectarines (Agusti et al., 1998) advanced maturity, enabling earlier commercial harvest. The accumulation of NSC above the girdle is believed to provide additional energy for fruit maturation and ripening processes (Seager et al., 1995).

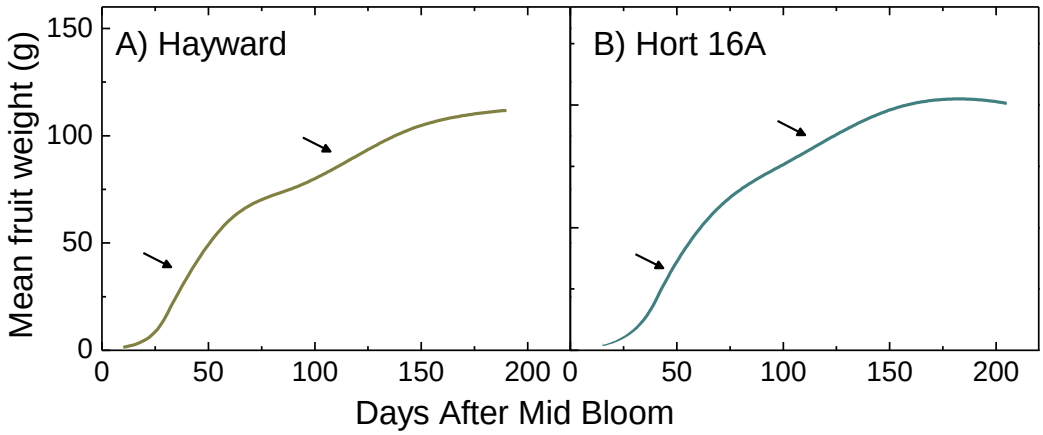

Figure 1.4 Growth curve from A) 'Hayward' and B) 'Hort16A' kiwifruit showing the double-sigmoidal pattern of fresh weight accumulation. The two periods of rapid growth are illustrated with arrows. Used with permission from Hall et al., 2002.

Girdling can have positive effects for fruit growers, but the technique is only sustainable if it does not adversely affect other sinks such as shoots and roots. As girdling becomes part of the annual vine management strategy for kiwifruit growers, it is necessary to gain more understand of what the consequences could be for the whole plant. If resources are allocated to fruit at the expense of roots and shoots then it is possible that leaf and root function will be adversely affected if transport of resources from canopy to roots is continually interrupted.

Plant responses to girdling. Many of the documented responses to girdling are the opposite of the typical plant responses to C starvation described in Section 1.2.1. Instead of increased shoot growth, girdling is sometimes accompanied by reduced shoot growth (Goren et al., 2004) which is seen in a range of crops including peach (Dann et al., 1984), and apple (Pretorius et al., 2002). Girdling resulted in reduced leaf photosynthesis in, for example, kiwifruit (Black et al., 2012) and grape (Roper and Williams, 1989; Harrell and Williams, 1987b). Leaves from girdled peach branches had lower SLA, smaller LA and senesced and abscised sooner than leaves on intact branches (Dann et al., 1984). Similar results have been found in other fruit crops such as mango where leaf starch concentrations increased, photosynthesis rates decreased, nitrogen status, measured on a DW concentration and leaf area basis, decreased in leaves from girdled mango branches compared with leaves on intact control branches (Urban and Alphonsout, 2007). The above results all suggest that whole-plant assimilate production is likely to be reduced in girdled plants: less shoot growth, smaller individual leaf area, reduced leaf photosynthesis and advanced leaf senescence are all likely to reduce plant carbohydrate status.

Less information is available on the effect of girdling on root growth and function than it is on shoots. Girdling stops carbohydrate transport to the roots during the time that the girdle is open (Roper and Williams, 1989). The consequences of this may vary depending on total root reserves, the length of time that the girdle is open, and the time of girdling relative to fruit and shoot growth. For example girdled pine trees had sufficient reserves to avoid immediate root mortality (Högberg et al., 2002). Root elongation ceased for two weeks after girdling in grape-vines (Yamane and Shibayama, 2006). Consequences of slowed or reduced root growth could include

reduced production of root-synthesised hormones such as cytokinins (Havelange et al., 2000), although it is also possible that girdling affects the production of root-derived hormones because auxin transport from shoots to roots is interrupted (Lockard and Schneider, 1981).

Mixed results have been reported on the effect of girdling on plant nutrient status. Bangerth (2008) reported that concentrations of Ca, K, Mg and P were lower in xylem exudates sampled from trunk-girdled apple trees 18 days after girdle application compared with ungirdled control trees sampled on the same day. The author suggested that reduced assimilate supply to the roots could be responsible for the decreased nutrient uptake. Trunk-girdling decreased apple fruit Ca concentrations (Arakawa et al., 1997). Wargo et al. (2004) saw no effect of mid-summer trunk-girdling on apple leaf or fruit N concentrations. Trunk-girdling of young peach trees resulted in lower N and Ca contents in the plant parts above the girdle and higher N and Ca contents in the parts below the girdle, relative to the ungirdled control trees. Whole plant P, Mg and K contents were lower in the trunk-girdled peach trees than ungirdled controls (Sharif Hossain et al., 2004). Ringed sour orange seedlings had lower concentrations of N, P and K in roots and leaves sampled a month after ringing compared to roots and leaves of ungirdled plants (Wallerstein et al., 1978).

Without knowing the total weight and DMC of different plant parts it is difficult to make conclusions about the effect of girdling on nutrient uptake and distribution from concentration values alone. If girdling increases fruit DMC, for example, then lower fruit Ca concentrations might be reported, but as a consequence of dilution from the higher fruit DMC, and not reduced Ca uptake by the plant. If nutrient uptake is affected by girdling, it is not known if some nutrients would be affected more than others. It is possible that severing the phloem connection between the roots and canopy would not affect the transport of nutrients such as calcium that travel predominantly in the xylem (White, 2001). In the young peach trees described above (Sharif Hossain et al., 2004), the within-plant distribution of Ca and N was affected by the girdle, whereas girdling reduced total uptake, but not within-plant distribution of P, Mg and K.

The adoption of trunk girdling in kiwifruit orchards raises several questions. In particular, what are the consequences of trunk girdling vines each year for overall vine health and performance, and does girdling affect fruit composition, maturity and storage performance. Little information is available on how girdling affects fruit storage performance. Results from crops such as apple are inconsistent. Wargo et al. (2004) found that trunk-girdling did not affect the incidence of apple storage disorders. Conversely, Elfving et al. (1991) found that apples from scored trees had better firmness retention after storage and reduced incidence of the storage disorder brown core relative to fruit from ungirdled trees. It is not known how annual trunk girdling affects nutrient uptake and allocation within the plant; which mineral nutrients will be most affected and what symptoms might develop over time.

To help answer these questions one experiment described in this thesis involved carrying out repeated extended trunk girdling for several years in an attempt to induce or exacerbate symptoms of vine decline.

1.2.3 Modified nitrogen input

Many long-term (3 or more seasons) field-trials have been carried out on 'Hayward' kiwifruit vines, looking particularly at the effect of insufficient N on FW, crop loads and return bloom. Nitrogen input rates typically ranged from 0 to 200 kg N ha^{-1} (Buwalda et al., 1990), 0 to 450 kg N ha^{-1} (Tagliavini et al., 1995, Costa et al., 1997; Johnson et al., 1997; Vizzotto et al., 1999), or 0 to 750 kg N ha^{-1} (Buwalda and Meekings, 1993). The main findings from these experiments were that the vines receiving no added N often had reduced yields, either based on fruit number, fruit FW or a combination of the two, relative to vines that received fertiliser N. Fruit DMC was not measured in any experiments and fruit maturity at harvest (SSC and firmness) was either not measured or not affected by N input. Fruit storage performance, if affected by N input, tended to be poorer with higher N input. For example, Johnson et al. (1997) reported that firmness after a set period in storage was negatively correlated with N input, and fruit receiving higher N input softened sooner in coolstore than fruit receiving lower N inputs (Vizzotto et al., 1999). Prasad and Spiers (1991) also found a negative association between fruit or leaf N status and time to soften to a specific firmness in storage.

Plant responses to altered nitrogen input. Across a large range of plants, increased N input is associated with an increase in above-ground growth relative to root growth (Hermans et al., 2006; Xia and Wan, 2008). In 'Hayward' vines, increased N input increased individual shoot length (Costa et al., 1997; Buwalda and Meekings, 1993), although vigour responses were not detected in all N inputs trials (Tagliavini et al., 1995). Low plant N status is often associated with a reduction of plant photosynthetic rates. High carbohydrate levels in the leaves repress photosynthesis and the N contained in the photosynthesis enzyme Rubisco is released (Paul and Driscoll, 1997). In addition to enhanced shoot growth, increasing N input resulted in larger individual leaf area and increased leaf photosynthetic rates in 'Hayward' vines (Costa et al., 1997; Buwalda and Meekings, 1993). It is possible that any effects of N input on kiwifruit quality are related to the canopy growth response. Insufficient N can limit fruit FW accumulation and reduce fruit numbers in 'Hayward' vines (Buwalda et al., 1990; Buwalda and Meekings, 1993). Addition of N results in increased shoot growth, increased individual leaf photosynthetic rates and therefore increased assimilate production, resulting in increased fruit FW. If the canopy becomes too dense then it is possible that fruit storage performance could be affected.

Little work has been carried out on the effect of N fertiliser on 'Hort16A' fruit. In one published experiment, increased N input tended to result in lower fruit DMC and delayed fruit maturation, based on colour change (Mills et al., 2008). High N inputs are associated with delayed fruit maturation in a range of fruit crops including grapes (Christensen et al., 1994) and apples (Neilsen et al., 1984). Little information is available on the effect of N on fruit DMC, although Saenz et al. (1997) found that withholding N fertiliser from peach trees increased fruit DMC from 15.2 % to 16.3 % and advanced commercial maturity, measured by flesh pressure and background colour, by around 10 days relative to fruit from trees that received 200 kg N ha^{-1}.

There may be potential to modify N input to affect source-sink relationships within 'Hort16A' kiwifruit vines, possibly improving fruit quality. Reduced N inputs could minimise pruning costs by reducing canopy vigour, although too much vigour reduction could adversely affect fruit quality and return bloom in the following season. Advanced fruit maturity would enable fruit to be harvested earlier,

minimising the time that fruit are exposed to potential hail and frost damage. There is a need to gain better understanding of how N input affects the various aspects of kiwifruit productivity: yield, fruit quality and DMC, harvest criteria, storage performance and vigour management and how growers could optimise N nutrition.

1.3 PROBLEM STATEMENT AND OBJECTIVES

The information summarised above raises a number of questions about the effects of different orchard management techniques on fruit quality and long-term vine health and productivity. If orchardists are to adopt these techniques with confidence then a better understanding of how the vines will respond to increased productivity is required.

The specific focus of the research described in this thesis is to investigate how orchard management practices, designed to alter source-sink relationships within mature field-grown 'Hort16A' kiwifruit vines, affect fruit maturity, composition and storage potential, and to determine if these practices are sustainable in terms of continued productivity and vine health.

The overall objective of this programme is:
To understand how manipulating whole vine source-sink relationships affects fruit quality and long-term vine health and productivity.
A range of orchard management techniques that influence source-sink relationships, within kiwifruit vines were investigated using mature field-grown 'Hort16A' kiwifruit vines. Specific research questions addressed in each of the experimental chapters are:

- What is the best way to subsample fruit so that results can be compared with results obtained using a range of different fruit sub-sampling methods? (Chapter 3).
- How does the vine respond to whole-vine carbohydrate depletion caused by excessive pruning and carrying high crop loads? (Chapter 4).
- How does the vine respond to extended trunk girdling that isolates the roots from the canopy for several months of the year? (Chapter 5).
- How do changes in canopy pruning affect fruit quality and return bloom in vines of different vigour generated by different nitrogen inputs? (Chapter 6).

The main outcomes of the programme will be: a better understanding of 'Hort16A' vine responses to modified leaf-to fruit ratios, summer trunk-girdling, pruning and N nutrition. Such information will enable growers to make more informed decisions about adopting and continued use of these techniques.

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2 GENERAL MATERIALS AND METHODS

Methods described in this chapter are common to more than one of the experiments reported in this thesis.

2.1 VINES

2.1.1 Plant material Field work was carried out on mature 'Hort16A' kiwifruit vines growing in Te Puke in the Bay of Plenty (37° 48 'S, 176° 19 'E) and trained on a pergola growing system. Vine age and orchard practices are described in the relevant chapters.

2.1.2 Canopy management techniques The canopy can be divided into three zones, each of which is managed differently (Figure 2.1).

- The leader zone (generally around 40 cm either side of the central leader) is where the vigorous replacement canes are retained for next season.
- The edge zone is usually pruned to give a small gap between the end of the female canopy and the adjacent male vines.
- The remaining region, which carries most of the crop, is known as the fruiting zone.

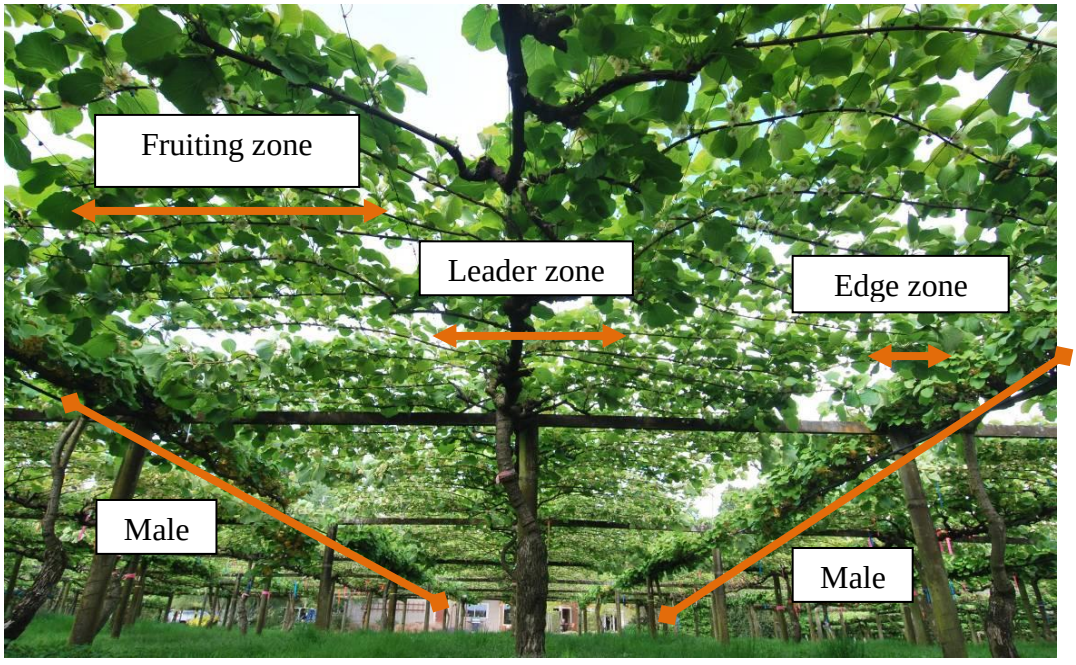


Figure 2.1 'Hort16A' vine canopy showing the leader zone, fruiting zone and edge zones and the male vines which occupy a small proportion of the canopy area.

The example illustrated in Figure 2.1 is from an orchard where male vines grow in alternate rows with female vines, a system known as strip male, the alternative system is known as opposing female where approximately every seventh vine in a row is a male vine (Thorp et al., 2011).

The pruning techniques used in Chapters 4 to 6 were:

Standard pruning. A fruiting shoot is cut back to 4 to 6 leaves after the last fruit. This technique is becoming less common now as the pruning cut generates a new growing point, which can compete with the fruit for resources (Minchin et al., 2010; Patterson and Currie, 2011), and has been replaced with some of the techniques discussed below.

Zero-leaf pruning. A technique used to control vegetative growth that involves cutting a vigorous shoot directly above the last fruit so there are no axillary buds, and hence no regrowth (Gardiner et al., 2005; Figure 2.2).

A) B)

C)

Figure 2.2 A) A vigorous fruiting shoot is cut back directly above the last fruit, B) a healed zero-leafed shoot, showing the lack of regrowth, and C) regrowth from an incorrectly zero-leafed shoot.

Blind shoot removal. The entire shoot is removed, usually by hand. This technique is used to eliminate vigorous vegetative shoots, also known as 'blind shoots' that could compete with fruiting shoots for resources.

Leader pruning. All vigorous vegetative shoots close to the central leader (Figure 2.1) are removed throughout the growing season. This technique is used to reduce vigour and promote the development of less vigorous replacement canes to be tied down in the following season (Miller et al., 2001).

Stubbing. A vigorous shoot is cut back to a leaf 1-5 cm from the base of the shoot, in the leader zone. In this technique a growing point is retained whereas in zero-leaf pruning, or blind shoot removal, no growing point is retained. Stubbing can be used to retain a growing point in the leader zone that could be used as a replacement cane later in the season if necessary (Figure 2.2C).

Tip-squeezing. The growing tip of a vigorous shoot is gently crushed so that growth stops, but not so hard that secondary shoots are activated (Figure 2.3B). This technique enables extra leaf area to be retained in the canopy but without vigorous growth or generation of regrowth.

A) B)

Figure 2.3 A) Regrowth from a stubbed (cut) shoot, and B) the tip of a vigorous shoot is squeezed so as to stop further growth but without generating new regrowths.

2.1.3 Components of yield Components of yield is a measure of a vine's potential productivity before any crop load adjustments are made. It is not the same as final yield or productivity of mature fruit, as defined in Table 1.1. Shortly after flowering, four canes per vine were selected and the following components were counted: total buds, buds that broke and commenced growth in spring forming shoots, the number of shoots that were floral and the number that were vegetative, the number of flowers (separated into king flowers and lateral flowers; Figure 2.4). From these data the following were calculated: percent bud break (BB), percent floral bud break (FBB), percent vegetative bud break (VBB), where BB = FBB + VBB; king flowers per floral shoot (F/FS) and king flowers per winter bud (KF/Bud).

Figure 2.4 Fruitlets originating from the central king flower (black arrow) and two lateral or side flowers (red arrows). Lateral flowers are often removed as they tend to produce smaller fruit than king flowers.

2.1.4 Flowering date Flowering date was measured using the techniques described in Boyd et al. (2008). Regions of each vine canopy, measuring approximately 1.2 m x 1.6 m, were marked with flagging tape and photographed from beneath at intervals of 2 to 4 days during October when flowering occurred (Figure 2.5). Each date, the number of flowers that had opened (petals had opened to allow bee entry, approximately 5 mm opening) was counted from the photograph.

When all flowers had opened, the proportion of flowers open at each date was calculated. A sigmoid curve was fitted to data from each vine (Figure 2.6) and solved for the date (in days after 30 September) when 10 %, 50 % and 90 % of flowers were open. The following definitions were used:

- start of flowering = date when 10 % of flowers were open
- mid bloom = date when 50 % of flowers were open

- end of flowering = date when 90 % of flowers were open
- duration of flowering = end - start (in days).

The effect of different treatments on start of flowering, mid-bloom and duration of flowering was determined using analysis of variance.

A) B)

Figure 2.5 The same part of a 'Hort16A' kiwifruit canopy photographed on A) 1 October, and B) 15 October 2008 when 0 % and 45 % of flowers were open respectively.

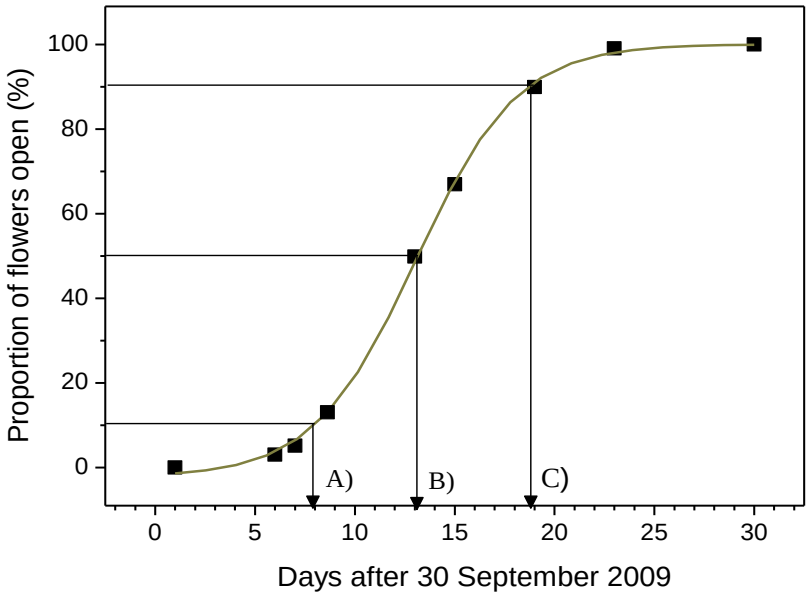


Figure 2.6 A sigmoid curve fitted through flowering data from an individual 'Hort16A' kiwifruit vine, illustrating the days when A) 10 %, B) 50 % and C) 90 % of flowers had opened.

2.1.5 Canopy development and architecture The number of leaves and fruit in each 1.2 m x 1.6 m region of canopy was counted at approximately monthly intervals from flowering (October) through to fruit harvest (May). The number, length and type of shoots in each 1.2 m x 1.6 m region was recorded in late November through until early January using methods described by Seleznyova et al. (2002). Briefly, each shoot was characterised as short, medium or long, where short shoots were terminated with ≤ 9 nodes, medium shoots were terminated with 10 - 18 nodes and long shoots were non-terminated with > 18 nodes. Shoot length and number of internodes was determined on a subset of 1 - 3 short and medium shoots per vine, enabling mean internode length to be determined.

- **2.1.6 Leaf sampling** Mature leaves and petioles (8 - 12 per vine) were sampled from fruiting shoots (1 - 3 leaves past the last fruit) approximately 1 m to 1.5 m from the central leader. Leaves and petioles were separated and placed in separate polyethylene bags. An 18 mm diameter cork borer was used to take samples from each leaf; these were immediately cooled in liquid nitrogen then held at -80ºC until analysis for carbohydrates and chlorophyll. Individual leaf blade area (LA) was determined using a LA meter (LI 3100C, Li-Cor Inc., Lincoln, NE, USA) and petiole length was measured. Leaf and petiole fresh weight (FW) and dry weight (DW) were determined after drying to constant weight at 65 °C. For the leaf blades LA, FW and DW were corrected for the area removed in the sampling process. Leaf area index (LAI) was calculated from vine leaf counts and individual leaf area. LAI data for Chapter 6 was calculated from hemispherical photographs taken from ground level under each vine in April 2007.
- Specific leaf weight (SLW) was calculated where SLW = dry weight per unit area (mg cm^{-2}). [Note that SLW is also referred to as leaf mass per area [LMA] (Poorter et al 2009) but SLW was the preferred term used in this thesis]. Oven-dried leaves and petioles were ground in a Fritsch grinder before being analysed for inorganic nutrients.

2.1.7 Leaf gas exchange measurements Leaf net carbon exchange rate (NCER) was measured using a portable photosynthesis system (LI 6400 Li-Cor, Inc., Lincoln, NE,

USA). Measurements were taken at approximately monthly intervals from November to April on sunny days between 11am and 2pm. Measurements were made of fully-expanded leaves on fruiting shoots located 50 - 80 cm from the central leader. Three measurements were made on each vine before moving onto the adjacent vine.

2.1.8 Fruit sampling A random sample of 8 - 18 fruit per vine was sampled from across the entire fruiting canopy at regular intervals, depending on the particular experiment. Fruit FW was determined, then a longitudinal quarter of each fruit was taken and combined to give one bulked sample per vine. The quarters were weighed then placed into liquid nitrogen, and stored at -80 ºC. The samples were freeze-dried, reweighed and ground before sub-samples were taken.

2.2 ANALYSIS

2.2.1 Fruit quality at harvest When fruit were approaching commercial harvest (April - May), 12 fruit per vine were sampled at regular intervals (3 - 10 days). Fruit quality was assessed by measuring fresh weight (FW), dry matter concentration (DMC; DW as a percentage of FW), soluble solids concentration (SSC), flesh firmness and flesh hue angle: DMC was determined on a 3-mm equatorial slice taken from each fruit which was oven-dried at 65°C for 24 h; SSC was measured with a refractometer (Atago Co. Ltd., Tokyo, Japan) using two drops of juice squeezed from the stem and stylar ends of each fruit, combined to give one value per fruit; flesh firmness was measured at the fruit equator on the flat and rounded sides of each fruit using an Effegi penetrometer (Facchini, Alfonsine, Italy) with a 7.9 - mm probe after a 1-mm slice of skin had been removed; flesh hue angle was measured using a Minolta chromameter (Minolta, Ramsey, NJ, USA) using a D65 light source after a 2-mm layer of skin and flesh had been removed from the equator of the fruit.

2.2.2 Fruit storage quality At each harvest date a random sample 120 fruit from each vine was picked directly into 30-count single-layer trays lined with a polyliner. Fruit were transported to Auckland by refrigerated courier as soon as practicable after harvest and were stored at 1.5 ºC. Fruit softening during storage was determined by measuring firmness on 3 fruit per vine at 5 to 28 day intervals depending on stage of softening. Before firmness was measured, fruit were held at 20 ºC for 24 hours. Fruit

were assessed after 18 to 22 weeks in storage by recording the presence or absence of rots and storage disorders on each fruit.

2.2.3 Analysis of mineral nutrients Samples were sent to RJ Hill Laboratories, a commercial laboratory where they were analysed for Ca, Mg, K, P, S, Fe, Mn, Zn, Cu and B using nitric/perchloric acid digestion followed by inductively coupled plasma - optical emission spectroscopy (ICP - OES; Integra XL, GBC, Hampshire IL, USA). Nitrogen was analysed using combustion analysis (vario MAX CN Macro Elemental Analyser, Elementar, Hanau, Germany).

Leaf mineral nutrient concentrations were compared with the 'normal' range provided by RJ Hill Laboratories in their Crop Guide which is based on their data for 'Hort16A'. No recommended leaf mineral nutrient ranges have been published for 'Hort16A' kiwifruit leaves, therefore comparison with the normal range gives an indication if any results are exceptionally low.

2.2.4 Analysis of carbohydrates Adonitol was added to each freeze dried, ground, tissue sub-sample as an internal standard. The sample (~ 50 mg) was extracted using 80 % ethanol for 1 h at 60 ºC. Samples were centrifuged and the supernatant decanted off. The residue was re-suspended in 80 % ethanol re-spun and supernatants combined and used for sugar analysis. The insoluble residue was analysed for starch using the method described by Smith et al. (1992). Briefly, the residue was washed into a conical flask and autoclaved for 2 h. The solution was then incubated with amyloglucosidase (Sigma chemicals) in actetate buffer (pH 4.5) for 1 h. Glucose was measured colourimetrically using a coupled glucose oxidase/peroxidase reaction (Trinder, 1969) that measured the peroxide released as the glucose was oxidised.

Soluble sugars were determined on a sub-sample of the 80 % ethanol supernatant obtained during the starch extractions. The sub-sample was dried using a stream of nitrogen gas and dissolved in ultrapure water. Sugars were analysed by high - performance anion - exchange chromatography coupled to an ECD detector using a Dionex IC-3000 Reagent-Free^{TM} IC system (Dionex Corp., Sunnyvale, CA, USA) equipped with a CarboPacPA-20 column and an amino trap guard column. The

eluent was KOH solution at a flow rate of 0.5 mL min^{-1} starting at 9 mM KOH increasing to 40 mM followed by a 100 mM column wash. Peaks were identified by co-elution with known standards of fructose, galactose, glucose, *myo*-inositol, raffinose, stachyose, and sucrose.

2.2.5 Analysis of chlorophyll Duplicate freeze-dried, ground samples (~25 mg) were extracted in dimethylsulphoxide (DMSO: 5 mL) at 65ºC for 30 min. as described by Richardson et al. (2002). Extracts were washed, filtered and made to 10 mL. Absorbance was measured at 645 nm (A_{645}) and 663 nm (A_{663}) using a visible spectrophotometer (Novaspec III, Biochrom Ltd, Cambridge, England). Chlorophyll (Chl) concentration was calculated using Arnon's (1949) equations:

$$\text{Chl}a\ (\text{g L}^{-1}) = 0.0127A_{663} - 0.00269A_{645}$$

$$\text{Chl}b\ (\text{g L}^{-1}) = 0.0229A_{645} - 0.00468A_{663}$$

$$\text{Total Chl} = 0.0202A_{645} + 0.00802A_{663}$$

Results were converted to mg g^{-1} DW or µg cm^{-2}.

2.2.6 Canopy and whole-vine biomass All material from summer pruning and fruitlet thinning (generally carried out in November and January), winter pruning (July) and leaf fall (April to June) was collected from each vine and a dry weight was obtained. At the end of the 2009/2010 season entire vines were excavated using the methods described by Clark and Smith, 1992 and Boyd et al., 2010. Each vine was dismantled and separated into its component parts (Figure 2.7):

Annual canopy growth - fruit, live leaves, senesced leaves (collected from within and beneath the canopy), and shoots.

Perennial framework - canes, leader, trunk and crown.

Roots - fibrous roots (< 2 mm diameter) and structural roots.

When comparing treatments vines were typically divided into annual canopy growth and perennial reserves: the perennial frame work and the roots. Woody samples from shoots, canes, leader, trunk, crown and structural roots were passed through a chipper before subsampling. Total FW of each component was measured and a subsample of 1-5 kg was taken for DW determination. An additional subsample of ~ 200 g was placed immediately into liquid nitrogen, and held at -80ºC until analysis for minerals

and carbohydrates. Vine excavation was carried out with assistance from staff from the Te Puke Research Orchard.

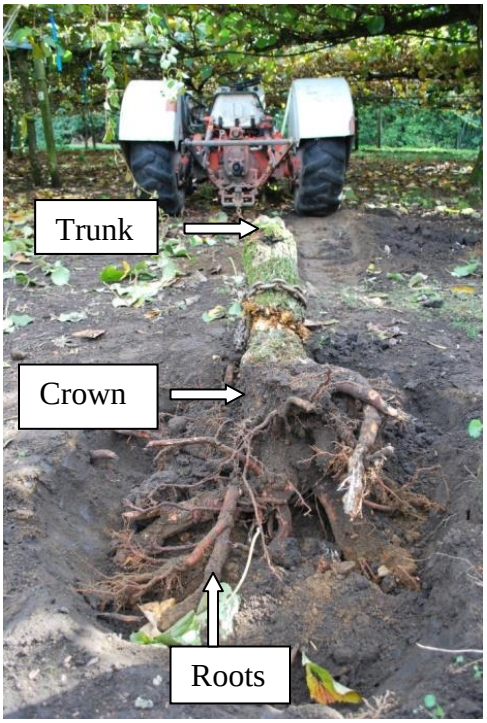
2.2.7 Data analysis Analysis of variance was carried out using GenStat Release 9.2 PC/Windows XP, Lawes Agricultural Trust Rothamsted Experimental Station; Harpenden, UK. Data were checked for normality before analysis and log transformed if necessary. If data were missing, then mixed models using restricted maximum likelihood were used. Proportion data derived from counts were subjected to angular transformation before analysis.

A)

B)

C)

D)



E)

Figure 2.7 A) A section of the canopy immediately before it was separated into its various components: fruit, live leaves, abscised leaves, senesced leaves, shoots and canes, B) the canopy being dismantled, C) the leader is cut into sections before being chopped and passed through a chipper, D) once the canopy and leader have been removed, the trunk, crown and some of the roots are pulled out, E) the remaining roots were exposed by digging, or by applying a stream of high-pressure water.

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3 FRUIT SUB-SAMPLING

3.1 INTRODUCTION

Most kiwifruit vine management techniques are designed to increase resource allocation towards fruit production without compromising consumer acceptability and storage potential. Fruit composition affects its taste (McMath et al., 1992; Rossiter et al., 2000; Crisosto and Crisosto, 2001; Harker et al., 2009) and storage performance (Tagliavini et al., 1995; Ferguson et al., 2003; Gerasopoulos and Drogoudi, 2005; Feng et al., 2006), so it is important to understand how the whole vine treatments affect fruit composition. For example, there are concerns within the kiwifruit industry that trunk girdling adversely affects fruit storage performance: fruit from trunk-girdled vines are believed to soften in storage more rapidly than fruit from intact vines (Currie et al., 2007). Low fruit calcium (Ca) concentration and/or low Ca to N ratios have previously been linked to poor storage of kiwifruit (Tagliavini et al., 1995; Ferguson et al., 2003; Gerasopoulos and Drogoudi, 2005; Feng et al., 2006). Fruit from girdled apple trees had lower Ca concentrations than their ungirdled counterparts (Arakawa et al., 1997), although fruit from girdled 'Hort16A' vines had the same flesh Ca concentrations as fruit from un-girdled control vines (Boyd and Barnett, 2011).

This raises the question of how to sub-sample fruit to measure composition. In this thesis, fruit composition was measured at the vine level, and the effect of different treatments on fruit composition during development and at harvest was determined. At each sampling date, a combined sample of 12 to 18 fruit per vine was taken at random from across the entire fruiting canopy. The within-fruit sub-sampling method needs to enable accurate whole-vine resource budgets to be calculated, and also needs to be comparable with results from existing publications and industry standards. Mineral nutrients, acids and carbohydrates are unevenly distributed within a kiwifruit. Differences occur among different tissue types (e.g. skin, flesh and seeds), and within the flesh (MacRae et al., 1989a). Longitudinal and transverse concentration gradients have been found for a range of fruit components (Ferguson, 1980; Clark and Smith, 1988, MacRae et al., 1989a). Within-fruit composition and relative distributions can change as fruit develop and ripen (MacRae et al., 1989b). Fruit composition is rarely determined on entire fruit, more typically a sub-sample is taken and analysed. A range of fruit sub-sampling methods is summarised in Table 3.1. There can be valid reasons why fruit are sub-sampled, and why different sub-sampling methods are used:

1. Variability among fruit is large for many attributes, meaning that relatively large numbers of fruit are sampled from a population. For example, a 90-fruit sample is used to measure maturity on an orchard block, known as a maturity area, to determine quality and when to harvest (Anon. 2009). A 10-fruit sample per vine was used to measure Ca status across ten vines (Ferguson et al., 2003). The logistics of transporting, homogenising and analysing large numbers of entire fruit can be limiting, so sub-samples are taken.
2. Several destructive tests are carried out on the same fruit, limiting which part of the fruit is available for analysis. Standard kiwifruit maturity testing for 'Hort16A' kiwifruit (Anon. 2009) includes measuring flesh colour on one face of the fruit at the equator with 2-mm deep piece of skin removed, measuring firmness on two faces of the fruit at the equator each with a 1-mm deep slice of skin removed, SSC is either measured on juice generated from the firmness measurement, or from juice squeezed from the stem and beak end of the fruit, and DMC is measured on an equatorial slice (Figure 3.1).
3. There is a strong relationship between two fruit attributes. For example, the concentration of Ca in the flesh just below the skin is related to the incidence of the storage disorders bitter pit in apples (Turner et al., 1977) and physiological pitting in kiwifruit (Ferguson et al., 2003). Therefore sub-samples of flesh from just under the skin, known as flesh plugs, are taken (Figure 3.2) in an attempt to predict which lines of fruit are more susceptible to the disorder.

A) B)

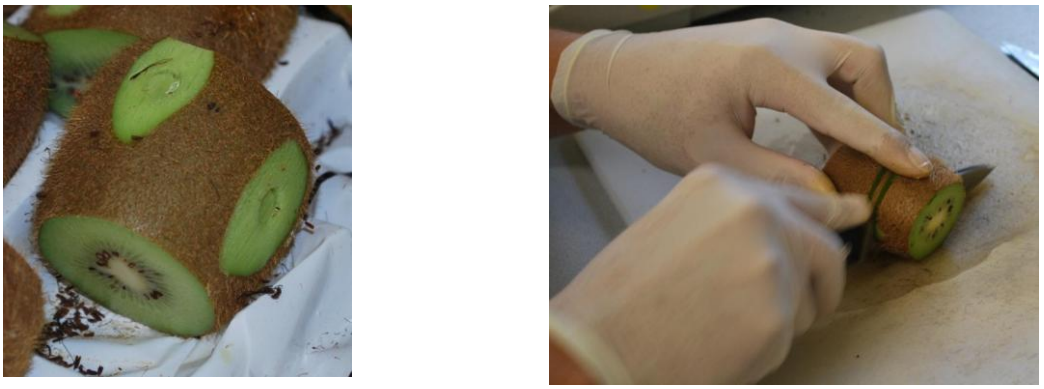

Figure 3.1 A) 'Hayward' fruit with both ends removed for soluble solids concentration determination, two 1-mm deep skin slices have been removed showing puncture marks from firmness measurement, and B) an equatorial slice being removed to measure dry matter concentration.

It is not known if results from different experiments are comparable if the fruit are sub-sampled differently. For example, inorganic nutrient concentrations have been measured on flesh plugs (Ferguson et al., 2003; Thorp et al., 2003; Afshar-Mohammadian and Rahimi-Koldeh, 2010) and equatorial slices (Mowat, 2003; Feng et al., 2006; Mills et al., 2008), including the dried slices left over from DMC analysis. It is also not known if results from either sub-sample accurately reflect the nutritional status of the whole fruit.

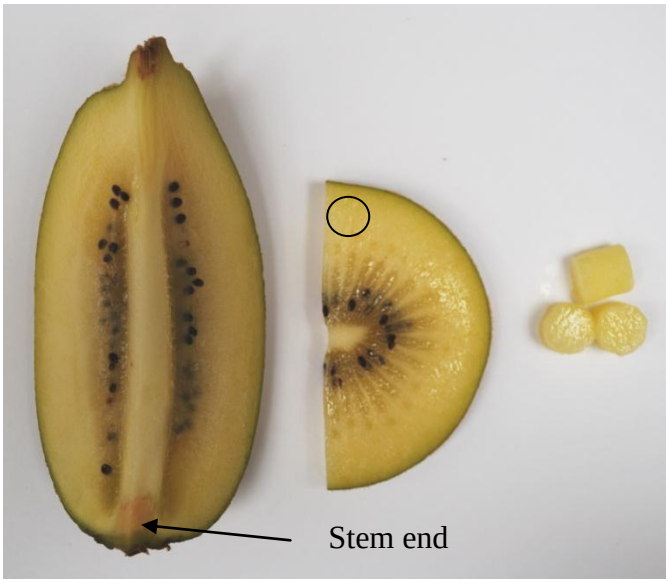

Figure 3.2 The three sample types used to compare sampling procedure: longitudinal quarter (left) equatorial slice (cut in half; centre), flesh plugs (right) taken from the remainder of the equatorial slice from just under the skin, as illustrated by the black circle. Relative sample fresh weights were ~ 50:10:1 in the longitudinal, equatorial and plug samples respectively (~594, 117 and 12 g per bulked sample).

The aims of the work described in this chapter were to determine which fruit sub-sampling technique should be used in the experiments described in this thesis, and how to compare and interpret results if fruit were sub-sampled differently. The specific questions were:

- Do equatorial slices accurately estimate nutrient and DM accumulation during fruit development?
- Are flesh plugs and equatorial slices accurate estimators of fruit composition in mature fruit?
- What are the limitations for comparing results from experiments that have used different sampling methods?

Table 3.1 Summary of fruit sub-sampling methods in a selection of papers on kiwifruit quality.

Analyte(s)	Fruit sub-sample (and units)	Purpose of research	Reference (s)
Inorganic nutrients	Equatorial slice	Relationship with storage potential	Feng et al., 2006; Mowat, 2003
	Outer pericarp (mg g^{-1} DW)	Developmental and cultivar differences	Afshar-Mohammadian and Rahimi-Koldeh, 2010
	Outer pericarp (mg 100 g^{-1} FW)	Relationships with disorder incidence	Ferguson et al., 2003,Thorp et al., 2003; Gerasopoulos and Drogoudi, 2005
DMC	Equatorial slice (g 100g^{-1} FW)	Treatment (heating) differences	Snelgar et al., 2005
		Sensory evaluation	Burdon et al., 2004
		Fruit development	Morandi et al., 2010
	Whole fruit when small, then equatorial slice (g 100g^{-1} FW)	Treatment (N input) differences over development	Mills et al., 2008
SSC	Combined juice from each end	Treatment (rootstock) differences,	Thorp et al., 2007
		Treatment (heating) differences; postharvest changes	Snelgar et al., 2005; MacRae et al., 1992
	Juice from equator	Treatment (source-sink ratio) differences	Famiani et al., 1997
		Relationship with storage potential	Feng et al., 2006
Carbohydrates	Outer pericarp (mg g^{-1} FW)	Developmental and cultivar differences	Afshar-Mohammadian and Rahimi-Koldeh, 2010
	Outer cortex (µmol g^{-1} FW)	Postharvest changes	MacRae et al., 1992
	Whole peeled fruit separated into core and outer pericarp	Developmental changes	Bielski et al., 1997
	Whole peeled fruit (% FW)	Developmental and postharvest changes	Reid et al., 1982
	Whole fruit (mg g^{-1} DW)	Developmental changes	Boldingh et al., 2000

DMC = dry matter concentration; SSC = soluble solids concentration, DW = dry weight; FW = fresh weight.

3.2 MATERIALS AND METHODS

3.2.1 Fruit harvest and sampling

Fruit were harvested from mature 'Hort16A' kiwifruit vines growing at the Te Puke Research Orchard, or a nearby commercial orchard in the Bay of Plenty, New Zealand (37°49'S, 176°19'E). Fruit were collected and sub-sampled as follows.

3.2.1.1 Longitudinal gradients in developing fruit. One fruit was sampled at random from the fruiting zone of five vines in December, January, February, March and early May, 44, 63, 101, 136 and 212 days after mid bloom (DAMB), respectively. Each fruit was cut into transverse slices approximately 7 mm thick, generating either 5 or 7 slices, depending on the fruit size (Figure 3.3). For each slice FW (fresh weight), DW (dry weight) and concentration of Ca, Mg (magnesium), K (potassium) and P (phosphorus) were determined. Mineral nutrient analysis was not carried out on the fruit sampled 136 DAMB due to a technical problem.

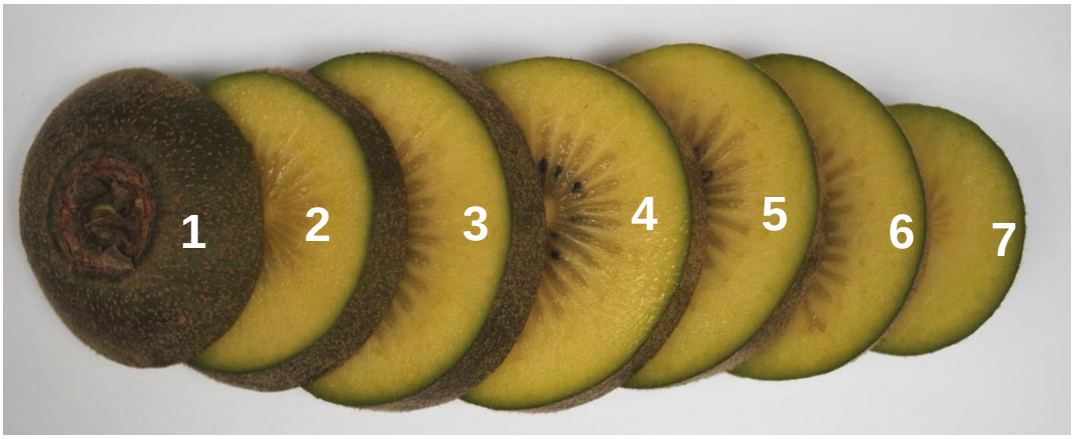

Figure 3.3 'Hort16A' kiwifruit cut into 7 transverse slices. The 1st slice is the stem end of the fruit the 4th slice is an equatorial slice and the 7th slice is at the beak end of the fruit.

3.2.1.2 Flesh plugs and equatorial slices for estimating mature fruit composition. A random sample of 20 fruit was taken from each of 10 'Hort16A' vines located on a commercial orchard in Te Puke in the Bay of Plenty. The fruit were weighed and sub-sampled within 24 hours of harvest. Mean FW was determined and three types of analytical sub-sample were taken from each fruit:

- a longitudinal quarter (a representative sample of whole fruit composition),
- an equatorial slice from half the fruit,

- flesh plugs (with no skin or seeds) taken from an equatorial slice from the remaining longitudinal fruit quarter (Figure 3.2).

Each sample type was combined to give one set of longitudinal quarters, one set of equatorial slices and one set of flesh plugs per vine. The sub-samples were placed in liquid nitrogen, freeze-dried, weighed and ground. Portions of the ground material were sent to RJ Hill Laboratories where they were analysed for mineral nutrients (see Chapter 2.2.3 for methods). Each sub-sample was also analysed for starch and soluble sugars using the methods described in Chapter 2.2.4.

3.2.2 Data analysis

3.2.2.1 Longitudinal gradients in developing fruit. For each harvest date the amount of DM and mineral nutrients in each slice were plotted against slice position and longitudinal trends were recorded. The concentration of analyte in each fruit was plotted against the concentration of that analyte in the equatorial slice.

3.2.2.2 Flesh plugs and equatorial slices as estimators of mature fruit composition. Regression analysis was used to compare the composition of the whole fruit (A_{fruit} calculated from the longitudinal quarter) with composition estimated from flesh plugs (A_{plug}) and equatorial slices ($A_{equatorial}$), collectively referred to as A_{est}.

1) Initially the null hypothesis that there was no relationship between A_{est} and A_{fruit} was tested. If no significant relationship was found ($P > 0.05$; Figure 3.4A) then no further testing was carried out.

2) If a significant relationship with a positive slope was found, then the null hypothesis that the slope of the line was 1 was tested (e.g. Figure 3.4.B and C).

3) If the slope of the line was not different to 1 then, further testing was carried out to determine if the relationship between the A_{est} and A_{fruit} was unbiased (Figure 3.4B), or has a systematic bias (Figure 3.4C).

4) If the slope of the line was not equal to 1 then the relationship between A_{est} and A_{fruit} had a non-systematic bias (Figure 3.4D).

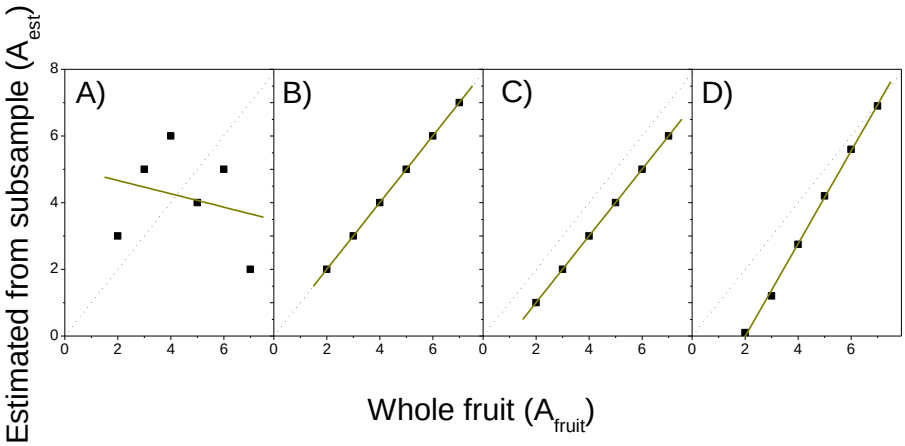

Figure 3.4 Relationships between composition of a whole fruit and the composition estimated from a sub-sample, A) no relationship, B) an unbiased relationship, C) a systematic bias and D) a non-systematic bias. Dashed line is a 1:1 line and solid line is line of best fit.

A worked example for the relationship between the whole fruit sulphur (S) status and that estimated from flesh plugs is given in Appendix 3.6.1.

3.3 RESULTS

3.3.1 Longitudinal gradients in developing fruit

Throughout fruit development DMC was highest in slices located at the stem and beak ends of the fruit (Figure 3.5A-E). When DMC_{fruit} was plotted against $DMC_{equatorial}$ the line of best fit sat below the 1:1 line by 0.34 to 0.74 percent-units depending on the sampling date (Figure 3.5F-J).

Based on the values in Figure 3.5, extrapolating from equatorial slices would underestimate DMC of developing 'Hort16A' kiwifruit by 0.34 to 0.74 percent-units, depending on the sampling date. A systematic underestimation of say 0.5 percent units would create proportionately larger inaccuracies in low DM fruit. For example up to around 100 days after mid-bloom (DAMB) an equatorial slice would underestimate whole fruit DMC by approximately 8 %, and this degree of underestimation would drop to around 2.5 % nearer maturity (Figure 3.6).

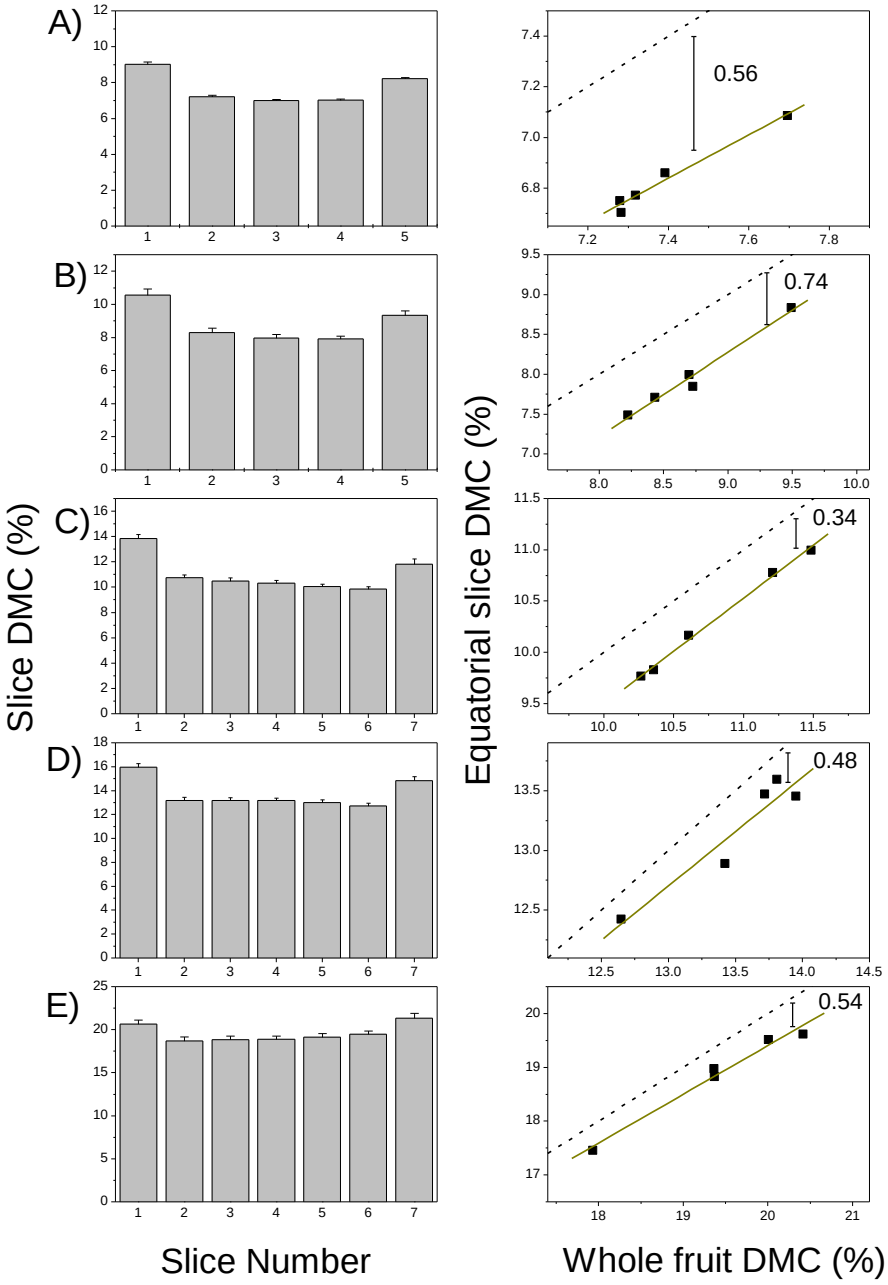

Figure 3.5 Longitudinal dry matter concentration (DMC) gradients in 'Hort16A' kiwifruit sampled A) 44, B) 63, C) 101, D) 136 and E) 212 days after mid-bloom; *n* = 5 + *SE*), and DMC in an equatorial slice plotted against the whole fruit DMC (right column) F) 44, G) 63, H) 101, I) 136 and J) 212 days after mid-bloom; solid line = the line of best fit; = dashed line = 1:1 line; slice 1 = from stem end of fruit; vertical bar (and associated numbers) = average bias.

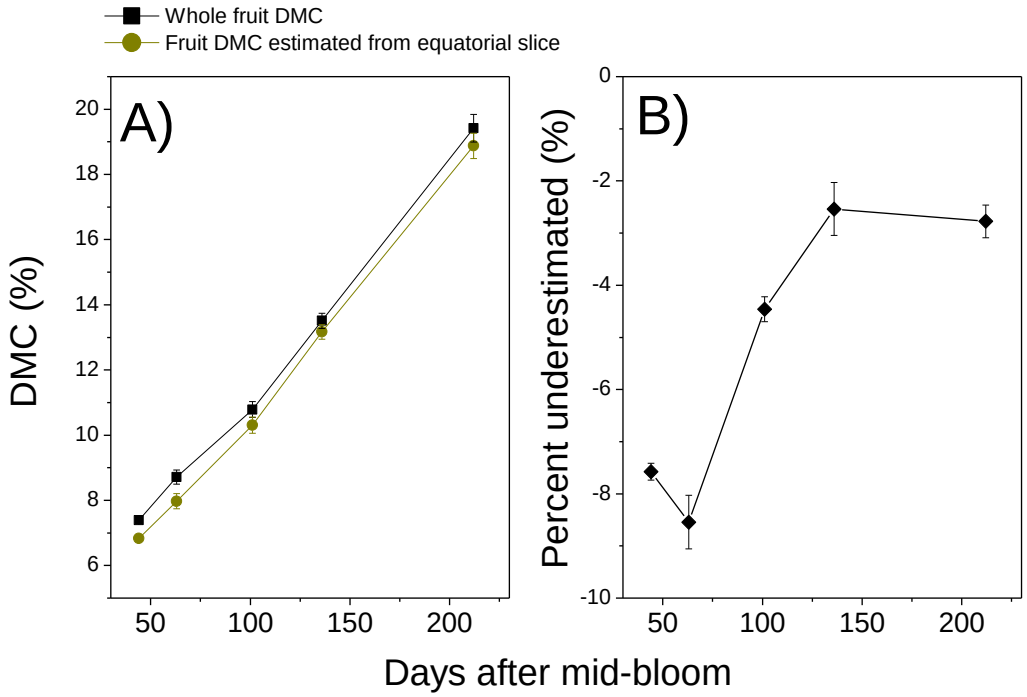

Figure 3.6 A) 'Hort16A' kiwifruit dry matter concentration (DMC) calculated on whole fruit (black line) and estimated from an equatorial slice taken from the same fruit (red line), during fruit growth and development; *n* = 5 ± *SE*; and, B) and the percentage underestimation when calculating whole fruit DMC from an equatorial slice.

Calcium concentration remained highest at the stem end of the fruit throughout development, was generally lower in the mid-region of the fruit and then increased at the beak-end of the fruit (Figure 3.7). The longitudinal distribution of K within the fruit was similar to that of Ca, but without the late-season increase at the beak-end of the fruit (Figure 3.7). Early in the season Mg and P concentrations were highest at the stem end of the fruit, by 101 DAMB the concentration increased in the mid-region of the fruit relative to the ends (Figure 3.7).

Longitudinal gradients in the distribution of mineral nutrients would also affect whole fruit values extrapolated from equatorial slices, although the degree of inaccuracy would depend on both the nutrient and the time of sampling. For example equatorial slices taken from fruit 44 DAMB underestimated Mg concentrations by approximately 3.7 %, and overestimated fruit Mg concentration by 7.9 % when sampled 212 DAMB.

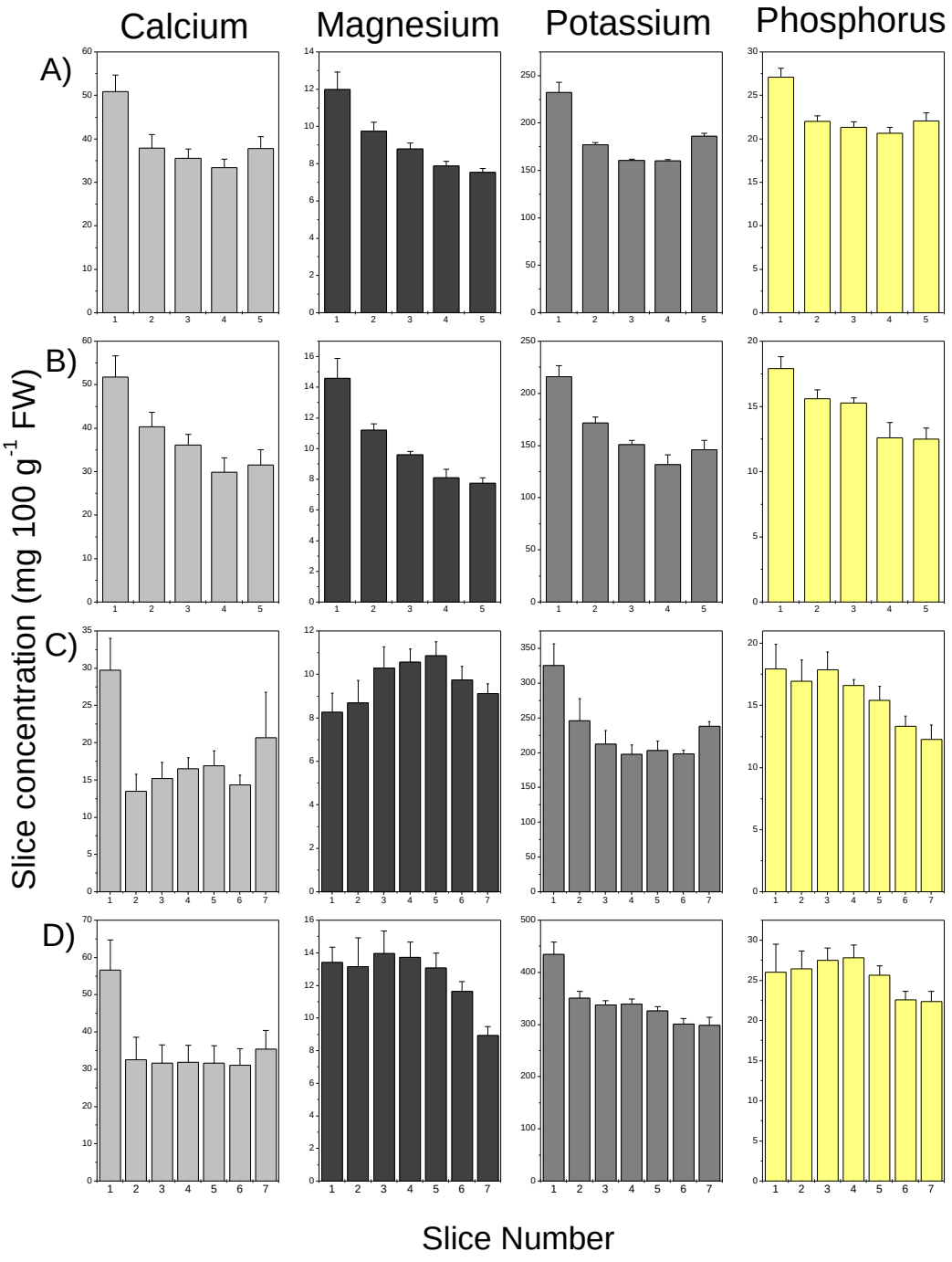

Figure 3.7 Longitudinal gradients in concentrations of calcium, potassium, magnesium and phosphorus in 'Hort16A' kiwifruit sampled A) 44, B) 63, C) 101, and D) 212 days after mid-bloom, *n* = 5 + *SE*; slice 1 = stem end.

3.3.2 Comparison of plugs and equatorial slices with whole fruit

Dry matter and mineral nutrients: flesh plugs. Significant positive relationships were found between A_{plug} and A_{fruit} for DM, Ca, K, Mg, N, P, S, Mn and Ca/N ($P \leq 0.05$), weaker relationships for Fe and B ($P \leq 0.10$) and no relationship for Zn and Cu ($P = 0.688$ and 0.383 respectively; Figure 3.8; Table 3.2). All significant relationships had some bias and A_{plug} was consistently lower than A_{fruit}. Systematic biases were detected between A_{plug} and A_{fruit} for DM, K, Mg and S, and non-systematic biases found for Ca, N, P, Mn and the ratio Ca/N.

Dry matter and mineral nutrients: equatorial slices. Positive relationships were found between $A_{equatorial}$ and A_{fruit} for DM and mineral nutrients (including Ca/N) with the exception of Zn ($P = 0.805$; Table 3.2). For P, Fe and Cu $A_{equatorial}$ was an unbiased estimator of $A_{fruit,}$ whereas $A_{equatorial}$ systematically underestimated whole fruit DMC, Ca, K, N, S, B and Ca/N and overestimated whole fruit Mg. There was a non-systematic bias between $A_{equatorial}$ and A_{fruit} for Mn (Figure 3.8; Table 3.2).

Starch and sugars. Fructose, glucose and sucrose were the main sugars present in the fruit. Positive relationships were found between A_{plug} and A_{fruit} and $A_{equatorial}$ and A_{fruit} for starch, fructose, glucose and sucrose and their combinations total sugars (fructose, glucose and sucrose) and total carbohydrates (starch, fructose, glucose and sucrose; Figure 3.10). Flesh plugs systematically overestimated fruit composition of total sugars, fructose, glucose and total carbohydrates (Figure 3.10; Table 3.2). Plugs provided an unbiased estimate of fruit starch contents and underestimated fruit sucrose in non-stystematic manner. Equatorial slices were unbiased estimators of total carbohydrates, starch, total sugars, fructose and glucose, and systematically underestimated the amount of sucrose in the whole fruit (Figure 3.10; Table 3.2).

Table 3.2 Test statistics used to define the relationship between two sub-sample types and whole fruit value of mineral nutrients and non-structural carbohydrates (NSC).

	Flesh plug			Equatorial slice		
		F-test			*F*-test	
Analyte	*P*-value[a]	Test 1[b]	Test 2[c]	*P*-value	Test 1	Test 2
DM	< 0.001	**0.65**	166 (-)	< 0.001	**1.67**	10.5 (-)
Ca	< 0.001	24.0	-	< 0.001	**0.795**	9.9 (-)
K	< 0.001	**1.30**	366 (-)	0.004	**0.163**	38.7 (-)
Mg	0.003	**0.096**	112 (-)	0.007	**2.59**	10.50 (+)
N	0.013	9.9	-	< 0.001	**4.25**	7.22 (-)
P	0.013	9.2	-	< 0.001	**0.027**	**0.408**
S	< 0.001	**0.139**	175 (-)	< 0.001	**1.21**	8.19 (-)
Fe	0.089	-	-	0.023	**1.16**	**1.50**
Mn	< 0.001	249	-	< 0.001	87.4	-
Zn	0.688	-	-	0.805	-	-
Cu	0.383	-	-	< 0.001	**3.66**	**1.92**
B	0.053	-	-	0.005	**0.325**	**4.48**
Ca/N	0.001	8.02	-	< 0.001	**4.04**	6.02 (-)
Total NSC	0.001	**1.42**	39.6 (+)	0.006	**1.08**	**3.21**
Sugars	< 0.001	**2.12**	56.7 (+)	< 0.001	**0.385**	**2.22**
Starch	0.011	**0.301**	**3.90**	0.014	**2.28**	**2.36**
Fructose	< 0.001	**0.729**	104 (+)	< 0.001	**1.83**	**0.917**
Glucose	< 0.001	**1.94**	279 (+)	0.001	**0.189**	**0.636**
Sucrose	0.004	14.6	-	0.002	**1.63**	7.99 (-)

[a]P-value for linear regression between sub-sample and whole fruit value, if P > 0.05 then no further testing;

[b]test that regression line has a slope of 1, if F-test < 5.3 then the slope was not different to 1 at the 5% level (highlighted in bold); if F-test ≥ 5.3 no further testing was done as the sample had a non-systematic bias

[c]test of regression line compared to a 1:1 line, if F-test < 4.5then the regression line was not different to a 1:1 line at the 5% level (highlighted in bold); if F-test ≥ 4.5 then direction of bias is given in brackets if the bias underestimates then (-),

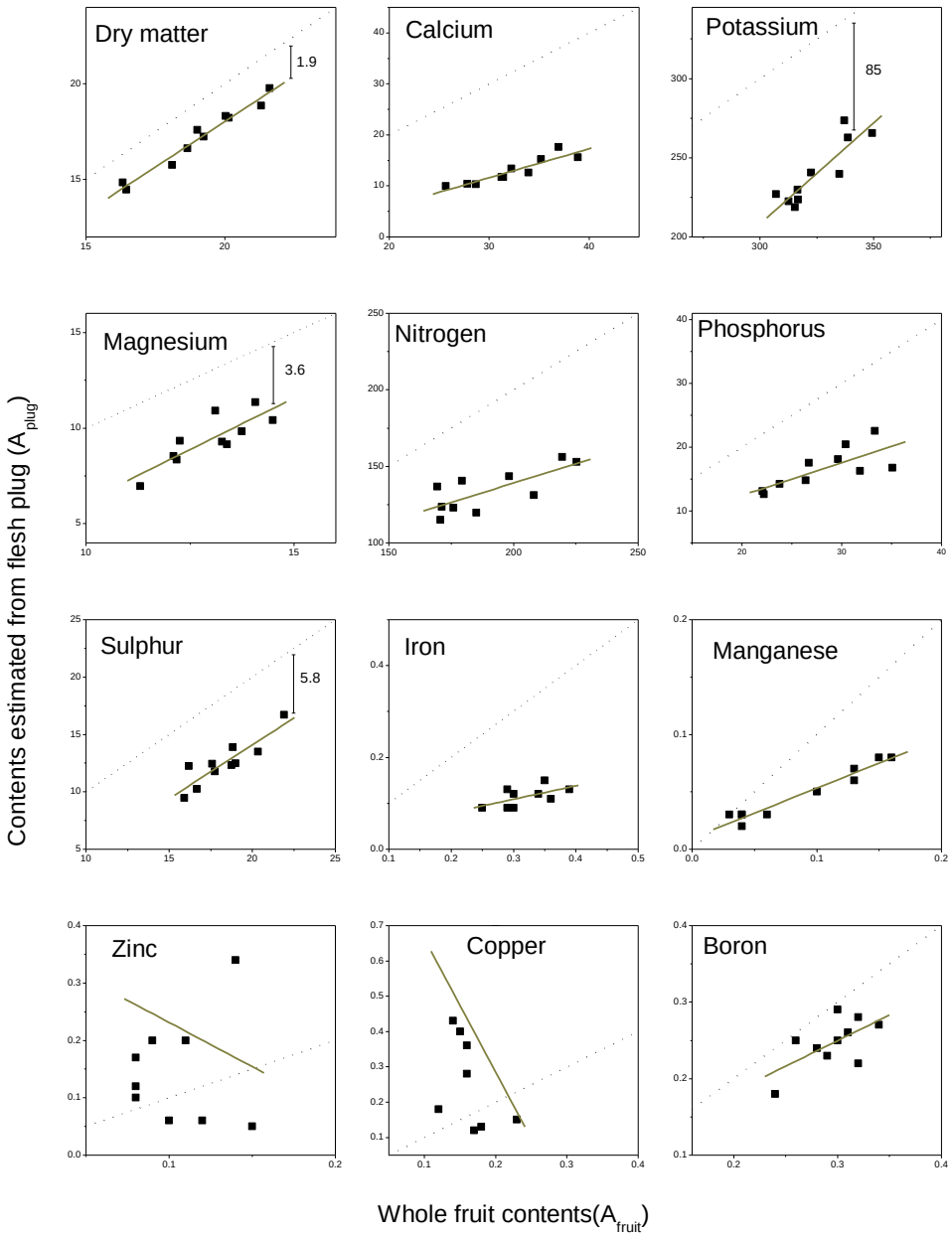

Figure 3.8 Relationship between whole fruit contents of analyte ($A_{fruit;}$ dry matter or mineral nutrient) and the contents estimated from a flesh plug (A_{plug}). Each datum point was derived from a composite sample of 20 fruit from an individual plant vines. Solid line = least squares line of best fit; dashed line = 1:1 line; vertical bar and number = degree of systematic bias, where found.

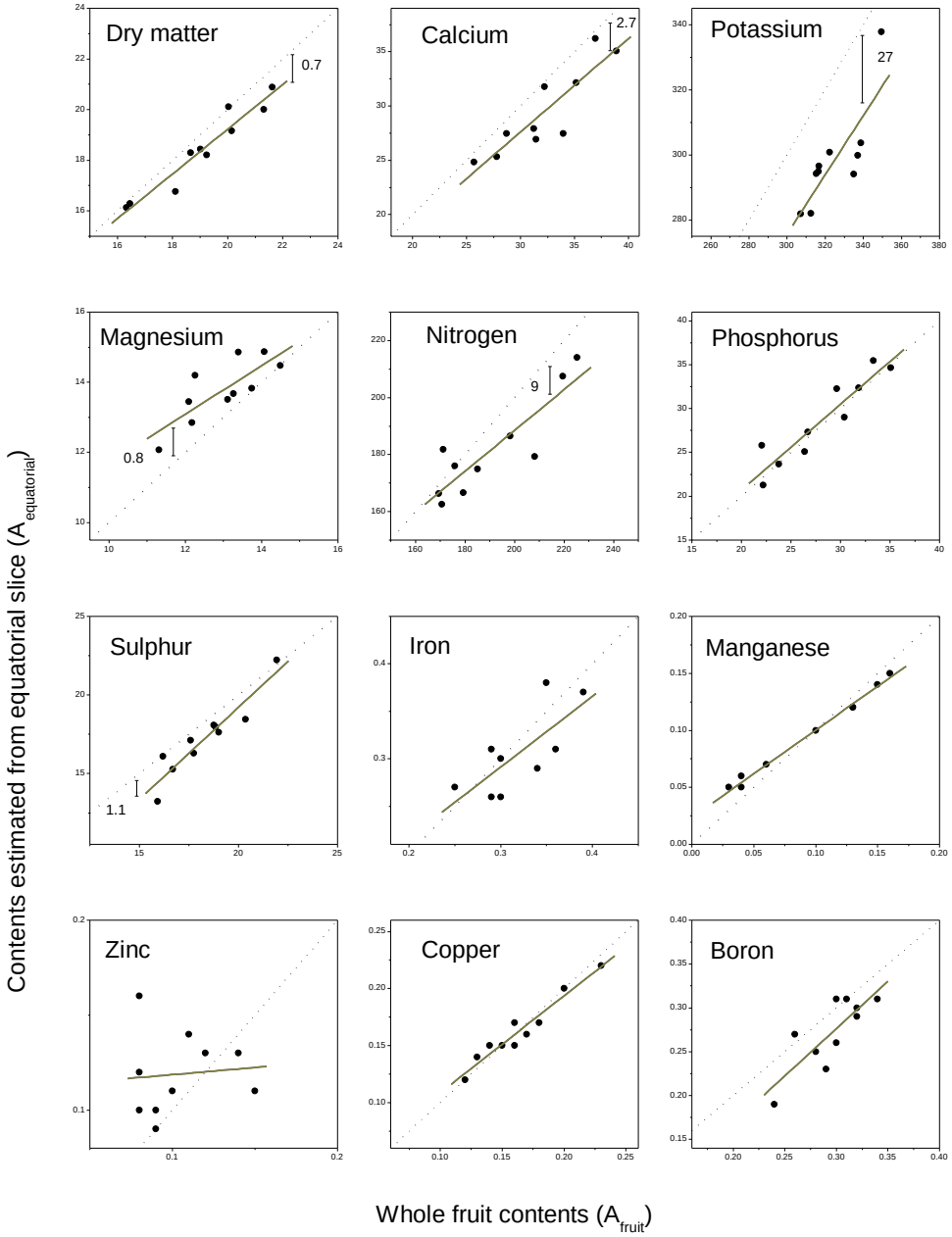

Figure 3.9 Relationship between whole fruit contents contents of analyte ($A_{fruit;}$ dry matter or mineral nutrient) and the contents estimated from an equatorial slice ($A_{equatorial}$). Each datum point is the mean of 20 fruit from 10 individual vines. Solid line = least squares line of best fit; dashed line = 1:1 line; vertical bar and number = degree of systematic bias, where found.

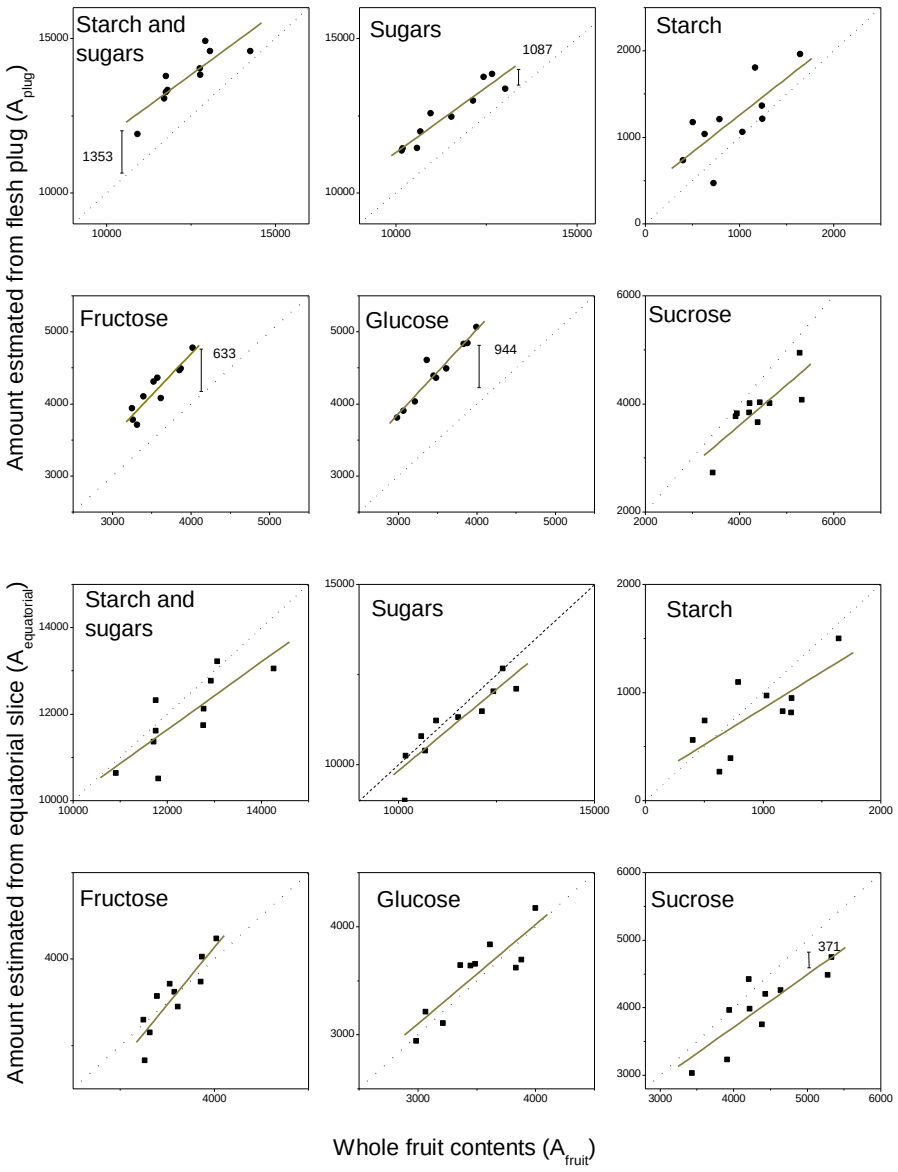

Figure 3.10 Relationship between whole fruit contents of analyte (A_{fruit}; non-structural carbohydrates) and contents estimated from a flesh plug (A_{plug}) or an equatorial slice ($A_{equatorial}$). Each datum point is the mean of 20 fruit from 10 individual vines. Dashed line = 1:1 line, solid line = least squares line of best fit, vertical bar and number = degree of systematic bias, where found.

Results extrapolated from a sub-sample with a negative systematic bias will be lower than they should be. This bias would proportionately affect fruit with lower concentrations of the analyte than fruit with higher concentrations. In the example below (Figure 3.11) flesh plugs would underestimate whole fruit S concentrations by 5.8 mg 100 g^{-1} FW. For a fruit with low S concentrations of say 15 mg 100 g^{-1} FW the flesh plug underestimates by around one third (Figure 3.11), if a fruit had a concentration nearer 24 mg 100 g^{-1} FW then the degree of underestimation would be nearer one quarter.

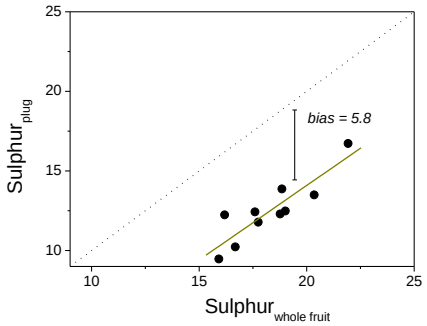

Figure 3.11 Relationship between sulphur concentrations in flesh plugs taken from 'Hort16A' kiwifruit compared with whole fruit S concentration. Dashed line = 1:1, and solid line = line of best fit.

The positive relationship between the Ca/N ratio in sub-samples and whole fruit (Figure 3.12) means that results obtained from different sampling methods can at least be ranked or compared.

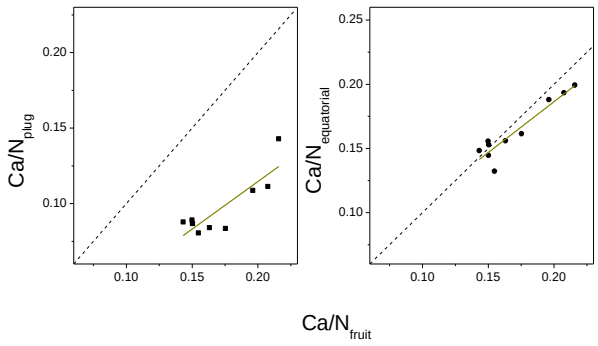

Figure 3.12 Relationship between Ca/N ratio in A) flesh plugs and, B) equatorial slices sub-sampled from 'Hort16A' kiwifruit compared with whole fruit Ca/N ratio. Dashed line = 1:1, and solid line = line of best fit.

3.4 DISCUSSION

3.4.1 Developing fruit

Longitudinal gradients in mineral nutrients and DM have previously been reported for mature fruit. Calcium content was highest at the stem end of 'Hayward' kiwifruit, whereas K and Mg did not show such marked patterns (Ferguson, 1980). In apple fruit, Ca concentrations, measured on a FW basis, declined from the stem end to the calyx end (Perring, 1989). In persimmon fruit, Ca concentration, measured on a DW basis, was highest at the stem end of the fruit in both the skin and flesh, whereas Mg, K and P concentrations showed little or no longitudinal gradient in either tissue type (Clark and Smith, 1990). There is a need for more information about how longitudinal gradients change as fruit develop. Clark and Smith (1990) measured longitudinal gradients in developing persimmon fruit and found that the magnitude of gradients depended on the time of sampling, with gradients generally declining as fruit developed. The implications for sub-sampling of changing gradients are that a whole fruit value extrapolated from an equatorial slice could have a bias, and the magnitude of the bias could change during fruit development.

The sample numbers used in the current experiment (n = 5) were too small to make an accurate estimation of the bias, but the message from this work was that it would be better to take whole fruit or longitudinal quarters, rather than equatorial slices, as sub-samples for any developmental work.

3.4.2 Mature fruit

Mature fruit composition was estimated from a longitudinal quarter taken from each fruit and bulked across the 20 fruit from each sub-sample. Extrapolating from a longitudinal fruit quarter does not take into account any gradients in composition that may exist around an individual fruit. For example, Perring and Wilkinson (1965) reported that higher concentrations of some constituents were found on the blushed side of apple fruit. Preliminary work on citrus showed that Ca concentration was different in sub-samples taken from the shaded and unshaded sides of individual fruit (Storey and Treeby, 2000). In the work described in this chapter, all 20 longitudinal quarters were combined to give one bulked sample per vine and no attention was paid to the exposure of the quarters when sub-samples were taken. This means that the bulked sample should give an accurate estimate of the population, but this method may not be suitable for measuring individual fruit composition.

With the exception of Zn, there was a correlation between composition of an equatorial slice ($A_{equatorial}$) and whole fruit composition (A_{fruit}). For Mn there was a non-systematic bias in the relationship between $A_{equatorial}$ and A_{fruit}. Equatorial slices were unbiased estimators of whole fruit P, Fe, Cu, B and most of the non-structural carbohydrates (total NSC, total sugars, starch, fructose and glucose). This could be because these analytes are evenly distributed down the fruit, or that longitudinal gradients exist but are cancelled out. For example concentrations of P in mature fruit tend to be highest in the mid-region of the fruit, but lower towards the beak end of the fruit (Figure 3.7D).

Equatorial slices were defined as biased estimators of fruit DMC, Ca, K, Mg, N, S and sucrose. With the exception of Mg, equatorial slices underestimated whole fruit composition. Concentrations of DM, Ca and K in mature fruit were higher at one or both ends of the fruit than at the equator (Figures 3.5E and 3.6D), longitudinal gradients in N and S were not measured, in this experiment. Equatorial slices overestimated whole fruit Mg concentration by ~ 0.8 mg 100 g^{-1} FW. The range of Mg concentrations in this experiment was around 11 to 15 mg 100 g^{-1} FW, so the magnitude of overestimation was 5 - 7 %. During fruit development, Mg concentrations increased in the mid-region of the fruit relative to either end of the fruit (Figure 3.6).

Although longitudinal gradients can be used to explain the relationship between the composition of an equatorial slice and that of a whole fruit, different nutrients accumulate in fruit in different ways. Calcium is believed to be preferentially translocated in the xylem and majority of Ca accumulation in the fruit occurs in the first two months of fruit development (Clark and Smith, 1988; Thorp et al., 2007). Calcium concentrations are higher in the skin than the flesh (Clark and Smith, 1988) so it is not surprising that Ca concentrations are higher at one or both ends of the fruit (the proportion of skin in a slice from either end is higher than a slice from the equator) and that this pattern largely remains throughout fruit development. Accumulation of Mg, K and P into developing fruit continues until shortly before harvest, with phloem being the main supply route for K and P throughout the season, and Mg in the latter part of the season (Clark and Smith, 1988). Phosphorus and Mg accumulated in the mid-region of the fruit in the second half of the season increased, changing the longitudinal distribution of these nutrients, so that an equatorial slice would closely represent fruit composition. Conversely K concentrations remained higher at the stem end of the fruit, even

late in the season so that an equatorial slice would underestimate whole fruit K contents. It is not known why these differences occur, possible explanations are that 1) P and Mg accumulate more than K late in the season, and phloem unloading occurs preferentially around the mid-section of the fruit, 2) the same amount of K, P and Mg accumulate in the fruit late in the season but they move within the fruit to different degrees, or 3) same amount of K, P and Mg accumulate in the fruit late in the season, but there is some part of the fruit, e.g. the woody tissue just below the fruit stalk at the stem end of the fruit (see Figure 3.2) that is particularly high in K and masks late-season K accumulation in the mid-region of the fruit. Detailed analyses of seasonal changes for all mineral nutrients would be required to understand whether some phloem-mobile nutrients accumulate differently into fruit late in the season.

Flesh plugs were less likely than equatorial slices to reflect whole fruit composition. This is not surprising because flesh plugs do not represent the range of tissues present in a whole fruit (seeds, skin, and core) whereas equatorial slices do, albeit in slightly different ratios to the entire fruit. Estimates derived from flesh plugs were unrelated to whole fruit composition of Fe, Zn, Cu and B (Figure 3.5; Table 3.2). There several reason why this might be the case:

- There is a relationship, but it may be weak and the cut-off value used ($P \leq 0.05$), has excluded it. This could be the case for B where the relationship between A_{plug} and A_{fruit} had a P - value of 0.053
- For some micronutrients the concentration in flesh plugs might be close to the level of detection, and/or the spread among samples might be smaller than can be detected. This this might be the case for Fe, but further work would be needed to test this possibility
- There was large range of Cu and Zn values for A_{plug}, including some values higher than the 1:1 line. Brass is an alloy of Cu and Zn, and brass cork borer used to take the plug samples may have contaminated the samples.
- The analyte may have very low levels in the flesh and high levels in, say, the seeds and therefore the flesh composition does not reflect the fruit composition.

3.5 CONCLUSIONS

The aims of this work were 1) to determine what fruit sub-sampling method should be used in the whole vine resource allocation studies, and 2) to gain background information that would enable interpretation of results and comparison with industry standards and other published results, as necessary. The following questions were asked:

Are equatorial slices accurate estimators of nutrient and DM accumulation during fruit development?

No. There was a positive correlation between equatorial slices and whole fruit concentrations for the analytes measured (DM, Ca, Mg, K and P) at each date, but the longitudinal distribution of each analyte changed during the season. This means that extrapolating from an equatorial slice to a whole fruit could add a bias to the results. As the fruit grew, biases tended to change. In experiments that examine the effect of different treatments on resource allocation to fruit, such as DM accumulation in developing fruit, or the amount of mineral nutrients lost to the vine during fruit thinning it is better to use whole fruit or longitudinal quarters.

Can the composition of mature fruit be estimated from flesh plugs and equatorial slices?

The concentration of most analytes in flesh plugs and in equatorial slices is positively correlated with whole fruit composition. Usually flesh plugs and equatorial slices have lower concentrations of analytes, therefore extrapolating from this type of sub-sample could underestimate whole fruit composition. Longitudinal quarters were used to estimate fruit composition in the work carried out in Chapters 4 to 6.

Is it possible to compare results from fruit when they are sampled and reported on different bases?

For most analytes, including the ratio Ca/N which is sometimes used in relation to fruit storage performance, there is a positive correlation between results obtained from a flesh plug or equatorial slice and the whole fruit, and so relative ranking is valid. This means that qualitative comparisons can be made, even if quantitative ones cannot. The most common type of extrapolation is from the industry standard DMC taken from an equatorial slice and

whole fruit DMC: in mature fruit the degree of underestimation is relatively small, but will affect fruit with lower DMC fruit more than high DMC fruit.

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3.7 APPENDIX

3.7.1 Regression analysis

Test 1: That the line of best fit has slope = 1. If the slope of the line was 1 then $A_{fruit} - A_{est}$ = constant (C) and the sum of squares (SS) of the differences $\{(A_{fruit} - A_{est}) - C\}^2$ would be relatively small compared with SS from the original regression analysis. The *F*-statistic was calculated. If the *F*-statistic was small then the hypothesis is not rejected. The *P*-values were determined with 1 degree of freedom (df) on the numerator and 8 df on the denominator ($F < 5.3$ means $P > 0.050$). In this case (Figure 3.4), $P = 0.719$ and the hypothesis was not rejected, i.e. the slope of the line was not different to 1.

Test 2: That the line of best fit has slope 1 and intercept a = 0. If true, then $A_{fruit} - A_{est}$ = 0 and its SS (***F***) would be small relative to the residual SS of the original regression. The ratio of these two SS provides an *F*-statistic with 2 on 8 df. In this case the F-statistic was 175 giving $P < 0.001$ and the hypothesis was rejected, so S_{plug} is a biased estimator of S_{fruit} (Figure 3.4).

Where:

A = sum of the squares (SS) of error terms from the original regression

B = the mean square error (MS), i.e. ***A***/df

C = mean (A_{fruit} and A_{est})

D = SS around the mean $\{(A_{fruit} - A_{est}) - \boldsymbol{C}\}^2$

$\boldsymbol{E^1}$ = no. parameters with error terms (Test 1)

G = MS around the mean i.e. $\boldsymbol{D}/2\text{-}\boldsymbol{E^1}$

$\boldsymbol{E^2}$ = no. parameters with error terms (Test 2)

F = SS around zero $(A_{fruit} - A_{est})^2$

H = MS around zero, i.e. $\boldsymbol{F}/(2\text{-}\boldsymbol{E^2})$

A worked example is given on the following page.

Example of calculations used to test if A_{plug} is a good estimator of A_{fruit} for sulphur (see text for details).

Sulphur (mg/fruit)			c.f. zero	c.f. mean difference	y = a + x		y = x
A_{est}		A_{fruit}	$A_{fruit} - A_{est}$	$(A_{fruit} - A_{est}) - mean$	$\{(A_{fruit} - A_{est}) - mean\}^2$		$(A_{fruit} - A_{est})^2$
10.23		16.68	6.45	0.65	0.42		41.60
13.49		20.35	6.86	1.06	1.12		47.06
9.47		15.92	6.45	0.65	0.42		41.60
12.48		19.01	6.53	0.73	0.53		42.64
12.3		18.77	6.47	0.67	0.45		41.86
12.24		16.19	3.95	-1.85	3.42		15.60
12.43		17.6	5.17	-0.63	0.40		26.73
11.78		17.75	5.97	0.17	0.03		35.64
13.87		18.85	4.98	-0.82	0.67		24.80
16.72		21.93	5.21	-0.59	0.35		27.14
			5.80		TEST 1		TEST 2
			Mean ***C***		7.82		344.68
					SS_about mean ***D***		SS_about 0 ***F***
Error_SS ***A***	7.686						
Df	8				*test b = 1*		*test a = 0 & b = 1*
Error_MS ***B***	0.961						
			Δ error	***D - A***	0.133	***F - A***	337.0
			# parameters	***E***	1	E^1	0
			Δ error/(2-***E***)	***G = (D-A)/(2-E)***	0.133	$H = (F-A)/(2-E^1)$	168.5
			F-test	**G/B**	**0.139**	***H/B***	**175.3**

4 WHOLE VINE CARBOHYDRATE STATUS

4.1 INTRODUCTION

In kiwifruit vines, around half of the dry weight in annual canopy growth is allocated to fruit (Clark and Smith, 1992; Boyd et al., 2010). Increasing the production of high dry matter fruit DMC may involve altering the vegetative/reproductive balance towards fruit production. A great deal of work has been published on the effect of altering source-sink balances in kiwifruit (Burge et al., 1987; Lai et al., 1989; Cooper and Marshall, 1991; Minchin et al., 2010; Patterson and Currie, 2011). However the long-term effects of altering the source-sink balance so that a greater proportion of carbohydrates (from sources: leaves and reserves), are allocated to fruit (sinks) are not well understood in kiwifruit vines. Any recommended change to kiwifruit grower practice should be ideally accompanied with an understanding of the potential long-term consequences of the practice. 'Hort16A' vines typically produce higher crop loads of high DMC fruit than 'Hayward' vines, and the long-term effects of allocating more carbohydrates towards fruit production have rarely been explored in detail. If leaves are unable to provide sufficient carbohydrates then it is possible that vine carbohydrate reserves will be depleted. Reserve depletion could adversely affect productivity in subsequent seasons and could compromise nutrient uptake if root starvation occurred (Koch, 1996). It is not known if fruit composition and, potentially, fruit storage quality could be affected if source-sink relationships are altered over consecutive seasons.

Source-sink balances have been altered in horticultural crops by a range of experimental treatments (Table 4.1). Some experiments are designed to explore the mechanisms behind short-term responses to specific source-sink manipulations. In girdled peach shoots, for example, low sink demand caused by fruit removal led to leaf carbohydrate buildup and reduced photosynthesis (Li et al., 2007). The reduction in photosynthesis was not associated with reduced activity of the enzymes associated with leaf carbohydrate export, but was associated with stomatal closure which led to increased leaf temperature, resulting in damage to the photosynthetic apparatus. In some instances source sink relationships may change depending on the plant carbohydrate content; excess carbohydrate might result in generation of new sinks while depleted carbohydrate might lead to delay in leaf senescence enabling further C acquisition. In perennial crops the effect of one season's treatment application will have consequences in subsequent seasons therefore the effect of any management practice must be assessed for impact upon plant performance in the subsequent season (Howell et al.,

1994). It is important to understand how horticultural crops compensate for management changes imposed upon them (Küppers et al., 1988), and how compensation can interact with ongoing management practice to generate secondary consequences. For example, if plants compensate for harsh pruning by compensatory vegetative growth at the expense of reserve accumulation, will mineral nutrient uptake and allocation within the plant be affected? If the plant response is to sequester resources (primarily carbohydrates and N) in the roots then is leaf N status and photosynthesis affected? Sultan (2000) highlighted the importance of testing plant responses across a range of ecologically relevant situations rather than arbitrary sets of contrasting conditions, and suggested that the range of experimental environments could include extreme or unrealistic experiments to test the potential limits of the plant responses.

Table 4.1 Experimental approaches to source-sink manipulation in horticultural crops.

Treatment	Example(s) and reference
Altered leaf-to-fruit ratios	Girdled peach shoots (Li et al., 2007)
	Potted grapevines (Candolfi-Vasconcelos et al., 1994)
	Field-grown apple trees (Palmer, 1992); grapes (Hunter et al., 1995)
Whole plant shading	Potted tomato (Baldet et al., 2002), field-grown kiwifruit (Buwalda and Meekings, 1993)
Whole plant illumination	Potted sour cherry (Layne and Flore, 1995)

Perennial plant responses to altered carbohydrate availability have been discussed in depth in the forestry and general ecology literature. Some representative examples are summarised in Table 4.2, along with information from kiwifruit vines. Plants respond to carbohydrate depletion by changing metabolic activity, carbohydrate resource partitioning and plant form (Koch, 1996). Woody plant responses to defoliation (whether by herbivory, frost or harsh pruning) have been extensively studied and reviewed (Karban and Myers, 1989; Nykänen and Koricheva, 2004). Responses can include compensatory vegetative growth at the expense of reproduction or allocation to reserves. In other cases, plants respond by increasing allocation to reserves (resource sequestration). Plants respond to abundant carbohydrates by increasing sink size, generating new sinks, buildup of starch in leaves and/or feedback inhibition of photosynthesis (Schaffer et al., 1986; Paul and Foyer, 2001).

Leaf traits such as specific leaf weight and chlorophyll to nitrogen ratio can change in response to altered light availability (reviewed by Rozendaal et al., 2006; Poorter et al., 2009).

The aim of the work described in this chapter was to determine how mature field-grown 'Hort16A' kiwifruit vines responded to long-term carbohydrate depletion, and how this affected fruit production, fruit attributes (fresh and dry weight, maturity, mineral nutrient composition and quality maintenance during storage) and overall vine performance. Vines were managed with the aim of depleting vine carbohydrates by keeping crop loads high, maintaining low leaf area to support fruit growth and by allowing vigorous new vegetative growth to compete for a longer period than normal with fruit before pruning. This strategy was collectively referred to as the 'famine' treatment. The vines were treated in this way for six consecutive seasons and were compared with control vines which received standard crop load and pruning and with vines where crop loads were kept low and leaf area high, the 'feast' treatment. In the first three seasons of experimentation, variation in fruit quality attributes (mineral nutrient concentrations, maturity and the incidence of storage disorders) were measured and have been published (Boyd and Barnett, 2011).

In the final three seasons of the experiment, which are reported in this chapter, whole vine resource (biomass, carbohydrate and mineral nutrient) allocation was measured. The hypothesis was that vines would respond to whole-vine carbohydrate depletion, (famine treatment), by upregulating photosynthesis, delaying leaf senescence and allocating additional carbohydrate resources towards shoot growth at the expense of fruit and root growth, relative to the feast vines. In addition, it was hypothesised that longer term depletion of carbohydrate reserves would lead to leaf nutrient deficiencies that would be exacerbated with time as both nutrient reserves and root growth were impaired by carbohydrate depletion. Finally the hypothesis that maturity at harvest was the main factor affecting fruit storage was tested by harvesting fruit from different treatments at a range of different harvest dates and measuring their storage performance.

Table 4.2 Examples of whole plant responses to defoliation/low light in a range of perennial plants including kiwifruit.

Plant response	Examples and reference	Examples in kiwifruit
Overall growth	Reduced (meta-analysis of woody plants Nykänen and Koricheva, 2004)	
Allocation to reproductive structures	Fewer flowers; fewer seeds per fruit, increased fruitlet abortion; delayed fruit maturation (Obeso, 1993)	Reduced return bloom (Buwalda and Smith, 1990; Cooper and Marshall, 1991; Cruz-Castillo et al., 2010); decreased fruit fresh weight (Snelgar and Thorp, 1988; Buwalda and Smith, 1990; Cruz-Castillo et al., 2010)
Allocation to reserves	Reduced: re-translocated from roots and trunk to ripening fruit (grape Candolfi-Vasconcelos et al., 1994)	Reduced NSC concentrations in bark and shoots, not roots (Cruz-Castillo et al., 2010); reduced fine root growth (Buwalda and Smith, 1990)
	Increased: sequestration of resources (Orians et al., 2011)	-
Allocation to leaf growth	Increased at the expense of trunk and root growth	Shaded vines had increased LAI in season 1, decreased LAI in season 2 (Buwalda and Meekings, 1993)
	Increase in proportion of long shoots in subsequent year (mountain birch Karlsson and Weih, 2003)	-
Leaf function	Increased leaf photosynthesis and increased leaf N (meta-analysis of woody plants Nykänen and Koricheva, 2004)	No effect in defoliated vines (Dick, 1987; Buwalda and Smith, 1990); shaded vines had decreased photosynthesis (Buwalda and Meekings, 1993)
	Responses to low light = low SLW, high chl. conc, high chl.:N ratio, reduced leaf thickness (Rozendaal et al., 2006)	Chl. conc unaffected by shade, chl a:b ratio higher in exposed leaves (Grant and Ryugo, 1984a)
	Delayed leaf senescence (in defoliated *Prunus* Layne and Flore, 1995)	Premature senescence (Buwalda and Smith, 1990)

LAI = leaf area index; SLW = specific leaf weight; N = nitrogen; conc = concentration; chl. = chlorophyll; NSC = non-structural carbohydrates.

4.2 MATERIALS AND METHODS

4.2.1 Vines and treatment application

Treatments were applied to mature 'Hort16A' kiwifruit vines (*Actinidia chinensis* Planch. var. *chinensis*) growing in three adjacent rows at the Te Puke Research Centre, Plant and Food Research, in the Bay of Plenty, New Zealand (37° 49'S; 176° 19'E). Vines were planted in 1993 and the 'Hort16A' scion was grafted onto existing rootstocks in 1996. The vines had been trained onto a pergola system and each vine had an allocated canopy area of 30 m^2 with a leader length of 6 m and canopy width of 5 m. Vines fully occupied this area during the study period. Treatments were assigned in a randomised design with 7 vines per treatment. Treatments were first applied late spring (December) in the 2003/2004 growing season through until winter of the 2009/2010. The experiment described in this chapter was carried out over three growing seasons: 2007/2008 (referred to as year 1 here), 2008/2009 (year 2) and 2009/2010 (year 3) although note that the experiments for this thesis were undertaken 4, 5 and 6 years respectively since the original treatments were applied.

The control treatment represented typical vine management techniques used at the Te Puke Research Orchard when the treatments were first applied in 2004. The treatment application schedule for 2009-2010 is typical of those used throughout this experiment (Table 4.3).

- **Control.** Crop load was reduced to approximately 40 fruit per m^2 by thinning in late November or early December. Fruiting shoots were pruned to 4 leaves past the last fruit (Figure 4.1). In January and February, any new vegetative growth was removed from the canopy (Figure 4.2A).
- **Famine.** Crop loads were maintained at high levels (typically 60 to 70 fruit per m^2 depending on the season) by carrying out little or no fruit thinning. Leaf-to-fruit ratios were kept low by pruning to the first leaf past the last fruit (Figure 4.1) and cutting back all non-fruiting shoots to a new growing point. Pruning was delayed 7 to 10 days, relative to other treatments allowing shoots to continue utilizing vine resources before removal (Figure 4.2B).
- **Feast.** Leaf-to-fruit ratios were kept high by pruning fruiting shoots to approximately 6 leaves past the last fruit in mid-November (Figure 4.1) and by thinning fruit to 1 per shoot in late November or early December.

None of the treatments received trunk or cane girdling. The feast and famine terminology is from Koch (1996).

Table 4.3 Schedule of 'Hort16A' kiwifruit of canopy management and vine treatments in year 3.

Date	Activity
6 - 24 Oct.	Flowering
13 Nov.	Pruning - leader zone (all treatments), remove unwanted shoots from leader zone, retaining 1 vigorous shoot per cane to be tied down next season
25 Nov.	Fruitlet thinning: control: thin fruit to 40 fruit per m^2, feast: remove fruit so that there is only 1 fruit per fruiting shoot
26 Nov.	Pruning - main canopy (control and feast vines), cut fruiting shoots back to 4 and 6 leaves past last fruit
10 Dec.	Pruning - main canopy (famine vines), remove all blind (non-fruiting) shoots, cut fruiting shoots to 1 leaf past last fruit
8 Jan.	Fruitlet thinning - remedial
12 Jan.	Pruning - main canopy (control and feast vines), remove all regrowth
4 Feb.	Pruning - main canopy (famine), stub all regrowth
18 Feb.	Pruning - main canopy (control and feast vines) remove all regrowth
24 Feb.	Pruning - main canopy (famine) stub all regrowth

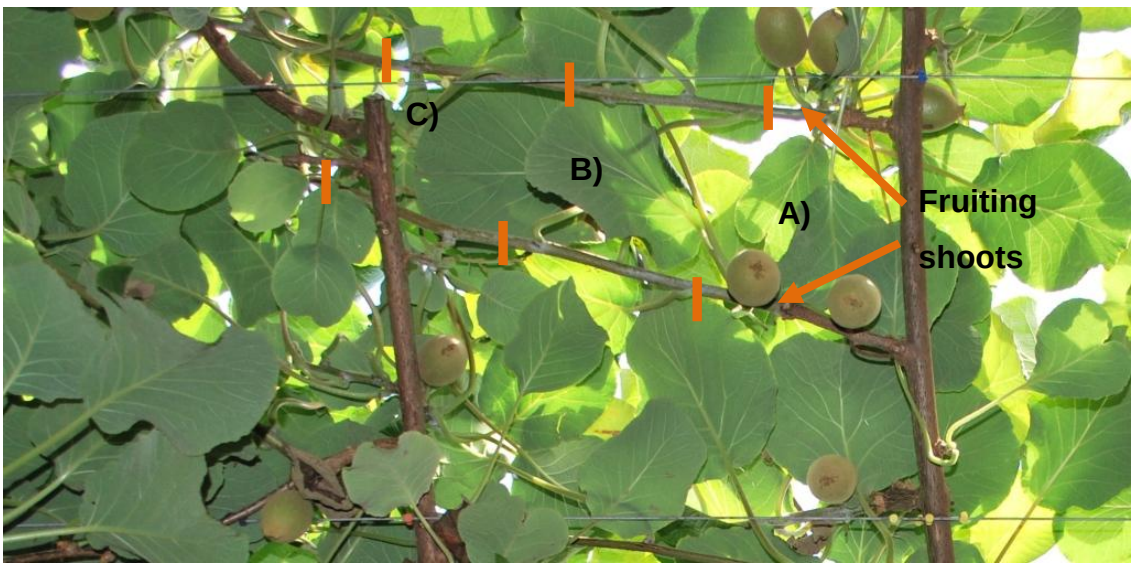

Figure 4.1 November pruning: fruiting shoot cut back to A) 1 leaf past the last fruit in famine treatment, B) cut to ~ 4 leaves past the last fruit in control, and C) to ~ 6 leaves past the last fruit in feast vines.

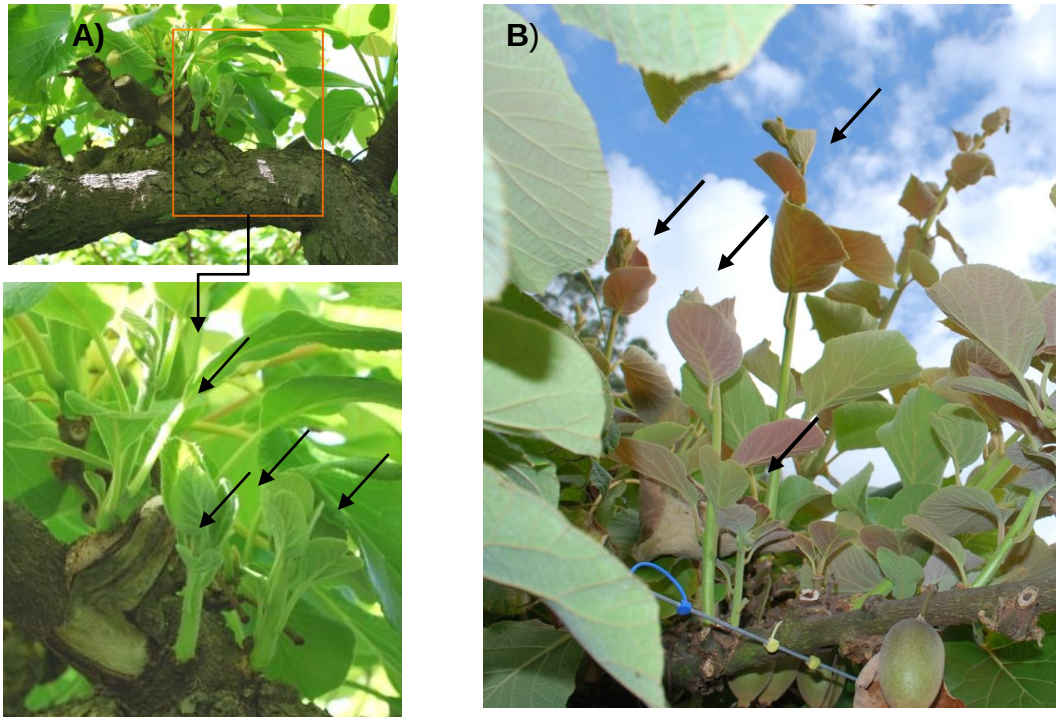

Figure 4.2 A) Summer pruning in control and feast treatments, small non-fruiting shoots, (arrows) were completely removed by hand, B) Summer pruning famine treatment, non-fruiting shoots (arrows) were allowed to grow longer before being cut back (stubbed) to allow regrowth.

4.2.2 General measurements

The experiment was carried out over three years: 1 (2007/2008), 2 (2008/2009) and 3 (2009/2010). The following measurements were made using individual vines as replicates. Unless stated otherwise, measurements were made on all 7 vines per treatment.

- Components of yield, bud break (BB); floral bud break (FBB), vegetative bud break (VBB); flowers per floral shoot (F/FS), and flowers per winter bud (KF/Bud) were measured on 4 canes per vine each spring (October).
- Flowering date for each vine was determined on a subsample of the fruiting canopy measuring ~ 1.2 m x 1.6 m by counting the number of flowers that had opened at 2 to 4-day intervals in seasons 2 and 3 during October. Timing of bud break was measured using the same technique, but in only in August year 3.
- Crop load was determined by counting the number of fruitlets per vine each November.

- Shoot type was determined on the same subsample of the fruiting canopy used for flowering, on 4 vines per treatment. Shoot types were determined in December and January in years 2 and 3 only.
- Leaf attributes (leaf area, leaf number, petiole length; specific leaf weight (SLW, dry weight per unit area, also referred to as leaf mass per are LMA (Poorter et al 2009)) and leaf composition (mineral nutrients, chlorophyll and non-structural carbohydrates (NSC)) were measured on a combined sample of 10 fully-expanded blades and petioles per vine. Samples were taken every 4 to 5 weeks throughout years 1 and 2 and fortnightly in year 3. Individual leaf area and petiole length as a function of node number were measured on 2 shoots per vine sampled destructively in January (years 2 and 3). Chlorophyll, SLW and NSC were measured in years 2 and 3 only. Mineral nutrient remobilisation from leaves in autumn was estimated by comparing leaf contents of each nutrient with those of leaves sampled in January.
- Leaf gas exchange measurements were made on a subsample of 4 vines per treatment at approximately 4 to 6 weekly intervals during years 2 and 3 from late spring (December) until March.
- Fruit mineral nutrient contents were measured on a combined sample of 12 fruit per vine. Fruit were sampled in November/December, February and April in years 1 and 2 and fortnightly between November and April in year 3. Not all samples were analysed.
- Fruit fresh weight, dry weight, firmness, soluble solids concentration and flesh hue angle were measured on a sample of 18 fruit per vine sampled randomly from across the entire canopy at regular intervals as close as possible to commercial harvest each year. Sampling dates were 5, 16 and 21 May in year 1, and every 4 to 5 days between late April and mid-May in years 2 and 3.
- In season 1, fruit storage performance was measured on 90 fruit per vine, sampled randomly from across the entire fruiting canopy at approximately 10-day intervals. In years 2 and 3, an extra 30 fruit per vine were sampled for destructive measurement of fruit softening during storage. Storage samples were taken every 4 to 8 days in year 2 and once or twice as close as possible to 103º hue in year 3.

- Canopy growth was estimated by collecting and weighing all material removed from each vine during pruning and leaf abscission.
- Total vine biomass was measured by excavating 3 vines per treatment between mid-May and mid-June 2010.

Experimental methods are described in Chapter 2.

4.2.3 Statistical analysis

Analyses were carried out using analysis of variance (ANOVA) with individual vines as replicates (see Chapter 2.14 for more details). Data were checked for normality and were log-transformed if necessary. Proportion data were subjected to angular transformation before analysis. Vine excavation data included the factor 'block', to account for the time difference (5 to 7 days between blocks) in excavating vines during leaf-fall. To determine if there was a seasonal change in famine or feast vine attributes compared with control vines, the effects of treatment, year and their interactions were analysed.

A Boltzmann equation (1) was fitted to the relationship between firmness and flesh hue angle on the vine to determine if the relationship was affected by the different treatments.

$$y = A_1 + \frac{(A_2 - A_1)}{1 + e^{(\frac{x - x0}{dx})}} \qquad (1)$$

Where:

y = firmness, x = hue angle, $x0$ = hue at maximum slope; dx = slope at maximum

A_1 = lower asymptote, A_2 = upper asymptote

4.3 RESULTS

4.3.1 Canopy composition

4.3.1.1 Components of yield. The general trend was for the famine vines to produce fewer flowers than the feast vines, with the control vines intermediate between the two (Figure 4.3). The difference in productivity appeared mainly due to changes in the number of flowers per floral shoot (F/FS), rather than changes in the relative amounts of floral and vegetative BB. In seasons 1 and 2 the feast vines produced more king flowers per winter bud than the famine vines, in the third year, there were no treatment differences in flower numbers. Overall there was a trend for reduced BB across the three years whereas F/FS remained relatively consistent across all three years in the control and feast vines, at around 2.5 and 2.8, respectively (Figure 4.3E). In the famine vines, F/FS dropped from 2.2 in season 1 to 1.9 in season 2 (Figure 4.3F) before recovering in year 3.

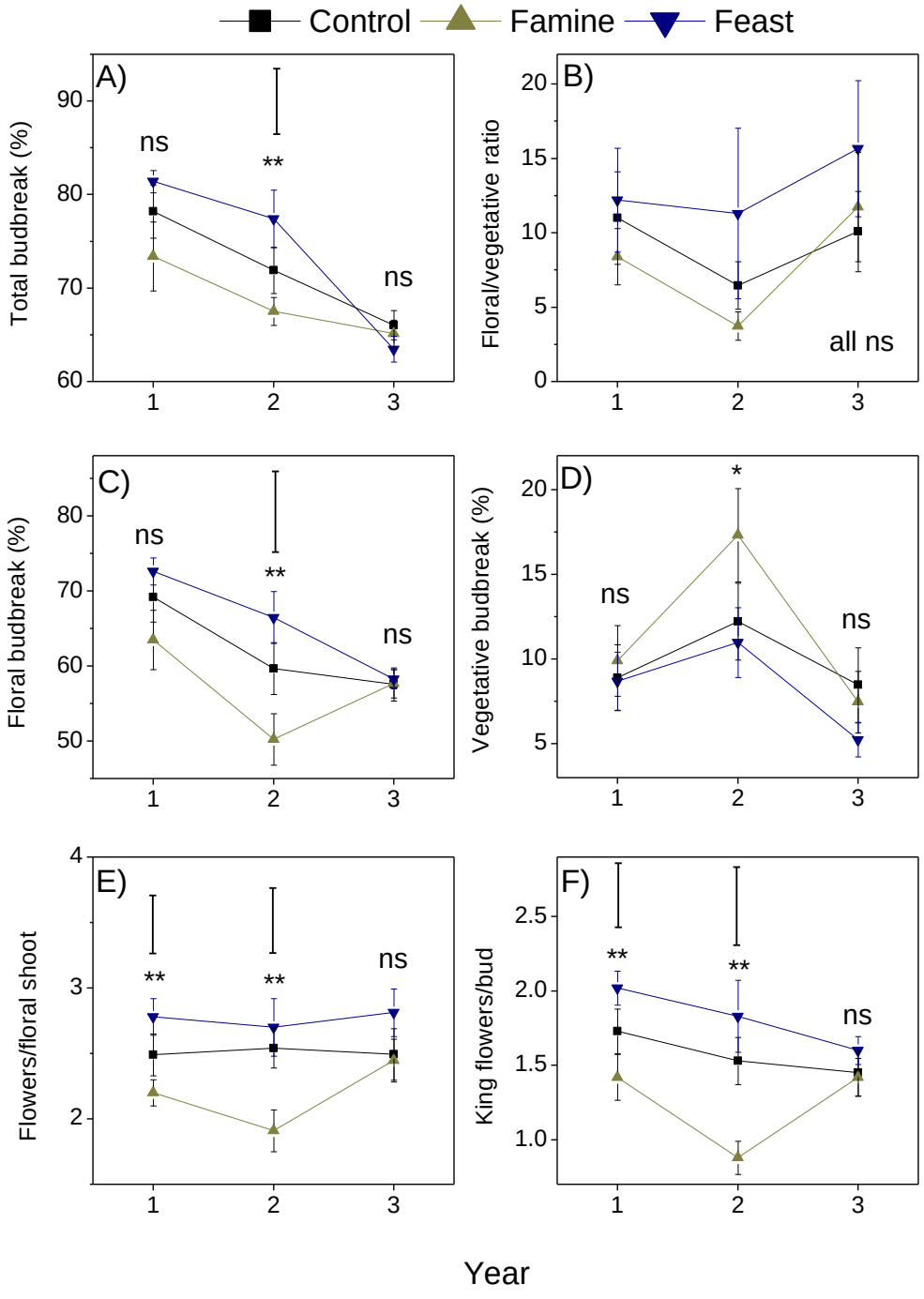

Figure 4.3 A) Total bud break (%); B) ratio of floral to vegetative bud break; C) floral bud break (%); D) vegetative bud break (%); E) flowers per floral shoot and F) king flowers per winter bud, measured over three years in 'Hort16A' vines receiving control, famine and feast treatments; *n* = 7 ± *SE*; ** = *P* ≤ 0.05, * = *P* ≤ 0.10, and ns = *P* > 0.10; vertical bar = $LSD_{0.05}$.

Fruitlet counts carried out before thinning confirmed the lack of treatment differences in productivity in year 3 (Figure 4.4A). It was not possible to maintain high crop loads in the famine vines in season 2, because fruitlet numbers were too low (Figure 4.4B).

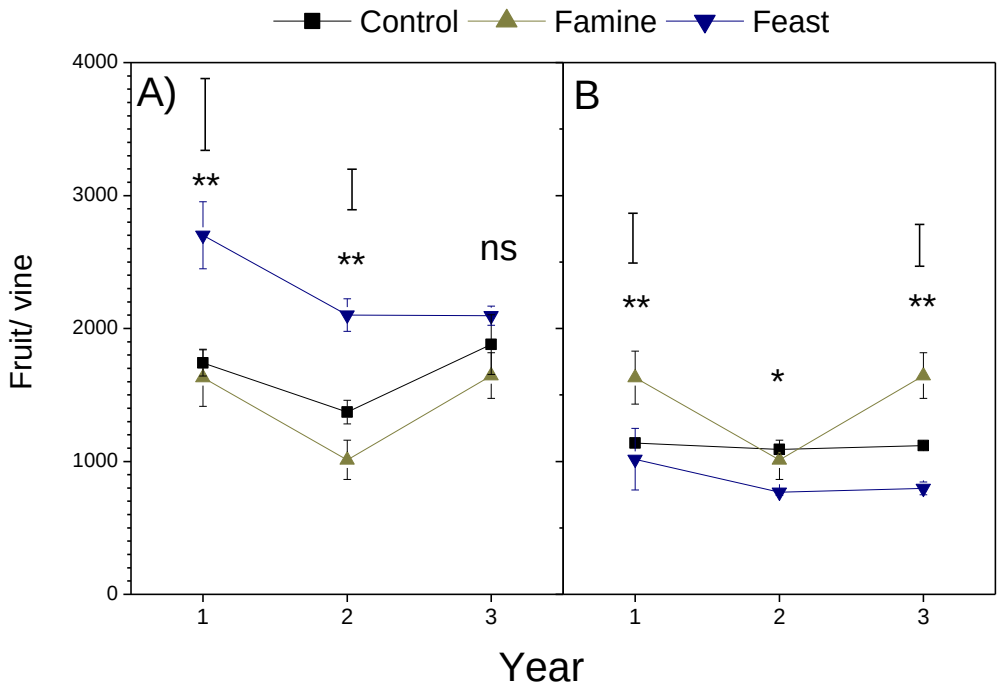

Figure 4.4 Number of fruit counted in 'Hort16A' kiwifruit vines receiving control, famine and feast treatments A) before, and B) after crop load adjustment; *n* = 7 ± *SE*; ** = *P* ≤ 0.05, * = *P* ≤ 0.10, and ns = *P* > 0.10; vertical bar = $LSD_{0.05}$.

4.3.1.2 Shoot types. In season 2, the treatments had no significant effects on the proportion of short, medium or long shoots. In season 3, the famine vines had more short shoots than the feast and control vines and fewer long shoots than the control vines (Table 4.4). Shoot types were not measured in season 1.

Table 4.4 Percentages of different shoot types measured in years 2 and 3 in the canopies of 'Hort16A' kiwifruit vines receiving control, famine and feast treatments.

Shoot type	Year 2			Year 3		
(%)	Control	Famine	Feast	Control	Famine	Feast
Short	57.4 ± 4.6a	61.1 ± 9.0a	57.5 ± 0.9a	**57.2 ± 1.5b**	**70.1 ± 3.0a**	**60.7 ± 1.9b**
Medium	19.5 ± 2.7a	12.3 ± 2.5a	10.3 ± 3.2a	8.9 ± 0.7a	6.2 ± 1.8a	10.7 ± 2.1a
Long	23.1 ± 5.4a	26.6 ± 7.8a	32.2 ± 4.0a	**33.9 ± 1.8a**	**23.5 ± 3.1b**	**28.6 ± 1.4ab**

n = 4 ± SE; values in any row within any one year accompanied by different letters are significantly different (P ≤ 0.05), data highlighted in bold for clarity; short ≤ 9 nodes; medium = 10 - 18 nodes; long > 18 nodes.

4.3.1.3 Key findings – canopy composition.

- In years 1 and 2 the famine vines were less productive than the feast vines, with controls intermediate. Total BB tended to decrease with season across all three treatments. In the control and feast vines, F/FS remained relatively consistent each year at ~ 2.5 and ~ 2.8, respectively. In the famine vines F/FS dropped to 1.9 in year 2 and recovered in year 3, when there were no treatment differences in productivity.
- In season 3, the famine vines had more short shoots and fewer long shoots that the control and feast vines, although in season 2 there were no treatment differences in shoot type. Shoot type was not measured in year 1.

4.3.2 Leaf attributes

4.3.2.1 Leaf physical characteristics. Leaf area and petiole length were unaffected by treatment (Figure 4.5). Petioles were longer in season 3 than in season 2 (Figure 4.5B). The relationship between node number and leaf area or petiole length appeared to be consistent across the treatments (Figure 4.6).

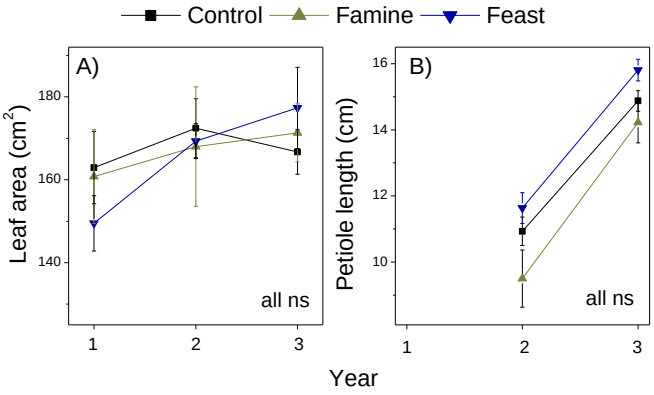

Figure 4.5 A) Mean leaf area, and B) mean petiole length in leaves sampled from 'Hort16A' kiwifruit vines receiving control, famine and feast treatments; *n* = 4 ± *SE*; ns = *P* ≥ 0.05.

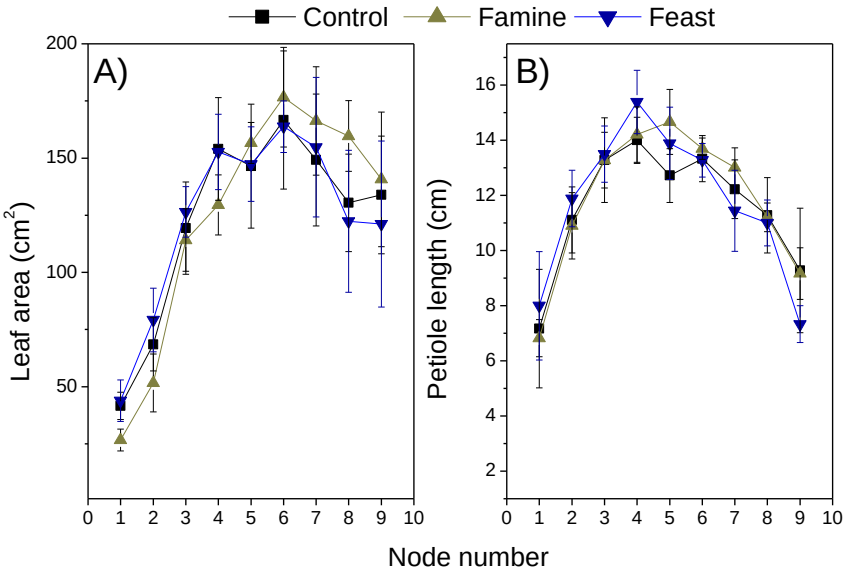

Figure 4.6 Mean leaf area (A), and mean petiole length (B) with node number in short shoots sampled in year 2 from 'Hort16A' kiwifruit vines receiving control, famine and feast treatments; *n* = 4 ± *SE*.

There was a trend for the blades of the famine leaves to have higher DMC and SLW than leaves from the feast vines with the control leaves intermediate between the two (Figure 4.7).

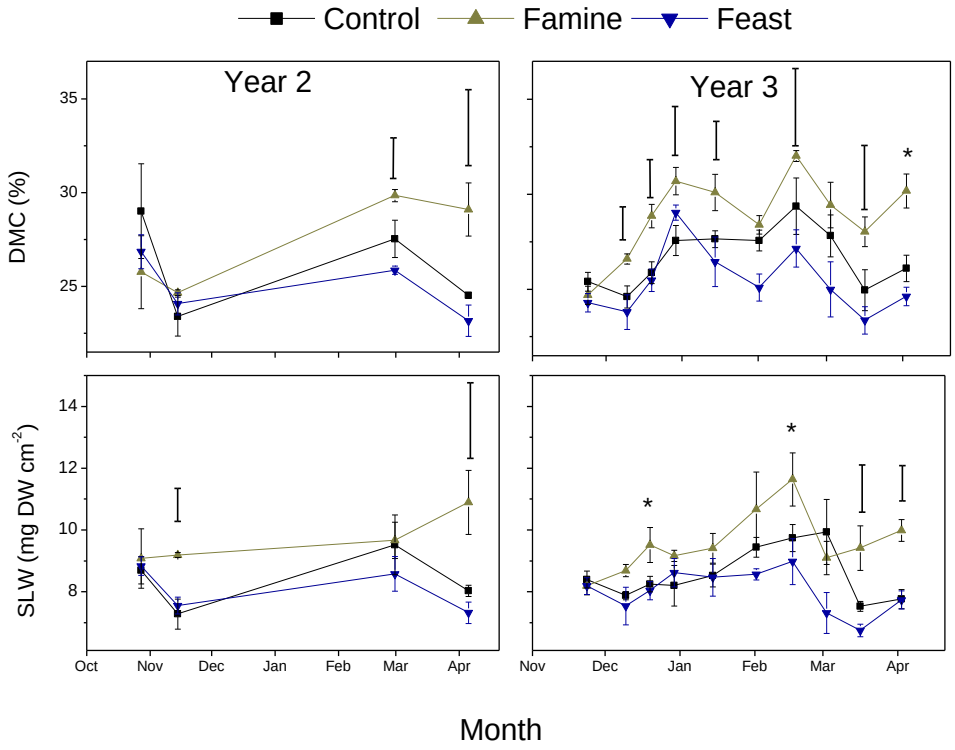

Figure 4.7 Dry matter concentration (DMC) and specific leaf weight (SLW) in leaf blades sampled in years 2 (left) and 3 (right) from 'Hort16A' kiwifruit vines receiving control, famine and feast treatments;; *n* = 4 ± *SE*; vertical bar = $LSD_{0.05}$ present only if $P \leq 0.05$; * = $P \leq 0.10$, if $P > 0.10$ data unaccompanied.

4.3.2.2 Leaf mineral nutrient status.

Standard industry method of sampling and analysis. The effect of treatment, sampling date and their interactions on seasonal accumulation of mineral nutrients in leaves and petioles was determined on the samples taken in the third season of the experiment (Table 4.5; Figure 4.7). Sampling date affected the concentration of all mineral nutrients (Table 4.5). The nutrients could loosely be classed into different groups depending on treatment effects and their interactions with sampling date:

- K concentration was highest in the feast leaves, lowest in the famine leaves throughout the season, more so at the end of the season.
- N, Ca and Mn were generally not affected by the treatments, but significance varied with sampling date. Specifically, N concentration was lower in the famine leaves than the feast and control vines (early-season only), Ca and Mn concentrations were higher in the famine leaves than leaves from the other treatments (late-season only).
- Concentrations of P, Cu and Fe were affected by treatment, and there were no significant interactions with date. Concentrations were higher in feast leaves than famine leaves with control leaves intermediate.
- Concentrations of S, Mg, Zn and B were not affected by the treatments.

Table 4.5 Effect of sampling date, treatment (control, famine or feast) and their interactions on mineral nutrient concentrations in 'Hort16A' kiwifruit vines.

	P-value		
Nutrient	Date	Treatment	Date x treatment
N	<0.001	0.138	**0.028**
P	<0.001	**0.010**	0.091
K	<0.001	**0.005**	**0.049**
S	<0.001	0.209	0.859
Ca	<0.001	0.594	**0.003**
Mg	<0.001	0.889	0.231
Mn	<0.001	0.634	**<0.001**
Zn	<0.001	0.661	0.215
Fe	0.001	**0.035**	0.191
Cu	<0.001	**0.011**	0.169
B	<0.001	0.074	0.127

Leaves and petioles were sampled across the 2009/2010 growing season, n = 4.

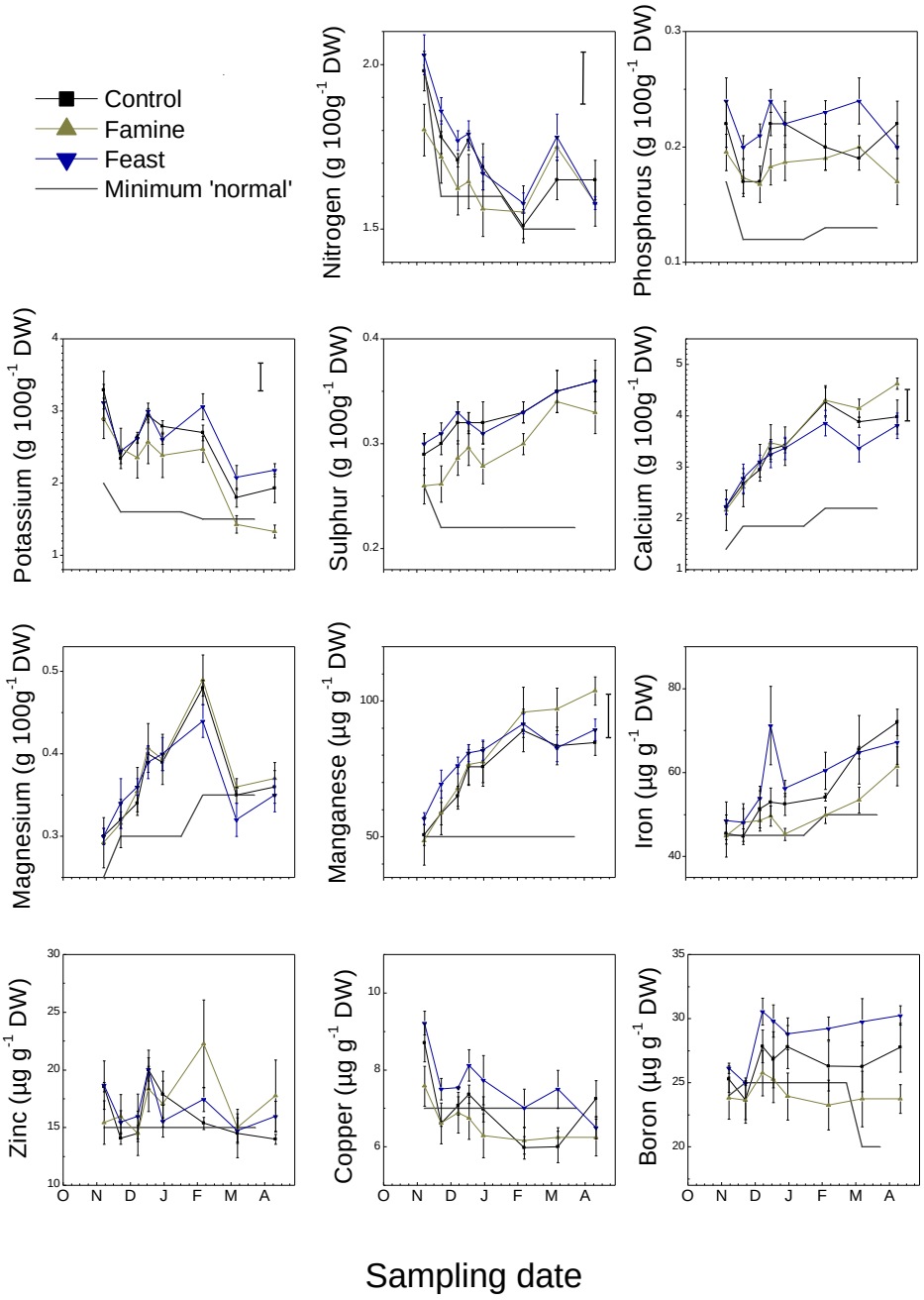

Figure 4.8 Mineral nutrient concentrations measured across year 3 in leaves from 'Hort16A' kiwifruit vines receiving control, famine and feast treatments; *n* = 4 ± *SE*. The solid line is the minimum 'normal' for leaf samples from RJ Hill Laboratories). When significant interactions between treatment and sampling date occur, the vertical bar represents $LSD_{0.05}$.

Leaf concentrations of N in spring, and autumn leaf K, Ca and Mn concentrations were then compared across all three seasons to see if the differences detected in season 3 were consistent across all three seasons (Table 4.6; Figure 4.9). Concentrations of N, K, Ca and Mn were affected by season and treatment, but there were no interactions between season and treatment (Table 4.6).

Table 4.6 Effect of year, treatment (control, famine and feast) and their interactions, on nitrogen, potassium, calcium and manganese concentrations in 'Hort16A' kiwifruit leaves.

Mineral nutrient and time of year	*P*-value		
	Year[a]	Treatment	Year x treatment
Nitrogen (spring)	**< 0.001**	**0.008**	0.509
Potassium (autumn)	**0.003**	**<0.001**	0.128
Calcium (autumn)	**< 0.001**	**0.027**	0.119
Manganese (autumn)	**< 0.001**	**0.016**	0.218

[a]*Measured in years 2 and 3.*

Spring N concentrations were consistently lowest in leaves from the famine vines (Figure 4.9A). In season 3 when concentrations were lower in all three treatments, the famine N concentrations were below the normal range. April K concentrations in the famine leaves were at or below the normal range each season (Figure 4.9B). Leaves from the famine vines in April displayed some symptoms typical of K deficiency (curling and necrosis of the leaf margins; Smith et al., 1987a; Figure 4.10A). April Ca and Mn concentrations were consistently highest in leaves from the famine vines (Figure 4.9C and D).

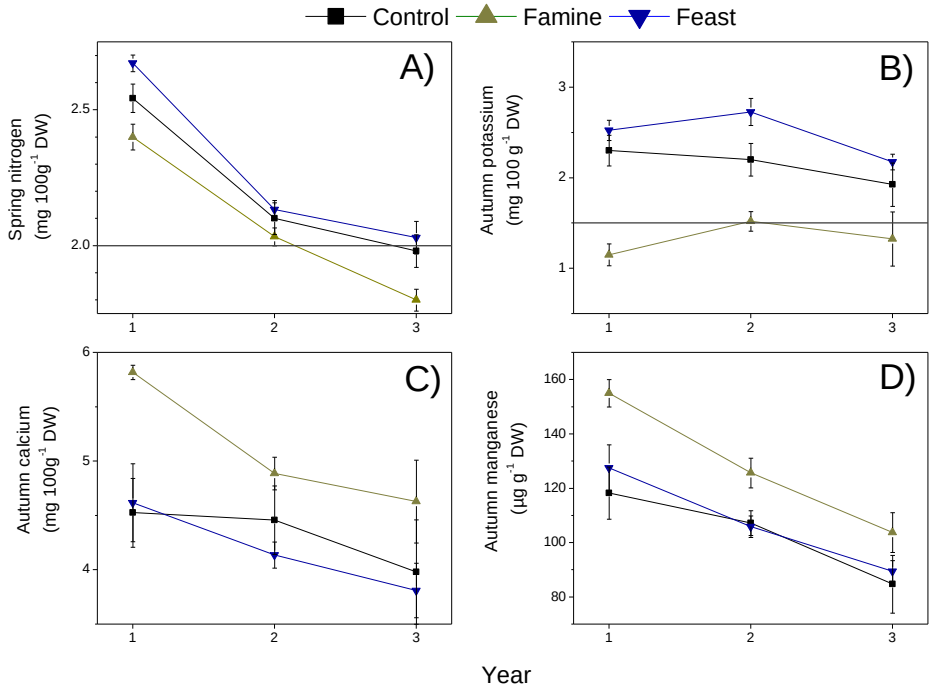

Figure 4.9 Mineral nutrient concentrations of leaves sampled in years 1 to 3 from 'Hort16A' kiwifruit vines receiving control, famine and feast treatments; *n* = 4 ± *SE*. The spring sample was taken in November and the autumn samples in April. The horizonal line is the minimum 'normal' recommended by RJ Hill Laboratories.

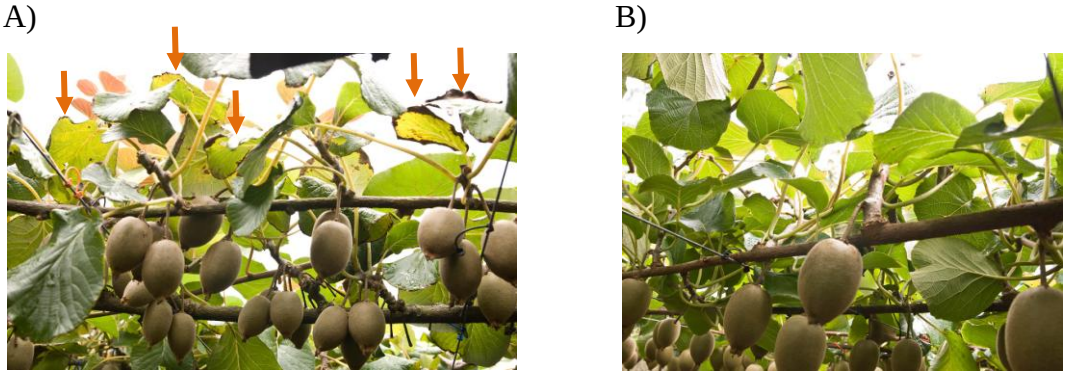

Figure 4.10 A) Possible symptoms of potassium deficiency, curling and necrosis in leaf margins (arrows), visible in the famine vines in April year 1, compared with B) canopy of the adjacent feast vine photographed on the same day.

Mineral nutrients expressed on a leaf area basis. Several treatment differences were consistent across the two seasons (Table 4.7; Figure 4.11), leaf blade P per unit area was lower in the famine vines than the feast and control vines early in the season, but not in autumn (Figure 4.11). The famine vines had consistently higher N, Ca, Mg and Mn per unit area than leaves from the other treatment vines at the end of the season but not at the start of the season. There were no consistent differences in K per unit area (Table 4.7).

Table 4.7 Effect of control, famine and feast treatments on leaf blade mineral nutrient contents expressed on a leaf area basis in 'Hort16A' kiwifruit vines.

	P-value			
	Year 2		Year 3	
Nutrient	Nov/Dec	April	Nov/Dec	April
N	**0.029**	**0.010**	**0.062**	**0.003**
P	**0.033**	0.423	**0.004**	0.512
K	**0.074**	0.304	0.971	**0.069**
S	0.483	**0.019**	**0.035**	0.117
Ca	0.244	**0.002**	0.828	**0.002**
Mg	0.156	**0.004**	0.710	**0.020**
Mn	0.435	**0.006**	0.119	**<0.001**
Zn	**0.038**	**0.053**	0.305	**0.074**
Fe	0.473	0.790	0.730	0.252
Cu	0.742	**0.052**	0.189	**0.099**
B	**0.053**	0.511	0.859	0.536

n = 4; P-values ≤ 0.10 are highlighted in bold.

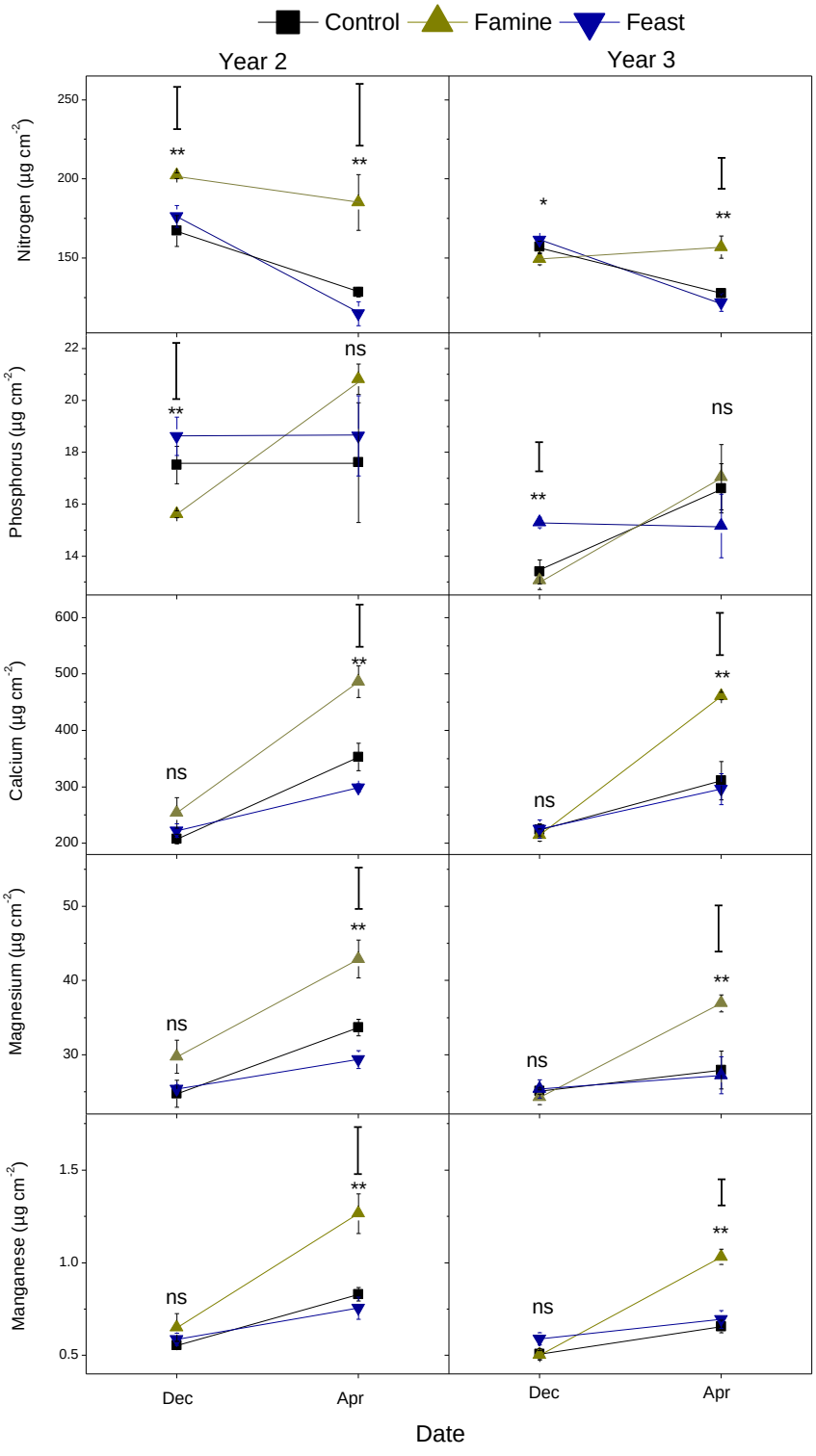

Figure 4.11 Nitrogen, phosphorus, calcium, magnesium and manganese contents (calculated on an area basis) in leaf blades sampled in spring and autumn of years 2 (left) and 3 (right) from 'Hort16A' kiwifruit vines receiving control, famine and feast treatments, *n* = 4 ± *SE*; ** = *P* ≤ 0.05,* = *P* ≤ 0.10, and ns = *P* > 0.10; vertical bar = $LSD_{0.05}$.

Late season mineral nutrient remobilisation and leaf senescence. There was a tendency for leaf blade and petiole N and P contents to decrease more and K less, in feast vines than the famine vines towards the end of the season (Figure 4.12).

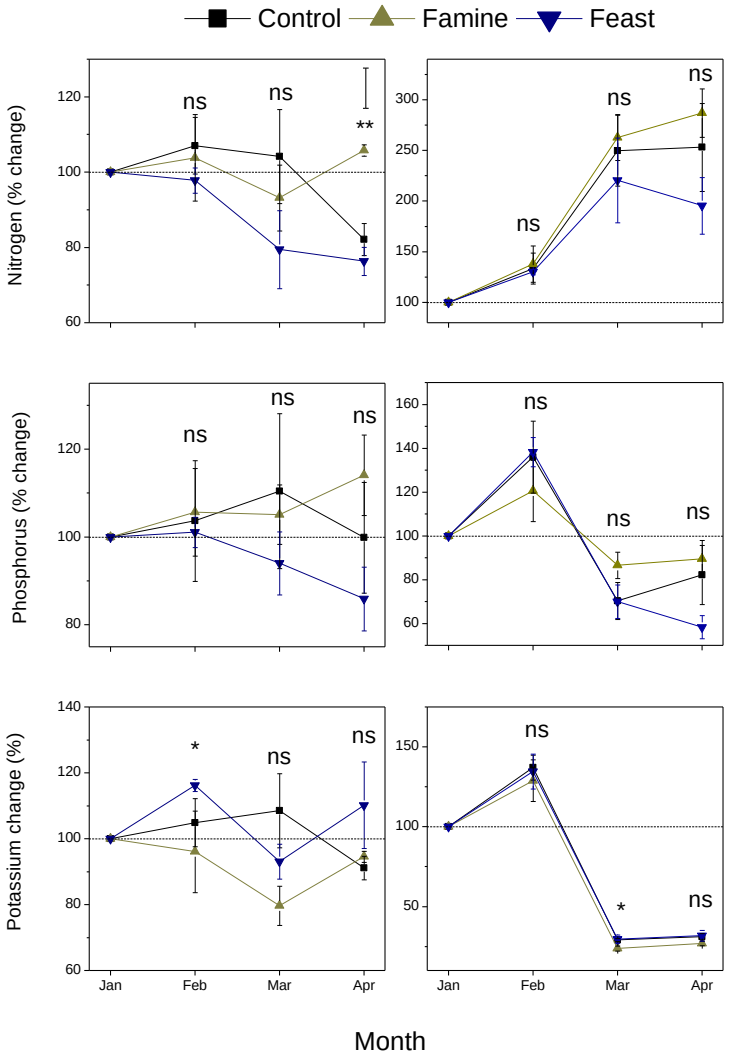

Figure 4.12 Change in leaf blade (left) and petiole (right) contents of nitrogen, phosphorus, and potassium in year 3 relative to contents in January (set at 100%) from 'Hort16A' kiwifruit vines receiving control, famine and feast treatments; *n* = 4 ± *SE*; ** = *P* ≤ 0.05,* = *P* ≤ 0.10, and ns = *P* > 0.10; vertical bar = $LSD_{0.05}$

4.3.2.3 Gas exchange measurements. No treatments differences in net carbon dioxide exchange rate (NCER), stomatal conductance (g_s) and internal CO_2 concentration (C_i) were detected at ambient conditions (Figure 4.12).

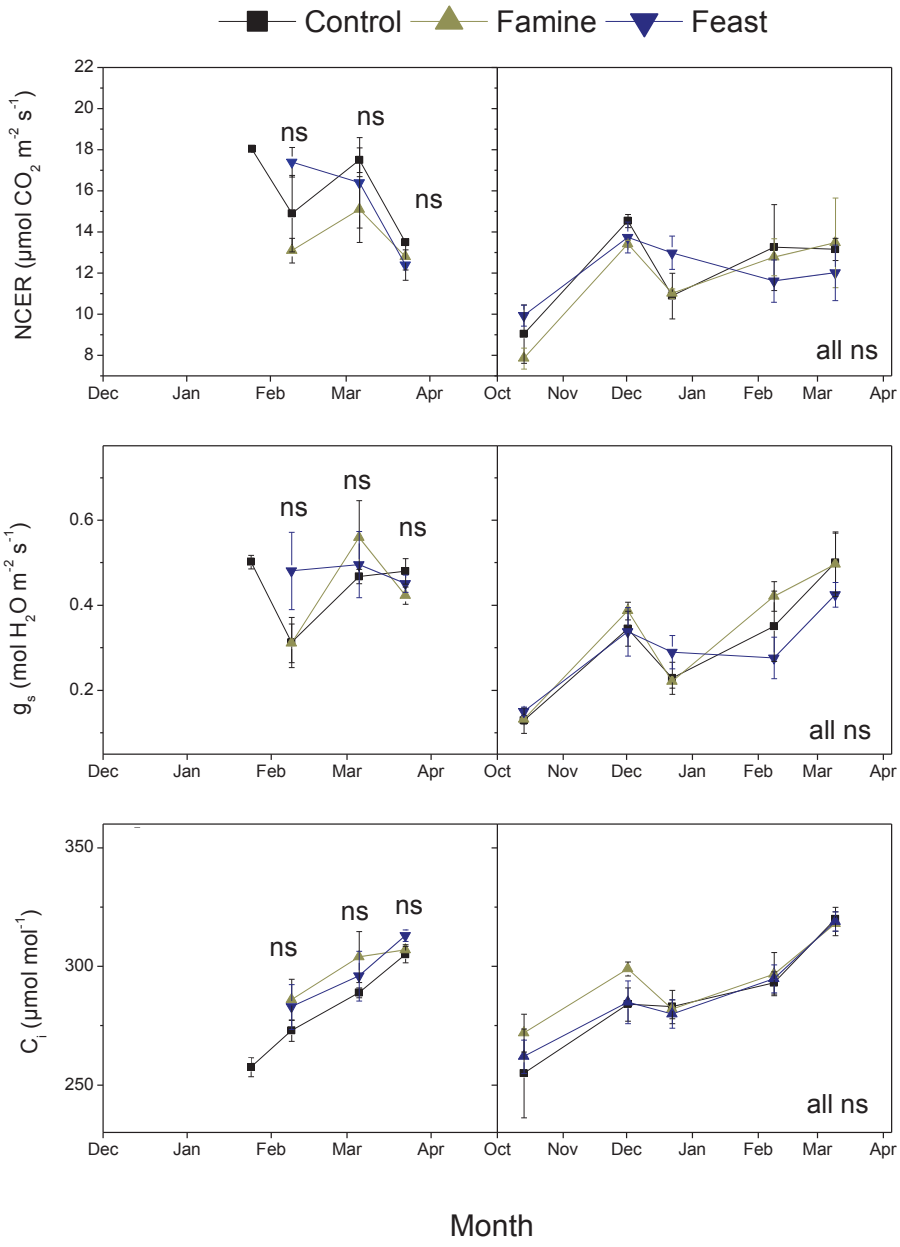

Figure 4.13 Net carbon dioxide exchange rate (NCER), stomatal conductance (g_s) and internal CO_2 concentration (C_i) measured in years 2 (left) and 3 (right) in leaves from 'Hort16A' kiwifruit vines receiving control, famine and feast treatments; $n = 4 \pm SE$; ns = $P > 0.100$.

4.3.2.4 Carbohydrates and chlorophyll. There were no consistent differences in leaf carbohydrate concentrations between treatments (Figure 4.14). In late-summer of year 3, starch concentrations tended to be higher than in year 2, and sugar concentrations lower in the famine leaves than the control and feast leaves (Figure 4.14). This trend was not seen in year 2 when starch concentrations, in particular, tended to be low in all leaves throughout the season.

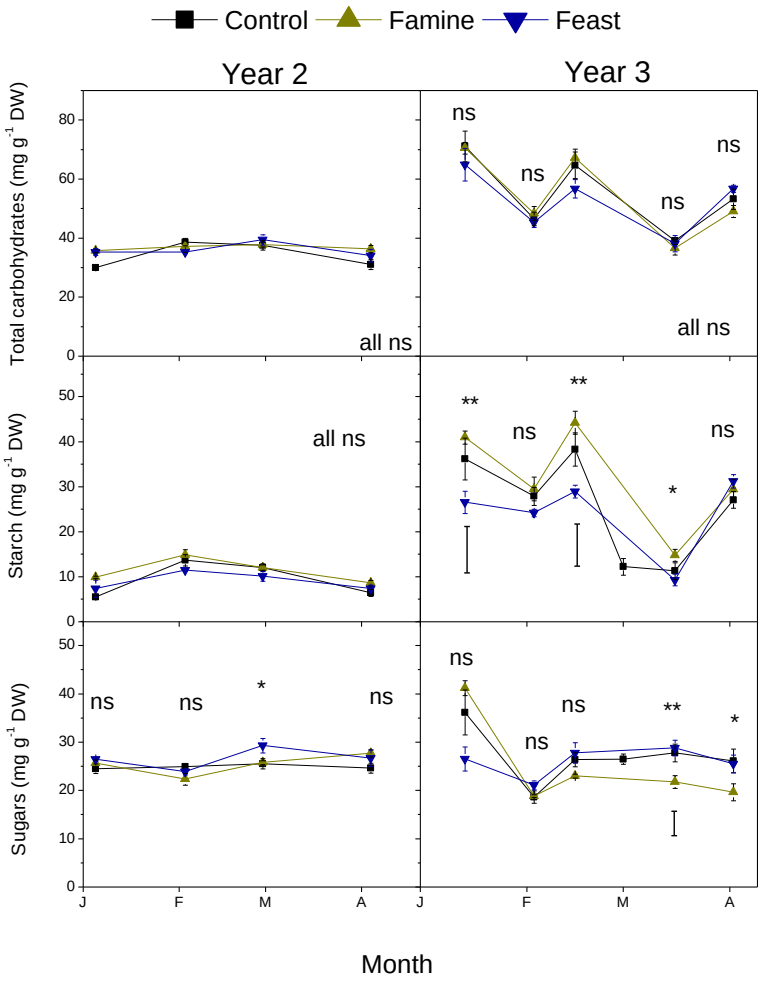

Figure 4.14 Concentrations of A) non-structural carbohydrates (starch and soluble sugars), B) starch, and C) soluble sugars, measured in years 2 and 3 in leaves sampled from 'Hort16A' kiwifruit vines receiving control, famine and feast treatments; ** = $P \leq 0.05$,* = $P \leq 0.10$, and ns = $P > 0.10$; vertical bar = $LSD_{0.05}$.

Leaf chlorophyll contents (measured on a leaf area basis) were not affected by the treatments (Table 4.8). When leaf chlorophyll was measured on a concentration basis alone, and as a ratio to total N concentration in the leaves, differences occurred towards the end of the season when both the chlorophyll concentrations and chlorophyll/N ratio were lower in the famine leaves than the feast leaves, with leaves from the control vines intermediate between the two (Table 4.8).

Table 4.8 Chlorophyll contents in leaf blades sampled from 'Hort16A' kiwifruit vines receiving control, famine and feast treatments.

	Year 2			Year 3		
	Control	Famine	Feast	Control	Famine	Feast
Date	Chlorophyll (μg cm^{-2})					
Nov.	28.8 ± 3.2a	33.4 ± 5.5a	30.5 ± 0.6a	20.7 ± 1.5a	19.9 ± 0.3a	21.1 ± 1.3a
Jan.	32.4 ± 5.7a	29.4 ± 1.5a	34.6 ± 2.3a	30.3 ± 0.3a	28.7 ± 1.4a	29.9 ± 5.2a
Apr.	27.3 ± 0.9a	30.8 ± 3.5a	29.1 ± 4.3a	30.6 ± 1.0a	31.2 ± 1.5a	34.7 ± 2.4a
	Chlorophyll (mg g^{-1} DW)					
Nov.	4.8 ± 0.9a	4.8 ± 0.6a	4.7 ± 0.9a	4.7 ± 0.5a	3.4 ± 0.3a	4.9 ± 0.6a
Jan.	7.0 ± 0.3a	4.8 ± 0.3a	6.8 ± 0.4a	5.1 ± 0.3a	4.5 ± 0.2a	5.3 ± 0.7a
Apr.	**3.4 ± 0.1ab**	**2.8 ± 0.1b**	**3.9 ± 0.4a**	**5.7 ± 0.4a**	**4.5 ± 0.3b**	**6.4 ± 0.4a**
	Chlorophyll/N ratio					
Nov.	0.18 ± 0.01a	0.17 ± 0.03a	0.17 ± 0.01a	0.22 ± 0.01a	0.17 ± 0.01a	0.21 ± 0.02a
Jan.	0.12 ± 0.02a	0.08 ± 0.01a	0.14 ± 0.01a	0.22 ± 0.02a	0.19 ± 0.02a	0.19 ± 0.03a
Apr.	**0.21 ± 0.01ab**	**0.17 ± 0.01b**	**0.25 ± 0.02a**	**0.26 ± 0.01a**	**0.19 ± 0.01b**	**0.30 ± 0.01a**

Data from any one sampling date accompanied by different letters are significantly different ($P \leq 0.05$) and are highlighted in bold for clarity; n = 4 ± SE.

4.3.2.5 Key findings – leaf attributes

- Treatments did not affect leaf area or petiole length in years 2 and 3.
- Leaves from the famine vines tended to have higher DMC and SLW than leaves from the feast vines with leaves from the control vines intermediate between the two.
- When measured using the industry standard method of leaf and petiole sampling and analysis, leaves from famine vines had lower concentrations of N in spring, lower concentrations of K in autumn and higher concentrations of Ca and Mn in autumn than leaves from feast vines with leaves of control vines

intermediate between the two. When measured on a leaf area basis the famine leaves had the same or higher N per cm^2 as leaves from the other treatments. At the end of the season the famine leaves had higher Ca, Mg and Mn per cm^2 than leaves from the other treatments.

- Leaf chlorophyll contents (calculated on an area basis), leaf concentrations of NSC, photosynthetic capacity, stomatal conductance and internal CO_2 concentrations were not affected by the treatments.
- In autumn, leaves from famine vines had lower chlorophyll concentrations, lower chlorophyll:N ratio and higher N per unit leaf area than leaves from the feast and control vines.

4.3.3 Fruit attributes

4.3.3.1 Fresh weight and dry weight. At harvest, fruit from the feast vines were larger with higher DMC than fruit from the famine vines, with the control fruit intermediate between the two (Figure 4.15). The difference between the feast and famine fruit FW was 20 to 30 g and ~ 1 % - unit for DMC. In year 1, control fruit FW was 10 g less than the feast fruit, and this difference was not detected in years 2 and 3. The FW of control fruit was 20 g greater than in the famine fruit in seasons 1 and 3, in season 2 the difference was 11g. In year 3 control fruit had higher DMC than the famine fruit, but not in years 1 and 2.

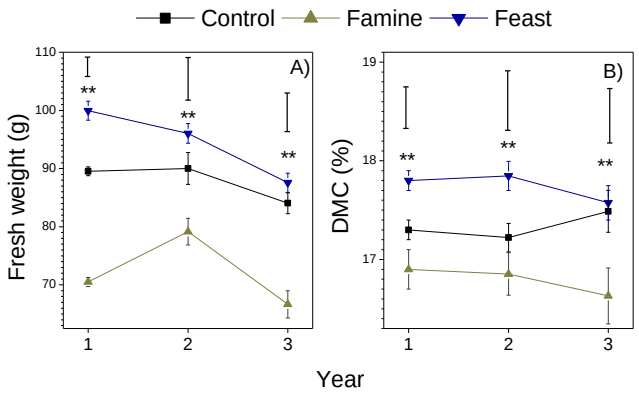

Figure 4.15 Fruit A) fresh weight and B) dry matter concentration (DMC) sampled at harvest over three years from 'Hort16A' kiwifruit vines receiving control, famine and feast treatments; *n* = 7 ± SE; ** = *P* ≤ 0.05, vertical bar = $LSD_{0.05}$.

4.3.3.2 Fruit mineral nutrient contents.

Calculated on a per-fruit basis. The main findings were (Figure 4.16):

- Fruit from the feast vines contained more N, P, K, S, Mg and Cu than fruit from the famine vines, with fruit from the control vines intermediate.
- Fruit from the feast vines tended to contain more Fe and B than fruit from the famine vines with the control vines intermediate, but differences were not consistent across the three seasons
- There were few differences in fruit contents of Ca, Mn or Zn among the treatments

Calculated on a concentration dry weight basis. The following patterns were observed (Figure 4.17):

- Ca, Mn and Zn concentrations were higher in the famine fruit than those from the feast or control vines.
- There were no consistent differences in the concentration of N, K, S, Mg, Cu and B.
- Concentrations of P and Fe were not affected by the treatments.

The Ca to N ratio was consistently higher in fruit from the famine vines than in fruit from the feast and control vines (Table 4.9).

Table 4.9 Ratio of calcium (Ca) to nitrogen (N) in mature fruit sampled over three years from 'Hort16A' kiwifruit vines receiving control, famine and feast treatments.

	Ca/N ratio			
Year	Control	Famine	Feast	*P*-value
1	0.126 ± 0.008b	0.171 ± 0.011a	0.104 ± 0.002b	< 0.001
2	0.142 ± 0.010b	0.189 ± 0.014a	0.104 ± 0.006c	< 0.001
3	0.123 ± 0.008b	0.173 ± 0.005a	0.099 ± 0.010b	0.002

n = 7 ± SE; values in any row accompanied by different letters are significantly different (P ≤ 0.05).

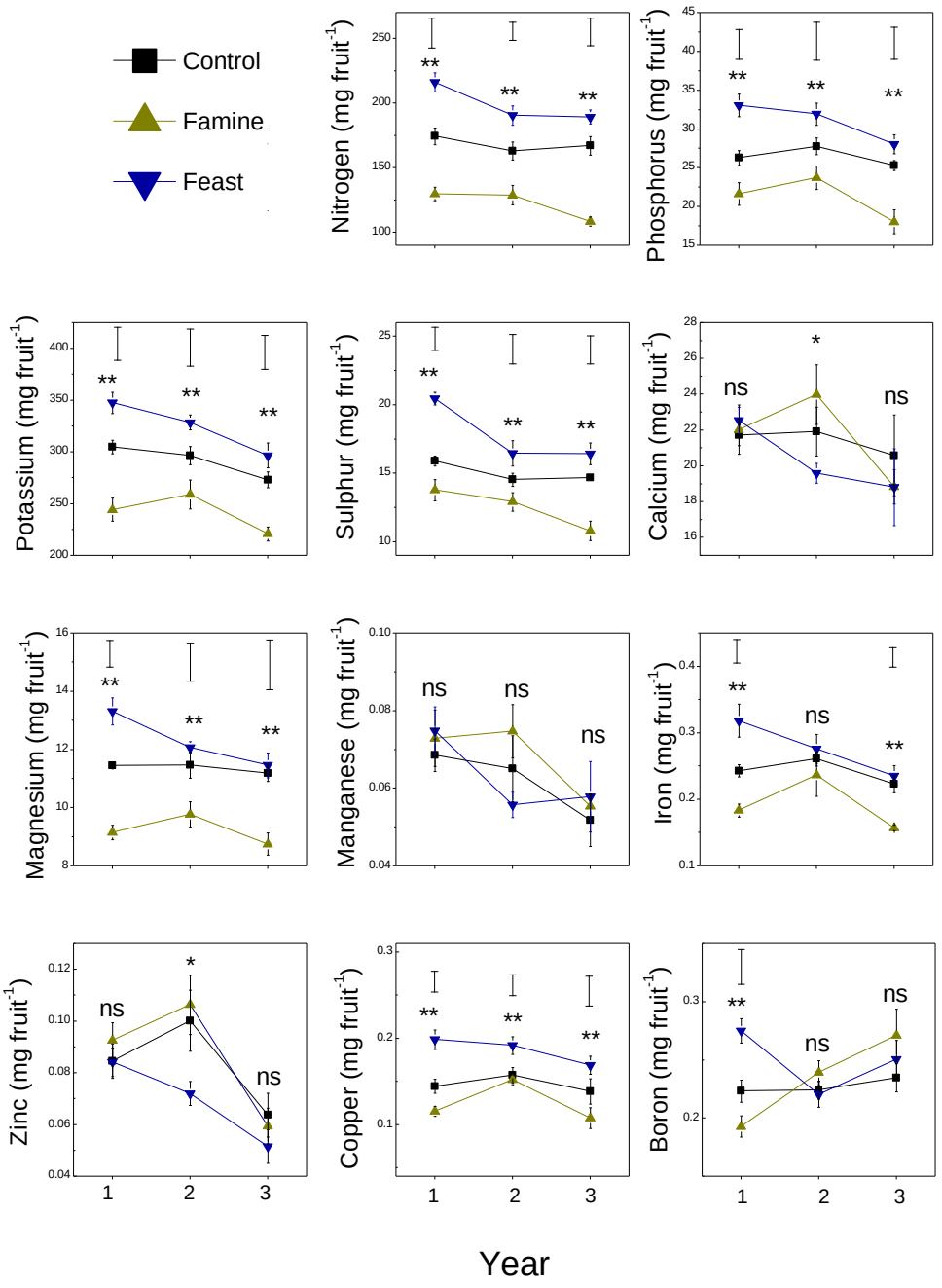

Figure 4.16 Mineral nutrient contents of mature 'Hort16A' kiwifruit sampled each year for three years from vines receiving control, famine and feast treatments; *n* = 7 ± *SE*; ** = *P* ≤ 0.05,* = *P* ≤ 0.10, and ns = *P* > 0.10; vertical bar = $LSD_{0.05}$.

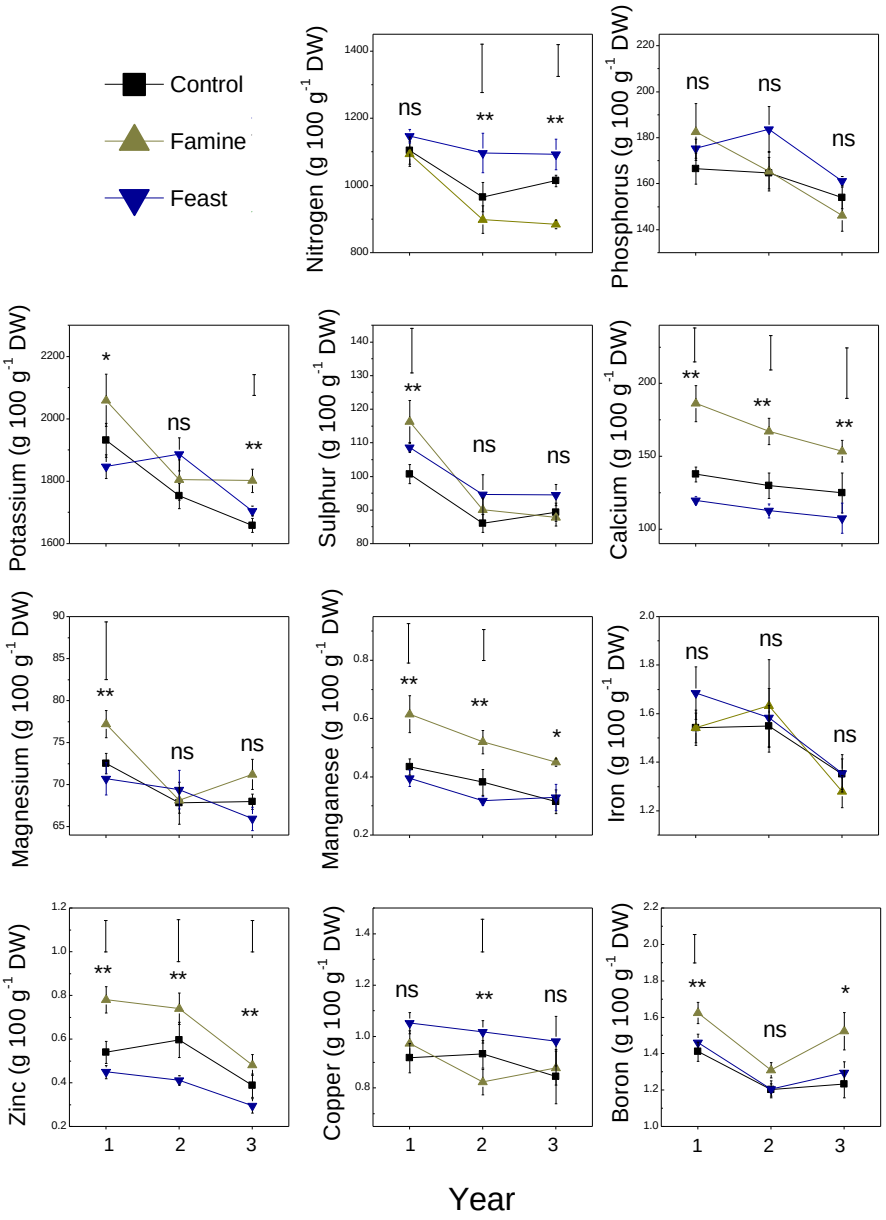

Figure 4.17 Mineral nutrient concentrations in mature 'Hort16A' kiwifruit sampled each year for three years from vines receiving control, famine and feast treatments; *n* = 7 ± *SE*; ** = *P* ≤ 0.05,* = *P* ≤ 0.10, and ns = *P* > 0.10; vertical bar = $LSD_{0.05}$.

4.3.3.3 Flowering. In season 2, treatments affected the timing, but not the duration, of flowering (Table 4.10). Mid-bloom occurred on 15, 17 and 19 October in the feast, control and famine vines respectively, and lasted approximately 8 days. In season 3, neither timing, nor duration of flowering was affected by the treatments (Table 4.10). In the first season the duration and timing of flowering was not measured.

Table 4.10 Timing and duration of flowering in years 2 and 3 in 'Hort16A' kiwifruit vines receiving control, famine and feast treatments.

		Time of flowering			
Treatment	Season	Start	Mid-bloom	End	Duration
Control	2	**13.3 ± 0.6c**	**17.3 ± 0.8b**	**21.5 ± 0.8ab**	7.9 ± 0.6a
Famine	2	**15.2 ± 0.4b**	**19.3 ± 0.4a**	**23.1 ± 0.6a**	7.8 ± 0.6a
Feast	2	**11.9 ± 0.4a**	**15.4 ± 0.2c**	**20.1 ± 0.4b**	8.2 ± 0.6a
P-value		**<0.001**	**<0.001**	**0.008**	0.911
Control	3	14.1 ± 0.8a	18.1 ± 0.8a	22.2 ± 0.8a	8.1 ± 0.5a
Famine	3	13.7 ± 1.1a	17.8 ± 0.9a	21.9 ± 1.1a	8.4 ± 1.1a
Feast	3	12.6 ± 0.9a	17.4 ± 0.5a	22.5 ± 0.4a	10.0 ± 1.1a
P-value		0.403	0.817	0.377	0.216

Values in any column for any season accompanied by different letters and highlighted in bold are significantly different P < 0.05; n = 7 ± SE. Start, mid and end are days after 30 September when 10, 50 and 90% of flowers are open respectively. Duration = number of days between start and end of flowering.

4.3.3.4 Fruit maturity at harvest. When fruit from all three treatments were harvested on the same day, as close as practicable to when the control fruit had degreened (mean hue angle ≤ 103^{o}), famine fruit were the least mature (lower SSC, higher hue and firmer), feast fruit were the most mature with control fruit intermediate between the two (Figure 4.18). The magnitude of difference between feast and famine treatments was approximately 2.3 to 3.6 % - units SSC, 2.2 to 6.2 hue degrees and 5 to 15 N, depending on the season.

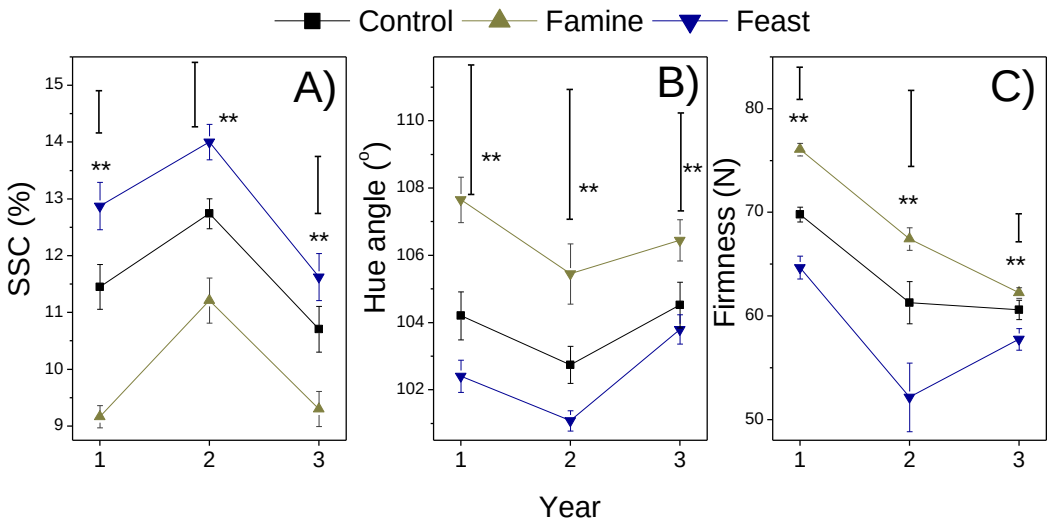

Figure 4.18 A) Soluble solids concentration (SSC) at normal harvest date for control fruit, B) flesh hue angle and C) firmness measured over three seasons in fruit from 'Hort16A' kiwifruit vines receiving control, famine and feast treatments; *n* = 7 ± *SE*; ** = *P* ≤ 0.05, vertical bar is $LSD_{0.05}$.

Fruit from the famine vines degreened, and were therefore cleared for main harvest, 5 to 9 days later than the control fruit depending on the season. This value was estimated from Figure 4.19, column B (summarised in Table 4.11). When cleared for harvest, fruit from the famine vines were likely to have similar SSC to the control vines (estimated from Figure 4.18, column A) and be as firm as, or softer than the control fruit (estimated from Figure 4.18, column C). In season 2, for example, the control fruit had a mean firmness 60 N and SSC 12.2 % on 7 May when they were cleared to pick. Nine days later the famine fruit were cleared for harvest with mean firmness 48 N and SSC 12.3 % (Table 4.11).

Similar estimates were not possible for fruit from the feast treatment: in seasons 1 and 2 sampling did not start until the feast fruit had already degreened, and in season 3 fruit sampling started early but there were insufficient fruit remaining on the feast vines to continue monitoring once fruit had degreened (Figure 4.19). From the

existing data in Figure 4.18, it was estimated that fruit from the feast vines degreened at least 5 days earlier than the control fruit in seasons 1 and 2 and within 5 days of the control fruit in season 3.

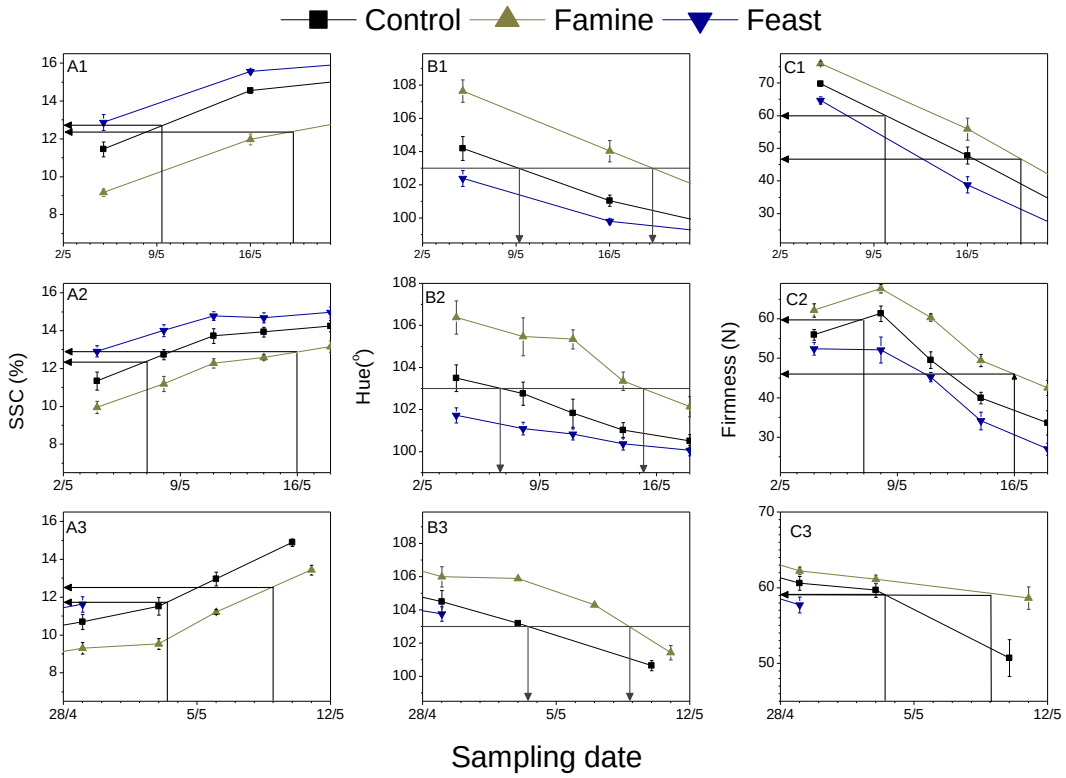

Figure 4.19 Soluble solids concentration (SSC; column A), flesh colour (column B) and firmness (column C) in fruit from 'Hort16A' kiwifruit vines receiving control, famine and feast treatments. Measurements were made across three years (rows 1 to 3 respectively); *n* = 7 ± *SE*. Downward arrow = estimated date when mean hue angle reached 103°, and left-facing arrow = estimated SSC and firmness when hue = 103°.

Table 4.11 Estimated harvest date[a], firmness and soluble solids concentration (SSC) at harvest in fruit sampled from 'Hort16A' kiwifruit vines receiving control, famine and feast treatments.

Treatment	Season	Degreening[a]		Firmness (N) when degreened[a]	SSC (%) when degreened[a]
		Date	Days after control		
Control	1	10 May	-	62	12.5
Famine	1	19 May	9	48	12.3
Feast	1	Before 5 May	(at least 5)	-	
Control	2	7 May	-	60	12.2
Famine	2	16 May	9	46	12.3
Feast	2	Before 4 May	(at least 5)	-	
Control	3	4 May	0	58	12.1
Famine	3	9 May	5	59	12.1
Feast	3	After 29 Apr	(less than 5)	-	-

[a]mean fruit hue angle = 103°, estimated from Figure 4.19; where - = unable to be estimated.

Based on the estimates in Table 4.11, fruit from the famine vines were likely to be as soft as, or softer than, fruit from the control vines when they reached commercial harvest. The relationship between colour change and firmness was determined for fruit from each treatment in seasons 1 and 2 when the spread of hue and firmness values was greatest (Figure 4.20). Fruit from the famine vines underwent rapid softening at higher hue angles than fruit from the control and feast vines (x_0 ~102.4°, 101.1° and 99.9° respectively; Table 4.12; Figure 4.20). Based on an assumed daily flesh colour change of ~ 0.25° per day (calculated from Minchin et al., (2003) and summarised in Appendix 4.6.1) the famine fruit would have been harvested 2 to 3 days before their maximum rate of softening, whereas control and feast fruit would have been harvested around 8 and 12 days before maximum softening. These results suggest that although famine fruit might be described as less mature, they are likely to be softer at commercial harvest, or the physiological changes associated with softening would be more advanced, than fruit from control and feast vines.

Table 4.12 Relationship between on-vine degreening (hue angle) and firmness of 'Hort16A' kiwifruit sampled vines receiving control, famine and feast treatments.

Treatment	Season	Rate of change (N/°) (dx ± *SE*)	Inflexion point (°) (x_0 ± *SE*)	R^2
Control	1	0.5 ± 0.2	101.1 ± 0.2	0.89
	2	0.8 ± 0.3	101.1 ± 0.3	0.69
Famine	1	1.9 ± 1.4	102.5 ± 1.8	0.82
	2	1.1 ± 0.4	102.3 ± 0.5	0.75
Feast	1	0.6 ± 0.3	100.0 ± 0.3	0.80
	2	0.9 ± 0.6	99.8 ± 0.8	0.68

Data are from Boltzmann equations fitted to the relationships as illustrated in Figure 4.20

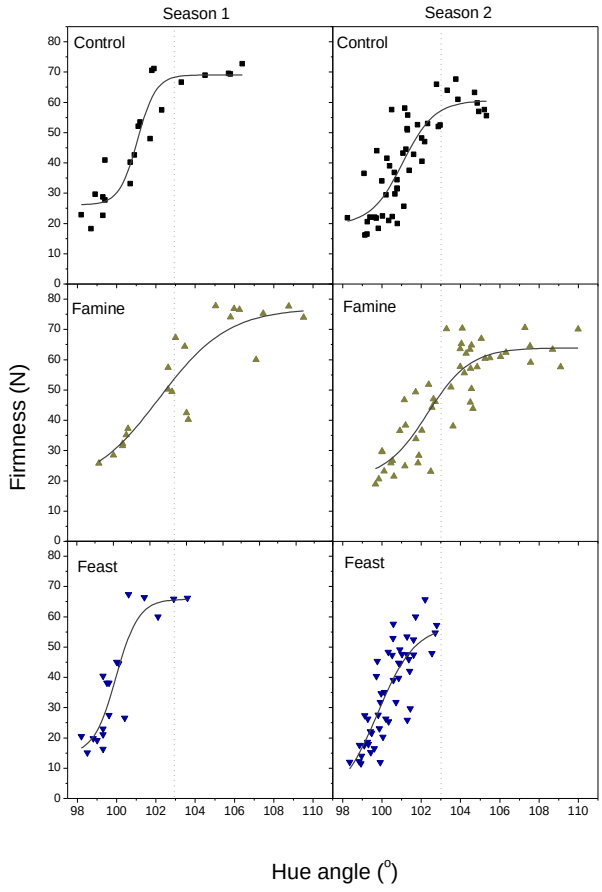

Figure 4.20 Boltzmann curves (solid lines) fitted to relationships between flesh colour change (mean hue angle = 103°, dashed vertical line) and softening in fruit from 'Hort16A' vines receiving control, famine and feast treatments. Each datum point is the mean value from an 18-fruit sample per vine.

4.3.3.5 Storage quality.

Firmness after storage. In seasons 1 and 2, firmness measured after 18 weeks at 1.5ºC was unaffected by treatment, there were few consistent differences in fruit firmness, and any differences were small, less than 1 N (Table 4.13). Firmness after a set time in coolstore was not measured in season 3.

Table 4.13 Average firmness (N) after18 weeks at 1.5 ^{o}C of 'Hort16A' kiwifruit from vines receiving control, famine and feast treatments.

Year	Harvest date	Treatment			*P*-value
		Control	Famine	Feast	
1	5 May	7.5 ± 0.1	-	7.2 ± 0.2	0.140
	16 May	**8.4 ± 0.3**	**9.3 ± 0.5**	**8.0 ± 0.2**	**0.060**
	26 May	8.7 ± 0.2	9.2 ± 0.4	8.2 ± 0.4	0.129
2	**4 May**	**7.7 ± 0.2**	-	**6.9 ± 0.3**	**0.030**
	8 May	6.5 ± 0.2	-	6.3 ± 0.2	0.485
	11 May	7.7 ± 0.3	-	7.1 ± 0.3	0.175
	14 May	7.2 ± 0.1	7.2 ± 0.2	-	0.737
	18 May	7.1 ± 0.4	7.2 ± 0.4	7.2 ± 0.4	0.924
	21 May	6.5 ± 0.4	7.2 ± 0.4	-	0.275

Fruit were stored at 1.5°C. At each harvest date fruit were harvested and stored for18 weeks; n = 7 ± SE; data with P ≤ 0.100 are highlighted in bold.

Softening during storage. In year 3, fruit from each treatment were harvested as close as practicable to degreening, rather than all on the same day as in the two previous years. Fruit from the control and famine vines softened to 20 N after approximately 17 days in storage whereas fruit from the feast vines reached 20 N after approximately 26 days.

If the control and famine fruit were harvested approximately 10 days earlier than in Figure 4.21A, they took longer to reach 20 N, ~ 32 days (Figure 4.21B), compared with 26 days when harvested later.

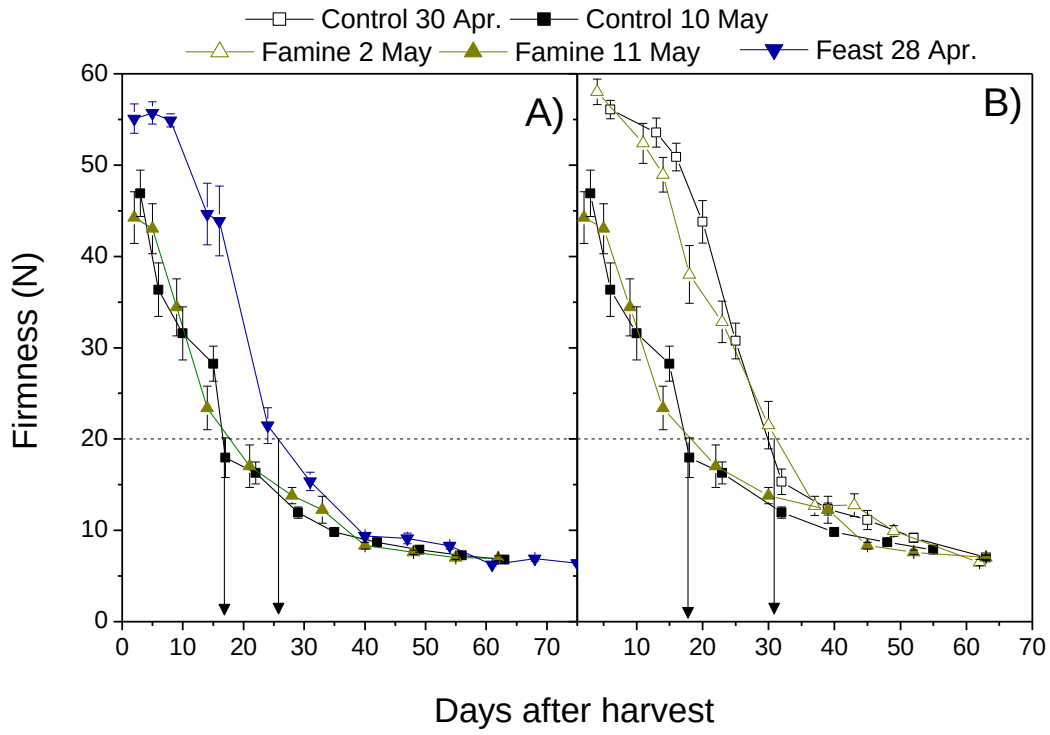

Figure 4.21 Firmness of 'Hort16A' kiwifruit during storage at 1.5°C sampled on A) 30/4, 28/4 and 2/5 from vines receiving control, famine and feast treatments respectively, and B) on 30/4 and 10/5 from control vines, and on 2/5 and 11/5 from famine vines. Dashed line is 20 N, an arbitrary value chosen for comparison purposes. Arrow used to estimate days after harvest until mean fruit firmness reached 20 N.

Storage disorders. Low temperature breakdown (LTB) was the only storage disorder seen in any quantity (> 4 % of fruit affected). In year 1, fruit were harvested at approximately ten day intervals for long-term storage, and there were clear differences in LTB incidence (Figure 4.22). Over 40 % of fruit from the famine vines harvested on 5 May were affected by LTB, with the incidence reducing to 10 % and 0.6 % in fruit from the same treatment harvested 11 and 21 days later, respectively. The famine fruit had the highest incidence of LTB at each sampling date except the last sampling date when LTB incidence was low in all treatments.

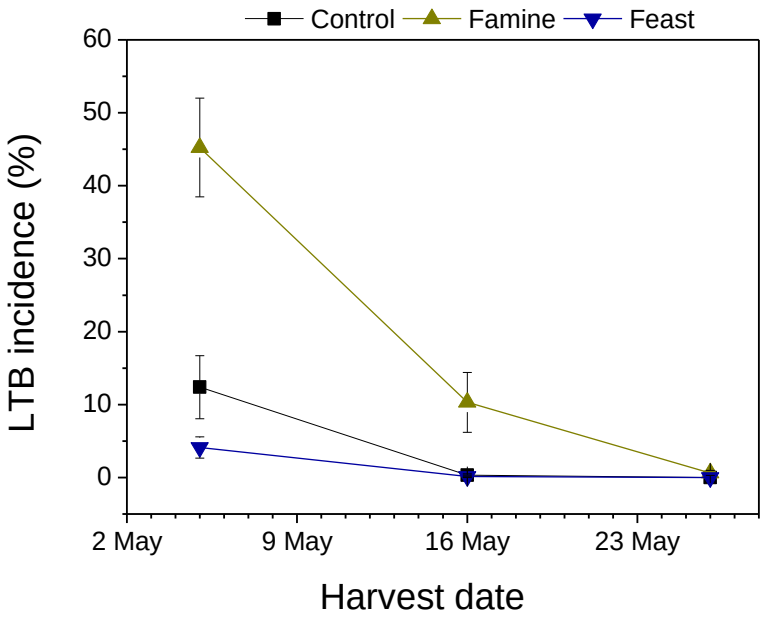

Figure 4.22 Incidence of low temperature breakdown (LTB) after storage in 'Hort16A' kiwifruit sampled on three dates from vines receiving control, famine and feast treatments; *n* = 7 ± *SE*.

4.3.3.5 Key findings - fruit attributes.

- At around harvest time, fruit from the feast vines had higher FW and DMC and were more mature (lower hue angle, higher SSC and softer) than fruit from the famine vines when harvested on the same day. Fruit from control vines were intermediate between the two.
- Based on flowering date, fruit from the feast treatment were up to 4 days older than fruit from the famine treatment, in season 2. In year 3, there were no treatment differences in the timing of flowering.
- The rate of change of different maturity indices were affected by the treatments, but to different degrees. Flesh colour change appeared to be advanced more than softening was, so fruit from a vine with delayed degreening could be softer at harvest than fruit from a vine with advanced degreening.
- The feast fruit contained more N, P, K, S, Mg and Cu than famine fruit, with control fruit intermediate between the two. Fruit Ca, Mn and Zn contents

were not affected by treatment, consequently fruit from the famine treatment had higher Ca/N ratios than fruit from the feast and control treatments.

- Harvest date influenced fruit softening in storage and the development of LTB, regardless of treatment. Fruit that were less mature at harvest were more susceptible to LTB than fruit that were more mature.

4.3.4 Whole-vine resource allocation

4.3.4.1 Biomass. Total vine biomass (DW) in the famine vines at excavation was 107.9 ± 2.7 kg, nearly 30 kg less than the feast vines (136.6 ± 6.7 kg) the control vines had a total biomass of 125.9 ± 5.0 kg (Figure 4.23). Roots and canes were the vine components most affected by the treatments (Table 4.14), the famine vines had approximately 16 kg less root biomass than the feast vines and less than half the 1-year cane biomass of the control and feast vines. The biomass of the remaining perennial vine components, and the new season's canopy growth were unaffected by the treatments (Figure 4.23; Table 4.14).

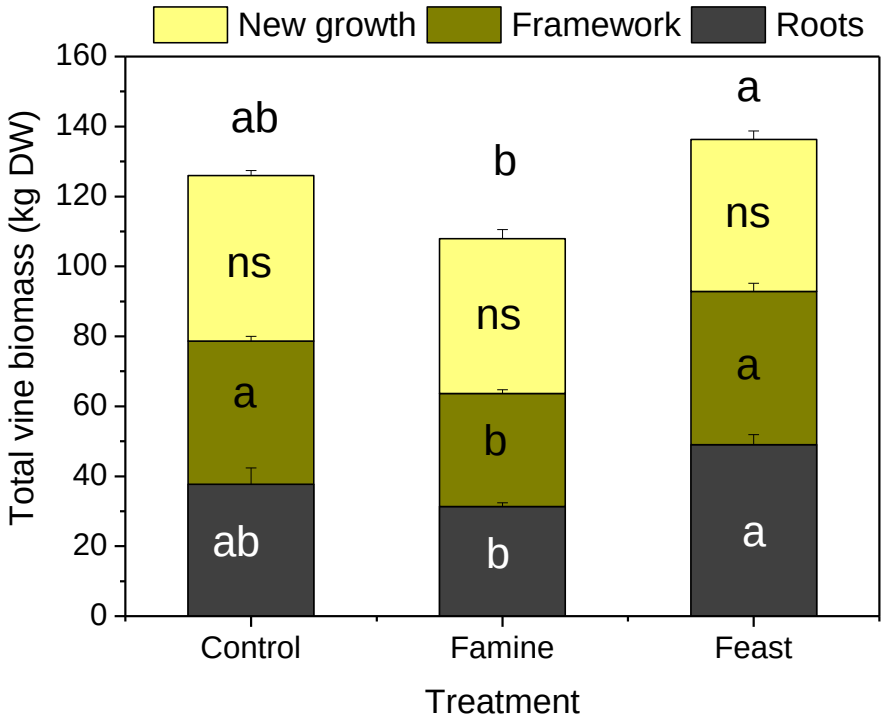

Figure 4.23 Biomass of 'Hort16A' kiwifruit vines receiving control, famine and feast treatments (n = 3 ± *SE*). Total biomass was divided into three components: new growth = annual canopy growth (pruned material, thinned fruit, senesced leaves and fruit, shoots and leaves still attached to the vines at excavation); framework = canes, leader, trunk and crown; roots = fibrous and structural roots. Biomass and/or components accompanied by letters are significantly different ($P \leq 0.05$).

There were no significant differences in the biomass allocated to the new season canopy growth, which was 45.0 ± 1.3 kg DW averaged across all three treatments (Table 4.14; Figure 4.23). The trend was for less DW allocated to fruit in the feast vines, as would be expected from treatment application (one fruit per shoot). Mature fruit total DW = 16.7, 19.1 and 11.5 kg in the control famine and feast vines respectively (P = 0.100; Table 4.14).

Table 4.14 Total and within-vine biomass allocation in 'Hort16A' kiwifruit vines receiving control, famine and feast treatments.

Component	Dry weight (kg vine^{-1})			*P*-value
	Control	Famine	Feast	
	Perennial components			
Canes (1 yr)	**8.8 ± 1.1a**	**4.3 ± 0.2b**	**9.8 ± 1.1a**	**0.005**
Canes (> 1 yr)	**13.9 ± 1.0**	**11.9 ± 0.5**	**15.6 ± 0.8**	**0.079**
Leader	13.3 ± 0.8	11.3 ± 0.5	13.2 ± 0.8	0.216
Trunk	2.2 ± 0.5	2.2 ± 0.4	2.8 ± 0.2	0.474
Crown	2.8 ± 0.2	2.5 ± 0.4	2.4 ± 0.4	0.640
Roots - structural	**36.1 ± 4.4**	**29.9 ± 0.8**	**46.6 ± 3.2**	**0.065**
Roots - fibrous[x]	**1.6 ± 0.2b**	**1.4 ± 0.2b**	**2.4 ± 0.5a**	**0.042**
Total perennial	**78.7 ± 4.9ab**	**63.6 ± 0.30b**	**92.8 ± 5.1a**	**0.017**
	New season's annual canopy growth			
Fruitlets thinned	**1.1 ± 0.4**	**0**	**0.96 ± 0.16**	**0.094**
Mature fruit	**16.7 ± 0.7**	**19.1 ± 3.0**	**11.5 ± 0.6**	**0.100**
Summer prunings	10.8 ± 0.5	10.6 ± 0.4	10.9 ± 0.8	0.948
Leaves - live	7.4 ± 1.8	5.0 ± 0.9	7.0 ± 1.4	0.107
Leaves - senesced	8.0 ± 1.9	4.7 ± 1.2	8.6 ± 2.0	0.104
Shoots	3.2 ± 0.9	4.5 ± 0.3	4.7 ± 0.3	0.192
Total annual	47.2 ± 1.4	44.2 ± 2.7	43.4 ± 2.4	0.562
Grand total	**125.9 ± 5.0ab**	**107.9 ± 2.7b**	**136.6 ± 6.7a**	**0.040**

n = 3 ± SE; values in any row accompanied by different letters are significantly different at P ≤ 0.05; rows where P ≤ 0.100 highlighted in bold.

Fruit. When DW allocation to mature fruit was measured across all seven replicate vines per treatment and not just the three that were excavated, the *P*-value was <

0.001 (n = 7), compared with P = 0.100 (n = 3), and the DW allocated to mature fruit in the feast vines was lower than that in the famine vines. In seasons 1 and 2 when mature fruit DW was collected from all 7 vines, treatment differences were not detected (Table 4.15). This result confirms the earlier findings (Figure 4.15) that treatment-induced differences in crop load and leaf to fruit ratio, affected individual FW and DW.

Table 4.15 Fresh and dry weight allocated to mature fruit in 'Hort16A' kiwifruit vines receiving control, famine and feast treatments.

Fruit attribute	Season	Control	Famine	Feast	*P*-value
Total FW	1	101.8 ± 2.8	115.0 ± 14.3	108.4 ± 7.4	0.628
(kg vine^{-1})	2	98.1 ± 4.5	80.8 ± 12.8	73.8 ± 2.9	0.115
	3	**90.7 ± 3.3ab**	**106.6 ± 12.3a**	**68.2 ± 3.9b**	**0.027**
Total DW	1	17.5 ± 0.5	18.2 ± 2.4	19.3 ± 0.6	0.743
(kg vine^{-1})	2	16.9 ± 0.8	13.7 ± 2.3	13.2 ± 0.5	0.162
	3	**14.9 ± 0.7ab**	**16.4 ± 1.9a**	**11.4 ± 0.6b**	**< 0.001**
	(3)[x]	**(16.7 ± 0.7)**	**(19.1 ± 3.0)**	**(11.5 ± 0.6)**	**0.100**
Individual FW	1	**89.5 ± 0.8b**	**70.5 ± 0.8c**	**99.9 ± 1.6a**	**< 0.001**
(g fruit^{-1})	2	**90.0 ± 2.7a**	**79.1 ± 2.3b**	**96.0 ± 1.6a**	**< 0.001**
	3	**84.0 ± 1.8a**	**66.7 ± 2.3b**	**87.6 ± 1.7a**	**< 0.001**
Individual DW	1	**15.4 ± 0.2b**	**11.1 ± 0.2c**	**17.8 ± 0.3a**	**< 0.001**
(g fruit^{-1})	2	**15.5 ± 0.5b**	**13.4 ± 0.5c**	**17.1 ± 0.4a**	**< 0.001**
	3	**14.7 ± 0.3a**	**11.1 ± 0.5b**	**15.4 ± 0.4a**	**< 0.001**

[x] *n = 3 ± SE, values from 3 excavated vines; for all others n = 7 ± SE; values in any row accompanied by different letters are significantly different (P ≤ 0.05); where P≤ 0.10, rows are highlighted in bold for clarity.*

Shoots. Direct comparison of the DW of shoots removed in pruning was not possible. This was because, as part of treatment application, the vines were pruned to a different degree at different times (as described in Table 4.3). The exception was the amount of unwanted regrowth removed from the canopy of the control and feast vines during summer, where the same procedure was followed. The feast vines produced more summer growth in some, but not all of the pruning events, specifically March of season 1 and February season 2 (Table 4.16).

Table 4.16 Dry weight removed during summer canopy pruning from 'Hort16A' kiwifruit vines receiving control, and feast treatments.

Season	Date	Control	Feast	*P* - value
1	31/1/2008	6.0 ± 0.5	6.7 ± 0.6	0.385
1	**17/3/2008**	**2.2 ± 0.3**	**3.3 ± 0.2**	**0.014**
2	**2/2/2009**	**8.0 ± 0.5**	**11.2 ± 0.9**	**0.009**
3	12/1/2010	0.7 ± 0.1	1.0 ± 0.2	0.139
3	18/2/2010	2.3 ± 0.2	2.3 ± 0.1	0.484

n = 7 ± SE.

Leaf abscission. There was no clear effect of treatment on timing of leaf abscission in the excavated vines (Figure 4.24). At each excavation date, however, the proportion of senesced leaves in famine vines was slightly lower than in feast vines. The first block of vines was excavated in mid-May and approximately 30 % of leaves in each vine had abscised compared with approximately 60 % of leaves in blocks 2 and 3, which were excavated 1 and 2 weeks after block 1, respectively (Figure 4.24).

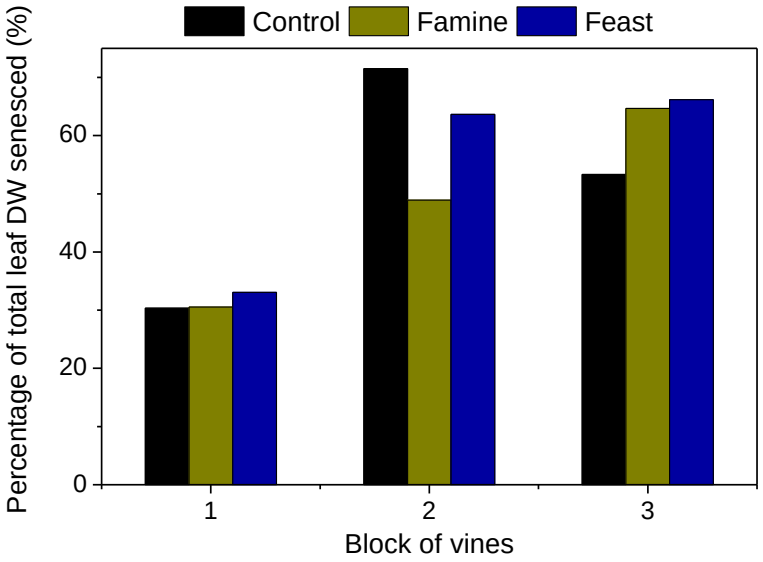

Figure 4.24 Percentages of total leaf biomass that had already abscised at the time of excavation of 'Hort16A' kiwifruit vines receiving control, famine and feast treatments; each block of vines represents 1 vine per treatment excavated in the same week.

4.3.4.2 Dry matter concentration. At excavation, canes from the famine vines had lower DMC than canes from the feast and control vines. Differences were not detected in the other vine components (Table 4.17). Data are not presented for fibrous roots because an accurate fresh weight could not be determined; subsamples were thoroughly washed and quickly patted dry before being placed into liquid nitrogen.

Table 4.17 Dry matter concentration of perennial components of 'Hort16A' kiwifruit vines receiving control, famine and feast treatments.

Component	Dry matter concentration (%)			*P*-value
	Control	Famine	Feast	
	Perennial components			
Canes (1 yr)	**36.6 ± 1.0a**	**33.0 ± 0.8b**	**36.6 ± 0.3a**	**0.021**
Canes (> 1 yr)	**40.7 ± 0.4a**	**38.5 ± 0.5b**	**40.1 ± 0.4a**	**0.034**
Leader	41.6 ± 0.2	40.4 ± 0.7	40.7 ± 0.5	0.296
Trunk	39.1 ± 0.2	37.9 ± 1.1	38.5 ± 0.5	0.540
Crown	36.0 ± 0.4	35.6 ± 1.0	34.8 ± 0.7	0.549
Roots - structural	27.6 ± 0.7	25.9 ± 0.9	26.3 ± 0.6	0.285
Roots - fibrous[x]	-	-	-	-
	New season's canopy growth			
Shoots	32.5 ± 1.1	29.6 ± 1.1	29.4 ± 0.7	0.106

Samples were taken from vines when they were destructively harvested in winter season 3; n = 3 ± SE, values in any row accompanied by different letters are significantly different (P ≤ 0.05); where P ≤ 0.10, rows are highlighted in bold for clarity.

4.3.4.3 Non-structural carbohydrates (NSC). There were no significant differences in the concentration of NSC in any of the perennial vine components (Table 4.18). There were some differences in the amount of NSC in different parts of the vine, for example the 1-year canes in the feast vines contained nearly twice as much NSC as those from the famine vines (0.21 kg compared with 0.48 kg, Table 4.18), and this difference reflected the total DW of 1-year canes: 9.8 and 4.3 kg DW in the feast and famine vines, respectively (Table 4.14).

Overall, the treatments did not significantly affect the total amount of perennial NSC reserves in the vines. The trend was: feast > control > famine with 9.44, 7.63 and 6.72 kg NSC vine^{-1} respectively (Table 4.18).

No significant treatment differences were found for the concentrations of starch or combined soluble sugars in any vine components (Appendix 4.6.2).

Table 4.18 Concentrations and amounts of non-structural carbohydrates (starch and soluble sugars) in perennial parts of 'Hort16A' kiwifruit vines receiving control, famine and feast treatments.

	Perennial components			
	Control	Famine	Feast	
Component	Concentration (mg g^{-1} DW)			*P*-value
Canes (1 yr)	52.8 ± 4.6	47.7 ± 0.9	48.3 ± 0.4	0.363
Canes (> 1 yr)	58.7 ± 5.5	46.3 ± 3.5	50.7 ± 2.9	0.185
Leader	41.5 ± 3.5	36.7 ± 2.5	38.1 ± 3.8	0.390
Trunk	57.9 ± 1.8	59.1 ± 7.7	66.1 ± 7.2	0.343
Crown	79.5 ± 9.5	86.0 ± 11.5	83.5 ± 8.3	0.612
Roots - structural	164 ± 9.6	176 ± 11.9	163 ± 16.1	0.744
Roots - fibrous	54.3 ± 3.4	54.1 ± 7.8	65.4 ± 5.6	0.501
	Amount (g $vine^{-1}$)			
Canes (1 yr)	**475 ± 181a**	**206 ± 10b**	**475 ± 56a**	**0.027**
Canes (> 1 yr)	**811 ± 120a**	**602 ± 20b**	**719 ± 30a**	**0.019**
Leader	547 ± 34	416 ± 42	510 ± 76	0.113
Trunk	126 ± 56	126 ± 21	187 ± 24	0.275
Crown	220 ± 38	209 ± 13	166 ± 46	0.569
Roots - structural	5840 ± 826	5285 ± 487	7713 ± 1297	0.272
Roots - fibrous	**87 ± 22b**	**78 ± 17b**	**150 ± 19a**	**< 0.001**
Total	7632 ± 691	6716 ± 485	9445 ± 1372	0.225

Values in any row accompanied by different letters (highlighted in bold) are significantly different (P < 0.05); n = 3 ± SE.

There was a trend for annual canopy growth in the feast vines to contain less NSC than the famine and control vines (6.9, 9.9 and 9.9 kg NSC $vine^{-1}$ respectively, P = 0.230). The trend reflected the lower crop loads in the feast vines rather than any differences in the composition of individual vine components (Table 4.19).

Table 4.19 Concentrations and amounts of non-structural carbohydrates in the new season's canopy growth of 'Hort16A' kiwifruit vines receiving control, famine and feast treatments.

Component	Control	Famine	Feast	*P*-value
	Concentration (mg g^{-1} DW)			
Fruitlets thinned	101 ± 8	(106 ± 6)[x]	108 ± 6	0.740
Mature fruit	503 ± 24	446± 23	457 ± 8	0.177
Leader pruning	72 ± 11	78 ± 16	86 ± 7	0.715
2nd main canopy pruning[z]	**86 ± 6a**	**65 ± 4b**	**94 ± 5a**	**0.016**
Leaves - live	41 ± 9	31 ± 6	40 ± 8	0.599
Leaves - senesced	5 ± 2	6 ± 1	5 ± 2	0.859
Shoots	63 ± 2	60 ± 3	67 ± 2	0.226
	Amount (g $vine^{-1}$)			
Fruitlets thinned	118 ± 44	0	106 ± 24	0.106
Mature fruit	8374 ± 216	8623 ± 1598	5273 ± 380	0.162
All summer pruning	843 ± 29	803 ± 74	926 ± 51	0.468
Leaves - live	315 ± 99	150 ± 30	269 ± 41	0.267
Leaves - senesced	38 ± 12	26 ± 6	33 ± 3	0.657
Shoots	**197 ± 51**	**272 ± 29**	**316 ± 32**	**0.098**
Total	9884 ± 320	9874 ± 1650	6923 ± 510	0.230

[x]no fruitlets thinned from this treatment, sample taken for comparison purposes only; [z]famine treatment usually pruned later than control and feast, so direct comparisons of any one pruning event across all three treatments treatment are not valid, examples given for comparison only; n = 3 ± SE; values in any row accompanied by different letters are significantly different at P ≤ 0.05, rows with P ≤ 0.100 are highlighted in bold.

4.3.4.4. Mineral nutrients. Perennial parts of the feast vines contained more N, P, K, S, Ca, Mg and B than the famine vines with the control vines intermediate between the two (Table 4.20). This pattern reflected the difference in total weight of the perennial parts of the vines (Table 4.20; Appendix 4.6.3). There were some concentration differences among different parts of the vines and for some mineral nutrients, but no clear patterns were detected (Appendix 4.6.4) and no nutrient was affected more than others. Several of the micronutrients, particularly Fe had large

standard errors, probably due to one sample being contaminated making interpretation of the results difficult, see for example Appendix 4.6.3.

Table 4.20 Mineral nutrient contents and dry weight (DW) of perennial parts of 'Hort16A' kiwifruit vines receiving control, famine and feast treatments.

	Amount (g vine^{-1})			
Nutrient	Control	Famine	Feast	*P*-value
N	**719 ± 65ab**	**582 ± 64b**	**1063 ± 109a**	**0.016**
P	**116 ± 7b**	**87 ± 2c**	**166 ± 9a**	**0.003**
K	**440 ± 31b**	**376 ± 19b**	**582 ± 41a**	**0.017**
S	**115 ± 10b**	**103 ± 10b**	**172 ± 15a**	**0.031**
Ca	**648 ± 34b**	**543 ± 9c**	**868 ± 20a**	**<0.001**
Mg	**152 ± 7b**	**125 ± 2c**	**197 ± 9a**	**0.020**
Mn	**1.75 ± 0.29**	**1.23 ± 0.12**	**1.98 ± 0.08**	**0.098**
Zn	6.14 ± 0.60	4.94 ± 0.48	6.61 ± 1.12	0.393
Fe	20.6 ± 12.2	6.30 ± 0.56	9.67 ± 0.78	0.428
Cu	0.86 ± 0.04	2.01 ± 0.13	1.06 ± 0.04	0.542
B	**0.67 ± 0.02b**	**0.52 ± 0.06c**	**0.81 ± 0.02a**	**0.001**
	Total DW (kg vine^{-1})			
Total DW	**78.7 ± 4.9ab**	**63.6 ± 0.30b**	**92.8 ± 5.1a**	**0.017**

Data accompanied by different letters are significantly different (n = 3 ± SE.), where P < 0.100 data are highlighted in bold

The concentration and amount of mineral nutrients in leaves and fruit were discussed in sections 4.3.2 and 4.3.3. In summary, leaf and fruit DMC were affected by the treatments, resulting in lower concentration of some mineral nutrients in high DMC such as fruit from the feast vines. On a per fruit basis, or a leaf area basis there were few treatment differences. In the combined new season's canopy growth (fruit, leaf, shoot, and pruned material) the feast and control vines contained more S, Ca and Mg than the famine vines (Table 4.21). Some values of micronutrients especially Zn and Fe had very high SE, making treatment comparison difficult, especially when n = 3.

Table 4.21 Mineral nutrient contents in the combined new season's canopy growth of 'Hort16A' kiwifruit vines receiving control, famine and feast treatments.

	Amount (g vine^{-1})			
Nutrient	Control	Famine	Feast	*P*-value
N	598 ± 26	483 ± 25	545 ± 28	0.111
P	90 ± 2	77 ± 6	83 ± 3	0.252
K	806 ± 60	769 ± 91	691 ± 45	0.326
S	**73 ± 3a**	**58 ± 4b**	**70 ± 2a**	**0.036**
Ca	**862 ± 74a**	**572 ± 40b**	**830 ± 15a**	**0.039**
Mg	**96 ± 7a**	**71 ± 4b**	**90 ± 6a**	**0.003**
Mn	1.91 ± 0.21	1.44 ± 0.18	2.00 ± 0.02	0.125
Zn	0.92 ± 0.08	0.82 ± 0.10	0.82 ± 0.37	0.344
Fe	1.99 ± 0.05	1.74 ± 0.13	1.88 ± 0.19	0.448
Cu	0.46 ± 0.02	0.44 ± 0.03	0.47 ± 0.03	0.777
B	0.79 ± 0.03	0.78 ± 0.05	0.81 ± 0.07	0.942

Values in any row accompanied by different letters are significantly different (P ≤ 0.05); If P ≤ 0.100 then row is highlighted in bold; n = 3 ± SE.

4.3.4.4 Key findings – resource allocation.

- Total DW of the famine vines was ~ 30 kg less than the feast vines (107.9 ± 2.7 kg compared with 136.6 ± 6.7 kg, respectively), control vines were intermediate with 125.9 ± 5.0 kg. Reduced root and cane biomass in the famine vines accounted for the majority of the difference.
- Concentrations of NSC and mineral nutrients in most perennial parts of the vines were unaffected by treatment, and overall perennial NSC and mineral nutrient reserve status reflected the biomass differences.
- Canes from the famine vines had lower DMC than canes from the control and feast treatments.
- In each treatment new season's canopy growth produced around 45 kg DW. There were few treatment differences in the total allocation of NSC and mineral nutrients to canopy growth, although allocation of some resources between fruit and leaves differed among treatments.

4.4 DISCUSSION

4.4.1 Vegetative/reproductive balance

The famine vines were expected to produce fewer flowers than the feast or control vines, the reduction in flowering potential was expected to manifest itself primarily as a high proportion of vegetative to floral shoots to compensate for carbohydrate depletion. This was not the case. Although the famine vines tended to have a higher ratio of vegetative to floral shoots, than the control and feast vines, the difference was not significant and between-vine variability was large. The number of flowers per floral shoot, was the attribute most affected by the treatment: reduced by 21 % and 35 % in the famine vines relative to feast vines in years 1 and 2, respectively (the number of flowers per floral shoot was unaffected by the treatments in the third and final season of the experiment). A similar result was found by Burge et al. (1987); 'Hayward' vines with a high crop load in one year had fewer flowers per floral shoot in the following year whilst total bud break and the proportion of floral bud break were not affected.

This finding suggests that in famine vines whole vine C depletion will result in a reduction in reproductive growth as a compensatory mechanism , although there was no accompanying increase in vegetative growth following this reduced C status. In the third and final year there were no differences between treatments. This finding suggests that the vines have the capacity to compensate for repeated carbohydrate depletion by reducing flower numbers then recovering, rather than continually declining in productivity.

In 'Hayward' kiwifruit vines, reduction in flower numbers in the season following defoliation treatments (Buwalda and Smith, 1990; Cooper and Marshall, 1991; Cruz-Castillo et al., 2010) was attributed to reduced assimilate supply (Buwalda and Smith, 1990). Entire field-grown 'Hayward' kiwifruit vines that were shaded in one year were less productive in the subsequent year than unshaded control vines (Buwalda and Meekings, 1993). Replacement canes that were shaded in the previous season produced fewer flowers per floral shoot than their exposed counterparts within the same 'Hayward' vine (Grant and Ryugo, 1984b). Cruz-Castillo et al. (2010) reported that reduced return bloom of defoliated 'Hayward' kiwifruit vines was related to

depletion of NSC, detectable as reduced NSC concentrations in shoots and trunk bark sampled in March (autumn). Defoliation in the previous season reduced starch concentrations in the trunk and roots of field-grown Chardonnay grapevines during the first 3-4 months after break in the following season and a reduction in reserve NSC concentration was closely associated with decreased numbers of inflorescences per shoot and flowers per inflorescence (Bennett et al., 2005).

It is also possible that other factors may be involved in the floral response to source-sink manipulations. Grant and Ryugo (1984b) found that exposed shoots were anatomically different to shaded shoots, having slightly thicker basal diameter, higher DMC and a greater proportion of dense lignified xylary tissue. Concentrations of NSC were not measured in their work, but it is possible that sun-exposure increased both localised NSC concentrations (by increased photosynthesis of the sun-exposed leaves) and the ability of the cane to attract reserves stored in other parts of the vine, as a result of their enhanced vasculature. In the present experiment the canes from the famine vines had lower DMC that canes from the feast or control vines despite being the most sun-exposed (a relatively large proportion of the leaf area was removed as part of the famine treatment). This result suggests that the structure of the cane might play a role in its productivity rather than just NSC concentration. Thorp et al. (2003) reported that large-diameter replacement canes were more productive, with more flowers per floral shoot than small-diameter canes. The authors hypothesised that the large-diameter canes had more available carbohydrates than smaller diameter canes, although the hypothesis was not tested. The large-diameter canes originated from long shoots that had been pruned to a manageable length (~ 2 m) in winter. The small-diameter canes were terminated and had not received any winter pruning. In Chapter 5 the effect of extended trunk girdling on productivity and reserve status will be explored, along with the potential role of hormones in floral induction.

4.4.2 Shoot architecture

In season 2, the proportion of short, medium and long shoots in the canopy was the same in the feast, famine and control vines. In season 3, the famine vines produced fewer long shoots and more short shoots than the control and feast vines. In season 2 the famine vines produced fewer fruit than in season 3, so it is possible that the higher crop load competed with shoot extension in the famine vines in season 3. Shoot

architecture was not measured in season 1, however if higher crop loads competed with shoot extension then it would be expected that there would have been a relatively high proportion of short shoots in the famine vines in season 1 as well as season 3.

Whether a shoot becomes short, medium or long is believed to depend on its initial growth rate. All shoots stop growing eventually. Short and medium shoots terminate growth sooner than long shoots that can continue growing to several metres (Seleznyova et al., 2002; Clearwater et al., 2006). Evidence that fruit compete with shoot extension was provided by Greer et al. (2003). Shoot growth and leaf expansion rate were depressed in fruiting 'Hayward' kiwifruit vines about 80 days after bud break, but this depression was not observed in non-fruiting vines. Clearwater et al. (2006) reported that there is also competition between shoots: the number of shoots that ceased growth and became terminated was reduced when neighbouring shoots were removed. The mechanism behind this competition is not clearly understood, it may be a simple competition for resources among growing shoots and fruit, or there may be an inhibitory effect.

The famine vines showed no tendency towards compensatory generation of extra leaf area in the form of more long shoots. Initial growth of long shoots is more rapid than that of short shoots, and long shoots have an initial carbon deficit relative to short shoots, but this is compensated for with greater carbon acquisition in the long run (Piller and Meekings, 1997). Results of the current experiment support the findings of Clearwater et al. (2006), that canopy architecture is more strongly affected by direct competition among fruit and shoots during the initial growth period rather than a response to carbohydrate reserve status.

4.4.3 Leaf function

Leaf photosynthesis, stomatal conductance and leaf internal CO_2 concentrations were not affected by the treatments. This confirms the findings of Buwalda and Smith (1990) that reducing the source-sink ratio in kiwifruit did not generate a compensatory increase in leaf photosynthesis. Altered photosynthesis is often, but not always, found as a result of source-sink manipulations in horticultural crops. Increased photosynthesis was observed in the remaining leaves of partially defoliated grapevines (Hunter et al., 1995) and potted sour cherry plants (Layne and Flore,

1995). Palmer (1992) found no clear relationship between photosynthetic rate and crop load among apple trees that had 0 to 90 % of their flowers removed, although mean photosynthetic rate was higher in fruiting trees than non-fruiting trees in July and August, the time of maximum fruit growth.

The feast treatment was designed to increase carbohydrate status in the vines, and these vines were expected to display some opposite responses to the famine vines. Plants typically respond to abundant carbohydrates by accumulation of starch in the leaves, inhibition of photosynthesis, increasing sink size or generating new sinks (Schaffer et al., 1986; Paul and Foyer, 2001). For example leaves of apple trees bearing little or no fruit accumulated starch and had reduced photosynthesis rates compared with fruiting trees (Wünsche et al., 2005). When all fruit were removed from mature field-grown plum trees during stage II of fruit growth (also known as pit-hardening, the period between cell division and cell-enlargement) and compared with intact control trees the following short-term responses were reported (Gucci et al., 1991):

- reduced photosynthesis 12 to 16 days after treatment.
- accumulation of starch, but not sugars, in the leaves 6 days after treatment.
- vigorous shoot growth around two weeks after treatment and coinciding with recovery of photosynthesis.

There was no evidence of reduced photosynthesis or accumulation of starch in leaves from the feast vines, despite the lower crop loads. In year 3, starch concentrations in the feast leaves tended to be the lowest of the three treatments. There was some evidence of additional shoot growth, in the form of increased summer pruning weight, in the feast vines relative to the control vines, but differences were not consistent across all three seasons.

4.4.4 Leaf plasticity

Individual leaf area (LA) was not affected by the different treatments in this experiment in any of the three seasons. Clearwater et al. (2006) reported that LA related to shoot type - the largest leaf on a short shoot was about 25 % smaller than leaves on the equivalent node of a medium or long shoot. In season 3 when the famine vines contained a higher proportion of short shoots than the feast or control

treatments, mean LA might have been expected to be lower in the famine vines, but no difference was detected.

Leaves from the famine treatment tended to have higher SLW than leaves from the other treatments. High SLW is often associated with accumulation of carbohydrates within the leaf, reduction of photosynthesis and with low crop loads (Marini and Sowers, 1990; Nii, 1997). In this experiment high SLW was found both early and late in the season and was not accompanied by reduced photosynthesis or leaf carbohydrate accumulation. Wilson et al. (1999) reported that variability across different species in SLW could be attributed to variation in leaf thickness, leaf DMC, or a combination of the two. Leaf thickness was not measured in the current experiment, but DMC tended to be higher in the famine leaves. Without detailed examination of leaf morphology it is difficult to explain why the famine leaves had higher SLW and DMC than leaves from the other treatments. There are several possible explanations which include:

- Leaves from the famine vines were less turgid than leaves from the feast or control vines. Gross leaf area, measured on the entire flattened leaf might remain relatively unchanged if the leaf was less turgid, DW would be the same and FW would be lower.
- Symptoms of possible K deficiency in the famine leaves (necrosis of leaf margins Figure 4.10A) mean that their FW would have decreased in April. This could explain the higher DMC, but not the higher SLW of the famine leaves.

Late in the season (April) the famine leaves had higher N per unit area and lower chlorophyll to N ratios than leaves from the feast or control vines. High N per unit area is usually associated with high photosynthetic capacity (Rozendaal et al., 2006) whereas low chlorophyll to N ratios are found in sun-exposed leaves where light capture is not limiting. The chlorophyll to N ratio is important in regulating the balance between the light and dark reactions of photosynthesis (Eichelmann et al., 2005). A high chlorophyll to N ratio means that a lot of N is invested in light-capture, and would be more common in shaded leaves. To further understand the physiology of leaves in the famine treatment, it would be necessary to compare changes in leaf

composition, including the chlorophyll, soluble proteins and Rubisco activity at approximately weekly intervals in the feast and famine leaves to determine how they differ (Bertamini and Nedunchezhian, 2002; Eichelmann et al., 2005).

Shading, defoliation or crop load manipulation treatments are typically used to generate whole-plant carbohydrate limitation. In the famine vines, high crop loads and low leaf numbers were used in combination to illustrate a worst-case scenario of inappropriate vine management with a high number of sinks and limited source availability. Leaf responses observed in the current experiment do not seem typical of much of the published literature and further experimentation would be valuable to determine the underlying physiology of the observed leaf responses.

4.4.5 Leaf senescence

It was expected that leaf senescence in the famine vines might have been delayed as a response to carbohydrate depletion. Leaf senescence proved difficult to assess in this experiment as hail, frost or both affected canopy health towards the end of season 2. In the final season there were no clear treatment differences in the proportion of abscised leaves at the time of excavation (May/June), but this was only estimated on the three excavated vines. There was an indication that leaves from the feast vines started to senesce sooner than vines from the famine vines, with a tendency for a greater proportion of N and P remobilised from the feast leaves in March and April, than from famine leaves. This might be a response to the denser canopy maintained in the feast vines. Leaves from kiwifruit vines with denser canopies tend to senesce sooner than leaves from vines with more open canopies (Michailides and Elmer, 2000).

In autumn, leaves from the famine vines consistently had lower K concentrations and higher Ca and Mn concentrations than leaves from the feast vines. Potassium and N are the nutrients most likely to be remobilised from leaves to nearby fruit in 'Hayward' vines (Smith et al., 1987b). In the current experiment there were indications of late-season K, but not N remobilisation from the leaves in the famine vines. The advanced senescence observed by Buwalda and Smith (1990) in defoliated vines may have been caused by K deficiency rather than senescence. Leaf senescence

processes typically involve N remobilisation (Wingler et al., 2006). There was evidence of advanced chlorophyll breakdown in the famine leaves compared with the control and feast leaves. If chlorophyll was breaking down without N remobilisation from famine leaves, then perhaps the N was being reallocated from light harvesting (chlorophyll) to photosynthetic enzymes such as Rubisco (Eichelmann et al., 2005).

Canopy senescence in apple trees was accompanied by reduced leaf photosynthesis, whilst leaf transpiration remained unaffected (Tartachnyk and Blanke, 2004). Phosphorus, K and N were remobilised from senescing leaves but Ca and Mg were not (Tartachnyk and Blanke, 2004). In this experiment kiwifruit leaves from the famine vines lost K, but not N and P, and gained Ca and Mn. This suggests that the famine leaves were transpiring more than the feast leaves, as the accumulation of Ca and Mn would be a function of preferential xylem transport (both are considered to be relatively immobile in the phloem) and hence transpiration rate (Clark and Smith, 1988).

4.4.6 Generation of alternative sinks

The feast vines with lower crop loads and ~ two leaves per shoot more than the control vines did not have reduced photosynthesis and carbohydrate accumulation in the leaves, some of the typical responses to carbohydrate abundance (Wünsche et al., 2005). It is therefore likely that additional assimilate was allocated to new or existing sinks.

Individual fruit FW and DW in the feast vines tended to be higher than fruit from the control vines, but the differences were small and were not always significant. Other potential new sinks include roots or shoot growth. If new shoot growth was generated in the feast treatment it might be detected in the January pruning, when new shoot growth was removed from the vines. More biomass was removed from the feast vines than the control vines during summer pruning, but the difference was rarely significant.

The feast vines had 50 percent more fine roots than the control vines (2.4 ± 0.5 kg DW compared with 1.6 ± 0.2 kg DW) at the time of excavation in late May/early June. This finding suggests that a proportion of the additional assimilate was

allocated to root growth. Similar findings were reported by Palmer (1992), increased crop loading led to reduced DM partitioning to roots. Conversely, treatments such as girdling (discussed in Chapter 5) and root pruning remove roots as a competitive sink for assimilates and resulting in increased allocation to fruit (Black et al., 2012).

4.4.7 Perennial reserves

The total amount of NSC and mineral nutrients present in perennial parts of the vine was significantly lower in the famine vines than the feast vines. Exceptions were Mn, Zn, Fe and Cu. Reserve contents of Mn and Zn showed similar patterns to other attributes, but the differences were not statistically significant. There was large variability among Fe and Cu measurements which made comparison among all three treatments difficult (see Appendix 4.7.3).

There were few significant treatment differences in the concentrations of NSC or mineral nutrients in any of the perennial vine parts, and little or no consistent trends or patterns. With few differences in concentration, changes in total reserve status reflected changes in biomass of different parts of the vine (see for example Appendix 4.6.3). One exception was that the famine vines had significantly lower NSC contents in the older canes despite having no significant differences in the biomass of older canes or concentration of NSC in them.

Destructive sampling by whole vine excavation gives a clear indication of both concentration and biomass at one time-point although it does not reflect changes that occur within or between seasons. The value of destructive sampling is that it gives information about long-term changes in biomass, which are needed to interpret results obtained by sub-sampling different parts of the vine. A disadvantage of this approach is the time involved to excavate a single vine: with only three replicates per treatment, any loss or contamination of a sample makes interpretation difficult. In addition, between-vine variability can mean that differences are not detected with statistical confidence - although consistent trends were still apparent for many of the analytes measured in the current experiment.

4.4.8 Fruit attributes

Fruit from the famine vines were smaller, with lower DMC and were slower to degreen than fruit from the feast and control vines, even once changes in flowering date, and therefore fruit age, were taken into account. Fruit from the control vines typically had FW, DMC and degreening times that were intermediate between feast and famine fruit. The trends were consistent across all three seasons, whether they were detected with statistical confidence was likely to be a consequence of seasonal variability, primarily in crop load and leaf to fruit ratios. For example, in season 2 when the famine vines only produced around 1000 fruit per vine, they would have had a similar crop load to the control vines, but the famine shoots would have only had 1 leaf per fruiting shoot compared with 4 leaves in the fruiting shoots of the control vines. In 'Hayward' vines leaf-to-fruit rations had a greater effect than crop load on fruit FW (Cooper and Marshall, 1991).

Source-sink manipulation affects FW in kiwifruit (e.g. Burge et al., 1987; Snelgar and Thorp, 1988; Cooper and Marshall, 1991; Buwalda and Smith, 1990; Cruz-Castillo et al., 2010; Minchin et al., 2010). Published results on the effect of source-sink manipulation of maturity and DW accumulation are less common, although Snelgar and Thorp (1988) reported no effect of partial defoliation on 'Hayward' fruit maturity (measured by SSC), and SSC was not affected by different crop-load treatments (Burge et al., 1987).

In peach, reduced crop load increased fruit FW and DW and DMC (Saenz et al., 1997). Reduced crop load increased DMC and advanced maturity in apple (Wünsche, 2005). Increased leaf area advanced maturity in table grapes (Kingston and Van Epenhuijsen, 1989). Defoliation had little effect on grape berry maturation, and this has been attributed to remobilisation of carbohydrate reserves or allocation of newly-assimilated carbohydrates to maturing fruit rather than replenishing reserves (Candolfi-Vasconcelos and Koblet, 1990; Candolfi-Vasconcelos et al., 1994; Bennett et al., 2005). The link between fruit maturation, fruit DMC and vine reserves in 'Hort16A' kiwifruit is explored further in Chapter 5 where trunk girdling is used to isolate the canopy from the majority of perennial reserves.

Results from this chapter suggest that flesh colour change (degreening) was affected more than softening by the source-sink manipulations. Fruit from the feast treatment would be firmer when cleared to pick than would famine fruit. Both fruit composition and maturity at harvest have been linked to storage performance in kiwifruit. Incidence of LTB was more common in fruit that were less mature (greener flesh hue) at harvest, so delaying harvest reduced LTB incidence. Delaying harvest of famine fruit by 3 weeks reduced LTB incidence from ~ 45 % to less than 1 %. Similar findings, that less mature fruit were more likely to have LTB, were reported by Clark et al. (2004) and Maguire et al. (2005).

4.5 CONCLUSIONS

Whole plant carbon depletion can result in compensatory responses which enable the plant to acquire more carbon: allocation to vegetative over reproductive or root growth, increased leaf photosynthesis, delayed leaf senescence, changes in plant architecture towards more long shoots, and larger leaves with lower SLW. Kiwifruit vines subjected to long-term whole-vine carbohydrate depletion did not show upregulated photosynthesis in the existing leaves. This finding is in agreement with other published reports on kiwifruit responses to altered leaf-to-fruit ratios. The famine vines showed no indication of additional vegetative growth (in the form of more medium and long shoots) as a means of compensating for carbohydrate depletion. Instead competition between fruit and shoot growth appeared to result in termination of shoot growth. This was seen in year 3 when fruit numbers were higher than in year 2. Flower numbers were reduced in famine vines, primarily as a result of a reduction in number of flowers per floral shoot rather than a reduction in number of floral shoots. Vines showed a tendency towards recovery in fruit numbers after one season of poor productivity.

Below-ground biomass was reduced in the famine vines, and this was balanced to some extent by reduced cane biomass. There was little or no evidence that root starvation had occurred and the only evidence of nutrient deficiency was late-season K loss which occurred in all leaves from all treatments, but to a greater extent in the famine than the control and feast vines. The necrosis associated with K depletion was not linked with earlier leaf senescence processes.

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4.7 APPENDICES

Appendix 4.7.1 Estimating change in hue angle

The following equation (from Minchin et al., 2003) was rearranged to determine the number of days for fruit flesh hue angle to change from 104° to 102°:

$$y = \frac{1}{1+ e^{(t-t0)\theta}}$$

where: $y = (hue - 97)/18$, t_0 = 174.8, and θ = 16.7,

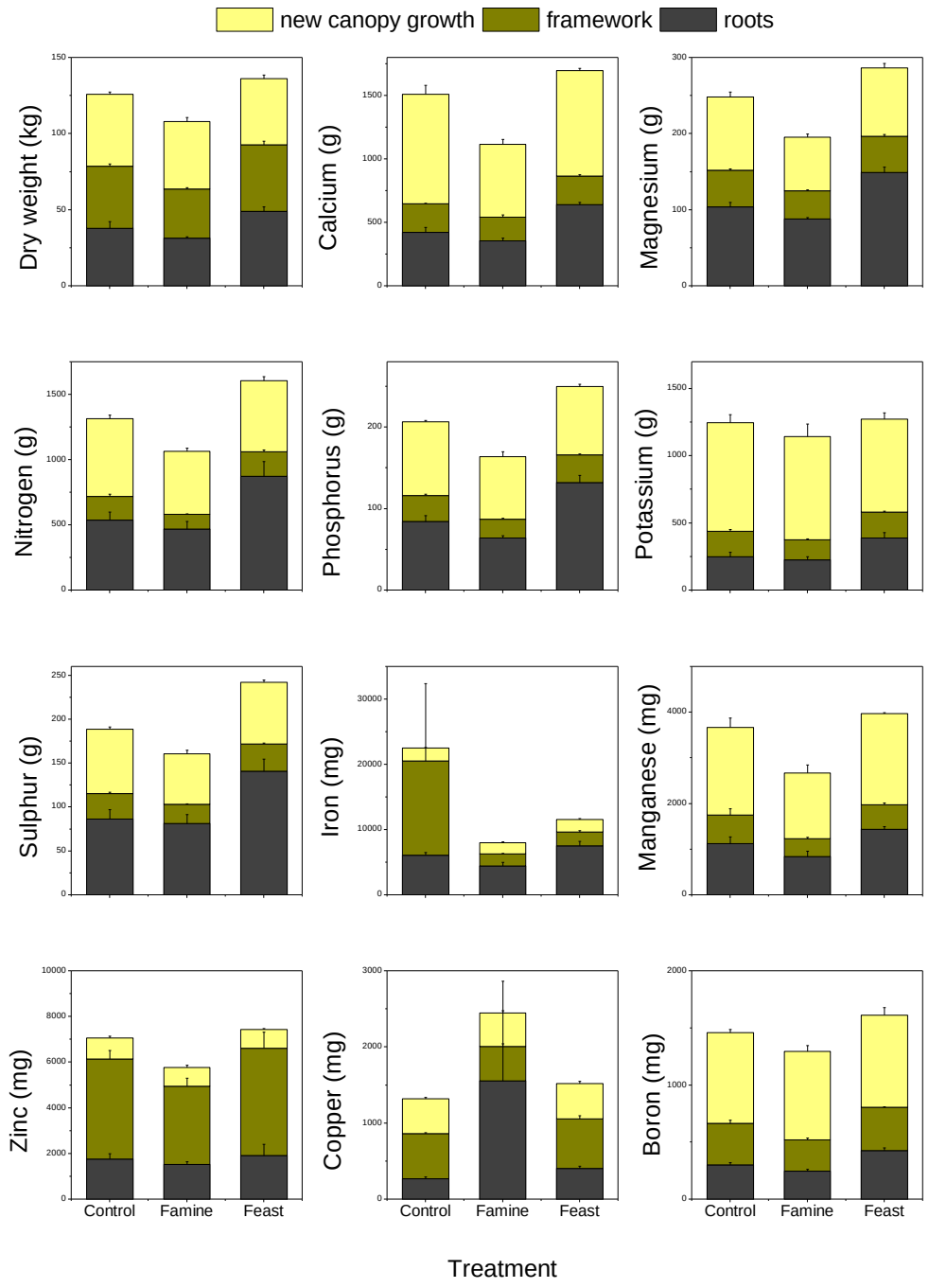
The result was 8.4 days, or a change of approximately 0.25° per day. The equation was also used to estimate commercial harvest if fruit were harvested too early or too late.

Appendix 4.7.2 Carbohydrate concentrations in perennial vine parts

Effect of source-sink manipulations on starch and soluble sugars concentrations in perennial parts of 'Hort16A' kiwifruit

	P-value	
Component	Starch	Soluble sugars
Canes (1 yr)	0.719	0.123
Canes (> 1 yr)	0.204	0.291
Leader	0.304	0.690
Trunk	0.400	0.924
Crown	0.623	0.865
Roots - structural	0.335	0.476
Roots - fibrous	0.582	0.212

Appendix 4.7.3 Whole vine mineral nutrient composition



Appendix 4.7.4 Mineral nutrient concentrations in perennial parts of the vine

Effects of different source-sink manipulations on the concentration of mineral nutrients in the perennial components of the vine

	Perennial part of vine						
	Canes					Roots	
Mineral nutrient	1-yr	≥ 2-yr	Leader	Trunk	Crown	Structural	Fibrous
	P-value						
N	0.309	0.134	0.621	0.160	0.871	0.274	0.549
P	0.751	**0.049**	0.250	0.132	0.934	0.287	0.165
K	**0.062**	0.444	0.309	0.111	1.000	0.549	0.395
S	0.269	0.250	0.269	0.234	0.716	0.436	0.229
Ca	0.134	0.210	0.873	**0.052**	0.225	0.306	0.827
Mg	0.156	0.160	0.963	0.129	0.526	0.507	0.447
Mn	0.353	0.621	0.698	**0.081**	0.622	0.442	0.537
Zn	0.151	0.187	0.910	0.488	0.483	0.220	0.447
Fe	**0.008**	0.556	0.435	0.249	0.814	0.482	0.982
Cu	0.805	0.659	0.923	0.871	0.188	0.448	0.750
B	0.502	0.145	0.569	**0.009**	**0.018**	0.621	0.871

P-values ≤ 0.100 highlighted in bold.

5 EXTENDED SUMMER TRUNK GIRDLING

5.1 INTRODUCTION

Trunk girdling temporarily stops the transport of carbohydrates and hormones from the canopy to the roots. Girdling in late summer is used by kiwifruit growers to increase fruit DMC. The girdle is usually applied in mid-February by removing a 5-mm wide strip of bark from the trunk (Figure 5.1), and can increase fruit DMC by 0.8 - 1.0 % - units in 'Hort16A' (Patterson and Currie, 2011).

Figure 5.1 A) Trunk and leader of 'Hort16A' kiwifruit vine that has received extended trunk girdles over several seasons, B) Close-up of the 'Hort16A' scion showing four healed girdles that were carried out in the previous four years (black arrows) and an unhealed (or open) girdle (brown arrow). The term extended trunk girdle refers to the experimental practice of keeping the girdle open for several months as it starts to heal.

The effects of girdling or related techniques, such as scoring, and ringing have been determined across a range of tree species (and summarised by Noel, 1970; Goren et al., 2004). The long-term effects of trunk girdling kiwifruit vines are not well-understood and there have been concerns from some quarters that girdling kiwifruit vines for consecutive seasons could have a negative affect on vine performance,

especially on the root system, and that fruit storage performance might be adversely affected.

Plant responses to girdling relative to ungirdled plants or branches, with examples, include:

- Increased flower numbers in the season after girdling; in apple (Arakawa et al., 1997), avocado (Lahav et al., 1971), grape (Caspari et al., 1998) and pear (Reynolds et al., 2005), but not in persimmon (Juan et al., 2009).
- Earlier flowering; in avocado (Lahav et al., 1971), although delayed flowering in peach (Dann et al., 1984).
- Advanced fruit maturity in the season of girdling; increased SSC in girdled apple trees (Elfving et al., 1991), advanced softening and colour development in loquat (Agusti et al., 2005), persimmon (Juan et al., 2009), and stone fruit (Fernandez-Escobar et al., 1987; Agusti et al., 1998).
- Increased fruit size; in avocado (Davie et al., 1995) and loquat (Agusti et al., 2005), but not in grape (Caspari et al., 1998) or apple (Elfving, 1991).
- Reduced leaf photosynthesis; in apple (Cheng et al., 2008), grape (Roper and Williams, 1989; Williams et al., 2000), kiwifruit (Black et al., 2012) and mango (Urban and Alphonsout, 2007).
- Poor leaf health; development of pale chlorotic leaves in avocado (Lahav et al., 1971), advanced leaf senescence and abscission in avocado (Lahav et al., 1971) and peach (Dann et al., 1984).
- Reduced canopy vigour; in apple (Pretorius et al., 2002), avocado (Lahav et al., 1971) and peach (Dann et al., 1984), although girdling did not affect shoot extension growth in grapes (Caspari et al., 1998).
- Reduced root growth; in peach (Sharif Hossain et al., 2006) and grape (Yamane and Shibayama, 2006).
- Reduced mineral nutrient uptake; in apple (Priestley, 1976a; Arakawa et al., 1997), avocado (Davie et al., 1995) and mango (Urban and Alphonsout, 2007).
- Reduced root reserves; reduced starch in citrus roots for 1 month after girdling (Wallerstein et al., 1978), and in grape vines 2 and 4 weeks after girdling (Roper and Williams, 1989).
- Poor girdle healing; in stone fruit (Fernandez-Escobar et al., 1987), and after several consecutive seasons in avocado (Trochoulias and O'Neill, 1976).

Responses to girdling are affected by timing of the girdle application, plant age, developmental stage, as well as plant environment and cultivar. Girdling in early spring can be used to enhance fruit set and flower bud formation (Rivas et al., 2006), mid-season girdling can enhance fruit size, and girdling later in the season can increase carbohydrate supply thereby increasing DMC and advancing maturity (Noel 1970; Goren et al., 2004).

The vines used in this study had already been trunk girdled for three consecutive years before the present study began. The aim of the work described in this chapter was to girdle the vines for a further three seasons and to determine if the vines were able to maintain productivity without adversely affecting vine health and fruit quality. The vines were girdled in late summer (February). For two months after the girdle was applied, it was regirdled when it started to heal, and after April girdles were allowed to heal. The aim was to extend the time when the canopy and roots were effectively isolated from each other. Vines were then managed the same as the control vines, with the same crop load and pruning treatment. Vine productivity, canopy development, leaf function and fruit attributes were monitored over three years. A subset of vines was then excavated to determine how treatments affected uptake and/or allocation of resources: dry weight, non-structural carbohydrates and mineral nutrients.

It was expected that isolating the canopy from the roots for an extended time would adversely affect root growth and therefore nutrient uptake. This would lead to gradual development of leaf nutrient deficiencies, reduced photosynthesis, reduced carbohydrate production, lower flower numbers and poor fruit size and reduced fruit DMC. Few studies have been carried out where the interactions among many of the reported consequences (see above) of girdling have been explored in one long-term experiment.

5.2 MATERIALS AND METHODS

5.2.1 Vines and treatment application

Treatments were applied to mature ‘Hort16A’ kiwifruit vines (*Actinidia chinensis* Planch. var. *chinensis*) growing in three adjacent rows at the Te Puke Research Centre

in the Bay of Plenty, New Zealand (37° 49'S; 176° 19'E). The vines were planted in 1993 and the 'Hort16A' scion was grafted onto the existing rootstock in 1996. The vines had been trained onto a pergola system and each vine had ~ 30 m^2 of canopy area with a leader length of 6 m and canopy width of 5 m. Treatments were assigned in a randomised design with seven vines per treatment. The extended trunk girdling treatment was first applied late spring in the 2003/2004 season through until winter 2010 when three vines per treatment were excavated for total biomass determination.

Control. Vines received standard management practices: sufficient vigorous shoots were retained in the leader zone (~ 40 cm either side of the leader; Figure 2.1) for replacement canes in the subsequent season. Excess growth was removed from the leader zone to minimise shading; vigorous fruiting shoots in the fruiting zone were cut back to ~ 4 leaves past the last fruit or zero-leafed (see Chapter 2 for definitions). New vegetative shoot growth was removed over summer (usually in February and March). Crop load was adjusted to ~ 40 fruit per m^2 by thinning in spring.

Extended trunk girdle (ETG). Vines received the same standard management practices as the control vines, except in February they were trunk-girdled by removing a 5-mm strip of bark from the scion of each vine (Figure 5.1). In March and April the girdles were inspected at weekly intervals and were re-girdled if they had begun to heal. After April the girdles were left to heal naturally. Healing did not occur until mid-October, around 8 months after girdle application (Figure 5.2).

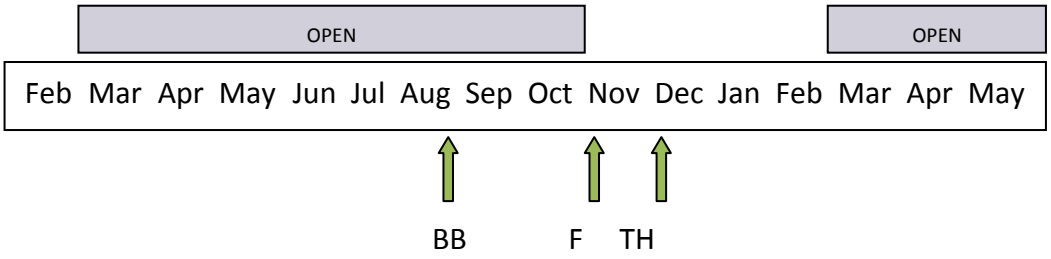


Figure 5.2 Time-line showing bud break (BB), flowering (F) and fruitlet thinning (TH) in relation to whether the girdle is open or not (top line).

As an example, the schedule of canopy management practices for the 2009/2010 season is presented in Table 5.1.

Table 5.1 Timing of canopy management and vine treatments in year 3 in relation to main phenological events.

Date	Treatment or event	Purpose of treatment
Mid-Oct	Flowering	
13 Nov	Leader pruning	Remove excessive growth in the leader zone, retain enough long shoots as next year's replacement canes
25 Nov	Fruitlet thinning	Remove fruitlets to give a crop load of ~ 40 fruit per m^2
30 Nov	1st canopy prune	Remove blind shoots in the main fruiting zone, cut long and medium shoots to 4 leaves past the last fruit, zero-leaf prune excessively vigorous fruiting shoots
8 Jan	Fruitlet thinning	Readjust crop load to 40 fruit m^2
12 Jan	2nd canopy prune	Remove new growth
18 Feb	3rd canopy prune	Remove new growth
19 Feb	Trunk girdle	ETG vines only
Feb - Apr	Check girdle	Remove new cambium to keep the girdle open
Late - Apr/May	Commercial fruit harvest	
Mid-May	Leaf-fall, Vine excavation	

5.2.2 Measurements

Vine attributes as listed below were measured each year to determine the effect of the ETG treatment on vine productivity, leaf and canopy health, fruit quality and resource allocation within the vines, and whether treatment differences became exacerbated over time.

The experiment was carried out over three years: 1 (2007/2008), 2 (2008/2009) and 3 (2009/2010). The following measurements were made using individual vines as replicates. Unless started otherwise, measurements were made on all 7 vines per treatment. More detailed canopy measurements were made on a sub-sample of 4 vines per treatment:

- Components of yield were measured on 4 canes per vine in spring each year.

- Flowering date for each vine was determined on a subsample of the fruiting canopy measuring ~ 1.2 m x 1.6 m by counting the number of flowers that had opened at 2 to 4-day intervals on all 7 vines in years 2 and 3. Bud break was measured on all 7 vines using the same technique, but in year 3 only.
- Shoot type was determined on the same sub-sample of fruiting canopy used for flowering photography on a subsample of 4 vines per treatment. Shoot types were determined in December year 2 and January year 3.
- Leaf attributes (leaf area, leaf number, petiole length (year 3 only; specific leaf weight) and leaf composition (mineral nutrients, chlorophyll and carbohydrates) were measured on a combined sample of 10 fully-expanded blades and petioles per vine. Samples were taken every 4 to 5 weeks. Leaf area and petiole length as a function of node number were measured on 2 shoots per vine sampled destructively in December/January.
- Leaf area index was estimated from leaf counts and individual leaf area.
- Leaf gas exchange measurements were made on a subsample of 4 vines per treatment at approximately 4 - 6 - weekly intervals during years 2 and 3.
- Fruit mineral nutrient contents were measured on a combined sample of 12 fruit per vine. Fruit were sampled in November/December, February and April each year.
- Fruit fresh weight, dry weight, firmness, soluble solids concentration and flesh hue angle were measured on a sample of 18 fruit per vine sampled randomly from across the entire canopy at regular intervals as close as possible to commercial harvest each year.
- In year 1, fruit storage performance was measured on 90 fruit per vine, sampled randomly from across the entire fruiting canopy at approximately 10-day intervals. In year 2 an extra 30 fruit per vine were sampled for destructive measurement of fruit softening during storage.
- Canopy growth was estimated by collecting and weighing all material removed from each vine during pruning and leaf abscission.
- Total vine biomass was measured by excavating 3 vines per treatment between mid-May and mid-June 2010.

Experimental methods are described in Chapter 2

5.2.3 Statistics

Analyses were carried out using analysis of variance (ANOVA) with individual vines as replicates (see Chapter 2.14 for more details). Attributes such as components of yield, flowering date and fruit maturity were measured on all seven replicate vines; more detailed canopy measurements such as shoot type, leaf gas exchange and leaf nutrient accumulation were measured on a subset of 4 vines per treatment. Total vine biomass was determined on three vines per treatment. Analysis of vine excavation data included the factor 'block', to account for the time difference (5 - 7 days) in excavating pairs of vines during leaf-fall. Most data were measured in individual years; to determine if there was a seasonal decline in ETG vine attributes compared with the control vines, the effects of treatment, year and their interactions were analysed. Data were checked for normality and were log-transformed if necessary. Proportion data from counts were subjected to angular transformation before ANOVA.

5.3 RESULTS

5.3.1 Canopy composition

5.3.1.1 Components of yield. Yield components were affected by year and treatment to different degrees (Table 5.2). Both total BB and FBB decreased across the three years (Figure 5.3A and B). No seasonal trends were observed for VBB (Figure 5.3C). The ETG vines had consistently higher BB and FBB and lower VBB lower than the control vines. The control produced ~ 2.5 F/FS each year, whereas this figure varied in the ETG vines: 3.3, 4.4 and 4.1 in years 1, 2 and 3 respectively. The ETG vines were more productive than the control vines (Figure 5.3E). The difference varied with year; in year 2 the ETG vines produced on average 2.8 times more fruit than the control vines (3872 ± 160 compared with 1371 ± 88; Figure 5.3F), whereas in years 1 and 3 the ETG vines produced 1.7 and 1.9 times as many fruit as the control vines respectively.

Table 5.2 Effect of year[a], treatment (extended trunk girdling or control) and their interactions on bud break and yield characteristics in 'Hort16A' kiwifruit vines.

	P-value					
Factor	BB	FBB	VBB	F/FS	KF/Bud	Fruitlet no
Year[a]	<0.001	0.011	0.348	0.009	<0.001	0.160
Treatment	0.025	<0.001	<0.001	<0.001	0.037	<0.001
Year x treatment	0.249	0.096	0.347	0.018	0.015	0.004

BB = bud break (%), FBB = floral bud break; VBB = vegetative bud break; F/FS[1] = number of flowers per floral shoot; KF/bud. [a]Data are for three consecutive years spring 2007 to winter 2010.

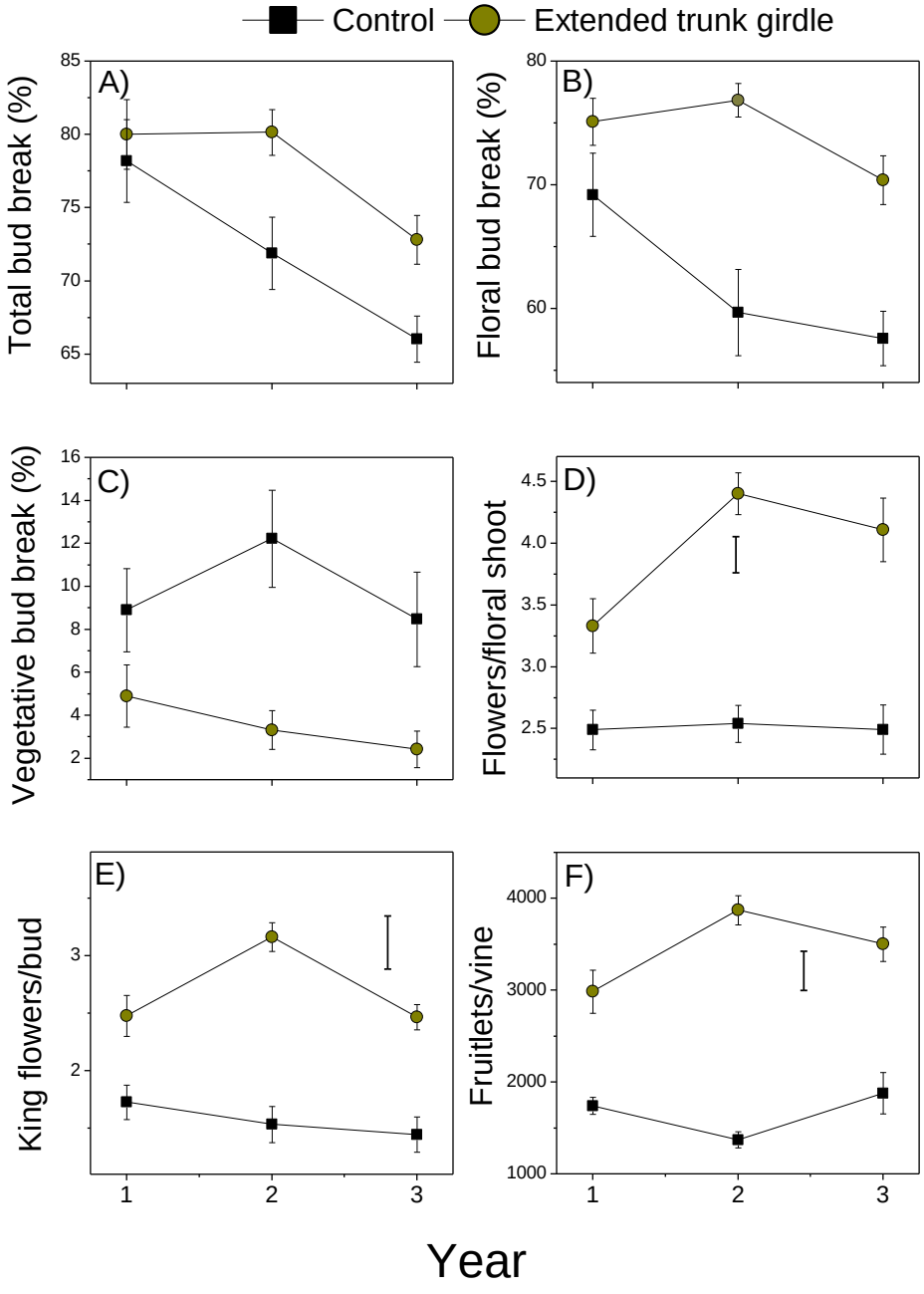


Figure 5.3 A) Total bud break (%), B) floral bud break (%), C) vegetative bud break (%), D) flowers per floral shoot, E) king flowers per winter bud, and F) total number of fruitlets per vine measured over three years in 'Hort16A' kiwifruit vines receiving extended trunk girdles and ungirdled control vines; *n* = 7 ± *SE*. Where significant interactions between treatment and year occurred, the vertical bar represents the $LSD_{0.05}$.

In year 3, 50 % of viable buds on the ETG vines were open on 8 August, whereas it took until 16 August for the similar percentage to be open in control vines (Figure 5.4). Duration of BB was unaffected by treatment (11.7 ± 1.0 and 10.5 ± 1.0 days respectively, $P = 0.395$). Timing of BB was not monitored in years 1 and 2.

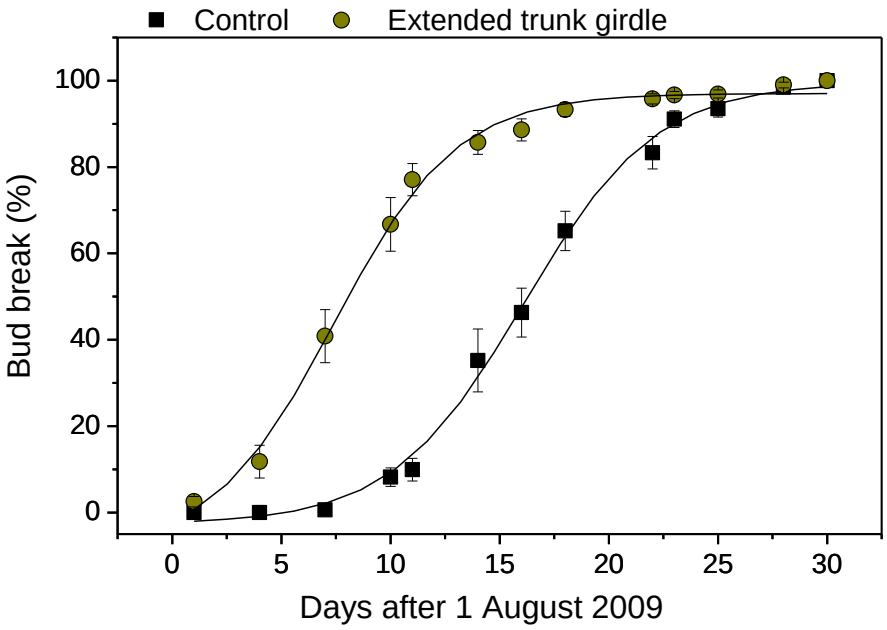


Figure 5.4 Percentages of viable buds open in 'Hort16A' kiwifruit vines receiving extended trunk girdles compared with ungirdled control vines; *n* = 7 ± *SE*. Solid lines are fitted sigmoid curves

5.3.1.2 Shoot types. Vines in both treatments were more vigorous in year 3 than in year 2, with more long shoots and fewer medium shoots (Tables 5.3 and 5.4). The proportion of short shoots was unaffected by year in either treatment (approximately 57 % in the control vines and 87 % in the ETG vines; Tables 5.3 and 5.4). Treatments affected the proportion of short shoots and long shoots, but not the proportion of medium shoots. In addition to more short shoots, ETG vines produced fewer long shoots than the control vines (Table 5.4).

Table 5.3 Effect of year[a], treatment (extended trunk girdling or ungirdled control) and their interactions on shoot types in the canopy of 'Hort16A' kiwifruit vines.

	P-value		
Factor	Short	Medium	Long
Year	0.444	0.038	0.029
Treatment	0.028	0.237	<0.001
Year x treatment	0.391	0.075	0.211

[a]Data are for two consecutive years 2008/2009 and 2009/2010.

Table 5.4 Percentages of different shoot types in years 2 and 3 in the canopies of 'Hort16A' kiwifruit vines receiving extended trunk girdles (ETG) and ungirdled control vines measured.

	Control		ETG	
Shoot type	Year 2	Year 3	Year 2	Year 3
Short (%)	57.4 ± 4.6	57.2 ± 1.5	88.0 ± 9.1	87.4 ± 4.9
Medium (%)	19.5 ± 2.7	8.9 ± 0.7	10.5 ± 7.6	7.7 ± 3.3
Long (%)	23.1 ± 5.4	33.9 ± 1.8	1.6 ± 1.6	4.9 ± 1.8

A short shoot is terminated with ≤ 9 nodes; medium shoot is terminated with 10 - 20 nodes; long shoot is non-terminated with < 20 nodes; n = 4 ± SE.

Many of short shoots in the ETG vine canopy showed little internode extension so that the average short shoot in the ETG canopy was shorter than a typical short shoot of the control vines (Table 5.5). To illustrate this, a sample of the canopy from one control and one ETG vine was separated out into its component shoots at the time of vine excavation (Appendix 5.7.1).

Table 5.5 Example of the different shoot lengths in the canopies of 'Hort16A' kiwifruit vines receiving extended trunk girdle (ETG) and an ungirdled control vine.

	Number of shoots (% of total in brackets)		Internode length (cm)	
Shoot length (cm)	Control	ETG	Control	ETG
< 5	3 (12)	30 (42)	0.93	nr
5 - 10	7 (27)	16 (22)	nr	1.11
10 - 20	8 (31)	17 (24)	1.61	1.68
20 - 30	7 (27)	5 (7)	2.21	2.65
30 - 40	1 (4)	4 (6)	2.75	3.33
< 40	26	72		

The two canopy sections are illustrated in Appendix 5.7.1; nr = not recorded.

5.3.1.2 Key findings – canopy composition.

- The ETG vines were consistently more productive than the control vines with higher FBB and more F/FS.
- The ETG vines produced more short and fewer shoots than control vines.
- The difference in productivity and shoot type was greatest in year 2 of the three year years of monitoring.

5.3.2 Leaf attributes

5.3.2.1 Physical characteristics. Leaves from the ETG vines had lower area per leaf (LA) and shorter petioles than leaves from the control vines (Table 5.6). The control vines had a relatively consistent LA of approximately 165 cm^2 each year, whereas LA varied from year to year in the ETG vines (Figure 5.5), being smallest in year 2 (mean = 105 ± 4 cm^2) and largest in year 3 (139 ± 6 cm^2). Petioles were about 4.7 cm shorter in the ETG vines than the control vines in both years (11.2 ± 0.8 cm compared with 15.9 ± 0.9 cm).

Table 5.6 Effect of year[a], treatment (extended trunk girdling and ungirdled control) and their interactions on leaf area and petiole length measured on leaves removed from Hort16A kiwifruit vines.

	P-value	
Factor	Leaf area	Petiole length
Year	0.013	0.068
Treatment	0.001	0.025
Year x treatment	0.009	0.178

[a]Leaf area was measured across 2007/2008, 2008/2009 and 2009/2010; petiole length was measured in the latter two years only.

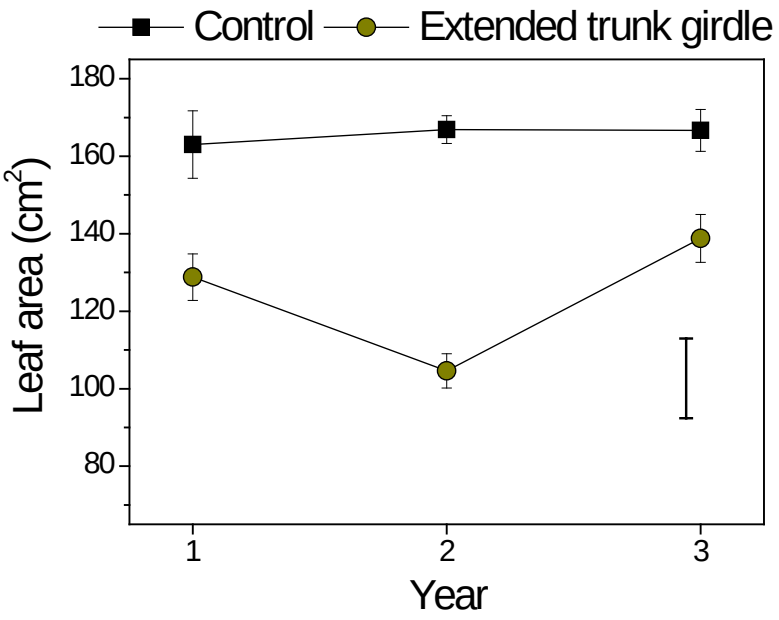


Figure 5.5 Individual leaf area measured over three years in 'Hort16A' kiwifruit vines receiving extended trunk girdles and control vines; *n* = 4 ± *SE* . Vertical bar = pooled $LSD_{0.050}$ for the interaction between treatment and year.

The ETG vines had smaller leaves than the control vines; when leaves from the same node position from the same type of shoot were compared they tended to be smaller and with shorter petioles than leaves from the same node from the same shoot type from the control vines (Figures 5.6 and 5.7).

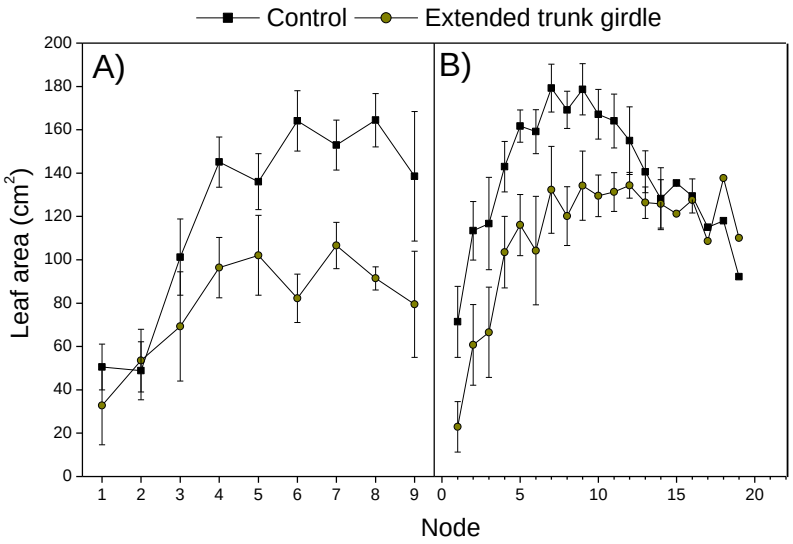


Figure 5.6 Area per leaf and node number measured in year 2 in A) short shoots (≤ 9 nodes) and B) medium shoots (10 to 18 nodes) from 'Hort16A' kiwifruit vines receiving extended trunk girdles and ungirdled control vines; *n* = 7 ± *SE*.

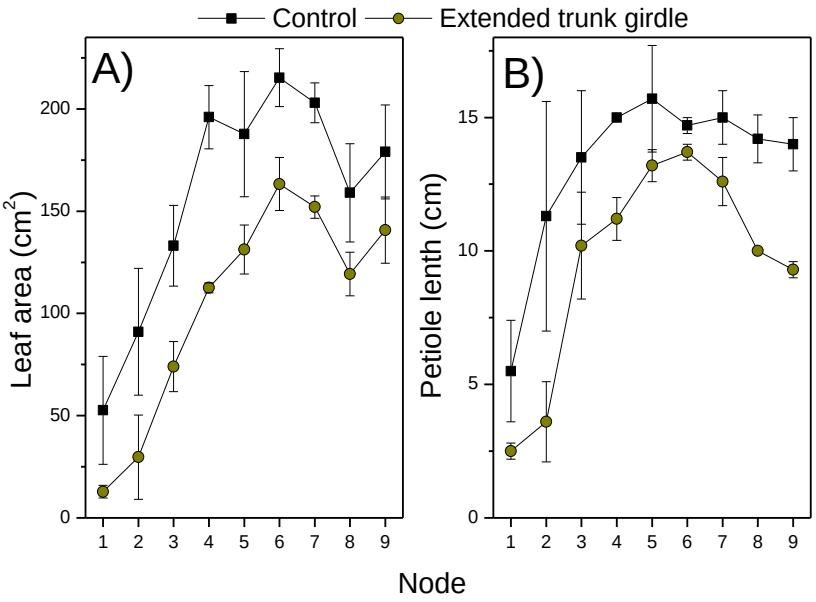


Figure 5.7 Area per leaf A) and petiole length B) as a function of node number in short shoots from 'Hort16A' kiwifruit vines receiving extended trunk girdles and ungirdled control vines; *n* = 7 ± *SE*. Shoots were sampled in January year 3.

Specific leaf weight (SLW) and leaf DMC were higher in the ETG vines regardless of whether the girdle was open or closed (Figure 5.8).

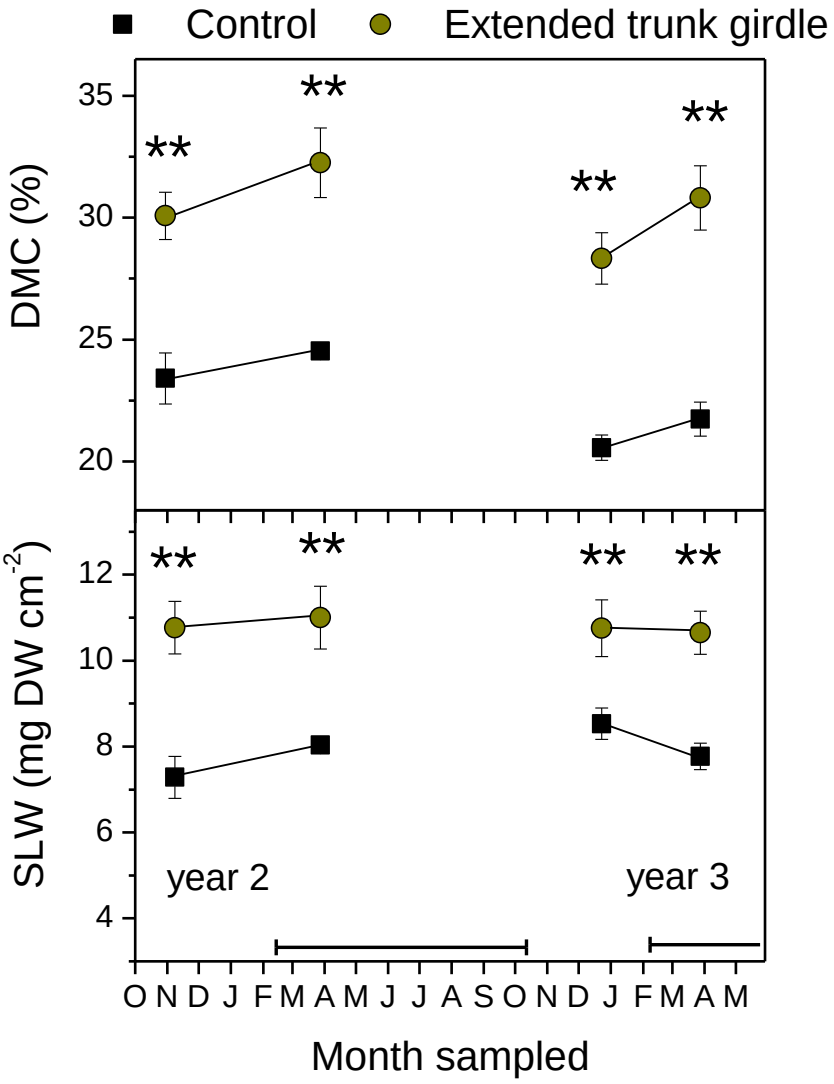


Figure 5.8 Dry matter concentration (DMC) and specific leaf weight (SLW) of leaves sampled from 'Hort16A' kiwifruit vines receiving extended trunk girdles and control vines; *n* = 4 ± *SE*; ** = *P* ≤ 0.05; horizontal bars = times when the girdle was open.

5.3.2.2 Leaf nutrient status – industry standard. The effect of sampling date, ETG and their interactions were analysed for year 3 (Table 5.7; Figure 5.9). The results were:

- Concentrations of Ca, Mg, S, Mn and Zn were affected by ETG and sampling date and there were no interactions between the two; concentrations of these nutrients were consistently lower in leaves from the ETG vines across the year regardless of whether the girdle was open or not.
- Concentrations of N, P, K, Cu and B were affected by sampling date and ETG, and there were interactions between the two factors. Concentrations of N, P, K and Cu were relatively constant across the year in the ETG vines, whereas in the control vines concentrations of these nutrients varied during the year, generally being higher at the start of the year.
- There were no consistent differences in in leaf Fe concentrations.

Table 5.7 Effect of sampling date, treatment and their interactions on 'Hort16A' kiwifruit leaf and petiole concentrations of mineral nutrients.

	P-value		
Nutrient	Date	Treatment	Date x treatment
N	< 0.001	0.004	< 0.001
P	< 0.001	< 0.001	0.001
K	< 0.001	0.001	< 0.001
S	< 0.001	0.002	0.810
Ca	< 0.001	< 0.001	0.350
Mg	< 0.001	< 0.001	0.182
Mn	< 0.001	0.004	0.199
Zn	< 0.001	0.013	0.274
Fe	< 0.001	0.455	0.008
Cu	< 0.001	0.002	0.013
B	< 0.001	0.033	0.047

Treatments were extended trunk girdles and ungirdled controls. Leaves were sample across year 3 every 4 to 5 weeks; n = 4.

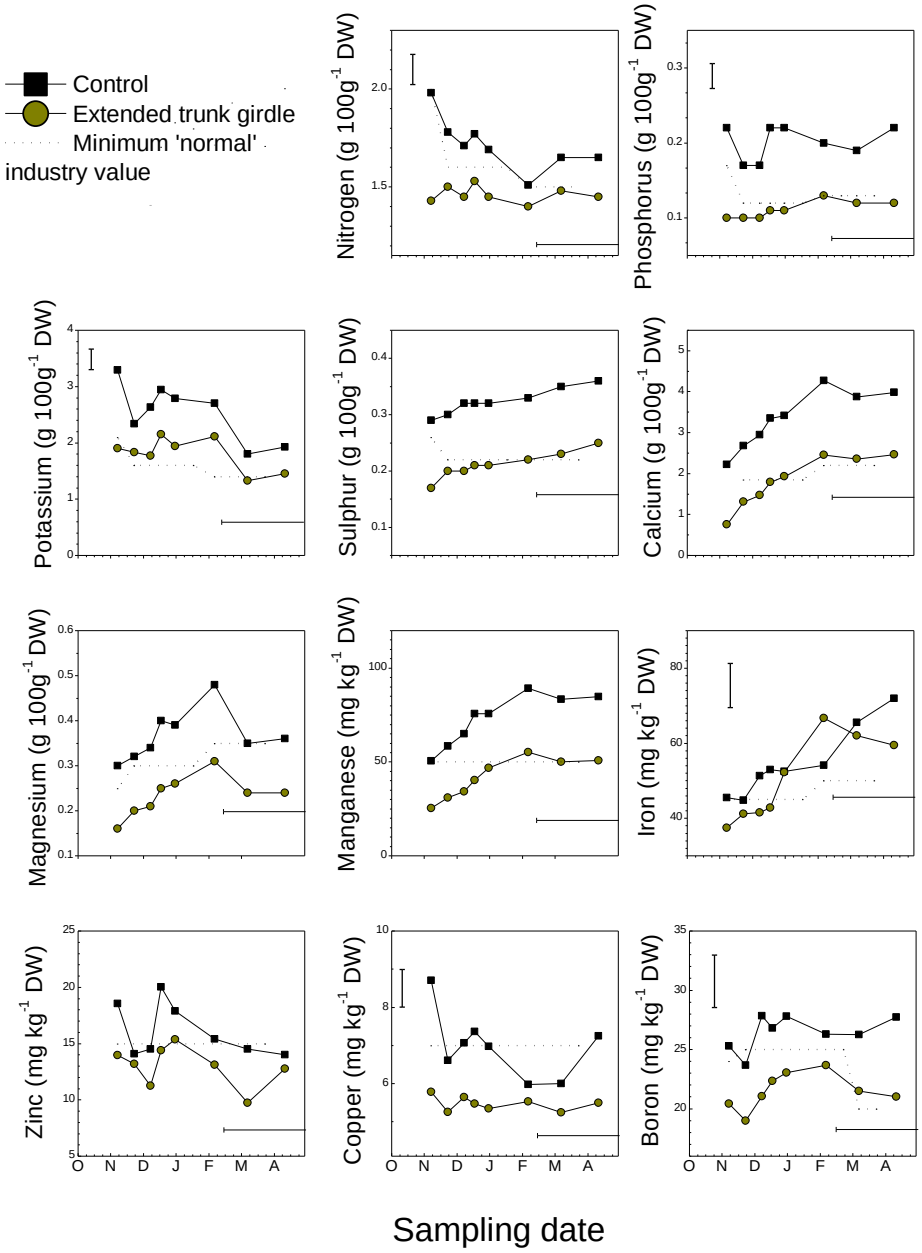


Figure 5.9 Leaf mineral nutrient concentrations measured across year 3 in 'Hort16A' kiwifruit vines receiving extended trunk girdles and ungirdled control vines; *n* = 4. Dashed line = minimum 'normal' value from RJ Hill laboratories, vertical bar = $LSD_{0.05}$ (when significant interactions between treatment and sampling date occurred), black horizontal bar = when the girdle was open.

In spring 2009, concentrations of all nutrients in leaves from the ETG vines leaves were at or below the normal range (recommended by RJ Hill Laboratories for optimal kiwifruit production) (Figure 5.9). Spring leaf nutrient concentrations were examined for all three years to determine if difference between control and ETG leaves was consistent each year (Figure 5.10). The November concentrations of most nutrients was consistently lower in the ETG leaves than the control leaves across all three years with the magnitude of the difference remaining relatively consistent across years.

Treatment differences in the April concentrations of leaf nutrients were also compared (Figure 5.11). For some minerals (N, Fe, Zn and Cu), no consistent differences were detected.

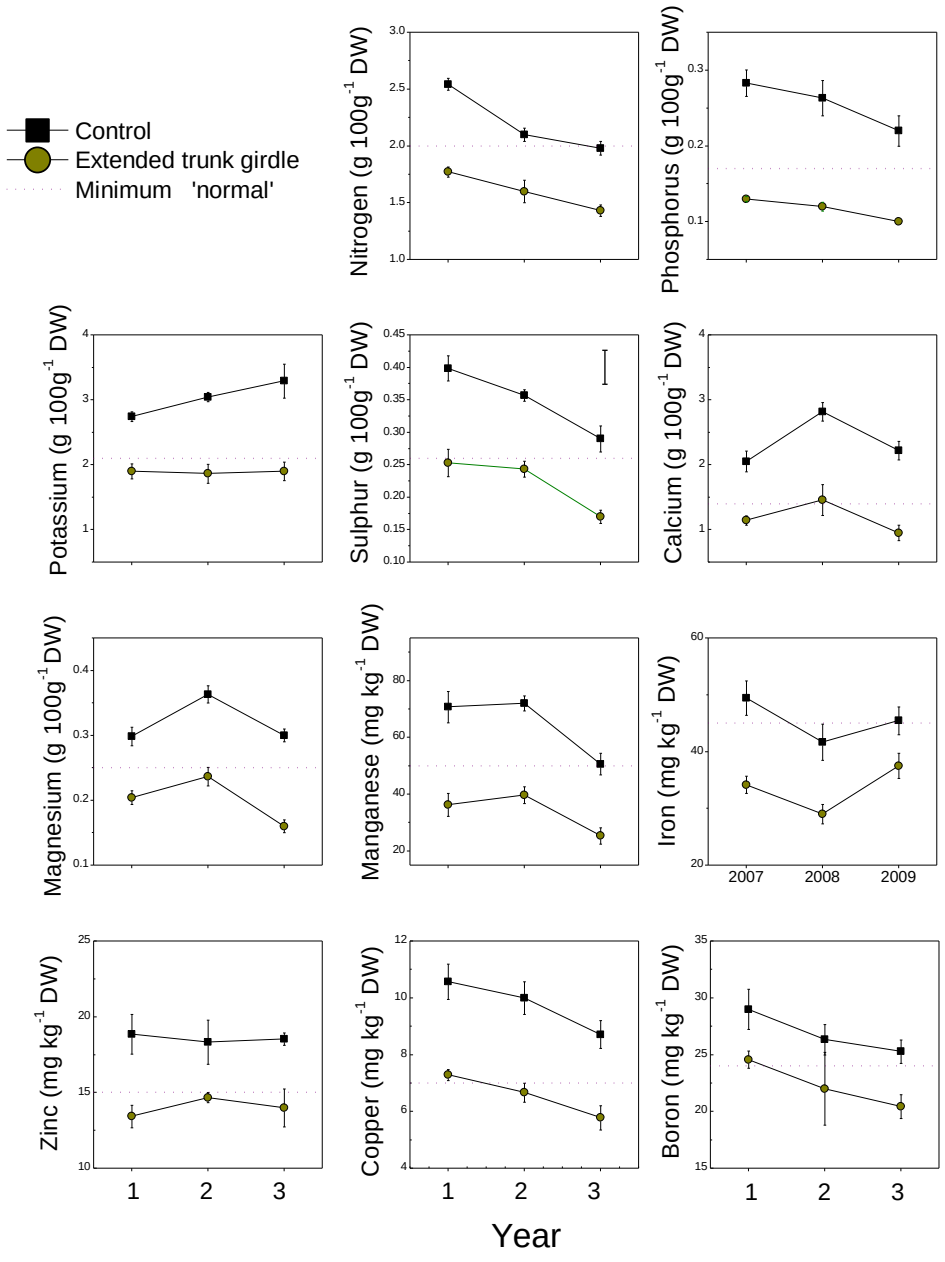


Figure 5.10 Leaf mineral nutrient concentrations sampled in November years 1 to 3 from 'Hort16A' kiwifruit vines receiving extended trunk girdles and ungirdled control vines; *n* = 4 ± *SE*. Dashed line is the minimum 'normal' for leaves sampled in November from RJ Hill Laboratories.

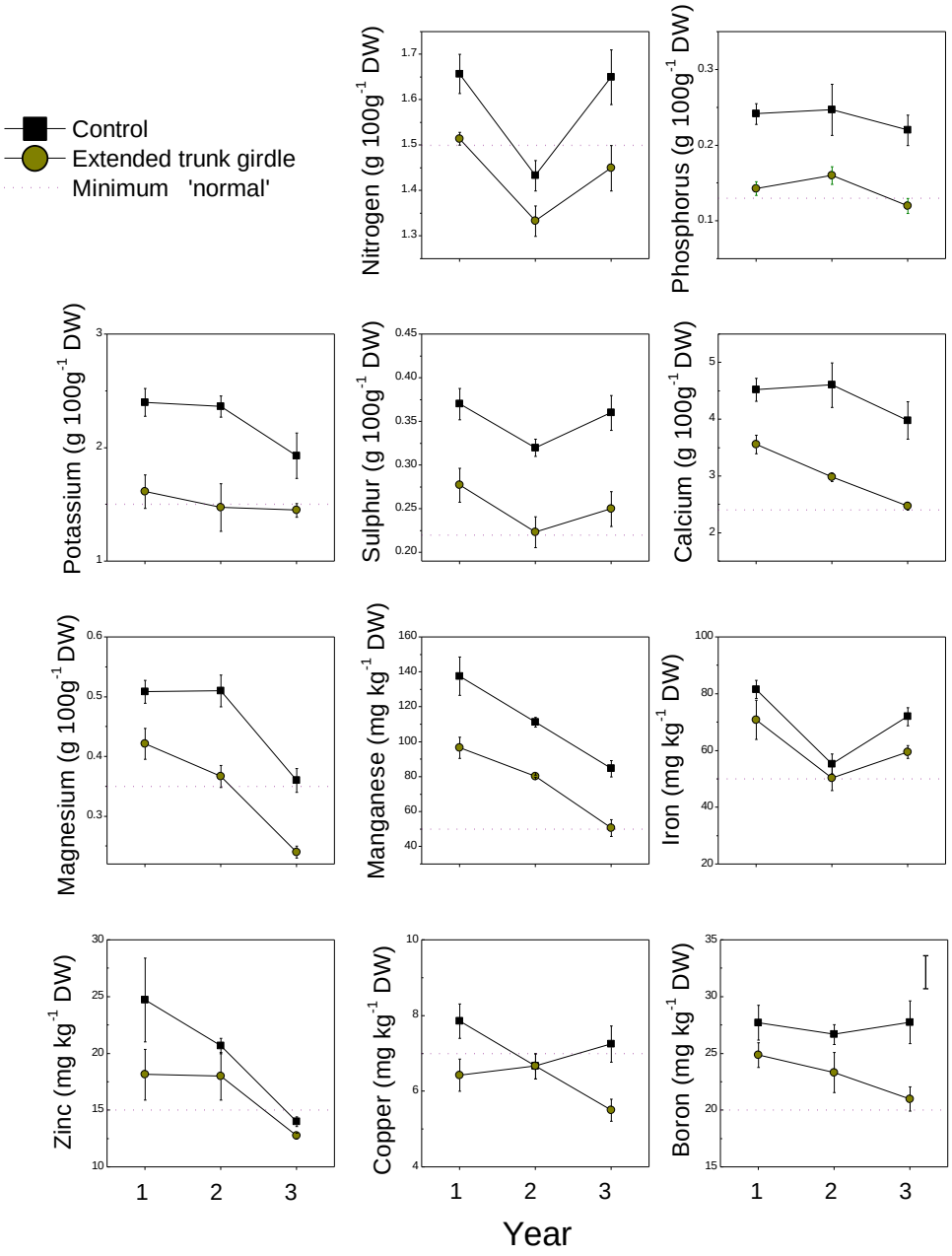


Figure 5.11 Mineral nutrient concentrations of leaves sampled in April years 1 to 3 from 'Hort16A' kiwifruit vines receiving extended trunk girdles and ungirdled control vines; *n* = 4 ± *SE*. Dashed line is the minimum 'normal' for leaves sampled in April from RJ Hill Laboratories.

5.3.2.2 Leaf nutrient status - per leaf blade area. When mineral nutrients in the leaf blade were calculated on a per cm^2 basis, there were no treatment differences in the amount of N, Zn, Cu and B (Figure 5.12; Table 5.8). Leaf contents of P, K, S, Ca, Mg, Mn and Fe were consistently lower in the ETG leaf blades than the control leaf blades.

Table 5.8 Effect of sampling date, treatment and their interactions on 'Hort16A' kiwifruit leaf blade mineral nutrient contents calculated on an area basis.

	P-value		
Nutrient	Date	Treatment	Date x treatment
N	< 0.001	0.797	0.625
P	< 0.001	< 0.001	0.994
K	< 0.001	0.029	0.781
S	< 0.001	< 0.001	0.808
Ca	< 0.001	< 0.001	0.224
Mg	< 0.001	< 0.001	0.281
Mn	< 0.001	< 0.001	0.580
Zn	< 0.001	0.451	0.201
Fe	< 0.001	0.003	0.118
Cu	< 0.001	0.643	0.888
B	< 0.001	0.272	0.923

Treatments were extended trunk girdles and ungirdled control vines. Leaves were sampled at 4 to 5 weekly intervals across year 3; n = 4.

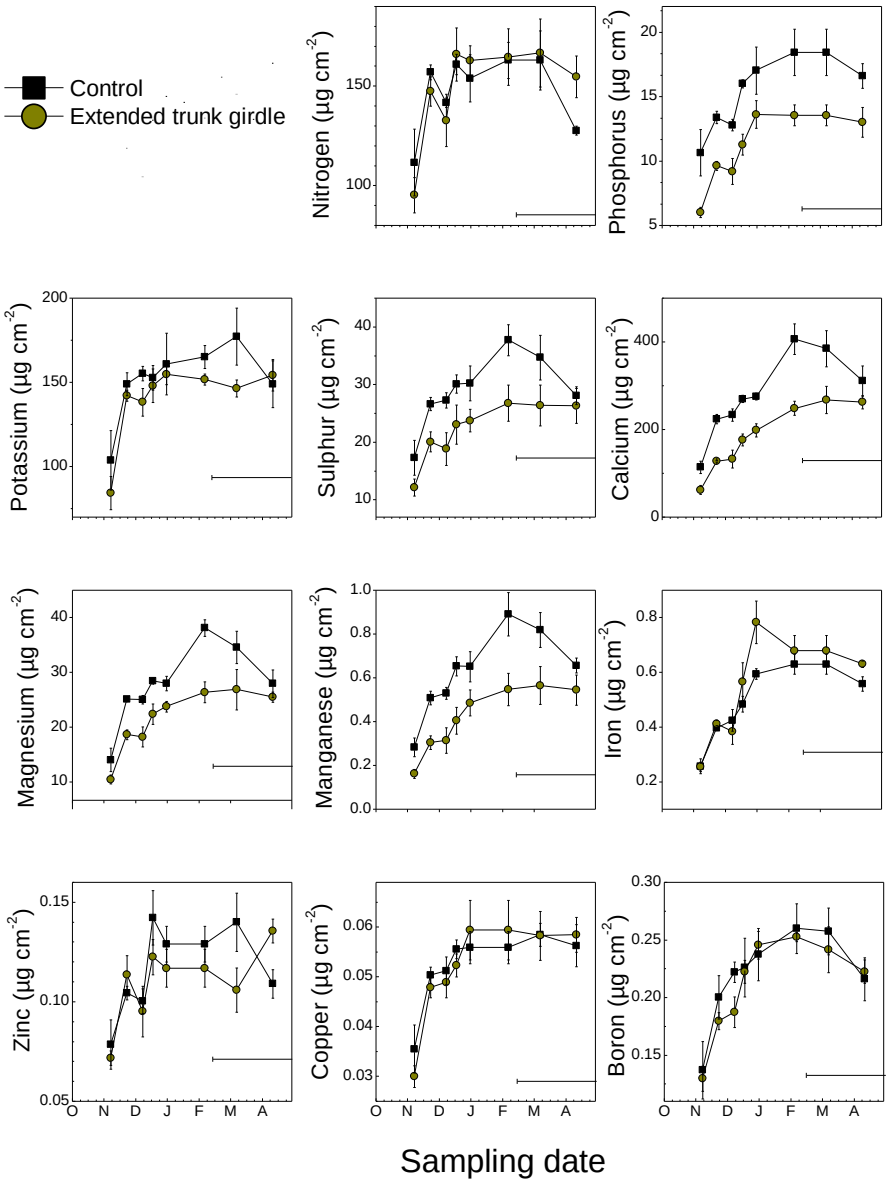


Figure 5.12 Leaf blade mineral nutrient contents measured on an area basis across year 3 in 'Hort16A' kiwifruit vines receiving extended trunk girdles and ungirdled control vines; *n* = 4 ± *SE*; the black horizontal bar represents the time when the girdle was open

5.3.2.3 Gas exchange measurements. There were no treatment differences or consistent trends in net carbon exchange rate (NCER; Figure 5.13). Stomatal conductance (g_s) was marginally higher in the ETG leaves in February and March 2008 (before and after girdle application), but not in 2009. Approximately 25 days after girdling in 2010 there was a trend towards lower g_s and lower NCER in the ETG vines, but this trend was not observed in the previous year (Figure 5.13). Leaf internal CO_2 concentrations (C_i) were higher in the ETG vines during the middle of the year both before and after girdling in both years.

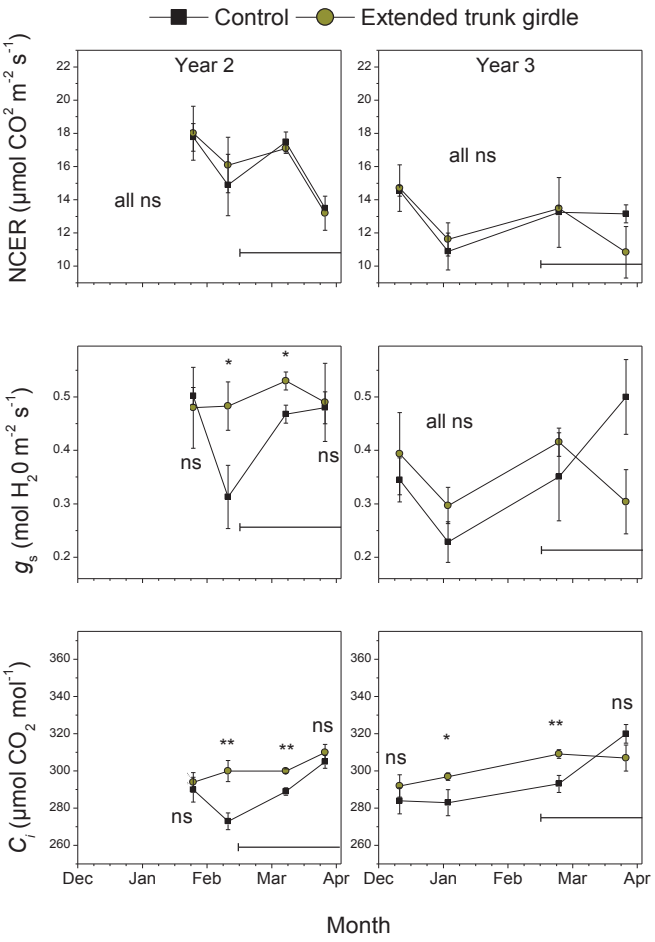


Figure 5.13 Net carbon dioxide exchange rate (NCER), stomatal conductance (g_s) and internal CO_2 concentration (C_i) in leaves from 'Hort16A' kiwifruit vines receiving extended trunk girdles and ungirdled control vines; measurements were made during years 2 (left column) and 3 (right column); n = 4 ± SE. Horizontal bars show when the girdle was open; ** = $P \leq 0.050$, * = $P \leq 0.010$, ns = $P > 0.100$.

5.3.2.4 Carbohydrates and chlorophyll. Before girdling, treatment differences in NSC concentrations were relatively small; starch and sugars concentrations were sometimes higher in leaves from the control vines but not consistently (Figure 5.14). After girdling, NSC concentrations in ETG leaves increased relative to control leaves. By mid-April (approximately 60 days after girdling), concentration of carbohydrates in the leaves of ETG vines was approximately 66 % higher than control leaves in year 2 and 50 % higher than control leaves in year 3. Concentration of NSC in abscised leaves from the ETG and control vines was the same, at 7 to 8 mg g^{-1} DW (data not presented), indicating that the accumulated NSC was remobilised to the vines before abscission. Leaf starch concentrations tended to be slightly higher in year 2 than year 3. Leaf chlorophyll per unit area was unaffected by the ETG except at November year 2 when it was lower in ETG leaves than leaves from control vines (Table 5.9). Chlorophyll concentrations tended to be lower in ETG leaves than in control leaves, but there were no consistent differences. The chlorophyll/N ratio was lower in ETG leaves in April of both years and concentrations measured in November, January and April were not affected by ETG treatment (Table 5.9).

Table 5.9 Chlorophyll contents of leaves sampled in years 2 and 3 from 'Hort16A' kiwifruit vines receiving extended trunk girdles and ungirdled control vines.

	Year 2		Year 3	
Sampling date	Control	ETG	Control	ETG
	Chlorophyll (µg cm^{-2})			
November	**28.8 ± 3.2a**	**22.2 ± 0.3b**	20.7 ± 1.5a	17.8 ± 1.6a
January	32.4 ± 5.7a	31.6 ± 2.9a	43.3 ± 0.4a	39.6 ± 6.8a
April	27.3 ± 0.9a	28.2 ± 1.5a	43.7 ± 1.5a	49.0 ± 3.6a
	Chlorophyll (mg g^{-1} DW)			
November	**4.2 ± 0.9a**	**2.1 ± 0.1b**	**4.7 ± 0.5a**	**2.9 ± 0.1b**
January	4.2 ± 0.3a	2.9 ± 0.2a	5.1 ± 0.3a	3.9 ± 0.5a
April	**3.4 ± 0.1a**	**2.6 ± 0.5b**	5.7 ± 0.4a	4.6 ± 0.3a
	Chlorophyll/N ratio			
November	**0.18 ± 0.01a**	**0.12 ± 0.01b**	0.21 ± 0.02a	0.20 ± 0.04a
January	0.12 ± 0.02a	0.08 ± 0.01a	0.22 ± 0.02a	0.20 ± 0.06a
April	**0.21 ± 0.01a**	**0.18 ± 0.01b**	**0.26 ± 0.01a**	**0.21 ± 0.01b**

Pairs of data accompanied by different letters and highlighted in bold are different ($P \leq 0.05$); $n = 4 \pm SE$.

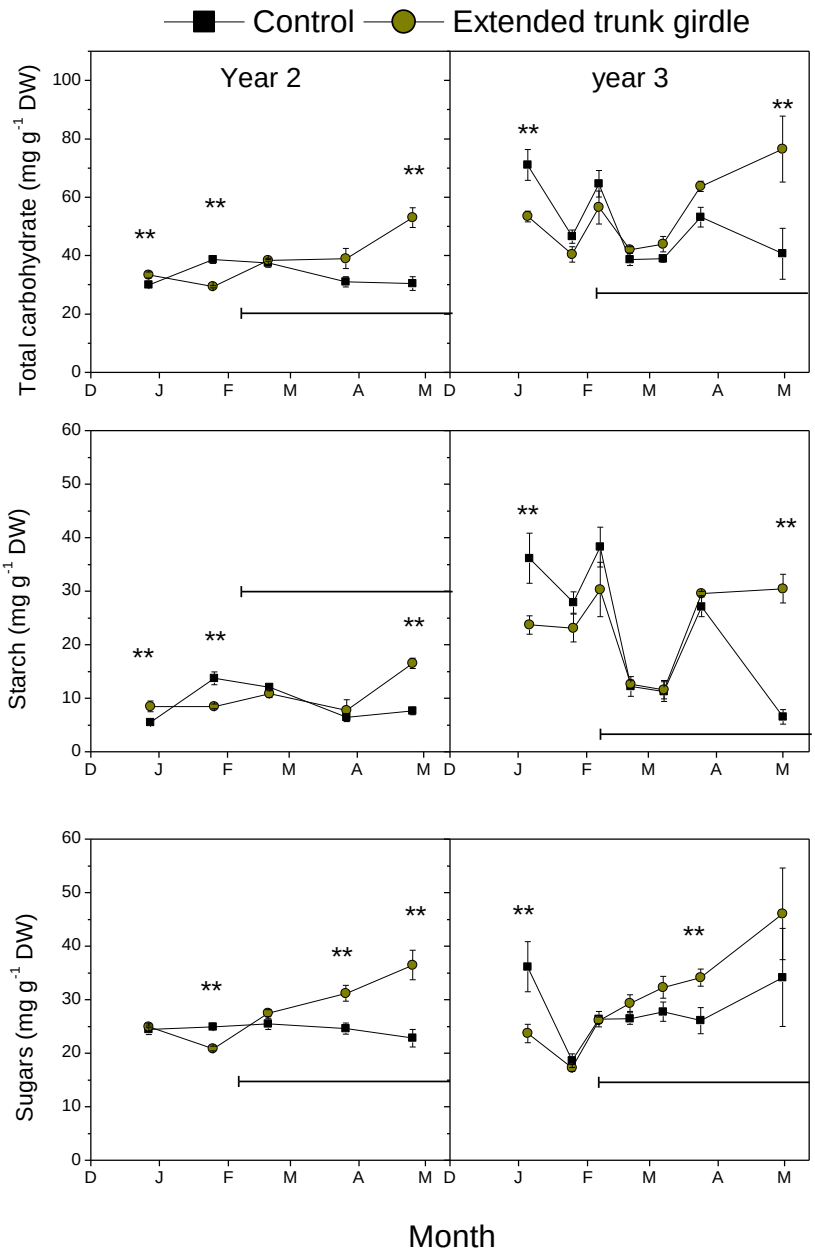


Figure 5.14 Non-structural carbohydrates (total starch and soluble sugars, starch and soluble sugars) concentrations in leaves from 'Hort16A' kiwifruit vines receiving extended trunk girdles and ungirdled control vines; *n* = 4 ± *SE*; ** = *P* ≤ 0.05; horizontal bars show when the girdle was open.

5.3.2.4 Key findings - leaf attributes.

- The ETG vines produced leaves with smaller blades and shorter petioles than the control vines. Area per leaf remained relatively constant in the control vines over the three years, the largest treatment difference occurred in year 2.
- Leaf blades from the ETG vines had higher specific leaf weight and DMC than the control vines both early and late in the year.
- Mineral nutrient concentrations, measured using the industry standard method, were lower in the ETG vines than the control vines, over most of the year, except for Fe.
- When leaf blade mineral contents were calculated on a per area basis there were few treatment differences, the main exceptions being that P, Ca and Mn were lower in the ETG leaves in November and N higher in the ETG leaves in April.
- Leaf photosynthetic capacity and stomatal conductance were not affected by the ETG treatment, internal CO_2 concentration in the ETG leaves increased around January and February in both years of measurement.
- Non-structural carbohydrates accumulated in leaves of the ETG vines in autumn.

5.3.3 Fruit attributes

5.3.3.1 Fresh weight and dry weight accumulation. In both treatments, fruitlets were thinned in spring to obtain crop loads of around 40 fruit per m^2. Approximately 3 times more fruitlet DW was thinned from the ETG vines than the control vines (Table 5.10). In year 1, individual fruit FW accumulation was slightly lower in the ETG vines by the time of fruitlet thinning (mean FW = 36.1 g in the control vines and 32.1 g in the ETG vines, approximately 11 % lower). In the following year both FW and DW accumulation into the ETG fruit was lower at thinning time, by approximately 28 % (for FW) and 33 % (for DW) than in the control fruit. In year 3, thinning took place earlier than in previous seasons (mean FW approximately 9 g), and no treatment differences in mean fruit FW or DW were detected.

Table 5.10 Mean fresh weights, dry weights and total biomass of fruitlets removed during spring thinning from 'Hort16A' kiwifruit vines receiving extended trunk girdles (ETG) and ungirdled control vines.

Year	Fresh weight		Dry weight	
	Control	ETG	Control	ETG
	Mean weight at thinning (g fruit^{-1})			
1	**36.1 ± 1.1a**	**32.1 ± 1.0b**	2.9 ± 0.1a	2.7 ± 0.1a
2	**34.5 ± 0.8a**	**24.5 ± 1.0b**	**2.7 ± 0.1a**	**1.8 ± 0.1b**
3	9.1 ± 0.5a	9.2 ± 0.8a	0.6 ± 0.1a	0.7 ± 0.1a
3[a]	57.2 ± 1.4a	54.0 ± 0.9a	6.1 ± 0.2a	6.1 ± 0.1a
	Total biomass removed (kg vine^{-1})			
1	22.1 ± 3.1	59.6 ± 6.4	1.7 ± 0.2	4.5 ± 0.5
2	16.9 ± 2.9	61.3 ± 4.6	1.3 ± 0.2	4.7 ± 0.4
3	6.8 ± 1.8	20.5 ± 2.0	0.6 ± 0.2	1.6 ± 0.3
3[a]	4.2 ± 1.2	28.1 ± 4.0	0.5 ± 0.3	3.7 ± 1.0

Pairs of means within any year accompanied by different letters are significantly different P ≤ 0.05, n = 7 ± SE; [a]a second round of thinning was carried out in year 3 as recounts indicated that crop loads were still above 40 fruit per m^2.

Mature fruit FW was unaffected by ETG (Table 5.11). Fruit from the ETG vines recovered from the delay in FW accumulation detected in spring of years 1 and 2 (Table 5.10). Mature fruit DMC was 1.7 to 2.5 % - units higher in the ETG fruit than the control fruit (Table 5.11), even in year 2 when early-season DW accumulation into ETG fruit was significantly lower than in control fruit (Table 5.10).

Table 5.11 Fresh weight (FW) and dry matter concentration (DMC) at harvest in fruit from 'Hort16A' kiwifruit vines receiving extended trunk girdles and ungirdled control vines.

Attribute	Year 1		Year 2		Year 3	
	Control	ETG	Control	ETG	Control	ETG
FW (g)	89.5 ± 0.8	96.0 ± 0.9^{ns}	90.2 ± 2.7	83.5 ± 4.4^{ns}	84.0 ± 1.8	90.2 ± 1.7^{ns}
DMC (%)	17.2 ± 0.2	19.2 ± 0.2**	17.2 ± 0.1	18.9 ± 0.2**	17.5 ± 0.2	20.0 ± 0.3**

*Pairs of values within any year accompanied by ** are significantly different (P ≤ 0.05); ns = P> 0.050; n = 7 ± SE*

5.3.3.2 Inorganic nutrient accumulation. Mature fruit concentrations of N, P, K, S and Mg, calculated on a DW basis, were significantly ($P \leq 0.05$) lower in the ETG fruit than control fruit. Concentrations of Ca, Mn, Zn, and Fe were unaffected by ETG, and concentrations of Cu and B tended to be lower in the ETG fruit, but not consistently (Figure 5.15).

When mineral nutrients were calculated on a per mature fruit basis, there were few treatment differences. Fruit from ETG vines tended to contain more Ca, Fe and Zn than fruit from the control vines, but differences were not significant in all three years (Figure 5.16). The ratio Ca/N tended to be higher in the ETG fruit than the control fruit, but this difference was not significant (Table 5.12).

Table 5.12 Calcium (Ca) to nitrogen (N) ratios in mature fruit from 'Hort16A' kiwifruit vines receiving extended trunk girdles (ETG) and ungirdled control vines.

	Ca/N ratio		
Year	Control	ETG	*P*-value
1	0.126 ± 0.008	0.134 ± 0.003	0.389
2	0.142 ± 0.010	0.165 ± 0.014	0.133
3	0.123 ± 0.008	0.142 ± 0.013	0.376

n = 7 ± SE.

Developing fruitlets in ETG vines had lower contents of some mineral nutrients than those from the control vines, although differences were not detected every season (Figure 5.17). In particular, ETG fruit contained less N, S, Mg, Mn and Cu in years 1 and 2 and less P and Fe in all three years. Fewer treatment differences were detected in fruitlets thinned in the third year when fruitlets were thinned earlier than the previous two years (mean FW in controls = 36.1 g, 34.5 g. 9.1 g in years 1, 2 and 3 respectively). The exception to this general trend was Zn: in years 1 and 3, fruitlets from the ETG vines contained more Zn than those from the control vines.

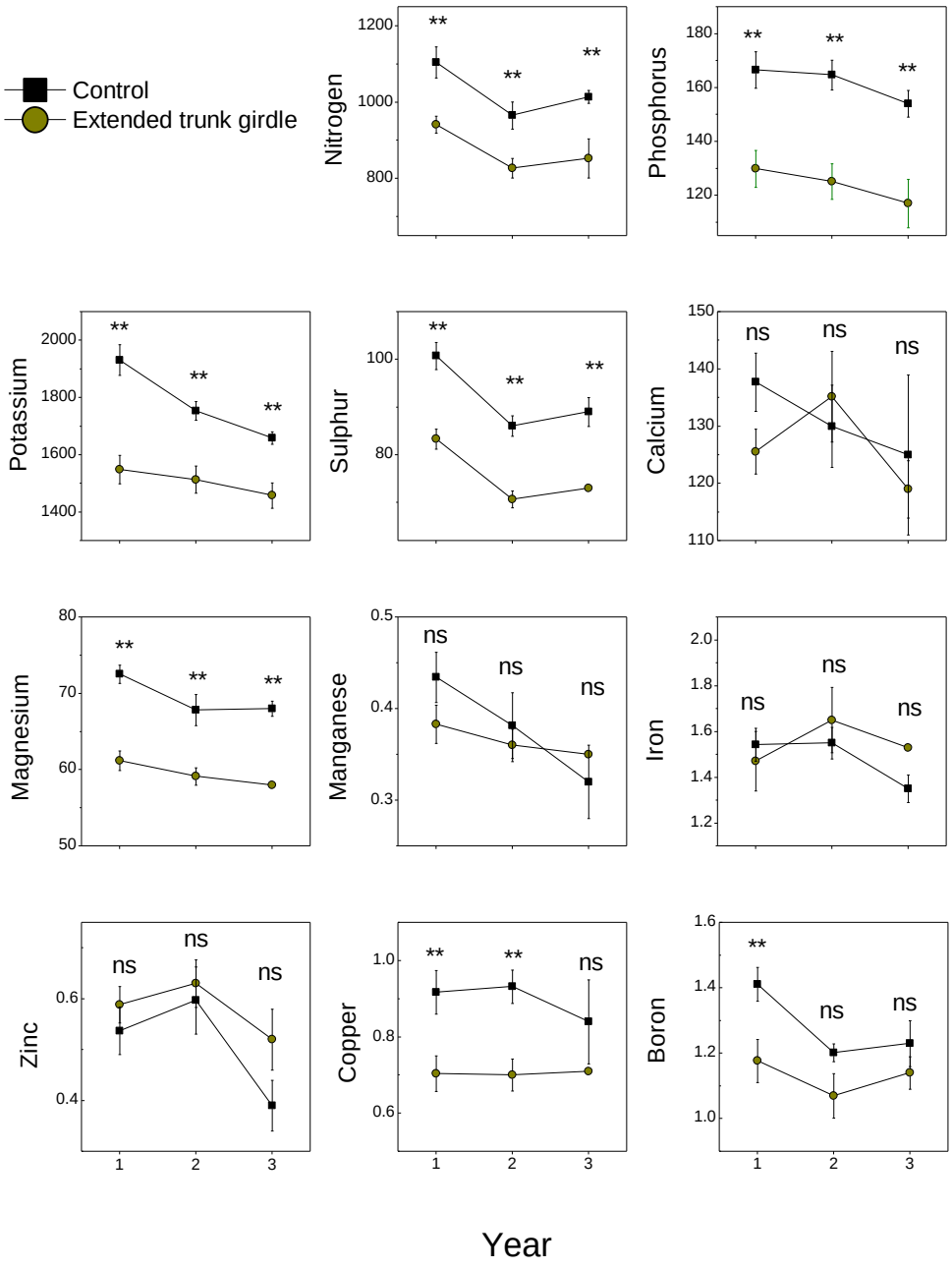


Figure 5.15 Mineral nutrient concentrations (in mg 100 g^{-1} FW) in mature fruit from vines receiving extended trunk girdles and ungirdled control vines; *n* = 7 ± *SE* in years 1 and 2, *n* = 4 ± *SE* in year 3; ** = *P* ≤ 0.050 and ns = *P* > 0.05.

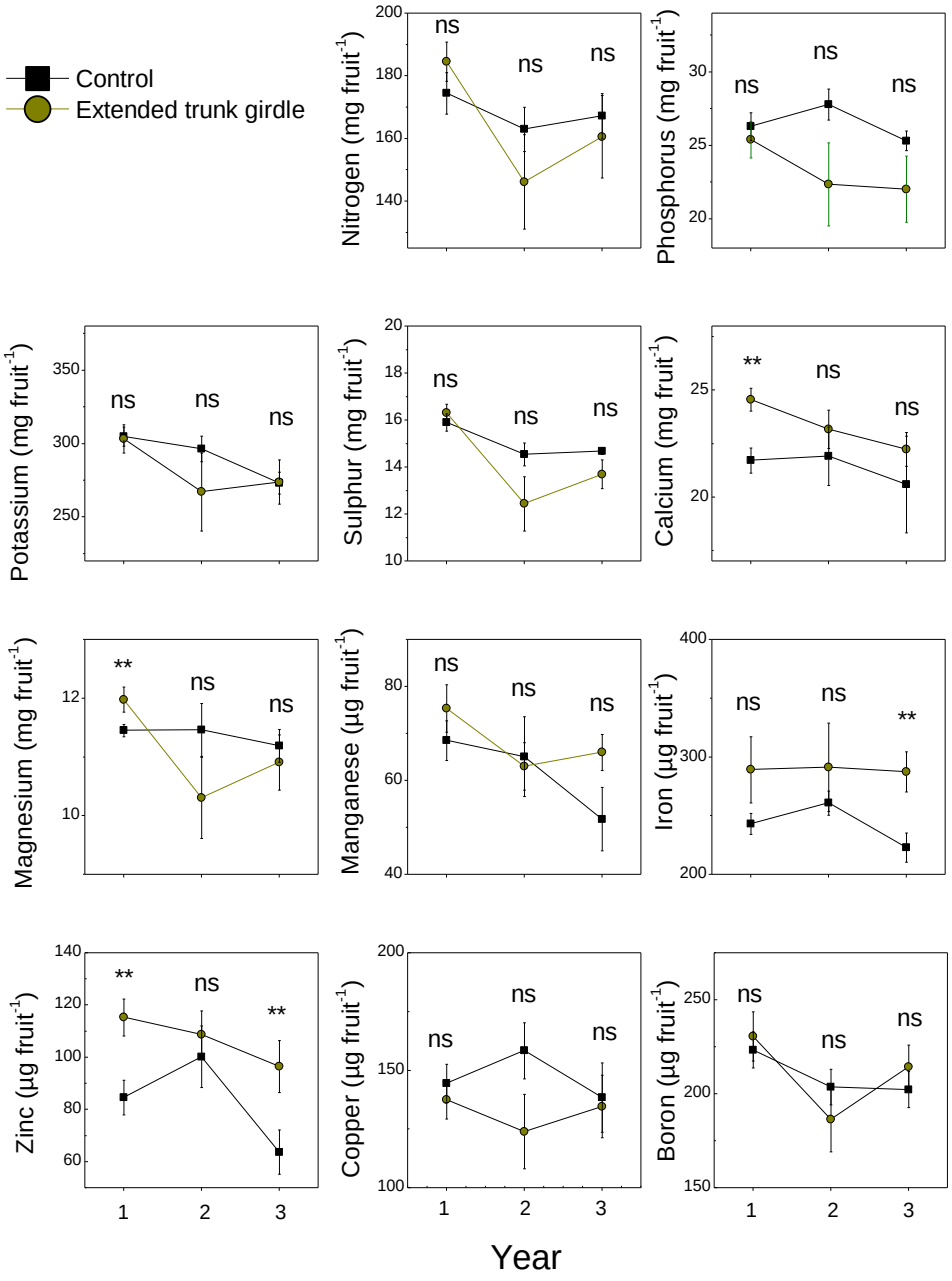


Figure 5.16 Mineral nutrient contents of mature fruit sampled from 'Hort16A' kiwifruit vines receiving extended trunk girdles and ungirdled control vines. Fruit were sampled in April in year 1 to 3; *n* = 7 ± *SE* in years 1 and 2, *n* = 4 ± *SE* in year 3; ** = *P* ≤ 0.050 and ns = *P* > 0.05.

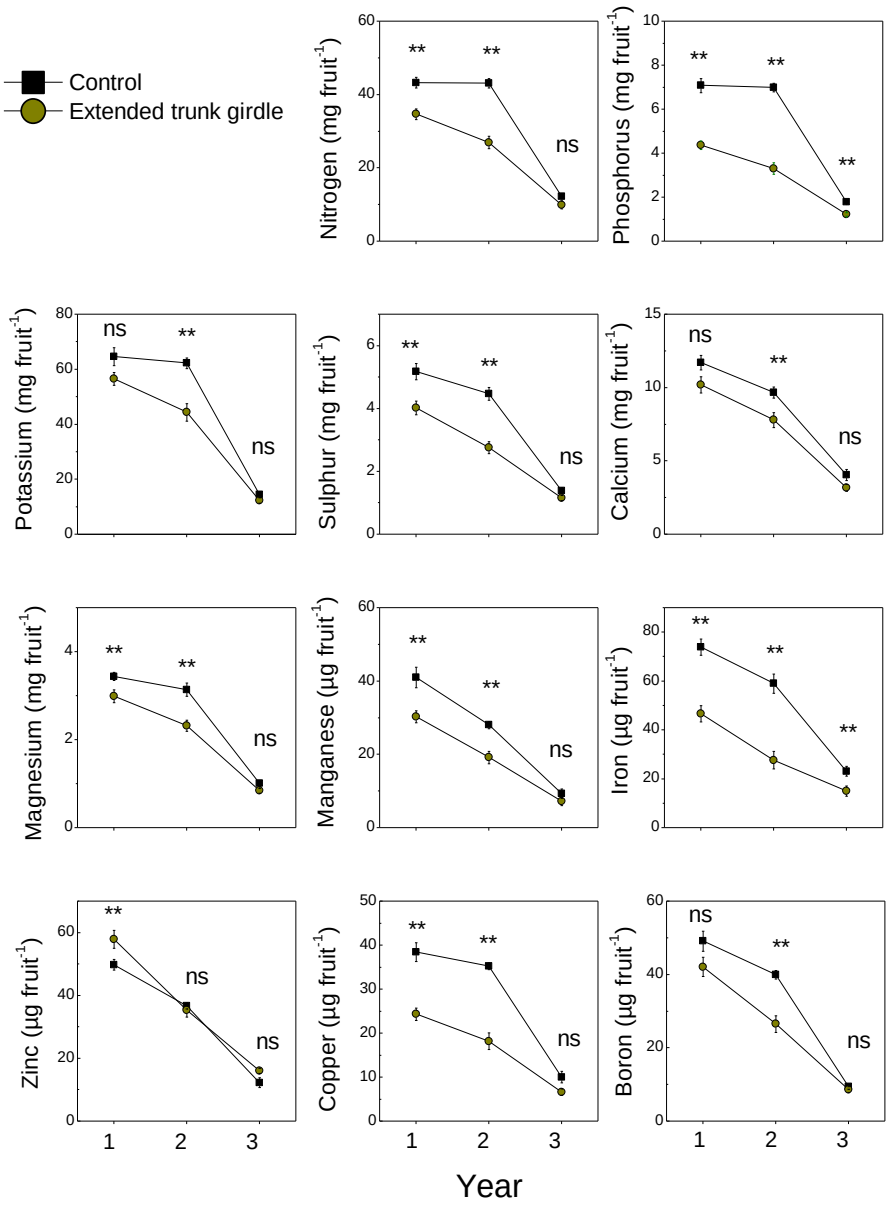


Figure 5.17 Mineral nutrient contents of fruitlets thinned during spring crop-load adjustments from 'Hort16A' kiwifruit vines receiving extended trunk girdles and ungirdled control vines. Fruit were sampled in November or December in year 1 to 3, *n* = 7 ± *SE* in years 1 and 2, *n* = 4 ± *SE* in year 3; ** = *P* ≤ 0.050 and ns = *P* > 0.05.

5.3.3.3 Flowering. In years 2 and 3, ETG affected timing, but not duration of flowering (Table 5.13). Flowering duration for both treatments was ~ 7.5 days. Mid-bloom occurred earlier in tETG vines than in control vines by 3.7 and 5.7 days in years 2 and 3 respectively. Flowering was not measured in year 1.

Table 5.13 Timing and duration of flowering in 'Hort16A' kiwifruit vines receiving extended trunk girdles compared with controls in years 2 and 3.

Flowering	Year 2			Year 3		
attribute	Control	ETG	*P*-value	Control	ETG	*P*-value
Start	13.6 ± 0.6	10.3 ± 0.3	< 0.001	14.1 ± 0.8	8.9 ± 0.8	< 0.001
Mid-bloom	17.3 ± 0.8	13.6 ± 0.4	0.001	18.1 ± 0.8	12.4 ± 0.5	< 0.001
Finish	21.5 ± 0.8	17.2 ± 0.6	< 0.001	22.2 ± 0.8	15.9 ± 0.7	< 0.001
Duration (days)	7.9 ± 0.6	6.9 ± 0.5	0.188	8.1 ± 0.5	7.0 ± 1.0	0.389

The start, mid and end of flowering are the dates when 10%, 50% and 90% of flowers were open, presented here as days after 30 September; duration = end - start, n = 7 ± SE.

5.3.3.4 Fruit maturity attributes. Each year fruit from control vines were sampled as close as practicable to degreening (mean hue angle ≤ 103^{o}), and compared with fruit from ETG vines sampled and tested on the same date. Each year, ETG fruit were more mature (higher SSC, lower flesh hue angle and softer flesh) than control fruit (Table 5.14; Figure 5.18). Mean hue angle of fruit from ETG vines was 1.3 to 3 degrees lower each year than controls indicating that ETG fruit would degreen sooner than control fruit, and be cleared to pick ~ 5 to 12 days earlier (Figure 5.19; Table 5.15).

Table 5.14 Effect of year, treatment (extended trunk girdling or ungirdled control) and their interactions on fruit maturity at harvest[a].

	P-value		
Factor	SSC	Hue	Firmness
Year	<0.001	0.023	<0.001
Treatment	<0.001	<0.001	<0.001
Year x treatment	<0.001	0.012	0.082

SSC = soluble solids concentration. [a]Fruit from both treatments were harvested when the mean hue angle of the control fruit was close to 103^{o} each year for three years.

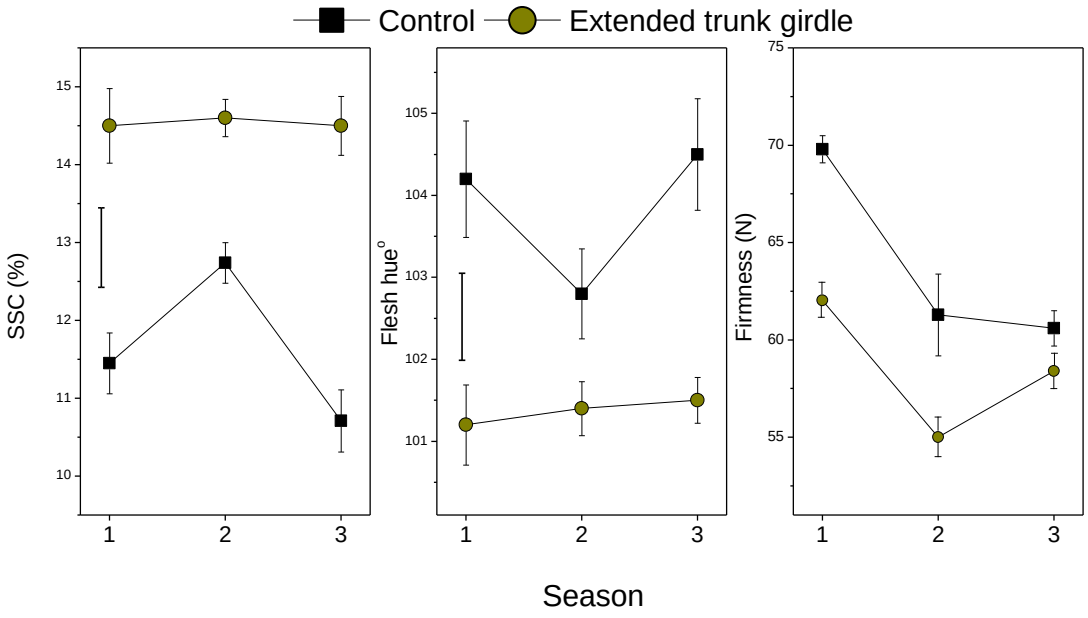


Figure 5.18 Fruit soluble solids concentrations (SSC), flesh hue angles and firmness in fruit sampled over three year from 'Hort16A' kiwifruit vines receiving extended trunk girdling and ungirdled control vines; *n* = 7 ± *SE*. Fruit from both treatments were sampled when the mean hue angle of the control fruit was as close as practicable to 103°. For attributes where was a significant interaction between treatment and year, the vertical bar represents $LSD_{0.05}$.

Fruit were sampled regularly in April/May to determine how treatments affected the rate change of fruit maturity attributes around commercial harvest (Figure 5.19). In years 1 and 2 regular sampling started too late and fruit from ETG vines had already degreened on the first sampling date (Figure 5.19). In year 1, control fruit degreened on approximately 9 May (estimated from Figure 5.19; and using Minchin et al. (2003; Appendix 4.6.1); summarised in Table 5.16). The ETG fruit would have degreened at least 6 days earlier as mean hue angle in ETG fruit was already 101 on 4 May. In year 2 control fruit degreened on 7 May, and ETG fruit would have degreened at least 4 days earlier as they were already at 102.3° on 4 May (Figure 5.19). In year 3, fruit from control vines degreened on 3 May and fruit from ETG vines degreened 10 days earlier on 22 April. The ETG fruit had a slightly lower SSC and were firmer on 22

April (11.6 % and 64 N) than control fruit were on 3 May (12.0 % and 58 N; Table 5.15).

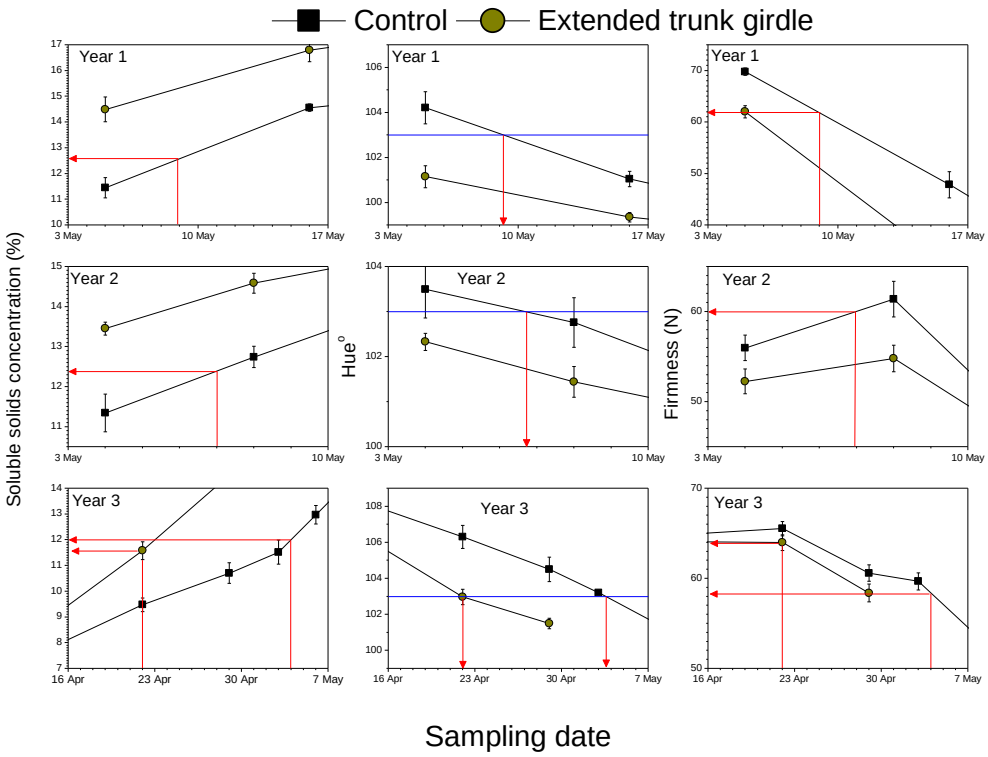


Figure 5.19 Soluble solids concentrations, flesh hue angles and firmness measured over three year in 'Hort16A' kiwifruit from vines receiving extended trunk girdles and ungirdled control vines; *n* = 7 ± *SE*. Blue line = 103° hue, downward arrows estimate date of degreening, upward arrows estimate firmness and SSC at degreening date.

Table 5.15 Soluble solids concentrations (SSC) and firmness at commercial harvest in fruit from 'Hort16A' kiwifruit vines receiving extended trunk girdles (ETG) and ungirdled control vines.

	Date degreened[a,b] (commercial harvest)		SSC when degreened (%)		Firmness when degreened (N)	
Year	Control	ETG	Control	ETG	Control	ETG
1	10 May	Before 4 May (30 Apr)	12.5	-	62	-
2	7 May	Before 3 May (30 Apr.)	12.4	-	60	-
3	4 May	22 Apr. (25 Apr.)	12.0	11.5	58	65

n = 7; [a]fruit have degreened when mean hue angle ≤ 103°; data estimated from Figure 5.19, [b]dates in brackets estimated using the equation in Appendix 4.6.1 (from Minchin et al., 2003).

5.3.3.5 Storage quality.

Softening. In all of the seven sampling dates listed in Table 5.16 fruit from the ETG vines tended to be softer after storage than fruit from control vines. Fruit from both treatments were picked on the same day and stored for 18 weeks at 1.5 ^{o}C. In four of the seven dates, significant differences were detected ($P \leq 0.05$). The magnitude of the difference was relatively small - in the range of 0.8 to 1.2 N, but for commercial purposes a batch of 'Hayward' kiwifruit can be rejected for export if softer than 10 N (Hopkirk et al., 1996), so small differences in firmness differences can have big implications.

In year 2, fruit were harvested at 3 to 5 day intervals to account for the ETG fruit being ~ 4 days older the than control fruit (based on flowering date table 5.13). If the firmness values from the ETG fruit after 18 weeks in storage are compared with control fruit harvested ~ 4 days later (see the arrows in Table 5.16), then the ETG fruit harvested on 4 May - although significantly softer than control fruit harvested on 4 May - would not be significantly softer than control fruit of the same age (those harvested on 8 May).

Table 5.16 Average firmness (N) of 'Hort16A' kiwifruit from vines receiving extended trunk girdles (ETG) or control vines after 18 weeks in coolstore.

		Treatment		
Year	Date	Control	ETG	*P*-value
2008	5 May	7.5 ± 0.1	7.1 ± 0.2	0.137
	16 May	**8.4 ± 0.3**	**7.2 ± 0.2**	**0.019**
	26 May	**8.7 ± 0.2**	**7.5 ± 0.2**	**< 0.001**
2009	**4 May**	**7.7 ± 2.9**	**6.9 ± 2.6**	**0.007**
	8 May	6.5 ± 2.4	6.2 ± 2.3	0.277
	11 May	8.3 ± 0.6	7.6 ± 0.4	0.366
	18 May	**7.1 ± 0.4**	**6.0 ± 0.1**	**0.031**

Fruit were stored at 1.5°C. At each date fruit from both treatments were harvested and stored for the same length of time; n = 7 ± SE; data with P < 0.050 are highlighted in bold.

When fruit from the control and ETG vines were sampled on the same date, the ETG fruit were softer than the control fruit (Figures 5.18 and 5.19), and softened during storage to threshold values of 20 N and 10 N sooner than fruit from the control vines (Figure 5.20; Table 5.17).

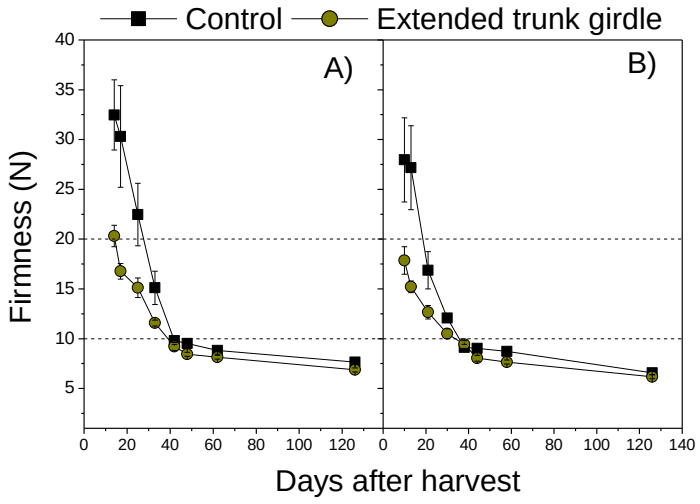


Figure 5.20 Softening curves for fruit from 'Hort16A' kiwifruit vines receiving extended trunk girdles (ETG) and ungirdled control vines. Fruit were harvested on A) 4 and B) 8 May year 2. Dashed lines are 20 N and 10 N for time of softening comparisons.

Table 5.17 Summary of harvest maturity and softening of fruit from 'Hort16A' kiwifruit vines receiving extended trunk girdles (ETG) and ungirdled control vines.

	4 May 2009		8 May 2009	
Attribute	Control	ETG	Control	ETG
Firmness at harvest (N)	61 ± 1	55 ± 1	56 ± 2	52 ± 1
Hue angle at harvest (°)	103.5 ± 0.6	102.3 ± 0.2	102.8 ± 0.6	101.4 ± 0.2
SSC at harvest (%)	11.3 ± 0.5	13.5 ± 0.2	12.7 ± 0.3	14.6 ± 0.2
Time to reach 20 N (days)	29	13	20	< 10
Time to reach 10 N (days)	42	39	35	33

n = 7 ± SE, softening times estimated from Figure 5.20.

To make a valid comparison of storage behaviour fruit from each treatment need to be harvested when the fruit would be cleared for commercial harvest. When ETG and control fruit were harvested as close as practicable to 103°, the control fruit softened more rapidly in store than the ETG fruit (Figure 5.21; Table 5.17).

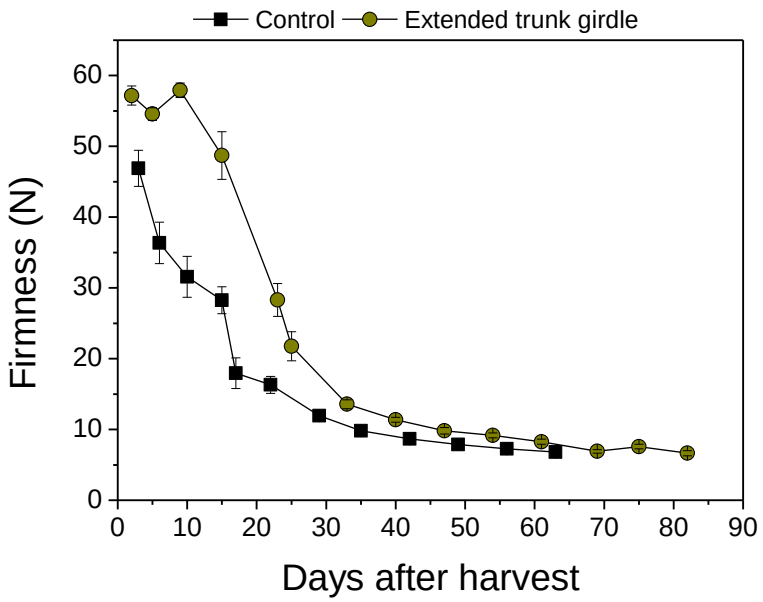


Figure 5.21 Softening curves of 'Hort16A' kiwifruit from vines receiving extended trunk girdles harvested at 103° hue and fruit from ungirdled control vines harvested at 102.8° hue.

Storage disorders. Fruit from the ETG vines showed little sign of low temperature breakdown (LTB) in years 1 and 2, and disorders were not assessed in year 3 (Figure 5.22). Incidence of LTB in the control fruit decreased with harvest date. Fruit from the ETG vines were more mature than fruit from the control vines, and were harvested too late to determine if they would develop LTB if harvested at the same hue angle as the control fruit.

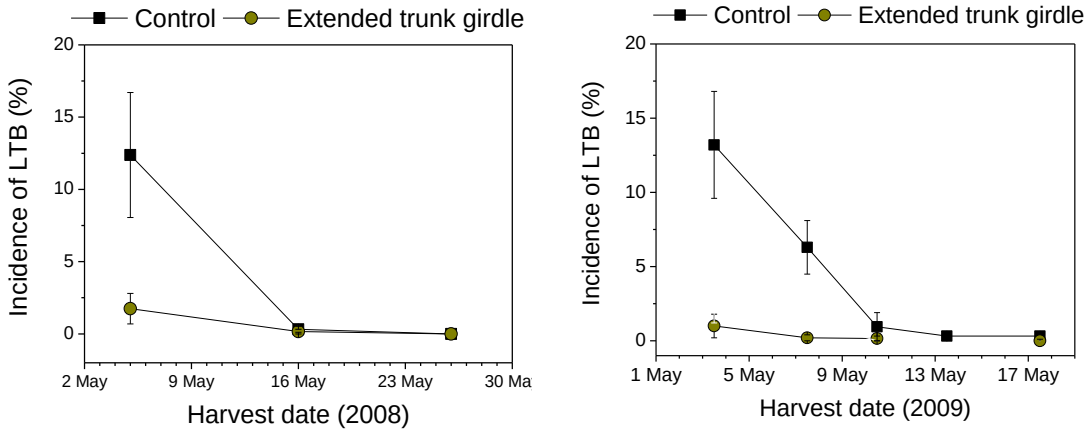


Figure 5.22 Incidence of low temperature breakdown (LTB) in fruit from 'Hort16A' kiwifruit vines receiving extended trunk girdles and ungirdled control vines. Fruit were stored at 1.5 °C for 18 weeks; *n* = 7 ± *SE*.

5.3.3.6 Key findings – fruit attributes.

- ETG vines carrying the same crop load (40 fruit per m^2) as control vines produced fruit with the same FW and higher DMC than the fruit from control vines.
- Mineral nutrient contents of mature fruit were unaffected by the ETG treatment.
- The ETG vines flowered approximately 4 - 7 days earlier than the control vines, depending on the year.
- Near commercial harvest, fruit from ETG vines were more mature (higher SSC, softer and lower flesh hue angle) than control fruit sampled on the same day.
- There were no clear treatment effects on softening during storage.

- Softening varied with harvest date, and there is some suggestion that when fruit from the ETG vines are cleared for harvest they are less likely to soften rapidly in storage - further work is needed to confirm this observation.

5.3.4 Resource allocation

5.3.4.1 Biomass. Total vine biomass was affected by extended trunk girdling. The mean total DW of the ETG vines was 102.0 ± 5.2 kg compared with 125.9 ± 5.0 kg in the control vines (Figure 5.23). The main difference was in the weight of the roots; the ETG vines had approximately 20 kg less root DW than the control vines (Table 5.18). A second difference in the perennial parts of the vine was that the proportion of older canes in the canopy was greater in the ETG vines (approximately 78 % of canes) than the control vines (approximately 60 %; Table 5.18). Overall the DW of the perennial vine parts was 28 % less in the ETG vines than the control vines primarily because of lower root biomass.

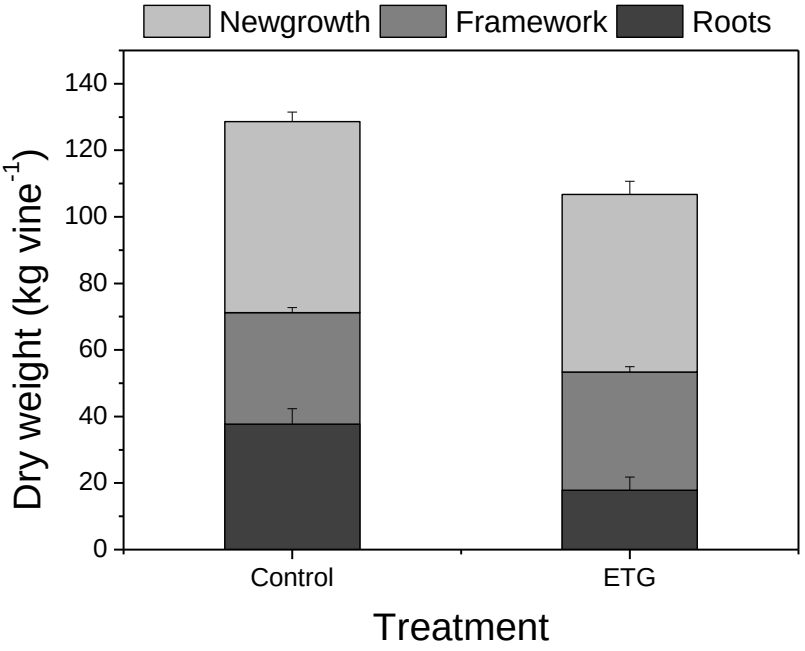


Figure 5.23 Total biomass of 'Hort16A' kiwifruit vines receiving extended trunk girdles (ETG) and ungirdled control vines; *n* = 3 ± *SE*. New growth = all annual canopy growth (pruned material, thinned fruitlets, abscised leaves and all fruit, shoots and leaves still attached to the vines at excavation); framework = canes, leader, trunk and crown; roots = structural and fine roots.

Table 5.18 Total and within-vine biomass allocation to 'Hort16A' kiwifruit vines receiving extended trunk girdles (ETG) and ungirdled control vines

	Dry weight (kg vine^{-1})			Change[b] %
Component	Control	ETG	*P*-value	
Perennial components				
Canes (1 year)	8.8 ± 1.1	4.6 ± 0.4	0.107	
Canes (> 1 year)	13.9 ± 1.0	15.9 ± 1.0	0.396	
Leader	13.3 ± 0.8	14.5 ± 0.3	0.361	
Trunk	2.2 ± 0.5	2.7 ± 0.8	0.217	
Crown	**2.8 ± 0.2**	**1.4 ± 0.2**	**0.054**	**50**
Roots - structural	**36.1 ± 4.4**	**17.0 ± 3.8**	**0.004**	**47**
Roots - fibrous[a]	**1.6 ± 0.2**	**0.9 ± 0.2**	**0.062**	**56**
Total perennial	**78.7 ± 4.9**	**57.0 ± 2.8**	**0.013**	**72**
New season's canopy growth				
Fruitlets thinned	1.1 ± 0.4	5.3 ± 0.7	**0.019**	
Mature fruit	16.7 ± 0.7	19.1 ± 0.8	0.266	
Summer prunings	10.8 ± 0.5	2.4 ± 0.6	**<0.001**	
Leaves - live	7.4 ± 1.8	7.5 ± 1.0	0.973	
Leaves - abscised	8.0 ± 1.9	9.2 ± 3.1	0.676	
Shoots	3.2 ± 0.9	1.5 ± 0.4	0.101	
Total new season's canopy growth	47.2 ± 1.4	45.0 ± 3.2	0.592	

[a]fibrous roots are short-lived and should not really be considered parts of the perennial vine structure, the classification is based on that of Clark and Smith (1992); [b]change = 100 % x ETG/Control; differences only presented if means that are significantly different at P < 0.10; n = 3 ± SE.

Total DW of the new season's canopy growth was unaffected by ETG treatment, but allocation between vegetative and reproductive growth was affected (Table 5.18). Higher fruitlet numbers in the ETG vines resulted in more DW removed in thinning fruitlets to 40 fruit per m^2 (5.3 kg DW per vine in the ETG and 1.1 kg DW in control vines; Table 5.18).

When total DW removed in mature fruit was compared across all seven vines and all three years, not just the three excavated vines in the final year, 0.8 to 3.7 kg DW extra was allocated to fruit in ETG vines depending on the year (Table 5.19).

Table 5.19 Dry weight (kg vine^{-1}) removed in mature fruit in three years from 'Hort16A' kiwifruit vines receiving extended trunk girdles (ETG) and controls.

Year	Control	ETG	*P* - value
1	17.5 ± 0. 5	21.2 ± 0.5	< 0.001
2	16.9 ± 0.8	17.7 ± 0.9	0.531
3	16.5 ± 0.7	19.7 ± 0.7	0.008

n = 7 ± SE

More than four times as much shoot growth was removed from control vines than from ETG vines during summer pruning (10.8 kg DW compared with 2.4 kg DW respectively; Table 5.18). Similar treatment differences were found for pruning biomass removed from all seven replicate vines across all three years (Table 5.20).

Table 5.20 Dry weight (kg vine^{-1}) removed during summer canopy pruning in three years from 'Hort16A' kiwifruit vines receiving extended trunk girdles (ETG) and controls.

Year	Date	Control	ETG	*P* - value
1[a]	26/10/2007	0.30 ± 0.05	0.07 ± 0.01	0.001
1[b]	31/1/2008	6.0 ± 0.5	1.5 ± 0.11	< 0.001
1[b]	17/3/2008	2.2 ± 0.3	1.3 ± 0.20	0.021
2[a]	25/10/2008	0.13 ± 0.03	0.04 ± 0.03	0.004
2[b]	2/2/2009	8.0 ± 0.5	0.9 ± 0.1	< 0.001
3[a]	13/11/2009	0.55 ± 0.8	0.01 ± 0.01	< 0.001
3[b]	12/1/2010	0.70 ± 0.11	0.15 ± 0.02	< 0.001
3[b]	18/2/2010	6.1 ± 0.2	1.4 ± 0.5	0.004

[a]leader pruning; [b]removal of new vegetative shoots; n = 7 ± SE

Both canopies had greater leaf area index (LAI) in year 3 than year 2 (Table 5.21). In control vines, spring LAI was 4.4 in year 2 and 7.2 in year 3. Before any canopy pruning took place, ETG vines had around half the LAI of control vines (2.2 and 3.8 in years 2 and 3 respectively).

In spring, before pruning and thinning, the control vines had 4 to 6 times more LA per fruit than ETG vines. After crop load adjustments and the first main canopy prune (November), LA per fruit in control vines was reduced by approximately 40 % in year 2 and increased (by 3 %) in year 3 when measured in Jan-Apr; this is because few fruit were thinned from the control vines in year 2. In contrast the LA per fruit in ETG vines more than doubled after thinning and pruning (Jan – Apr data) in both years (Table 5.21).

Table 5.21 Leaf area index (LAI) and leaf area (LA) per fruit in vines receiving extended trunk girdling (ETG) compared with control vines in years 2 and 3.

	Year 2		Year 3	
Ratio	Control	ETG	Control	ETG
LAI - Nov[a] (m^2 m^{-2})	4.4 ± 0.9	2.2 ± 0.5	7.2 ± 1.4	3.8 ± 0.5
LA per fruit-Nov (cm^2 $fruit^{-1}$)	1197 ± 285	174 ± 39	1080 ± 132	261 ± 37
LAI - Jan-Apr[b] (m^2 m^{-2})	2.5 ± 0.3	1.8 ± 0.4	4.3 ± 1.1	3.5 ± 0.5
LA per fruit -Jan-Apr (cm^2 $fruit^{-1}$)	711 ± 68	370 ± 83	1115 ± 256	730 ± 127

[a]Before summer pruning or fruitlet thinning ; [b]after main summer pruning and fruitlet thinning; n = 4 ± SE.

At excavation, there was no evidence of early leaf senescence (Figure 5.24) or earlier remobilisation of leaf N and P in the ETG vines (Figure 5.25).

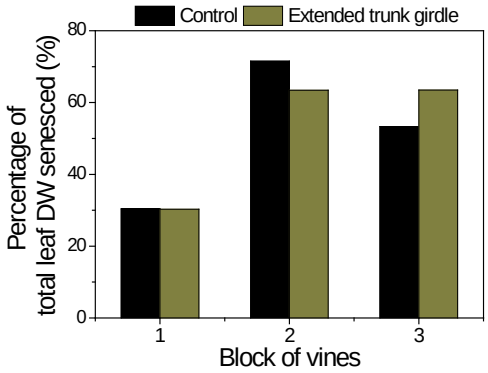


Figure 5.24 Abscised leaf dry weight (DW) as a percentage of total leaf DW collected from 'Hort16A' kiwifruit vines receiving extended trunk girdles and controls, each block represents 1 vine per treatment excavated in the same week. Blocks were exacavated at weekly intervals.

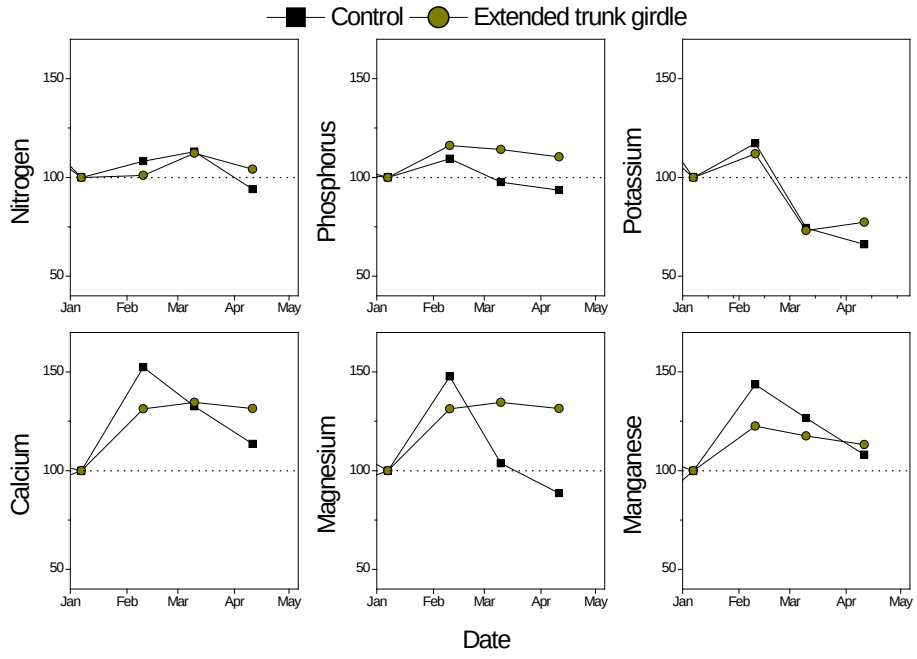


Figure 5.25 Leaf contents of nitrogen, phosphorus, potassium, calcium, magnesium and manganese relative to contents in January (set at 100%) from 'Hort16A' kiwifruit vines receiving extended trunk girdles compared with ungirdled control vines; *n* = 4.

5.3.4.2 Non-structural carbohydrates (NSC).

Perennial parts of the control vines contained almost twice as much NSC as the ETG vines (8.11 kg compared with 4.21 kg; Table 5.22). The main difference was in the crown and structural roots. The ETG vines contained 27 % and 29 % of the NSC in crown and structural roots of the control vines (Table 5.22). Concentrations of NSC were higher in the canes of the ETG vines than those of the control vines, and were lower in the trunk, crown and structural roots of the ETG vines than those of the control vines (Table 5.22).

Table 5.22 Amounts and concentrations of non-structural carbohydrate (starch and soluble sugars) in perennial parts of 'Hort16A' kiwifruit vines receiving extended trunk girdling (ETG) and ungirdled control vines.

Component	Control	ETG	*P*-value	% Change[a]
	Amount (kg vine^{-1})			
Canes (1 year)	0.48 ± 0.10	0.36 ± 0.03	0.455	
Canes (> 1 year)	0.81 ± 0.07	1.28 ± 0.17	0.115	
Leader	0.55 ± 0.02	0.65 ± 0.12	0.395	
Trunk	**0.13 ± 0.03**	**0.09 ± 0.03**	**0.011**	**72**
Crown	**0.22 ± 0.02**	**0.06 ± 0.02**	**0.045**	**27**
Roots - structural	**5.84 ± 0.48**	**1.73 ± 0.39**	**< 0.001**	**29**
Roots - fibrous	**0.09 ± 0.01**	**0.04 ± 0.02**	**0.043**	**44**
Total	**8.11 ± 0.29**	**4.21 ± 0.18**	**0.003**	**52**
	Concentration (mg g^{-1} DW)			
Canes (1 year)	**52.8 ± 4.6**	**77.3 ± 2.7**	**0.033**	
Canes (> 1 year)	**58.7 ± 5.5**	**80.5 ± 8.7**	**0.030**	
Leader	41.5 ± 3.5	44.9 ± 7.9	0.547	
Trunk	**57.9 ± 1.8**	**31.9 ± 3.0**	**0.022**	
Crown	**79.5 ± 3.1**	**42.2 ± 10**	**0.055**	
Roots - structural	**163.6 ± 9.6**	**102.6 ± 16.5**	**0.055**	
Roots - fibrous	53.4 ± 3.4	42.6 ± 14.1	0.401	

[a]change = 100 % x ETG/control, change only presented if P ≤ 0.100 for amounts; n = 3 ± SE

The ratio between starch and soluble sugars tended to increase toward the base of the vine in both ETG and control vines. The highest starch/sugar ratio was in structural roots and the lowest in 1-year-old canes. Lower starch/sugar ratios were found in the trunk and crown of ETG vines than in control vines (Table 5.23). Starch/sugar ratios in the remaining perennial parts of the vines were unaffected by ETG.

Table 5.23 Starch to soluble sugars ratio in perennial parts of 'Hort16A' kiwifruit vines receiving extended trunk girdles (ETG) and ungirdled control vines.

Component	Control	ETG	*P*-value
	Ratio starch/sugars		
Canes (1 year)	1.0 ± 0.1	1.0 ± 0.1	0.863
Canes (> 1 year)	2.2 ± 0.3	2.0 ± 0.2	0.571
Leader	2.0 ± 0.1	2.1 ± 0.4	0.822
Trunk	**5.3 ± 0.6**	**3.4 ± 0.8**	**0.040**
Crown	**7.1 ± 0.7**	**4.8 ± 1.2**	**0.098**
Roots - structural	10.0 ± 0.7	11.6 ± 3.4	0.634
Roots - fibrous	5.6 ± 3.4	8.2 ± 2.1.	0.318

In both treatments, around 10 to 12 kg of carbohydrates was allocated to annual canopy growth (Table 5.24). The main difference between the two treatments was the early-season allocation between reproductive and vegetative growth (Chapter 5.3.1). In spring, more DW was removed in pruning, and less in fruitlet thinning, from the control vines than the ETG vines. More than 4 times as much NSC were removed from the ETG vines than the control vines to thin the crop load to 40 fruit per m^2 (Table 5.24), and less than one third of carbohydrates were removed in unwanted canopy growth during summer pruning. The majority of NSC in the canopy was allocated to mature fruit at harvest; 8.4 of 9.9 kg (or 85 %) in the control vines and 10.2 of 11.9 kg (or 86 %) in the ETG vines (Appendix 5.7.2).

Table 5.24 Non-structural carbohydrate contents in the new season's canopy growth of 'Hort16A' kiwifruit vines receiving extended trunk girdling (ETG) and control vines.

Vine component	Non-structural carbohydrate (g vine^{-1})		*P*-value	% Change[a]
	Control	ETG		
Fruitlets thinned	**118 ± 44**	**614 ± 93**	**0.030**	**520**
Mature fruit	8374 ± 216	10238 ± 499	0.106	-
Summer prunings	**843 ± 29**	**256 ± 85**	**0.009**	**30**
Leaves - live	**315 ± 99**	**581 ± 134**	**0.074**	**184**
Leaves - abscised	38 ± 12	105 ± 35	0.146	-
Shoots	197 ± 51	111 ± 35	0.149	-
Total	9884 ± 320	11905 ± 447	0.111	

[a]*change = 100 % x ETG/control, only presented if P ≤ 0.100; n = 3 ± SE.*

5.3.4.3 Mineral nutrients. Perennial components of the ETG vines contained less N, P, K, S, Ca, Mg, Cu and B than the control vines (Table 5.25). Most notably, the ETG vines contained less than half the N, P, S and Mg of the control vines.

Table 5.25 Mineral nutrient contents of the perennial parts of the kiwifruit vine that received extended trunk girdles (ETG) and ungirdled control vines.

Nutrient	Amount (g vine^{-1})		*P*-value	% Change
	Control	ETG		
N	**719 ± 65**	**320 ± 31**	**0.011**	**45**
P	**116 ± 7**	**52 ± 9**	**0.010**	**45**
K	**440 ± 31**	**280 ± 11**	**0.019**	**64**
S	**115 ± 10**	**50 ± 7**	**0.005**	**43**
Ca	**648 ± 34**	**361 ± 26**	**0.004**	**56**
Mg	**152 ± 7**	**73 ± 6**	**0.009**	**48**
Mn	1.75 ± 0.29	1.20 ± 0.38	0.287	
Zn	6.14 ± 0.60	5.63 ± 1.09	0.427	
Fe	20.6 ± 12.2	45.4 ± 39.2	0.644	
Cu	**0.86 ± 0.04**	**0.79 ± 0.02**	**0.045**	**92**
B	**0.67 ± 0.02**	**0.50 ± 0.02**	**0.006**	**76**
DW (kg vine^{-1})	**78.7 ± 4.9**	**57.0 ± 2.8**	**0.013**	**72**

[a]*change = 100 x ETG/Control, only presented if P≤ 0.100; n = 3 ± SE.*

The ETG vines contained less N, P, K, S, Ca, Mg, Cu and B reserves than control vines, yet there was no part of the vine where concentration of these mineral was consistently lower in ETG vines than controls (Table 5.26). In other words, there was no part of the plant where a sub-sample would accurately reflect vine reserves. For example concentrations of N, S and Mg, but not P, K, Ca, Cu and B were lower in the structural roots of ETG vines than control vines (Table 5.27). Total vine Zn status was not affected by ETG, yet Zn concentrations were lower in fibrous roots and higher in structural roots of ETG vines than in control vines (Tables 5.26 and 5.27). Boron concentrations were higher in the trunk and crown of ETG vines than control vines despite overall vine B status being lower in ETG vines than control vines.

Table 5.26 Effect of extended trunk girdling (ETG) on the concentration of mineral nutrients in the perennial components of the vine compared with control vines

	Perennial part of vine						
	Canes					Roots	
Mineral	1-yr	≥ 2-yr	Leader	Trunk	Crown	Structural	Fibrous
nutrient	*P*-value						
N	**0.055**	0.158	0.116	0.374	0.768	**0.011**	**0.025**
P	1.000	0.116	0.374	0.678	0.561	0.131	0.859
K	0.288	0.678	1.000	0.116	0.116	0.205	0.234
S	0.230	0.374	0.374	0.643	0.349	**0.030**	**0.026**
Ca	0.685	0.567	0.625	0.134	0.398	0.125	0.382
Mg	0.345	0.643	**0.089**	**0.038**	0.106	**0.073**	**0.006**
Mn	0.150	**0.065**	0.624	0.468	0.442	**0.054**	0.124
Zn	0.554	0.909	0.761	**0.052**	0.294	**0.061**	**0.006**
Fe	0.394	0.470	0.550	0.432	0.524	0.448	0.851
Cu	0.725	**0.034**	1.000	1.000	**0.010**	0.374	0.113
B	0.189	0.492	1.000	**0.008**	**0.013**	0.492	0.116

P-values ≤ 0.100 highlighted in bold, if concentration higher in ETG than control P-value is also highlighted in grey, for all others concentration is higher in control than ETG.

Table 5.27 Concentrations of mineral nutrient in structural roots of 'Hort16A' kiwifruit vines receiving extended trunk girdles (ETG) and ungirdled control vines.

	Mineral nutrient										
	N	P	K	S	Ca	Mg	Mn	Zn	Fe	Cu	B
	Concentration in structural roots										
Trt.	% DW						mg kg^{-1} DW				
Control	1.43	0.23	2.23	0.21	1.11	0.26	21	22	142	5.7	8.0
ETG	0.77	0.14	2.00	0.12	0.74	0.18	11	33	124	5.0	7.3
P-value	**0.011**	0.131	0.205	**0.030**	0.125	**0.073**	**0.054**	**0.061**	0.488	0.374	0.492

n = 3; trt = treatment

There was little indication that minerals accumulated in tissues above the girdle. Except Mn and Cu concentrations were higher in 2-year plus canes (Table 5.28) and N concentration higher in 1-year canes in the ETG vines

Table 5.28 Concentrations of mineral nutrient in canes ≥ 2 years old in the canopies of 'Hort16A' kiwifruit vines receiving extended trunk girdles (ETG) and ungirdled control vines.

	Mineral nutrient										
	N	P	K	S	Ca	Mg	Mn	Zn	Fe	Cu	B
	Concentration in 2-year canes										
Trt.	% DW						mg kg^{-1} DW				
Control	0.50	0.08	0.53	0.07	0.54	0.10	12.3	102	52	15.3	9.3
ETG	0.60	0.07	0.57	0.07	0.58	0.10	16.0	104	68	19.7	10.0
P-value	0.158	0.116	0.678	0.374	0.567	0.643	**0.065**	0.909	0.470	**0.034**	0.492

n = 3; trt = treatment

There was a trend for less mineral nutrients to be allocated to annual canopy growth in ETG vines than in control vines. In particular the amount of Ca, Mn and Zn was lower in the canopy of ETG than in control vines ($P < 0.100$; Table 5.29).

Table 5.29 Mineral nutrient contents in the new season's canopy growth of 'Hort16A' kiwifruit vines receiving extended trunk girdles (ETG) and ungirdled control vines.

Mineral nutrient	Amount (g vine^{-1})		*P*-value	% Change[a]
	Control	ETG		
N	598 ± 26	465 ± 63	0.194	
P	90 ± 2	63 ± 10	0.130	
K	806 ± 60	671 ± 31	0.265	
S	73 ± 3	53 ± 7	0.193	
Ca	**862 ± 74**	**539 ± 89**	**0.097**	**63**
Mg	96 ± 7	71 ± 6	0.181	
Mn	**1.91 ± 0.21**	**1.24 ± 0.26**	**0.043**	**65**
Zn	**0.92 ± 0.08**	**0.60 ± 0.05**	**0.032**	**65**
Fe	1.99 ± 0.05	2.06 ± 0.11	0.695	
Cu	0.46 ± 0.02	0.34 ± 0.04	0.174	
B	0.79 ± 0.03	0.70 ± 0.07	0.359	

[a]*Change = 100 x ETG/control, only presented if P ≤ 0.100; n = 3 ± SE.*

5.3.4.4 Key findings - resource allocation.

- The ETG vines contained approximately 24 kg less DW than control vines (102.0 ± 5.2 kg compared with 125.9 ± 5.0 kg, respectively). Reduced root biomass accounted for the majority of the difference (19.8 kg).
- The ETG vines contained approximately half the NSC, N, P, S and Mg reserves of control vines, and significantly lower reserves of Ca, K, B and Cu.
- Total biomass, NSC and mineral nutrient (except Ca, Mn and Zn) allocation to annual canopy growth was unaffected by the ETG treatment.
- Resource allocation within the annual canopy growth was affected by the ETG treatment with allocation to fruit and leaves favoured over allocation to shoots (Appendix 5.7.2).

5.4 DISCUSSION

5.4.1 Vegetative/reproductive balance

The ETG vines consistently had more floral shoots and more flowers per floral shoot than the control vines, producing 70 %, 260 % and 100 % more flowers than the control vines in years 1, 2 and 3 respectively. Increased flower numbers (by 60 %) in the year after girdle application have previously reported for 'Hayward' kiwifruit (Davison, 1990), and other fruit crops such as citrus (Mataa et al., 1998), grape (Zabadal, 1992), pear (Reynolds et al., 2005), and persimmon (Steyn et al., 2008). The mechanism behind this response is not clear. Increased carbohydrate availability in the plant parts above the girdle and altered production of plant growth regulators have been implicated in the floral response to girdling. Grant and Ryugo, (1982) suggested that growing kiwifruit shoots could compete for NSC with flower production, or could export a signal which inhibited flowering in nearby shoots. Shoot growth was reduced in the ETG vines, suggesting that both competition between growing shoots and fruit, and the production of a potential inhibitory signal by shoots would be reduced in the ETG vines.

Trunk girdling severs the phloem connection between the canopy and roots, and can result in increased assimilate accumulation in the organs above the girdle (Noel, 1970; Goren et al., 2004). In the ETG vines, NSC concentrations were generally higher in plant parts above the girdle and lower below the girdle than the equivalent parts of the control vines. In winter, when the vines were excavated, NSC concentrations in the canes of the ETG vines were approximately 25 % higher than those in the control vines. The vines were excavated part way through leaf-fall, meaning that NSC concentrations could be even higher at the end of leaf fall if remobilisation of NSC from leaves to the vines had continued.

A link between plant carbohydrate status and flowering has been found in many fruit crops including kiwifruit. Shading and defoliation treatments devised to deplete carbohydrate status, reduced flower numbers in 'Hayward' vines (Buwalda and Smith, 1990; Cooper and Marshall, 1991; Cruz-Castillo et al., 2010). Piller et al. (1998) combined girdling and defoliation during the first few weeks after bud break in 'Hayward' kiwifruit shoots and found that floral apex development was affected by

localised carbon supply 3 to 4 weeks before anthesis. Fruitless mandarin branches girdled one month before flowering had higher leaf starch concentrations and more flowers than intact fruitless branches (Goldschmidt et al., 1985). Autumn girdling of mandarin branches increased starch concentrations in the stem bark and fruit set in the subsequent year, whilst girdling at other times of the year did not affect either attribute to the same degree (Mataa et al., 1998).

Bangerth (2006) suggested that auxins and gibberellins acted either independently or together to inhibit floral induction and cytokinins stimulated floral induction. The source of the cytokinins involved in this process, whether from roots and/or meristems above the girdle, is not clear. It is also not known how the signals interact to affect flowering. One theory is that a high local cytokinin/auxin ratio is needed for a bud to be reproductive rather than vegetative (Bangerth et al., 2000), and growing shoots inhibit flowering by producing high levels of auxin which is transported to the roots, reducing cytokinin production. Girdling and the application of auxin transport inhibitors such as 1-N-naphthylphthalamic acid (NPA) are believed to promote flowering by interrupting auxin flow to the roots, thereby increasing cytokinin production (Hegele et al., 2004). The small leaves in the ETG vines may be symptomatic of high cytokinin levels in the canopy. Overproduction of cytokinins, has previously been associated with reduced leaf size and internode length in kiwifruit (Honda et al., 2011).

Reynolds et al. (2005) suggested that the improved floral response in girdled pear trees was related to disruption of the basipetal (from the shoot apex downwards) auxin signal leading to:

1) Reduced apical dominance in the remaining shoots increasing their likelihood of becoming reproductive, and/or
2) Increased concentrations of cytokinins in the shoots of girdled plants making them more likely to be floral than vegetative.

The authors did not test these theories.

Kiwifruit flowers develop over two years, floral evocation (the process where a meristem becomes committed to a reproductive state) occurs in the first year and

floral differentiation occurs in the second year (Varkonyi-Gasic et al., 2011). The relatively long time between evocation and differentiation can render kiwifruit susceptible to reversion: switching from floral development back to vegetative development (Tooke et al., 2005). It is possible therefore, earlier bud break in the ETG vines resulted in less reversion and more flowers.

5.4.2 Shoot architecture

The ETG vines contained a higher proportion of short shoots (~ 88 % compared with ~ 57 %), and fewer long shoots (< 5 % compared with 23 to 34 %) than the control vines. It was also observed that a higher proportion of the short shoots in the ETG vines were less than 5 cm long and showed little sign of internode expansion, relative to the short shoots in the control vines. Individual LA and petiole length were lower in the ETG vines than the controls. Shoot type affects area per leaf in kiwifruit, short terminated shoots have smaller leaves than other shoot types, for example the largest leaves on short shoots were about 25 % smaller than leaves at equivalent nodes in medium or long shoots (Clearwater et al., 2006). In the current experiment the smaller LA in the ETG vines was not simply a function of the increased number of short shoots in the canopy. When shoots of the same type and length were compared, there was a trend that LA at a given node was smaller in the shoots from the ETG vines than the control vines.

Reduced canopy vigour is often observed as a result of girdling. Total shoot growth was reduced by 18 % in peach branches girdled at stage II fruit growth compared with ungirdled branches on the same tree (Cutting and Lyne, 1993). Reduced shoot growth, a combination of reduced node number and internode length, was apparent for about eight weeks after girdling until the girdle had healed over. Shoot growth was not measured after the girdle healed. Girdling during early stage I growth (rapid fruit growth) reduced shoot extension in nectarine (Day and DeJong, 1990). Decreased shoot growth was observed in apple trees girdled 2 to 6 weeks after full bloom (Pretorius et al., 2002).

Several factors could interact to generate the canopy differences between the ETG and control vines. Whether a kiwifruit shoot becomes short, medium or long is believed to depend on its initial growth rate (Seleznyova et al., 2002; Clearwater et al., 2006).

Initial growth of long shoots is more rapid than that of short shoots (Piller and Meekings, 1997). Temperature, competition from fruit or other shoots have been shown to reduce the rate of shoot growth (Piller and Meekings, 1997; Greer et al., 2003; Clearwater et al., 2006). The ETG vines broke bud approximately 8 days before the control vines in August so initial growth could be slower in the ETG vines because of lower temperatures (these were not measured in the current experiment). The higher crop loads carried by the ETG vines could have competed with shoot growth from mid-October when flowering occurred until fruitlets were thinned in late November or December. It is also possible that the increased number of short shoots in the ETG canopy produced less auxin thereby increasing flower numbers.

In the ETG vines, the previous season's girdle was still open at bud break and did not heal until mid-October when flowering started (Figure 5.26). During initial shoot growth, there was no phloem connection between the canopy and roots of the ETG vines.

Figure 5.26 Canopy development in 'Hort16A' kiwifruit vines photographed on 10 October 2008 just before flowering and just before the previous season's girdle healed; (left) control vine; (right) vine receiving extended trunk girdle.

It is not known exactly how girdling affects the transport and production of plant growth regulators (PGRs). Interrupting auxin transport to the roots is believed to reduce synthesis of PGRs in the roots (Goren et al., 2004), although PGRs were not measured in the current experiment. Bangerth et al. (2000) reported a strong mutual interaction between auxin production in the shoots and cytokinin production in the roots. Cytokinins and gibberellins are believed to interact to control shoot growth:

cytokinins are primarily involved in regulation of cell division and gibberellins are known to stimulate cell elongation (Taiz & Zeiger, 2002). Application of gibberellins to 'Hayward' kiwifruit vines increased shoot length and mean internode length (Vattiprolu et al., 2011). It is therefore possible that the reduced internode length, shorter petioles and smaller leaves observed in the current experiment were related to reduced production of cytokinins and gibberellins in the roots. There is limited evidence available to support this theory, and there is an opportunity to carry out further work on kiwifruit vines to gain better understanding. Generally it is believed that girdling is associated with reduced cytokinin production, but there is contradictory evidence. Transformation of kiwifruit using the *isopentyl transferase* gene (*ipt*) which overproduces cytokinin, resulted in reduced leaf size and internode length (Honda et al., 2011), a response similar to that observed in the ETG vines.

Girdling peach branches reduced existing shoot growth rates during the time that the girdle was open and only in the girdled branches (Cutting and Lyne, 1993). Shoot growth on intact branches on the same tree was the same as the rate of shoot growth on adjacent trees with no girdled branches. The reduced shoot growth in the girdled branches was accompanied by reduced concentrations of cytokinin and gibberellins in the xylem sap above the girdle compared with xylem sap in the intact branches from the same trees. If girdling affects the synthesis of PGRs in the roots, it might be expected that reduced xylem PGR concentrations, and reduced shoot growth would occur throughout the plant, and not just in the girdled branches.

Some plant responses have been found in both girdled and intact branches of the same tree, whilst others are more localised. Dann et al. (1985) girdled all but one of the branches on a peach tree and found that branch shrinkage occurred within 12 hours, on all branches including the intact branch. The rapid shrinkage was attributed to water loss from the bark tissue caused by reduced water uptake by the roots, although the mechanism for the reduced water uptake was not explored. Ringing branches of two-branched apple trees at full bloom reduced shoot extension growth (Priestley, 1976b). Ringing one branch did not reduce shoot growth on the intact branch.

Within 24 hours of ringing, concentrations of gibberellin-like substances decreased in rootlets of ringed citrus trees and increased in the shoots above the ring (Wallerstein et al., 1973). The authors suggested that either lack of sugar translocation to the roots reduced gibberellin synthesis in the roots, or that reduced translocation of canopy-synthesised gibberellins itself could occur. Increased concentrations of the auxin IAA (indole-3-acetic acid) were detected in the bark immediately above girdles in peach tree branches 1 day after girdling, whilst concentrations in the bark below the girdle decreased relative to ungirdled control branches (Dann, 1985). It is not clear if the increased IAA was caused by buildup of shoot-synthesised IAA that was unable to traverse the girdle, or if IAA was associated with the girdle healing process. Increased IAA concentrations were detected in the recovering bark of olive trees 2 days after girdling, and remained higher than levels in ungirdled controls until the girdle had healed (Mwange et al., 2003).

5.4.3 Leaf plasticity

Individual leaves from the ETG vines had smaller area and shorter petioles than leaves from the control vines. The ETG leaves had higher SLW and DMC than the control leaves. These differences were detected in both spring and autumn, and were therefore not a short-term response to the application of the girdle in February. An increase in SLW is often reported after girdling, and is caused by starch accumulation in, for example, apple (Schecter et al., 1994) and nectarine (Day and DeJong, 1990). Starch accumulated in the ETG leaves later in the year after the February girdle, but this does not explain why the ETG leaves had higher SLW in November/December before girdling. In addition, leaf DMC was higher in the ETG leaves in spring and autumn. This suggests that the ETG leaves were denser than the control leaves. It is also possible that the ETG leaves could have been thicker than the control leaves, but leaf thickness was not measured in this experiment. The ETG leaves developed whilst the girdle from the previous year was open and it is possible that cell expansion could have been limited by reduced availability of gibberellins. This might also explain the shorter petiole length in ETG leaves.

Higher leaf DMC can result in dilution of mineral nutrient concentrations, which can be sometimes misinterpreted as nutrient deficiencies. Calculation of leaf nutrient status on a leaf area basis enables better interpretation of leaf mineral nutrient results,

especially if SLW is affected by a treatment (see Section 5.3.2). Leaves from girdled apple shoots had higher SLW and nutrient concentrations in leaves were unaffected except that Zn and Fe concentrations were significantly higher in leaves from the girdled shoots (Schechter et al., 1994). When calculated on an area basis, leaves from girdled apple shoots had higher N, Cu, Fe, Zn and B per cm^2 than leaves from intact shoots. There is no clear reason why allocation of this group of mineral nutrients to leaves would be less affected by girdling than P, K, S, Ca, Mg, and Mn, these results will be discussed further in Section 5.4.7.

5.4.4 Leaf function

Net carbon dioxide exchange rates were not affected by the ETG treatment on any of the measurement dates in this experiment. Stomatal conductance (g_s) and internal CO_2 concentration (C_i) tended to be higher in ETG leaves in measurements taken before and after girdling, but not during the rest of the season. These results differ from many published leaf responses to girdling. Black et al. (2012) detected reduced leaf photosynthesis (*A*) 5 to 35 days after trunk-girdling in potted 'Hort16A' kiwifruit vines. Stomatal conductance was reduced during this time and lower *A* detected 4 to 5 weeks after girdling. *A* was reduced in trunk-girdled grape vines 4 days to 2 weeks after girdle application and NSC accumulated in the leaves 2 to 4 weeks after girdling (Roper and Williams, 1989). Wire girdling, or strangulation, of pumello trees reduced *A* (measured 1 month after girdling) and increased specific leaf weight, measured 1 to 3 months after girdling (Yamanishi et al., 1995). Seven days after girdling apple shoots, the leaves above the girdle had decreased *A*, g_s and C_i and increased starch concentrations relative to leaves from trees with ungirdled shoots (Zhou and Quebedeaux, 2003). Leaf responses to girdling have been attributed to stomatal closure and/or feedback inhibition of photosynthesis caused by starch accumulation in the leaves e.g. in apple (Zhou and Quebedeaux, 2003), citrus (Rivas et al., 2007), grape (Harrell and Williams, 1987), kiwifruit (Black et al., 2012) and mango (Urban and Alphonsout, 2007).

Leaves from the ETG vines did not show the responses described above, except that starch accumulated in the leaves several weeks after the girdle was applied. In the experiments described in the previous paragraph, the girdles and control plants were equivalent before treatment application, and any differences could be seen as a result

of the girdle treatment. In the current experiment there were already differences between the vines as a result of ETG in the previous years, specifically the ETG vines had smaller individual LA and higher SLW than leaves from the control vines. More detailed microscopic and biochemical analysis would be required to understand the differences between the ETG and control leaves, and below are some areas which could be explored further:

- The high internal CO_2 in the ETG leaves relative to the control leaves suggests that diffusion within the leaf (through intercellular air space and liquid resistance when moving into the chloroplast) may be reduced in the ETG leaves, possibly because they are denser and may have reduced intercellular space, they may also be thicker. Detailed microscopy would be required to determine if the internal structure within the leaves of the ETG vines was markedly different from the control leaves, or thicker. Usually stomatal resistance, rather than movement within the leaf is the main limiting factor to CO_2 uptake by leaves (Taiz & Zeiger, 2002), although intercellular gaseous diffusion is a substantial limitation to *A* in species with thicker leaves (Parkhurst, 1994; Smith et al., 1997), and also potentially in leaves from the same species which are thicker or perhaps denser.
- Higher mid-season C_i in the ETG leaves than the control leaves, but without an associated treatment difference in *A*, suggests that the relationship between *A* and C_i could be different in the ETG leaves. This relationship could be measured, although there are technical difficulties in making detailed measurements in mature kiwifruit vines growing on a pergola structure. The relationship between *A* and C_i provides information about the limiting factors of carbon fixation. At low C_i, *A* is limited by the capacity of the enzyme rubisco, as C_i increases and rubsico becomes saturated, the regeneration of ribulose-1, 5-bisphosphate becomes limiting.
- Are the ETG leaves respiring more than leaves from the ungirdled control vines? Strictly speaking the LiCor measures the net carbon exchange rate (NCER), which is CO_2 taken up in photosynthesis less CO_2 released by respiration.

$$NCER = A - CO_2$$

If ETG leaves respired more than control leaves, but NCER is unaffected, then *A* would be expected to increase in ETG leaves. It is not known if CO_2 generated from respiration would cause increased C_i.

They key message from these results is that leaves are capable of continuing to produce NSC even after several years of ETG. The higher SLW of ETG leaves relative to controls means that photosynthesis on a leaf dry weight basis is less efficient in ETG vines than control vines.

5.4.5 Leaf senescence

There was no evidence of accelerated leaf abscission in excavated ETG vines. At each of the three excavation dates a similar proportion of leaves had abscised in ETG and control vines. Dann (1994) reported early leaf senescence in girdled peach branches measured by leaf colour, decreased chlorophyll concentrations and advanced abscission. The chlorophyll/N ratio was significantly lower at the end of the season in ETG leaves compared with control leaves; reduced chlorophyll without N remobilisation can be an indication that light is not a limiting factor in photosynthesis, rather than an indication of leaf senescence. There was no evidence of enhanced N and P remobilisation from leaves of the ETG vines relative to control vines.

5.4.6 Generation of alternative sinks

As discussed in Chapter 4, carbohydrate abundance typically results in NSC accumulation in leaves, reduced *A* and generation of new sinks, or increased allocation to existing sinks (Paul and Foyer, 2001). In the both the ETG vines and the feast vines, there was no evidence of reduced *A*, but there was significant NSC accumulation in the leaves of the ETG vines, and not the feast leaves. This may be because the feast vines were able transport NSC to the roots whereas the girdle prevented NSC transport to the roots. In the ETG vines there were two main events when sink removal occurred: in late spring when crop load adjustments were made, and from February to October when trunk girdling effectively removed the roots as a sink. The ETG vines showed little or no tendency to generate new sinks in the form of new shoots; in the control vines 6 to 8 kg DW of new shoot growth per vine was removed during summer pruning compared with 0.9 to 1.5 kg DW per vine from the ETG vines. There was evidence of NSC accumulation in existing sinks:

- **Fruit.** At commercial maturity, the ETG vines had approximately the same crop load as the control vines, mean FW was the same in both treatments and DMC was higher in fruit from the ETG vines. Overall 0 to 3.7 kg DW per vine was allocated to fruit in the ETG vines.
- **Leaves.** By late-April, leaves from the ETG vines contained significantly more NSC than leaves from the control vines. When the vines were excavated in May/June, abscised leaves from the ETG vines had the same NSC concentration as abscised leaves from the control vines, suggesting that the extra NSC from the ETG leaves was remobilised back to the canopy before abscission.
- **Perennial reserves.** The ETG vines accumulated NSC in perennial parts of the vine above the girdle. Concentration of NSC was significantly higher in the canes of ETG vines than in control vines.

These results support the suggestions of Noel (1970) that carbohydrates accumulate above the girdle, in the fruit leaves and canes - either by direct accumulation or by remobilisation from the leaves before senescence. Additional losses from respiration may be higher in the tissues above the girdle than equivalent tissues in intact plants (Wang et al., 2006). Respiration losses were not measured in the experiment described in this chapter.

5.4.7 Perennial reserves

Dry weight. Total perennial DW was 28 % lower in the ETG vines than the control vines (78.7 and 57.0 kg DW per vine, respectively), almost all of the difference was in the root biomass (19.8 kg of the 21.7 kg). Total root DW in the ETG vines was 47.5 % of that in the control vines (17.9 ± 3.9 kg and 37.7 ± 4.6 kg per vine, respectively). It is not clear from these results if the lower root DW in the ETG vines was a result of slower root growth or increased root death. Black (2011) found that root numbers, cumulative root growth, and fine root growth tended to be lower in mature field-grown kiwifruit vines with a history of annual trunk girdling than ungirdled control vines, across the entire season not just when the girdles were open. A significant reduction on root DW was reported in girdled 1-year old peach trees (Sharif Hossain et al., 2006). Priestley (1976b) estimated that ringed young apple trees had lost 10 % of their dry weight relative to intact control trees, probably in the form of fine roots or

the surface of old roots being sloughed off. It is therefore possible that the reduced root biomass in the ETG vines was a combination of slower root growth and increased root death. Results from the current experiment suggest that kiwifruit vines have extra reserves in the roots and halving root biomass does not seriously impair plant health. This finding is supported by the work of Black et al. (2012) who removed, by pruning, 50 % of the root system from potted 'Hort16A' kiwifruit vines and found no visible effect on plant health or leaf area in the 5 weeks post-treatment. Longer-term effects were not reported.

Carbohydrates. Perennial parts of the ETG vines contained around half the NSC of the control vines. Carbohydrates can accumulate in the plant parts above the canopy because they cannot be translocated to the roots whilst the girdle is open (Goren et al 1994). The concentration of NSC was higher in canes from the ETG vines than the control vines, and lower in the trunk, crown and structural roots. Accumulation of NSC above the girdle and reduction below the girdle has been reported for many crops (Goren et al., 2004). Roots from girdled grape vines contained approximately 60 % of the NSC of control vines, measured 31 days after girdling (Roper and Williams, 1989).

Root starvation. The effect of girdling on root function is not well understood. Short-term changes, i.e. those which occur within days or weeks of girdle application, have been documented, but it is not always clear if longer-term changes also occur. For example root elongation in girdled grapevines ceased for the two weeks when the girdle was open and resumed when the girdle healed (Wallerstein et al., 1973; Yamane and Shibayama, 2006). Black (2011) found that root growth tended to be slower in girdled kiwifruit vines than in control vines across the entire season not just when the girdles were open. This finding suggests that there might be longer-term effects that continue after the girdle has healed, particularly if NSC were depleted during the time the girdle was open. Soil respiration from roots and associated symbiotic fungi was reduced in girdled beech trees a few days after girdling and 6 weeks after girdling in spruce trees. The slower response of the spruce trees suggests that they may have more available root reserves than beech trees which are more dependent on recent photosynthate (Andersen et al., 2005).

One concern about girdling is that it will affect mineral nutrient uptake and allocation within the plant. It is also not clear if all mineral nutrients would be affected equally by girdling. As girdling severs the phloem connection between the roots and canopy, it might be expected that uptake of xylem-mobile nutrients (Ca, Mn, Zn; Clark and Smith, 1988) would not be affected by girdling. Priestley (1976a) reported that apple leaf Ca was reduced more by ringing than N, P or K. Ringing inhibited Ca uptake into apple seedlings and Ca uptake was restored when sucrose was supplied below the ring (Faust, 1980). These finding suggest that either (a) Ca is phloem-mobile, or (b) girdling affects nutrient uptake by the roots, rather than just their physical transport within the plant. If fine root biomass and fine root turnover are slowed or stopped by girdling, then nutrient uptake might be limited by the available fine root surface area. Growing root tips are structurally different from the more mature region at the base of the root and different parts of the root are believed to take up different minerals, depending on the mineral and the plant type (Taiz & Zieger, 2002). Girdling might therefore be expected to affect uptake of some mineral nutrients more than others. For example the growing root tip of maize was found to take up more Ca than the base of the roots, whereas the opposite was true for P uptake (Ferguson and Clarkson, 1975). The authors also found that root Ca uptake occured by two processes: Ca uptake at the apex was inhibited when respiration was inhibited, whereas Ca uptake in the basal region was not (Ferguson and Clarkson, 1975). These findings suggest that processes such as girdling, which can affect root growth and root respiration, could affect the nutrient uptake to a differing degree. Bangerth (2008) found that concentrations of Ca, K, Mg and P, but not B and Zn, were reduced in the sap of girdled apple trees relative to intact trees (Table 5.30). Other mineral nutrient concentrations were not reported in Bangerth's paper.

Table 5.30 Concentration of macro- and micro-elements (mg L^{-1}) in xylem exudates of control and girdled 'Elstar' apple trees in 2005 (from Bangerth, 2008).

Treatment	Ca	K	Mg	P	B	Zn
Control	50.5a	96.2a	8.6a	8.5a	1.23a	1.08a
Girdled	26.0b	15.5b	4.3b	3.5b	0.95a	1.04a

**Mean values (n = 3) within a column followed by a different lower-case letter are significantly different at P ≤ 0.01 using Student's t-test. Samples were taken 18 days after girdling on 28 May.*

Interestingly, Schechter et al. (1994) also found that leaf contents of Zn and B (along with N, Cu, Fe) were the mineral nutrients least affected by girdling. In the current experiment, leaf status of the same five N, Cu, Fe, Zn and B were least adversely affected by ETG. It is not clear exactly why uptake and/or allocation to leaves of these five mineral nutrients was less affected by girdling than that of P, K, S, Ca, Mg and Mn. It would be worthwhile to collect xylem sap from girdled and intact kiwifruit vines and measure all 11 mineral nutrients to determine if there are clear concentration differences for some mineral nutrients, which would suggest that uptake might be affected.

There are several reasons why allocation of certain mineral nutrients to leaves might not be adversely affected by trunk girdling, these include:

- Nutrient uptake by roots is not affected by girdling, Bangerth's results (Table 5.30) suggest that this might be the case for Zn, and possibly B, but further work would be needed to confirm this.
- Reserve status is high and reserves can be remobilised when necessary. Perennial parts of the ETG vines contained less than half the N, P, S, and Mg (45%, 45%, 43% and 48% respectively), less than two thirds the amount of Ca (56%) and K (64%) and significantly less B (76%) and Cu (92%) than the perennial reserves of the control vines. No treatment differences were detected for Mn, Zn and Fe, although between-vine variability for these minerals was very high (Table 5.26). Long-term ETG reduced structural root DW by approximately 19 kg relative to the control vines, due to either slowed growth, or increased root death, or a combination of the two. If roots die and their mineral nutrient contents are not remobilised to the living parts of the vine, this would explain some of the large reductions in reserve status of the ETG vines. Particularly for nutrients like K whose concentration in the SR remained relatively consistent in the control and ETG vines (Table 5.27).
- The girdle prevents remobilisation of mineral nutrients from canopy to roots, increasing reserves in the leader and canes. For example, girdling prevented significant translocation of ^{35}S from above the girdle to the roots in maple and poplar trees, resulting in increased ^{35}S concentrations above the girdle and reduced ^{35}S concentrations in tissue below the girdle, relative to intact control

trees (Garten Jr., 1988). There was little evidence of this occurring in ETG vines, with the exception of N and Mn. Both mineral nutrients had higher concentrations in a tissue above the girdle (2 year plus canes for N and 1 year canes for Mn; Table 5.26) and lower concentrations in the SR than the control vines. This may indicate that the girdle blocked translocation of N and Mn to the roots, but this seems unlikely in the case of Mn which is relatively phloem-immobile in kiwifruit (Clark and Smith, 1988), but could be possible for N.

5.4.8 Fruit attributes

Productivity. The ETG vines produced fruit with the same FW and higher DMC than controls with the same crop load, of around 40 fruit per m^2. This crop load is lower than typical commercial orchards, which could produce nearer 65 fruit per m^2 (Chapter 6; Patterson and Currie, 2011). Without Benefit® application it was felt that 40 fruit per m^2 was all the vines were capable of while maintaining a realistic FW of ≥ 80 g (A. Barnett, personal communication; Cooper and Marshall, 1991).

It is unclear how the ETG vines would have responded to higher crop loads. Accumulation of NSC in the leaves and shoots of the ETG vines suggests that carbohydrates were available for additional fruit. In alternate-bearing citrus NSC only accumulated in the canopy of girdled trees in the 'off' years (Li et al., 2003), and NSC accumulated in the leaves of girdled mango shoots when crop load was low (Urban et al., 2004). Despite the girdle making NSC available to fruit late in the season, early season NSC, FW and fruit mineral accumulation may have been adversely affected if crop loads were any higher. Before crop load adjustments in spring there was evidence of reduced FW, DW and some mineral nutrient accumulation in the ETG fruitlets, suggesting that these were in limited supply during early fruit growth.

Fruit maturity. Fruit from the ETG vines underwent flesh colour change and could be cleared for main harvest several days sooner than fruit from the control vines. Advanced maturity as a result of girdling or similar techniques such as scoring and ringing has been reported in many crops such as apple (Elfving et al., 1991; Autio and Greene, 1994; Arakawa et al., 1997; Wargo et al., 2004), kiwifruit (Davison 1990), loquat (Agusti et al., 2005), peach and nectarine (Andrews et al., 1978; Agusti et al., 1998) and persimmon (Juan et al., 2009). Advanced fruit maturation resulting from

the current year's girdle is likely to be related to increased availability of carbohydrates above the girdle. Agusti et al. (1998) is was unlikely that ethylene generation linked to a wound response (the wound being the girdle) could be responsible for advanced fruit maturity, asthe length of the delay was such that it was unlikely that ringing (girdling) was the cause of enhanced fruit ethylene production. Comparison of the biochemistry of fruit from the feast and ETG treatments, both of which have advanced maturity relative to controls, could help elucidate any wound-related differences.

Much research on how plant management techniques affect fruit quality reports comparison of fruit attributes measured on the same day. In this chapter, for example, fruit from the ETG vines were more mature (higher SSC, lower hue angle and softer) than fruit from the control vines (Figure 5.18). To make useful recommendations to industry about harvesting and storage criteria for fruit from girdled plants, other factors need to be considered. For example if fruit mature sooner, has the maturation process occurred over a shorter time-frame, or has earlier flowering moved the entire fruit developmental and maturation process forward without changing it. The sample sizes and number of sampling dates in this experiment were insufficient to determine with certainty how different processes were affected by the treatment. Both mid-bloom and degreening were advanced by several days in the ETG vines, degreening appeared to be advanced more than mid-bloom. Fruit from kiwifruit vines girdled for the first time within the same growing season showed advanced degreening which supports the compressed maturation theory (Snelgar and Blattmann, 2012). In peach trees, Dann (1994) reported that girdling compressed fruit development and maturation: fruit matured earlier on girdled peach branches despite fruit set being later on the girdled branches. If harvest date is advanced by a treatment such as girdling, it is also important to determine if all maturity attributes are affected to the same degree. In Chapter 4 the relationship between flesh firmness and degreening was affected by the feast and famine treatments. As a consequence more fruit that mature early were likely to be firmer at harvest than later-maturing fruit. The relationship between flesh firmness and colour change was changed in peach by different treatments including shading and shoot girdling (Marini et al., 1991). A consequence of this was that fruit

with similar background colour harvested from different positions within the canopy may have different flesh firmnesses and/or storage life (Marini et al., 1991).

Mineral nutrients. At commercial maturity, fruit mineral contents were unaffected by the ETG treatment, except in some years when ETG fruit contained more Ca, Mg, Fe and Zn than control fruit. If mineral nutrients were measured on a concentration per DW basis, the higher DMC in ETG fruit resulted in significantly lower fruit concentrations of N, P, K, S and Mg in ETG fruit than in control fruit, and concentrations of Ca, Mn, Fe or Zn were not affected by the ETG treatment. Inconsistent results have been previously reported on how girdling affects mineral nutrient uptake and allocation to fruit. Girdled apple trees produced fruit with lower flesh Ca concentrations (measured on a DW basis) than intact control trees (Arakawa et al., 1997). No other mineral nutrient was measured in this study so it was not possible to if determine Ca specifically was affected. The authors suggested two reasons for the lower Ca concentrations: (1) reduced assimilate supply to the roots reducing Ca uptake, or (2) dilution of Ca concentration by higher fruit DW in girdled trees. Fruit concentrations of K and Mg, but not Ca, were reduced by ringing in apple trees (Autio and Greene, 1994). Elfving et al. (1991) found that apple flesh concentrations of K were unaffected and Mg and Ca were marginally reduced by scoring.

The results of the experiment described in this chapter are relatively consistent across the three years and indicate that ETG treatment did not adversely affect fruit mineral nutrient contents. This might be because (a) the girdle healed during flowering, possibly restoring 'normal' mineral nutrient uptake, and (b) there was little canopy growth during summer suggesting little competition between fruit and growing shoots for mineral nutrients.

Fruit storage quality. If fruit from the ETG and control vines were harvested on the same day they would behave differently during storage. Fruit from the ETG vines would soften more rapidly in storage than fruit from the control vines (using the criteria of time to soften to 20 N and 10 N), and fewer fruit from the ETG vines than the control vines would develop LTB. However fruit from the ETG and control vines

can both develop little or no LTB if harvested at the right time. Fruit from the ETG and control vines can both display the same softening behavior if harvested at the right time. The challenge lies in identifying the right time: the optimum harvest criteria to enable delayed initial fruit softening, whilst minimising LTB incidence later in storage. This will require understanding how the relationship between degreening and softening is affected by fruit age and fruit DMC.

5.5 CONCLUSIONS

The ETG vines showed many of the responses to trunk girdling that have been seen in other crops: increased flower numbers, earlier flowering, reduced canopy vigour and advanced fruit maturity. When fruit were thinned to the same crop load as the ungirdled control vines, fruit DMC was higher in the ETG vines and there was no evidence that fruit from the ETG vines had inferior storage performance to fruit from the control vines. Individual leaves from the ETG vines were smaller with shorter petioles and higher SLW than those from the control vines, but they were able to produce sufficient carbohydrates to maintain fruit growth and maturation. The extended trunk girdle severed phloem translocation between canopy and roots for approximately two thirds of the year, from late-summer until spring. The girdle was closed for most of fruit growth and development, and fruit attributes such as mineral nutrient accumulation were not affected by the ETG. However root biomass was greatly reduced, as were root-stored reserves of NSC and mineral nutrients.

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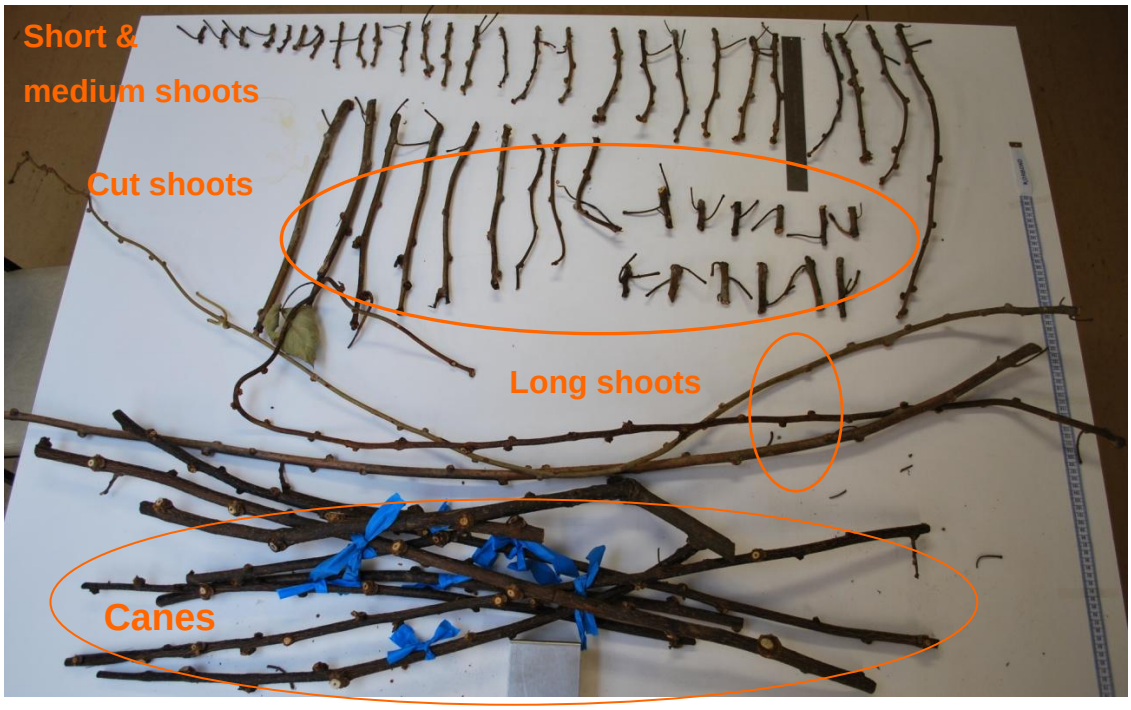
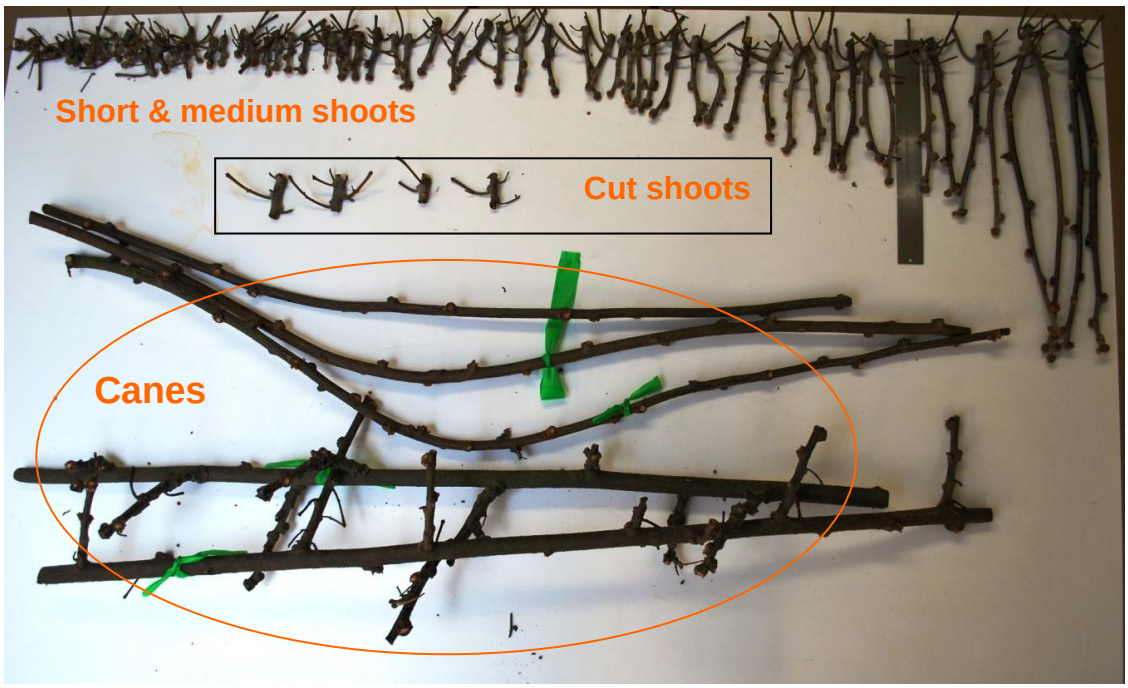
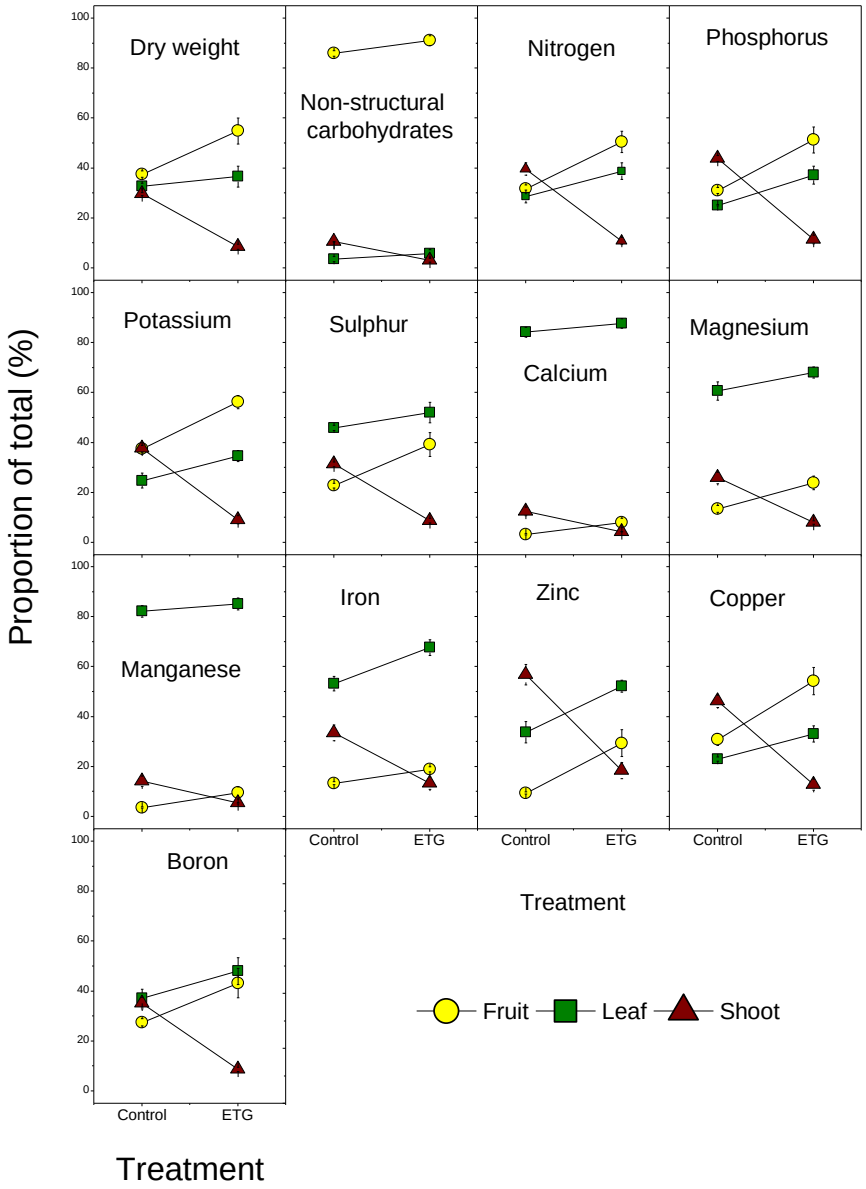
5.7 APPENDICES

Appendix 5.7.1 Canopy composition Dismantled section of canopy from ungirdled control 'Hort16A' kiwifruit vine (top) and a vine receiving an extended trunk girdle (bottom); a 30 cm ruler is at the top right of each photograph.





Appendix 5.7.2 Within-canopy resource allocation Proportion of each resource allocated to fruit, leaves and shoots within new season's canopy growth of 'Hort16A' kiwifruit vines that received extended trunk girdling (ETG) compared with control vines; $n = 3 \pm SE$.



6 EFFECTS OF PRUNING AND NITROGEN APPLICATION

6.1 INTRODUCTION

The canopy influences many aspects of kiwifruit production including fruit fresh weight (FW), maturity, composition and storage quality, and flower number in the subsequent season (Snelgar and Thorp, 1988; Buwalda and Smith, 1990; Cooper and Marshall, 1991; Tombesi et al., 1994). If canopy management is altered to improve one aspect of fruit quality then other fruit attributes can be affected. It is important to achieve a balance between too much and too little leaf area (LA). Tombesi et al. (1994) reported that 'Hayward' kiwifruit vines required at least 600 cm^2 of LA per fruit to obtain a commercially acceptable fruit size and quality whilst also maintaining regular vine growth. A similar result was reported by Snelgar and Thorp (1988), who suggested that LA of approximately 630 to 730 cm^2 was required to produce one 100 g 'Hayward' kiwifruit with little or no further effect of LA above 700 cm^2 per fruit. Excessive shading can affect productivity in the following season, as can too little LA. Shade-grown 'Hayward' shoots tend to have lower bud break and fewer flowers per floral shoot than exposed shoots (Grant and Ryugo, 1984a). Insufficient LA in the canopy can result in reduced flowering in the following season (Buwalda and Smith, 1990; Cruz-Castillo et al., 2010).

A more open canopy generated by summer pruning improves the microclimate around fruit and can reduce the incidence of 'Hayward' storage rots caused by *Botrytis cinerea* (Michailides and Elmer, 2000; Miller et al., 2001). Fruit grown in more shaded positions within the canopy or in denser canopies are generally smaller (Grant and Ryugo, 1984a) with lower DMC and poorer storage performance than those grown in less shaded zones (Snelgar et al., 1998; Tombesi et al., 1993). Shaded kiwifruit have lower calcium (Ca) concentrations than their more exposed counterparts (Biasi and Altamura, 1996; Montanaro et al., 2005). Low fruit Ca status has been linked to poor storage quality in 'Hayward' kiwifruit (Basiouny and Basiouny, 2000; Benge et al., 2000; Ferguson et al., 2003; Thorp et al., 2003a; Xie et al., 2003; Gerasopoulos and Drogoudi, 2005; Kazemi et al., 2011), although not always (Johnson et al., 1997; Boyd et al., 2006; Cooper et al., 2007).

'Hort16A' kiwifruit vines are more productive and more vigorous than 'Hayward' vines. In 'Hort16A' vines, extension of long shoots continues for a longer period into

late summer than is typical of 'Hayward' vines (Patterson et al., 2003). 'Hort16A' primary shoots often produce secondary shoots, whereas 'Hayward' has greater apical dominance. Relatively little work has been published on optimum leaf-to-fruit ratios, or the effect of canopy density on fruit quality in 'Hort16A' vines. Zero-leaf pruning and tip squeezing, described in Chapter 2, are being adopted by 'Hort16A' growers. These pruning techniques enable growers to remove leaf area or prevent rapid shoot growth without generating a new growing point. They also enable us to separate out effect of retaining extra leaf area in the canopy from competition between shoot regrowth and fruit development.

The aim of the work described in this chapter was to determine how changes in 'Hort16A' canopy management would affect fruit production (DMC, maturity, composition, storage) and vine health (specifically return bloom and leaf nutrient status) in vines with a history of relatively high or low vigour. The vines used in this experiment had been part of an earlier trial where individual rows of vines had received 0, 105 and 210 kg N ha^{-1} for several years (Green et al., 2007; Mills et al., 2008). In this earlier trial, more shoot biomass was removed from the vines receiving the highest N inputs than vines receiving 0 or 105 kg N ha^{-1} (~ 97 kg FW per vine compared with ~ 75 kg and ~ 61 kg respectively, Mills et al., 2008). This result suggested that the vines receiving 210 kg N ha^{-1} were more vigorous than those receiving 0 or 105 kg N ha^{-1}. Vines are pruned to maintain a uniform canopy, therefore pruning weight can be used to estimate vine growth (Spayd et al., 1993; Tagliavini et al., 1995).

The experiments described in Chapters 4 and 5 of this thesis, were carried out on a research orchard, whilst the experiment described in Chapter 6 was carried out on a commercial orchard. In the commercial orchard, hydrogen cyanamide (Hi-Cane®) was used to enhance bud break and the biostimulant Benefit® was applied to fruitlets during the first 4 to 6 weeks after flowering to increase fruit size (Patterson and Currie, 2011); these products were not used in the research orchard. Carrying out source-sink manipulations in a commercial growing environment enabled the information gained from the more extreme experiments in Chapters 4 and 5 to be transferred to a more realistic situation. The hypothesis was that with the same crop load, retaining extra leaf area in the canopy of the less vigorous no-N vines would be

beneficial to fruit quality, and that retaining extra leaf area in the canopy of the vigorous vines receiving 210 kg N ha^{-1} would be detrimental to fruit quality. In addition the options for optimising N input to maximise fruit quality were described.

6.2 MATERIALS AND METHODS

6.2.1 Vines and treatment application

The experimental plot used in the N/pruning trial was set up in 2003, primarily as a study of soil N leaching, and the N treatments were not fully replicated, i.e. the three N fertiliser treatments of 0, 105 or 210 kg N ha^{-1} were each applied to a single row of 16 female vines (Green et al., 2007; Mills et al., 2008). A consequence of this is that the effect of N on any of the responses measured in this experiment could not be detected with statistical confidence.

In the three years from 2007 to 2009, the vines continued to receive 0, 105 and 210 kg N ha^{-1}. Around half of the N fertiliser was applied to the ground as a solid in August/September and the remainder applied in October/November. Nitrogen was applied in two forms: calcium ammonium nitrate (CAN), containing 27 % N and 4 % Ca and Nitrabor™ (19.2 % Ca, 15.5 % N and 0.3 % B). The mid-range 105 kg N ha^{-1} was the standard fertiliser rate for the orchard.

In October 2007, at the start of the N/pruning trial described in this chapter, pairs of adjacent vines in each row were randomly assigned to one of two spring pruning treatments (Figure 6.1):

- **Conventional pruning (CP).** Standard practice of the grower. Leader zone pruned in November: potential replacement canes stubbed and unwanted vigorous shoots removed. Vigorous shoots in the fruiting zone were zero-leaf pruned in spring.

- **Minimal pruning (MP).** Less intensive plant management. November stubbing was carried out in the leader zone but unwanted vegetative shoots were retained rather than removed. Vigorous shoots in the fruiting zone were tip-squeezed in spring.

Summer and winter pruning was carried out by contractors, who pruned the vines to the same degree as the rest of the orchard and left the pruned material from each vine under that vine for weighing and sub-sampling. When the experiment started, contractors had already carried out flower thinning by removing entire floral shoots. The discarded floral shoots were collected from beneath each vine, dried and weighed (Table 6.1).

The initial plan was to carry out this experiment for two or three years, however the full experiment was only able to be carried out in year 1 (although some measurements were taken in all three years). In year 2, flower numbers were low and no flower thinning was carried out. This meant that crop loads were not uniform among the treatments. In addition, fruit quality was improved by the minimal pruning treatment after one year, so minimal pruning was adopted by the orchard manager as standard practice (i.e. the new conventional pruning). In the third year when it was agreed that the original pruning treatments would be applied to the experimental vines, the canopy produced few vigorous shoots so it was not possible to have two different degrees of pruning intensity.

Nth							
	210 kg N	**male**	**105 kg N**	**male**	**105 kg N**	**male**	**0 kg N**
	x		x		x		x
	201		101		x		001
	202		102		x		002
	203		103		x		003
	204		104		x		004
	205		105		x		005
	206		106		x		006
	207		107		x		007
	208		108		x		008
	209		109		x		009
	210		110		x		010
	211		111		x		011
	212		112		x		012
	213		113		x		013
	214		114		x		014
	x		x		x		x
x = female vine not used							
	Conventional pruning						
	Minimal pruning						

Figure 6.1 Vine layout for pruning/nitrogen (N) experiment. Each rectangle represents an individual 'Hort16A' kiwifruit vine.

Table 6.1 Schedule of canopy management in year 1 (2007-2008).

Date	Activity
Mid-October	Flowers thinned (by removing entire floral shoots) on all vines to give a crop load of ~ 65 fruit per m^2
Spring pruning	
Late-October	Potential replacement canes in leader zone were stubbed in all vines. Unwanted vigorous shoots growing in the leader zone were removed from the conventionally pruned vines and retained in the minimally pruned vines
Early-November	Vigorous vegetative shoots were removed from the canopy by zero-leafing in the conventionally-pruned vines and retained in the canopy with tip-squeezing to restrict growth in the minimally-pruned vines
Summer pruning	
January & March	Unwanted vigorous growth removed from all vines by removing entire shoots
Winter pruning	
July	Existing canes removed and replacement canes tied down

6.2.2 General measurements

Although the general experiment was carried out over three years: 2007/2008, 2008/2009 and 2009/2010 the nitrogen and pruning experimental focus for this chapter was based mainly on Year 1 (2007/20008). The following were recorded on individual vines:

- Components of yield, budburst (BB); floral budburst (FBB), vegetative budburst (VBB); flowers per floral shoot (F/FS), were measured on 4 canes per vine in spring of years 2 and 3 of the experiment. Measurements were not made in year 1 because flowering shoots had already been removed when the experiment started.
- Flowering date for each vine was determined (years 2 and 3 only) on a subsample of the fruiting canopy measuring ~ 1.2 m x 1.6 m by counting the number of flowers that had opened at 2 to 4-day intervals.

- Leaf mineral nutrient concentration was measured in a combined sample of 10 fully-expanded leaves and petioles per vine. Samples were taken every 4 to 5 weeks throughout year 1. Leaf fresh weight and dry weight and individual leaf area was measured on each sample.
- Fruit mineral nutrient contents were measured on a combined sample of 12 fruit per vine. Fruit were sampled in December, February and April of year 1.
- Fruit fresh weight, dry weight, firmness, soluble solids concentration and flesh hue angle were measured on a sample of 18 fruit per vine sampled randomly from across the entire canopy as close as possible to commercial harvest each year.
- In year 1, fruit storage performance was measured on 90 fruit per vine, sampled randomly from across the entire fruiting canopy. In year 2 an extra 30 fruit per vine were sampled for destructive measurement of fruit softening during storage.
- Canopy growth was estimated by collecting and weighing all material removed from each vine during pruning and leaf abscission.
- Leaf area index (for this chapter only) was calculated from hemispherical photographs taken on each vine in April 2007.

Details of the experimental methods are described in Chapter 2.

6.2.3 Statistical analysis

All variables were measured on a vine basis. The effect of pruning treatment within each N input was tested using analysis of variance (GenStat Release 8.2 [(PC/Windows XP) Copyright 2005, Lawes Agricultural Trust (Rothamsted Experimental Station)]). Mean separation tests were carried out using Fisher's Protected LSD at the 5% level of significance. General trends rather than effects of N treatment levels were reported (mean ± *SE*), but no hypothesis test of the effect of N input was done because of the lack of replication. Paired t-test was used to compare attributes from the same vines between seasons.

6.3 RESULTS

Results from the pruning/nitrogen experiment are presented first, then observations relating N input and vine productivity will be presented.

6.3.1 Pruning/nitrogen interaction.

6.3.1.1 Canopy biomass. The minimal pruning treatment resulted in an extra 0.33, 0.34 and 0.44 kg DW of shoot biomass being retained in the leader zone and 0.58, 0.72 and 0.87 kg DW of shoot biomass in the fruiting zone of the vines receiving 0, 105 and 210 kg N ha^{-1} respectively (Table.6.2).

Table 6.2 Total dry weight removed from 'Hort16A' kiwifruit vines during crop load adjustment and application of the pruning treatments in spring year 1.

	N input (kg ha^{-1})		
Pruning	0	105	210
treatment	Floral shoots (kg DW $vine^{-1}$)		
Conventional	0.19 ± 0.03	0.26 ± 0.05	0.20 ± 0.03
Minimal	0.23 ± 0.04	0.26 ± 0.03	0.16 ± 0.03
P-value	0.413	0.995	0.300
	Shoots removed from leader zone (kg DW $vine^{-1}$)		
Conventional	0.63 ± 0.07	0.77 ± 0.11	0.71 ± 0.09
Minimal	0.30 ± 0.04	0.43 ± 0.04	0.27 ± 0.04
P-value	0.001	0.013	<0.001
	Shoots removed from fruiting zone (kg DW $vine^{-1}$)		
Conventional	0.68 ± 0.09	0.86 ± 0.11	1.04 ± 0.20
Minimal	0.10 ± 0.02	0.14 ± 0.03	0.17 ± 0.04
P-value	<0.001	<0.001	<0.001

n = 7 ± SE.

Pruning treatment did not affect canopy growth during the remainder of the season. The amount of DW removed during summer and winter pruning, and the DW of abscised leaves collected from under each vine during autumn and winter was not affected by pruning treatment (Table 6.3).

Leaf area index (LAI) was higher in the MP vines receiving 105 kg N ha^{-1} than the CP vines receiving 105 kg N ha^{-1}. Pruning treatment did not significantly affect LAI in the vines receiving 0 and 210 kg N ha^{-1}.

Table 6.3 Total dry weight removed from 'Hort16A' kiwifruit vines during summer and winter pruning and leaf abscission, and leaf area index measured in April 2008 from 'Hort16A' kiwifruit vines receiving one of three different N inputs and two pruning treatments.

	N input (kg ha^{-1})		
Pruning	0	105	210
treatment	Summer pruning (kg DW $vine^{-1}$)		
Conventional	1.6 ± 0.2	2.2 ± 0.2	2.1 ± 0.3
Minimal	1.7 ± 0.1	2.3 ± 0.3	2.0 ± 0.4
P-value	0.773	0.688	0.941
	Winter pruning (kg DW $vine^{-1}$)		
Conventional	13.4 ± 0.7	15.6 ± 0.8	14.9 ± 1.0
Minimal	13.4 ± 0.7	16.3 ± 0.9	15.3 ± 1.1
P-value	0.988	0.590	0.762
	Abscised leaf (kg DW $vine^{-1}$)		
Conventional	10.3 ± 0.9	13.2 ± 0.6	11.4 ± 0.8
Minimal	9.9 ± 0.9	12.8 ± 0.8	12.1 ± 0.6
P-value	0.764	0.704	0.481
	Leaf area index (m^2 m^{-2})		
Conventional	5.1 ± 0.3	5.6 ± 0.2	5.4 ± 0.3
Minimal	5.8 ± 0.3	6.9 ± 0.3	6.0 ± 0.5
P-value	0.127	0.005	0.302

n = 7 ± SE.

6.3.1.2 Fruit attributes at harvest. Fruit FW was not affected by the pruning treatments (Table 6.4).

Table 6.4 Individual fresh weight of fruit harvested in April 2008 from 'Hort16A' kiwifruit vines receiving one of three different N inputs and two pruning treatments.

	Fresh weight (g fruit^{-1})		
	N input (kg ha^{-1})		
Pruning treatment	0	105	210
Conventional	117.0 ± 2.8	116.1 ± 1.8	114.6 ± 3.1
Minimal	118.4 ± 2.7	116.5 ± 2.8	114.4 ± 2.9
P-value	0.716	0.902	0.795

n = 7 ± SE.

Minimal pruning increased DMC and advanced maturity in the vines receiving 0 kg N ha^{-1} but not the vines receiving added N (Figure 6.2). Fruit from MP vines was softer than fruit from the CP vines, but results were not consistent across N inputs or sampling dates.

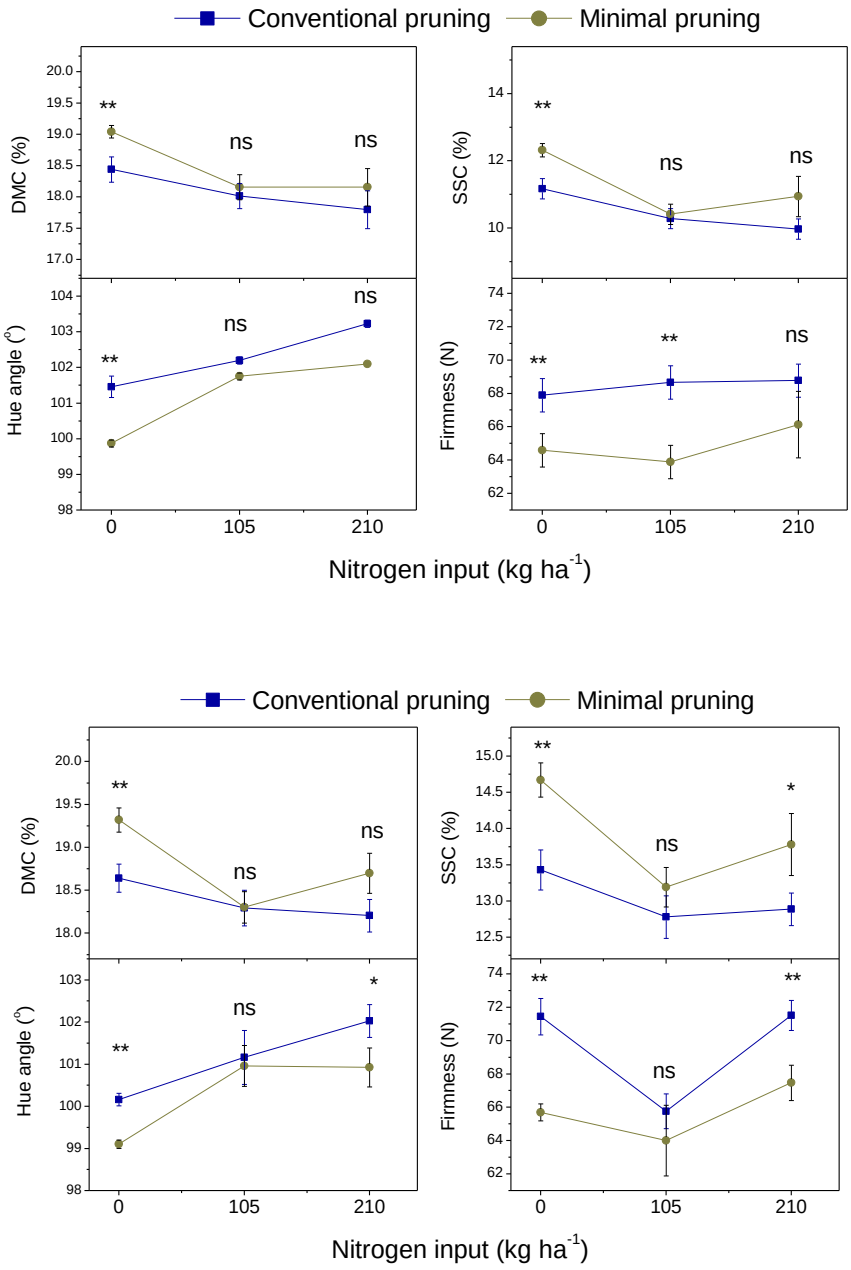


Figure 6.2 Dry matter concentration (DMC), soluble solids concentration (SSC), flesh hue angle and firmness of 'Hort16A' kiwifruit sampled on 28 April 2008 (top) and 8 May 2008 (bottom) from vines receiving three different nitrogen inputs and two different pruning treatments; *n* = 7 ± *SE*; ** = *P* ≤ 0.05, * = *P* ≤ 0.10, ns = *P* > 0.10.

6.3.1.3 Fruit mineral nutrient contents. Pruning treatment did not affect fruit mineral nutrient contents generally (Table 6.5). The exceptions were Cu in the vines receiving 105 kg N ha^{-1} (162 ± 6 and 141 ± 7 µg $fruit^{-1}$ in the CP and MP vines respectively, P = 0.041), and B in the vines receiving 210 kg N ha^{-1} (273 ± 6 and 247 ± 10 µg $fruit^{-1}$ in the CP and MP vines respectively, *P* = 0.041).

Table 6.5 Effect of pruning treatment (minimal or conventional) on mineral nutrient contents of mature 'Hort16A' fruit sampled from vines receiving three different nitrogen inputs.

Inorganic Nutrient	*P*-value		
	Nitrogen input (kg ha^{-1})		
	0	105	210
N	0.840	0.957	0.650
P	0.570	0.586	0.691
K	0.476	0.326	0.895
S	0.881	0.244	0.739
Ca	0.735	0.866	0.969
Mg	0.560	0.434	0.700
Mn	0.268	0.421	0.185
Zn	0.189	0.651	0.318
Fe	0.865	0.419	0.789
Cu	0.521	**0.041**	0.622
B	0.826	0.393	**0.041**

P-values < 0.100 are highlighted in bold font.

6.3.1.4 Fruit storage performance. Fruit firmness after storage was not affected by pruning treatment for any N input (Table 6.6).

Table 6.6 Firmness after 18 weeks at 1.5 °C of fruit harvested in April 2008 from 'Hort16A' kiwifruit vines receiving one of three nitrogen (N) inputs and two pruning treatments.

Pruning treatment	Firmness (N)		
	N input (kg ha^{-1})		
	0	105	210
Conventional	7.4 ± 0.2	7.2 ± 0.2	7.2 ± 0.2
Minimal	7.4 ± 0.1	7.7 ± 0.3	7.4 ± 0.2
P-value	0.861	0.216	0.348

n = 7 ± SE.

Less than 2 % of fruit in any treatment were affected by low temperature breakdown (LTB; Table 6.7). No statistical analysis was attempted because disorder incidence was very low and would be unduly influenced by one or two affected fruit (Ferguson et al., 2003).

Table 6.7 Percentage of fruit from 'Hort16A' kiwifruit vines receiving one of three nitrogen (N) inputs and two pruning treatmentsand affected by low temperature breakdown after storage at 1.5 °C for 18 weeks.

Pruning treatment	Low temperature breakdown (%)		
	N input (kg ha^{-1})		
	0	105	210
Conventional	0	1.6 ± 1.4	1.7 ± 1.6
Minimal	0	1.1 ± 0.9	0.8 ± 0.5

n = 7 ± SE.

6.3.1.6 Leaf mineral nutrient status. Early in the season (November and December), no differences in leaf mineral nutrient concentrations were detected between the pruning treatments for any of the three N inputs (see *P*-values in Appendix 6.7.1). As the season progressed, pruning treatment affected leaf concentrations of N, P, K, Ca, Mg and Mn. For most of these nutrients, significant differences were detected on one date only or were only marginally significant ($0.050 < P < 0.100$; Appendix 6.7.1). The exceptions were N and K. In the vines receiving 0 kg N ha^{-1}, leaf N concentrations were lower in the MP vines than the CP vines from February onwards (Figure 6.3A); the MP vines receiving 105 kg N ha^{-1} had lower N concentrations, but only in February (Figure 6.3B) and no differences were detected in the vines receiving 210 kg N ha^{-1} (Figure 6.4C). Later in the season, leaf K concentrations were lower in the CP vines than the MP vines (Figure 6.4), in the vines receiving 105 kg N ha^{-1} (and to a lesser extent in the vines receiving 210 kg N ha^{-1}).

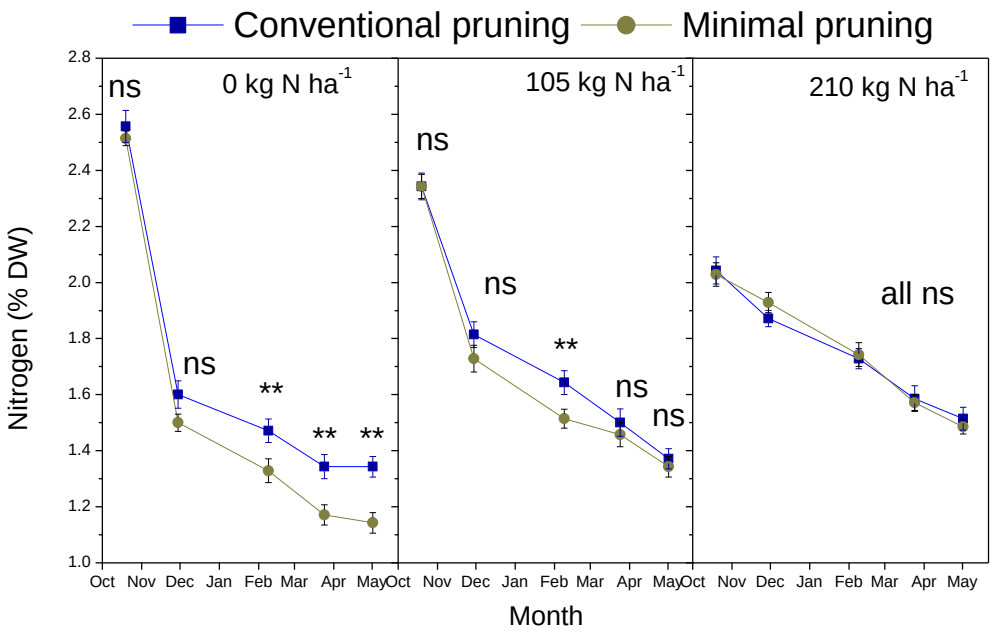


Figure 6.3 Leaf (blade and petiole) nitrogen concentrations sampled from 'Hort16A' kiwifruit vines that received different N inputs and pruning treatments; *n* = 7 ± *SE*; ** = *P* ≤ 0.05, * = *P* ≤ 0.10, ns = *P* > 0.10.

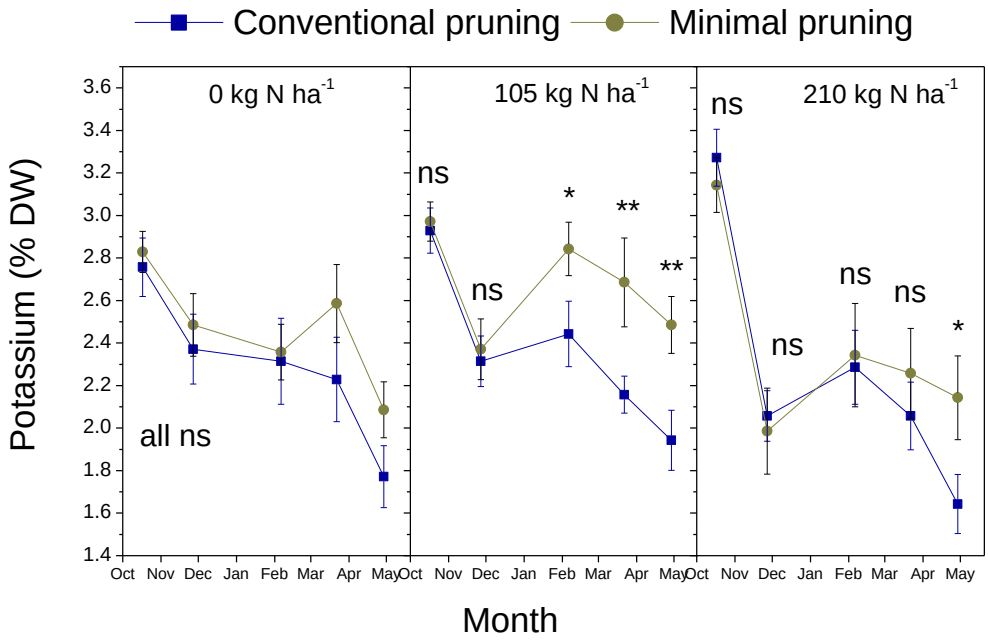


Figure 6.4 Leaf (blade and petiole) potassium concentrations sampled from 'Hort16A' kiwifruit vines that received different N inputs and pruning treatments; *n* = 7 ± *SE*; ** = *P* ≤ 0.05, * = *P* ≤ 0.10, ns = *P* > 0.10.

6.3.1.7 Components of yield. Pruning treatment affected some yield components in the year after treatments were applied. The CP vines receiving 105 kg N ha^{-1} tended to be more productive than the MP vines, although results were not consistent across the N application rates (Figure 6.5). Pruning did not affect components of yield in the vines receiving 0 kg N ha^{-1}.

Conventional pruning increased BB and FBB in the vines receiving 105 and 210 kg N ha^{-1} over MP (Figure 6.5). Minimally-pruned vines receiving 105 kg N ha^{-1} produced more flowers than CP vines (2.38 and 1.85 king flowers per winter bud, respectively Figure 6.5F). This effect on productivity was confirmed by counting fruit in all 14 vines receiving 105 kg N ha^{-1}; crop load in the CP pruned vines was 1505 ± 175, compared with 1299 ± 72 in the MP vines - a difference of 206 fruit per vine. No difference in flower numbers was detected in the vines receiving 210 kg N ha^{-1}.

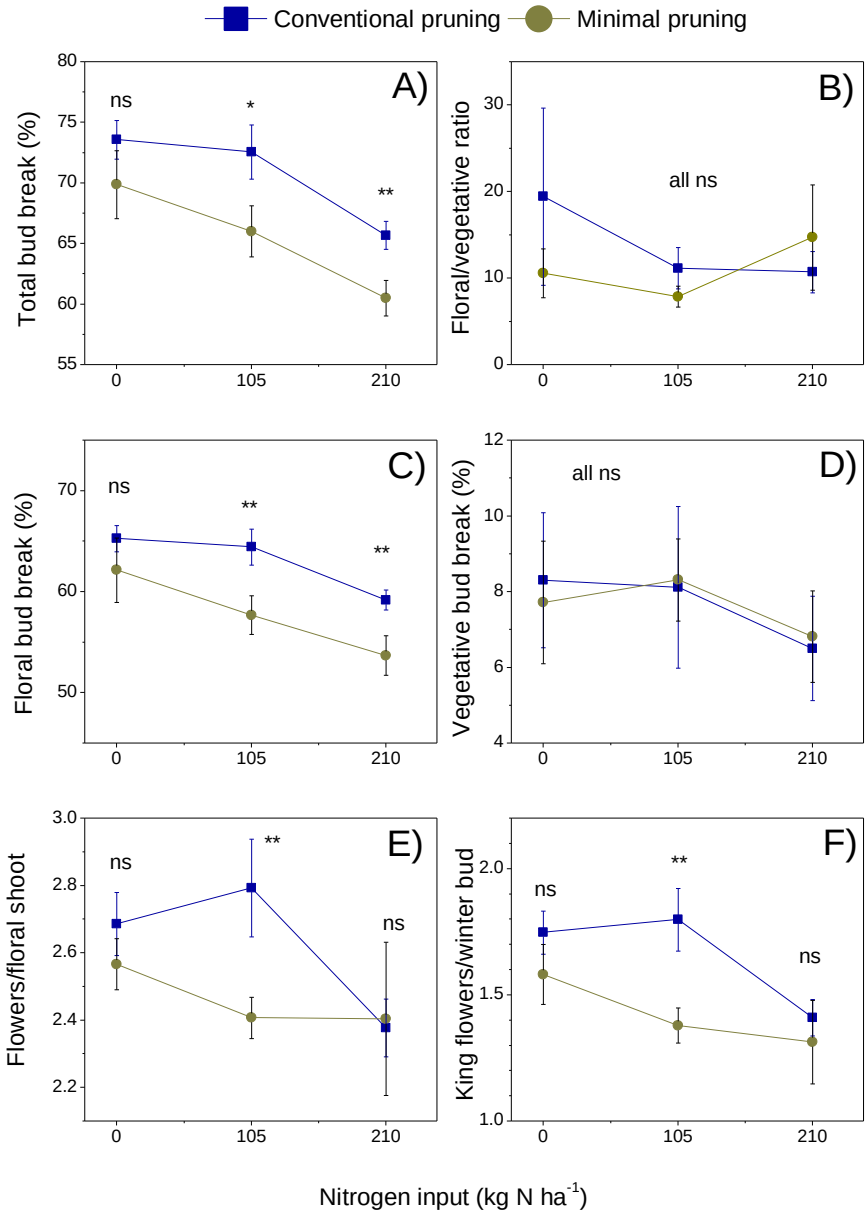


Figure 6.5. A) Total bud break, B) ratio of floral to vegetative buds, C) floral bud break, D) vegetative bud break, E), flowers per floral shoot ,and F) king flowers per winter bud measured in spring 2008 in 'Hort16A' kiwifruit vines that received three nitrogen inputs and two pruning treatments; *n* = 7 ± *SE*; ** = *P* ≤ 0.05, * = *P* ≤ 0.10, ns = *P* > 0.10.

6.3.1.8 Flowering date. The timing and duration of flowering was not affected by the pruning treatment applied in the previous year for any of the N inputs. There was a slight trend for the vines receiving CP to flower slightly later (Figure 6.6A) and over a shorter duration than the vines receiving MP (Figure 6.6D).

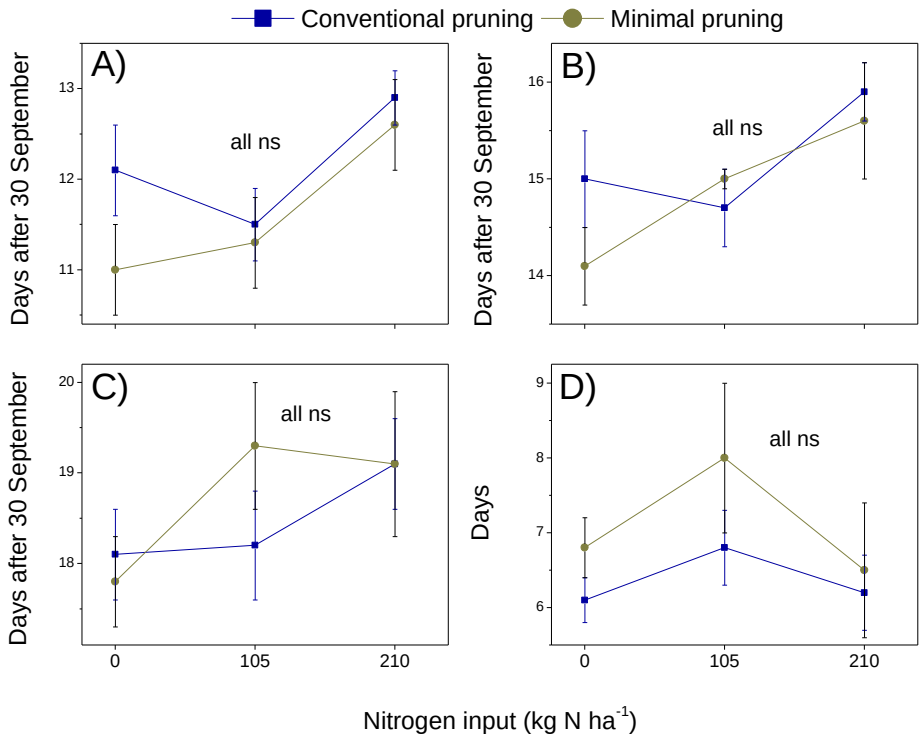


Figure 6.6 A) Start of flowering, B) mid-bloom C) end of flowering and D) duration of flowering measured in spring 2008 in 'Hort16A' kiwifruit vines that received three nitrogen inputs and two pruning treatments; *n* = 7 ± *SE*; ns = *P* < 0.10.

6.3.1.9 Key findings.

- Overall vine growth (estimated from all pruned material and abscised leaves) was highest in vines receiving 105 kg N ha^{-1} (32.6 ± 1.5 kg DW vine^{-1}), and lowest in vines receiving no added N (26.2 ± 1.1 kg DW vine^{-1}), with vines receiving 210 kg N ha^{-1} intermediate at 30.2 ± 1.5 kg DW vine^{-1}.

- In vines receiving no added N, retaining extra leaf in the canopy by minimal pruning resulted in fruit with higher DMC (by approximately 0.7 % - units) and advanced maturity relative to fruit from conventionally pruned (CP) vines.

Summer leaf N concentrations were lower in vines from the MP treatment. MP treatment did not affect return bloom in the following year.

- In vines receiving 105 kg N ha^{-1}, pruning treatment did not affect fruit attributes FW, DMC or maturity. Flower numbers in the following year were reduced by approximately 13.7 %.

- In vines receiving 210 kg N ha^{-1} fruit maturity was slightly advanced and productivity slightly reduced in the following year in MP vines compared with CP vines.

- Firmness after storage and incidence of low temperature breakdown were not affected by the pruning treatments.

6.3.2 Effect of Nitrogen input

In this section general trends with N input are described. The results cover three growing seasons from late spring 2007/2008 (year 1) through to winter pruning at the end of the 2009/2010 growing season (year 3). Means (± *SE*) are presented for the seven conventionally-pruned vines only as these represent the standard practice of the grower for that particular season.

6.3.2.1 Canopy biomass. More biomass was removed from the vines in year 2 (Figure 6.7), when approximately 40 kg DW of pruned shoots and abscised leaf was collected from the vines receiving 105 and 210 kg N ha^{-1}, compared with approximately 27 to 31 DW kg in years 1 and 3 (Figure 6.7). The increased vigour in year 2 was manifested less in the no-N vines than the vines receiving added N: the relative increase was around 20 % in the no-N vines (from ~ 25 kg DW to ~ 30 kg DW) and around 33 % in the vine receiving added N (from ~ 30 kg DW to ~ 40 kg DW).

Very little spring pruning was carried out in years 2 and 3 compared to year 1. This was partly because in year 1 the treatment with less spring pruning tended to produce higher DMC fruit and so was adopted by the grower as the standard practice in the following year, and also because little vigorous shoot growth was seen in the vines in year 3. Leaf area index was measured in year 1 only and ranged from 5.1 in the no-N vines to 5.6 and 5.4 in the vines receiving 105 and 210 kg N ha^{-1} (Table 6.3).

Table 6.8 Total dry weight of abscised leaf collected from under each vine in autumn and winter from 'Hort16A' kiwifruit vines receiving different N inputs.

Year	Abscised leaf (kg DW/vine)		
	N input (kg ha^{-1})		
	0	105	210
1	10.3 ± 0.9	13.2 ± 0.6	11.4 ± 0.8
2	18.8 ± 0.5	24.3 ± 0.9	25.1 ± 1.6
3	11.8 ± 0.5	13.0 ± 0.6	14.1 ± 0.6

n = 7 ± SE.

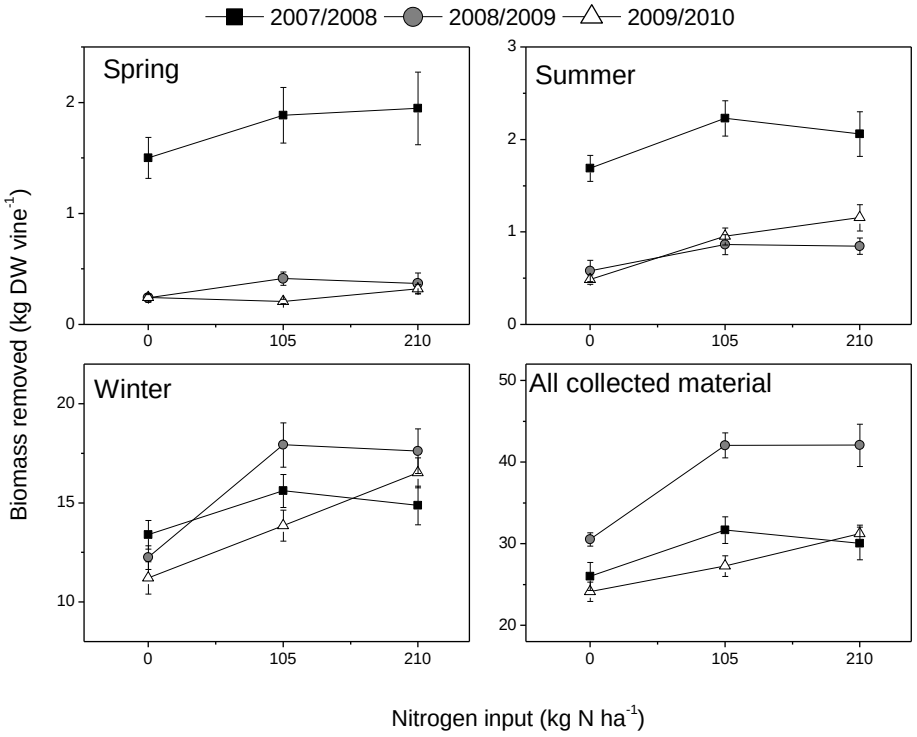


Figure 6.7 Total biomass removed from conventionally-pruned 'Hort16A' kiwifruit vines receiving 0, 105 and 201 kg N ha^{-1} in three consecutive years; *n* = 7 ± *SE*.

In year 1 DW allocation was lowest in vegetative growth of the no-N vines with no difference among the three nutrient treatments for reproductive growth (Figure 6.8). The main difference between the N treatments was in the amount of DW allocated to

vegetative growth, with DW allocated to fruit relatively consistent. This relationship was not established in years 2 and 3 as crop loads were not consistent across N inputs.

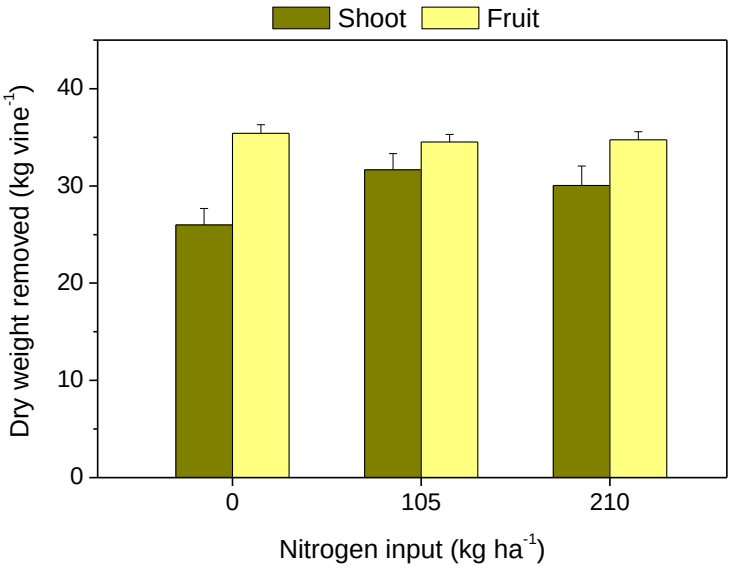


Figure 6.8 Dry weight of mature fruit and shoots removed from vines receiving different N inputs in the 2007/2008 growing season; *n* = 7 ± *SE*. Shoot dry weight is a combination of pruned shoots and abscised leaves collected from each vine.

6.3.2.2 Components of yield. Floral bud break, flowers per floral shoot and king flowers per winter bud tended to be higher in year 3 than year 2 across all three N inputs (Figure 6.9). Vines receiving 210 kg N ha^{-1} produced more flowers in year 3 than in year 2 (Figure 6.9F; 1.81 ± 0.06 and 1.41 ± 0.15 king flowers per winter bud, respectively, an increase of ~ 28 %). There were no other significant differences in productivity between years. The no-N vines produced fewer vegetative buds in year 2 than year 3 (Figure 6.9C and D), but this did not translate into significantly increased flower numbers.

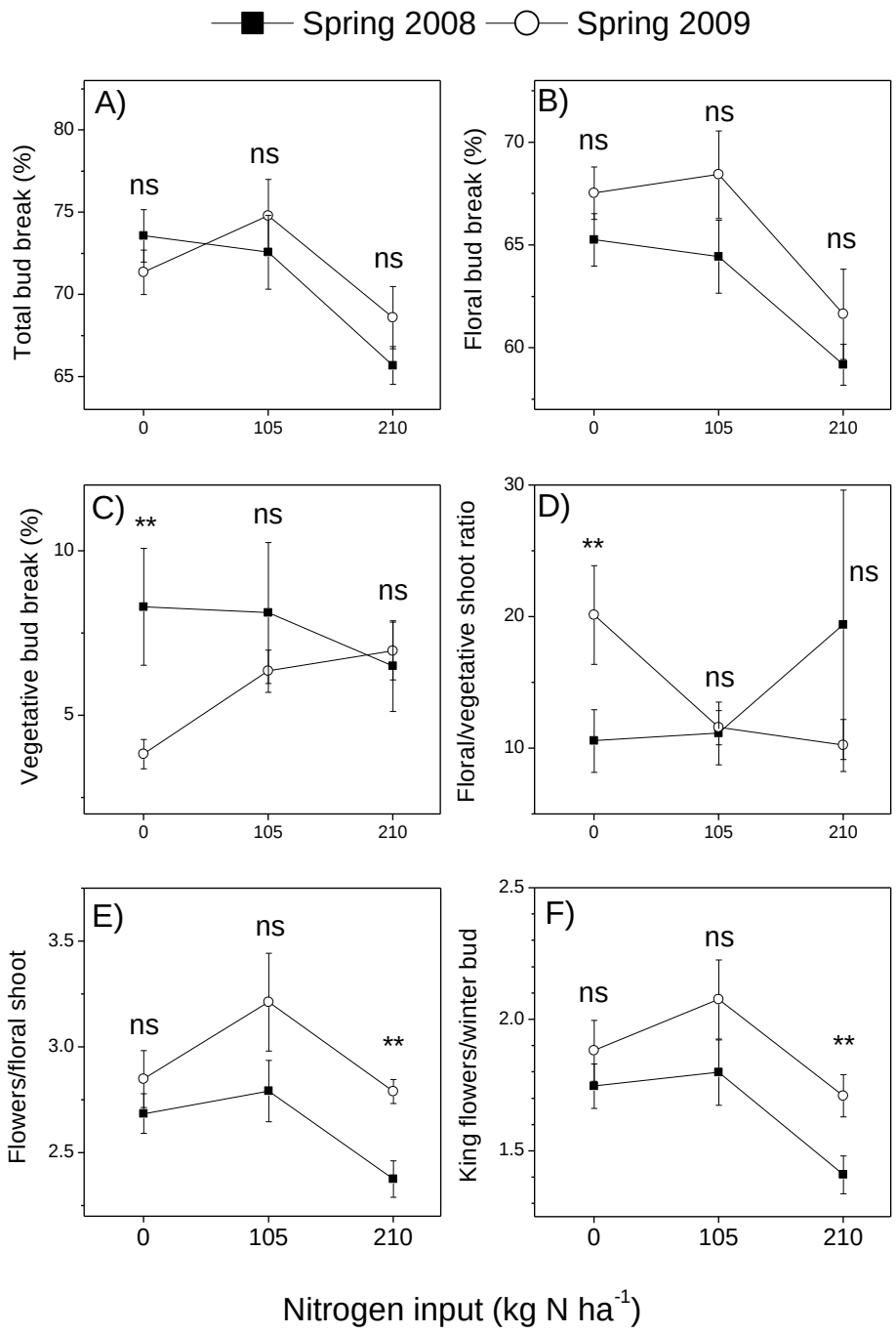


Figure 6.9 A) Bud break B), floral bud break C), vegetative bud break D) ratio of floral to vegetative buds, E) flowers per floral shoot and F) king flowers per winter bud measured in years 2 (spring 2008) and 3 (spring 2009) in 'Hort16A' kiwifruit vines that received three nitrogen inputs; *n* = 7 ± *SE*; ** = *P* ≤ 0.05, * = *P* ≤ 0.10, ns = *P* > 0.10.

6.3.2.3 Time of flowering. In spring 2008, vines receiving 210 kg N ha^{-1} tended to flower slightly later than those receiving 0 and 105 kg N ha$^{-1,}$ (Figure 6.10A). In spring 2009 vines receiving 105 and 210 kg N ha^{-1} tended to flower slightly later than vines receiving 0 kg N ha^{-1}. In year 3, heavy rain during flowering of that year meant that photographing flowering was only possible on 3 dates between 10 and 20 October, compared with 5 in year 2. In both years mid bloom was affected by ≤ 2 days.

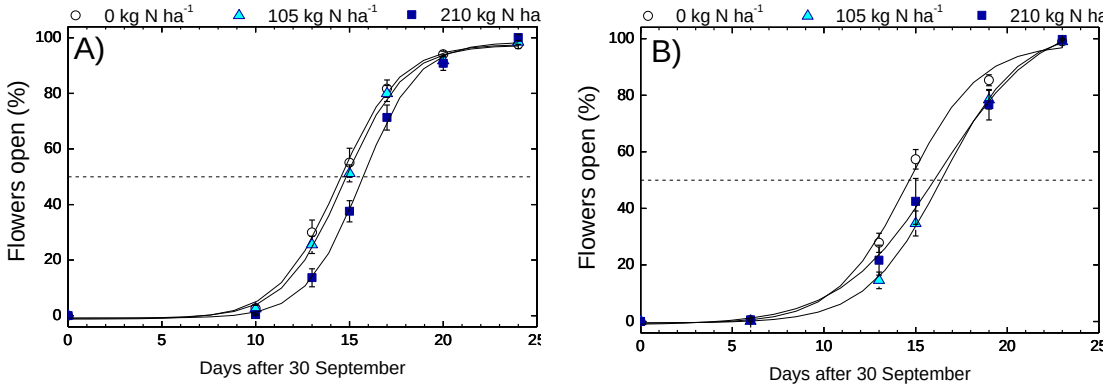


Figure 6.10 Percentage of flowers open in 'Hort16A' kiwifruit vines receiving 0, 105 and 210 kg N ha^{-1} in spring A) 2008 and B) 2009. Black lines are fitted sigmoid curves for each N rate; *n* = 7 ± *SE*.

6.3.2.4 Leaf nutrient status. Leaf mineral nutrient accumulation was only measured in year 1. The method used was the industry standard of analysing combined leaf and petiole and reporting on a concentration DW basis (Appendix 6.7.2). The main trends for leaves sampled mid-season (Figure 6.10) were:

- Leaf concentrations of N increased and those of S decreased with increasing N input.
- Vines receiving 0 kg N ha^{-1} had lower leaf Cu concentrations than leaves from vines receiving 105 and 210 kg N ha^{-1}.
- Leaves from the vines receiving 210 kg N ha^{-1} tended to have the highest concentrations of Mn and B.

Leaf N and B concentrations were below the minimum 'normal' range in vines receiving 0 kg N ha^{-1}, leaf P and B concentrations were below the minimum 'normal'

range in vines receiving 105 kg N ha^{-1}, and leaf P concentrations were below the minimum 'normal' range in vines receiving 210 kg N ha^{-1}.

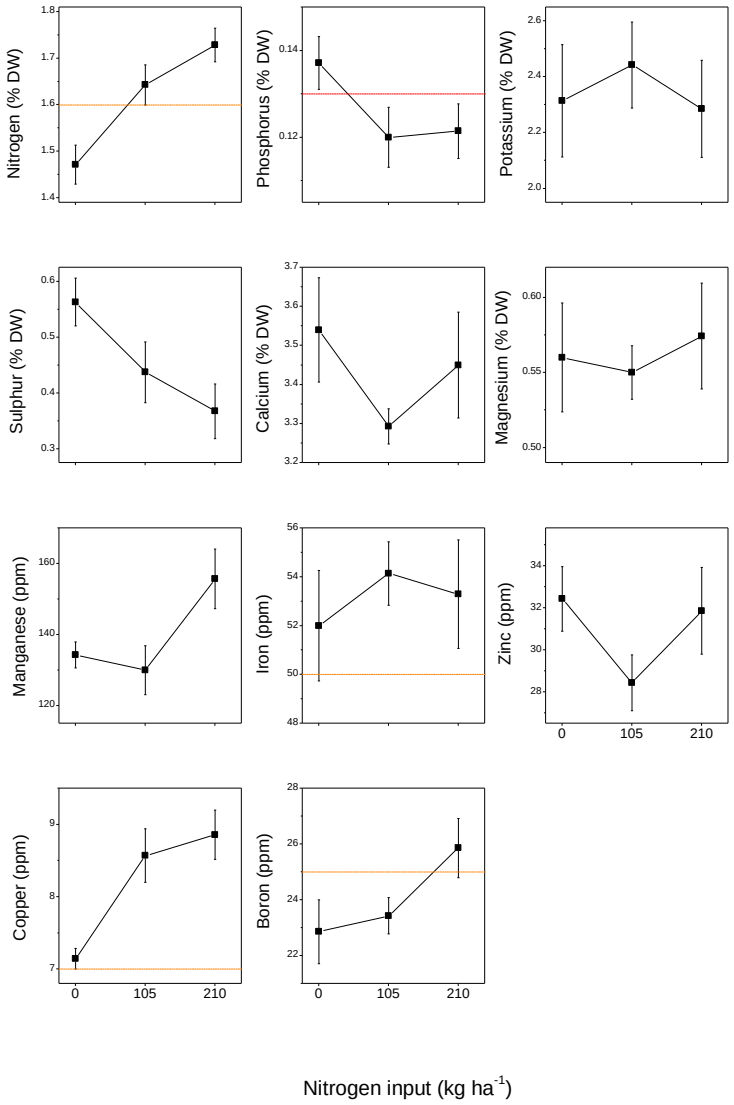


Figure 6.11 Leaf mineral nutrient concentrations measured in February 2008 in 'Hort16A' kiwifruit vines that received three N inputs *n* = 7 ± *SE*.

6.3.2.5 Fruit fresh and dry weight. There was no consistent trend with N input and fruit FW across the three years (Figure 6.12A). Fruit from the no-N vines tended to

have higher DMC than the vines receiving added N, the magnitude of the difference was greatest in year 2 (~ 0.7 % - units), and ~ 0.2 % - units in year 3.

In year 1 when crop loads in all vines were adjusted to ~ 65 fruit per m^2, FW was relatively consistent across the three N inputs (Figure 6.12A) with less than 6 g separating the treatments, and a slight trend for decreased FW with increased N input. Fruit DMC tended to be highest in the vines receiving no added N, by ~ 0.2 to 0.9 % - units over fruit from the vines receiving 105 and 210 kg N ha^{-1} (Figure 6.12B).

In years 2 and 3 when flower numbers were low, crop loads were not adjusted to the same level across all vines. The trend towards higher FW in the vines receiving 210 kg N ha^{-1} in years 2 and 3 might reflect the tendency of the high-N vines to produce fewer flowers - although high-N vines were probably the least productive in year 2 whereas the trend towards larger fruit with higher N input was greater in year 3.

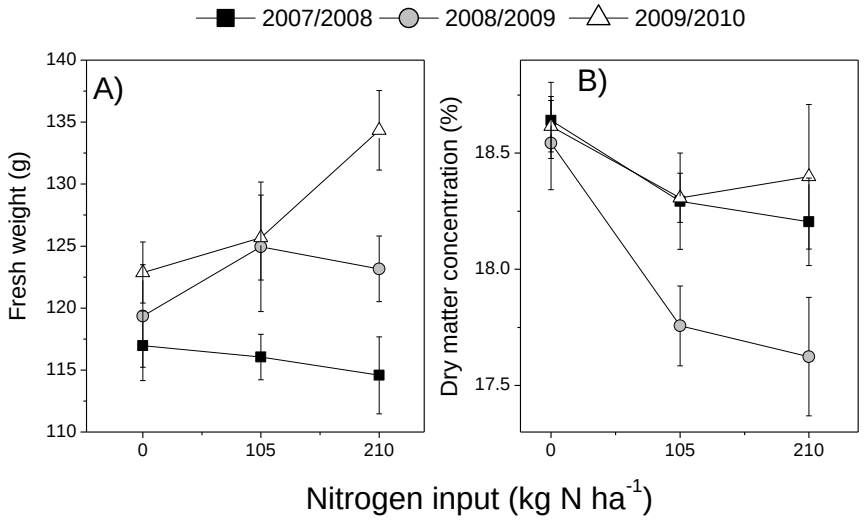


Figure 6.12 Fruit A) fresh weight and B) dry matter concentration measured over three consecutive years in mature 'Hort16A' kiwifruit vines receiving three different nitrogen inputs, *n* = 7 ± *SE*.

6.3.2.6 Fruit maturity attributes. Flesh hue angle tended to increase with N input, the magnitude of the difference was approximately 2.0º to 2.5º between the vines

receiving 0 and 201 kg N ha^{-1} each year (Figure 6.13A). There were no consistent trends with N input and fruit firmness (Figure 6.13B). Fruit with the lowest N input tended to have higher SSC than fruit from the vines receiving 105 and 210 kg N ha^{-1}, particularly in years 1 and 2 when fruit had higher SSC at the time of sampling (Figure 6.13C).

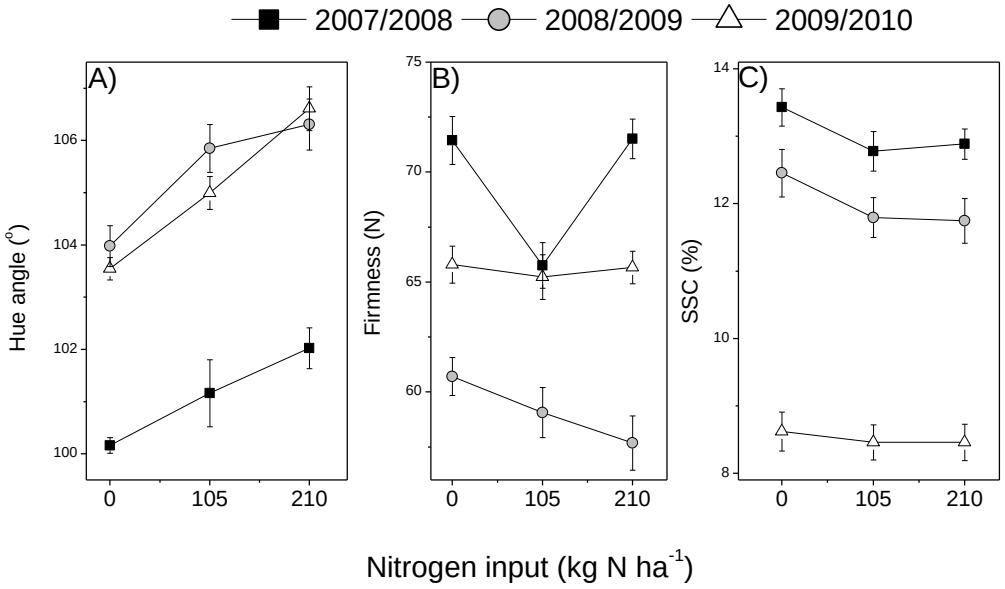


Figure 6.13 A) Flesh hue angle, B) firmness and C) soluble solids concentration (SSC) measured over three consecutive years in fruit from 'Hort16A' kiwifruit vines receiving different nitrogen inputs and conventional pruning treatment, *n* = 7 ± *SE*.

6.3.2.7 Fruit mineral nutrient status. At harvest date, fruit from vines receiving no added N tended to contain more P, Ca, Mg, Fe, Zn and B than fruit from vines receiving 105 or 210 kg N ha^{-1} (Figure 6.14). Fruit from the vines receiving 210 kg N ha^{-1} tended to contain the most N, and fruit from the vines receiving 105 kg N ha^{-1} contained the least Mn.

Fruit from the no-N vines had higher Ca/N ratios than fruit from the vines receiving 105 and 210 kg N ha^{-1} (0.154 ± 0.020, 0.113 ± 0.023 and 0.115 ± 0.012, respectively).

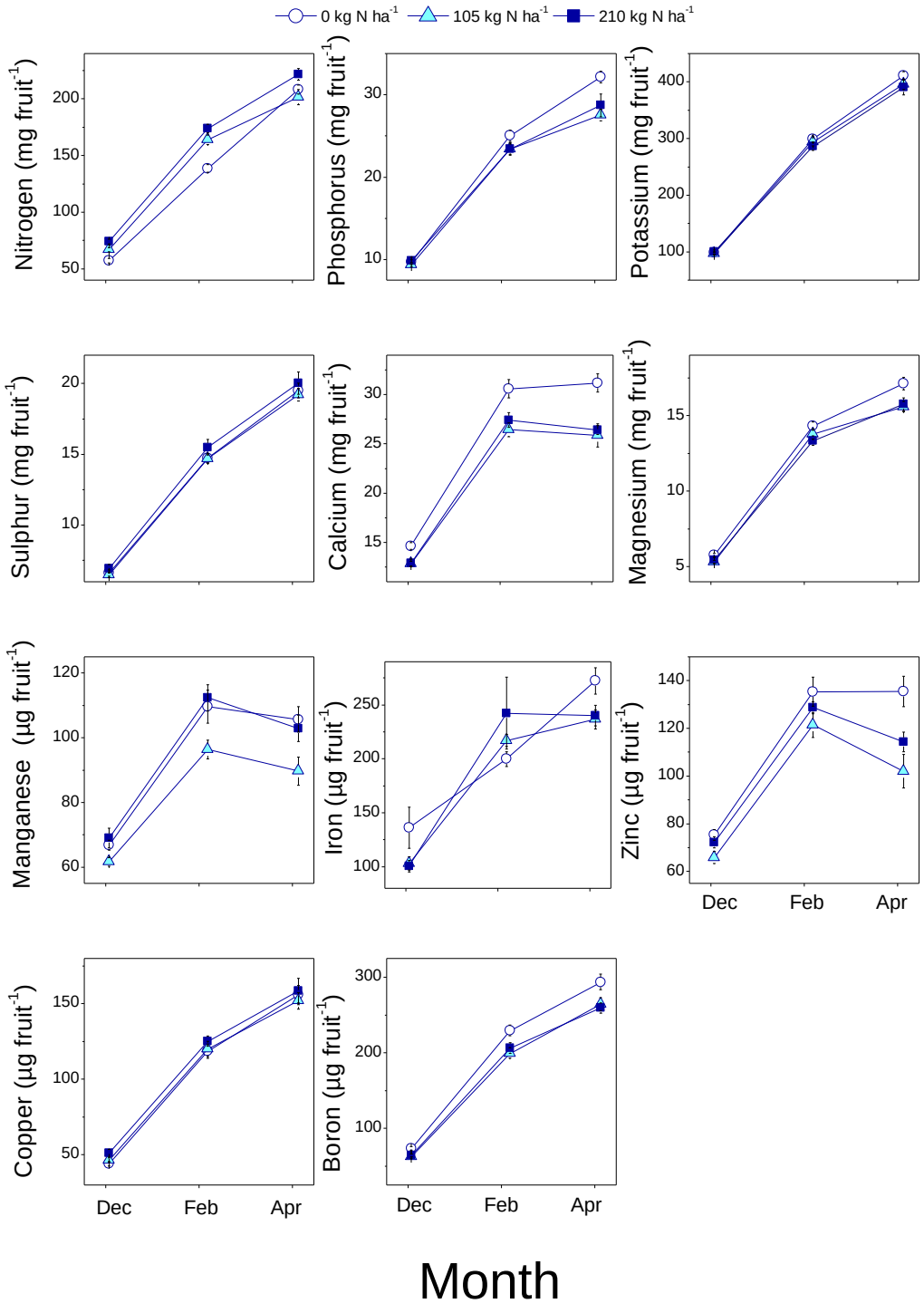


Figure 6.14 Mineral nutrient contents of fruit sampled from 'Hort16A' kiwifruit vines receiving different nitrogen inputs for Year 1 and measured in Autumn 2008; *n* = 7 ± *SE*.

6.3.2.8 Fruit storage performance. In year 1 there were no trends with firmness after storage, firmness after storage was 7.4, 7.2 and 7.2 N in the vines receiving 0, 105 and 201 kg N ha^{-1}, respectively.

In year 2, softening during storage was monitored in fruit harvested on 30 April. No clear trends were observed, although fruit from the vines receiving 210 kg N ha^{-1} softened to 20 N and 10 N slightly sooner (1 or 2 days) than fruit from the vines receiving 0 and 105 kg N ha^{-1} (Figure 6.15).

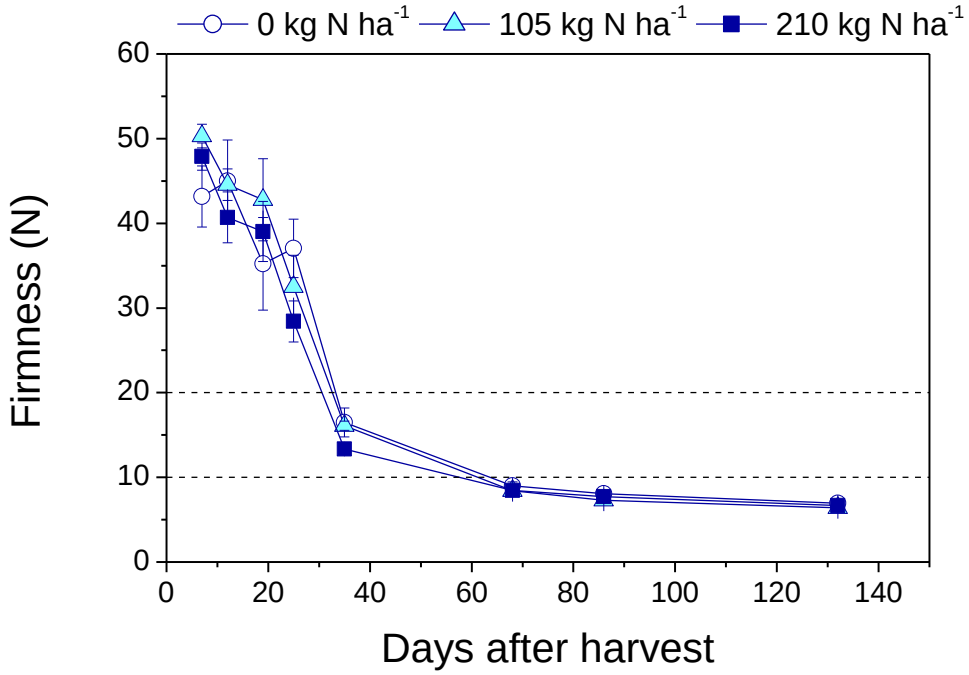


Figure 6.15 Firmness during storage of 'Hort16A' kiwifruit harvested on 30 April 2009 from vines receiving different nitrogen (N) inputs; *n* = 7 ± *SE*; dashed lines represent firmnesses of 20 N (newtons) and 10 N for comparison purposes.

Less than 2 % of fruit harvested in year 1 were affected by low temperature breakdown (LTB; Table 6.9). In year 2 more LTB was detected, and tended to be highest in the vines receiving higher N inputs (Table 6.9).

Table 6.9 Percentage of fruit from 'Hort16A' kiwifruit vines affected by low temperature breakdown after storage at 1.5°C for 18 weeks in years 1 and 2. Vines received different nitrogen (N) inputs.

	Low-temperature breakdown (%)		
	N input (kg ha^{-1})		
Year	0	105	210
1	0	1.6 ± 1.4	1.7 ± 1.6
2	1.0 ± 0.6	3.0 ± 0.7	8.4 ± 2.6

n = 7 ± SE.

6.3.2.9 Key findings N input and vine productivity.

The vines receiving no added N tended to:

- Have less canopy growth than the vines receiving 105 and 210 kg N ha^{-1}, especially in year 2 when canopies were most vigorous.
- Have lower leaf N concentrations and higher leaf S concentrations than the vines receiving 105 and 210 kg N ha^{-1}.
- Produce fruit with higher DMC and advanced maturity, relative to the vines that received N fertiliser.

Nitrogen input did not have any consistent effects on fruit FW or softening in storage. There were no consistent patterns with N input and return bloom , although the vines receiving the highest N input tended to be the least productive.

6.4 DISCUSSION

The results presented in this chapter illustrate: 1) how minor adjustments to canopy management can affect fruit quality in one year and productivity in the following year, and 2) the potential for optimising N fertiliser input to maximise vine productivity.

6.4.1 Pruning/nitrogen interactions

6.4.1.1 Fruit quality attributes. In the vines receiving no added N, the MP treatment increased fruit DMC and advanced fruit maturity. Vines receiving 201 kg N ha^{-1} showed a similar but less pronounced response to MP; maturity was advanced and DMC was unaffected. In the vines receiving 105 kg N ha^{-1}, MP did not affect fruit attributes. Minimal pruning had the greatest effect in the vines with the least overall canopy growth and a lesser effect as canopy growth increased. Total dry weight removed in pruning and leaf abscission in year 1 was 26.2 ± 1.1, 30.2 ± 1.5 and 32.6 ± 1.5 from the vines receiving 0, 210 and 105 kg N ha^{-1}, respectively. The relationship between vine growth and N input will be discussed in Section 6.4.2.

In the previous work of Mills et al. (2008), a greater FW of material was removed during summer pruning from the vines receiving 201 kg N ha^{-1} than the vines receiving 0 or 105 kg N ha^{-1}, suggesting that vigour was greatest in the vines receiving the highest N input. In the current experiment the total DW of pruned shoots and abscised leaf collected from the vines receiving 210 kg N ha^{-1} tended to be ≤ that removed from the vines receiving 105 kg N ha^{-1} (for example Figures 6.7 and 6.8), except in the final year when there was a trend for increased biomass removal with increased N input. Seasonal differences and the lack of replication make it difficult to determine if there is a clear association with the observed response and canopy vigour. The main finding is that in year 1, MP increased fruit DMC and advanced maturity in vines that tended to have the least vigorous canopies.

Based on the weight of material removed in the pruning treatment, the MP vines retained ~ 5 to 7 m^2 of leaf area per vine more than the CP vines (see calculations in Appendix 6.7.3). This would be equivalent to an additional LAI of 0.20 to 0.23 m^2 per m^2 of canopy. The pruning treatments were applied in spring before the canopy had closed over. A canopy is considered to be closed when the all available sunlight is

captured by leaf area and little or no direct sunlight is apparent on the ground beneath the vines. The extra leaf area retained in MP vines before canopy closure, probably increased assimilate supply to the vine. Once the canopy has closed over, any extra leaf area would be shaded, or would shade other leaves, and therefore would have much lower photosynthetic rates than sun-exposed leaves (Grant and Ryugo, 1984b) adding relatively little to whole-vine assimilate supply.

The most rapid FW accumulation occurs ~ 4 to 7 weeks after flowering in kiwifruit (Figure 1.4) and treatments designed to increase FW are often applied within 6 weeks of mid-bloom. For example, spring cane or trunk girdling is used to increase FW by up to 7 g (Patterson and Currie, 2011) with the largest size response occurring when the girdle is applied between 4 and 5 weeks after full bloom (Currie, unpublished). The fruit biostimulant Benefit®, which can increase 'Hort16A' FW by up to 30 g, is applied within 5 weeks of petal-fall (Patterson and Currie, 2011; Currie, unpublished). Plant growth regulators, primarily the cytokinin-active compound CPPU (N-(2-chloro-4-pyridyl)-N′-phenylurea), are applied ~ 3 weeks after flowering to increase FW in 'Hayward' (Iwahori et al., 1988, Patterson et al., 1993, Antognozzi et al., 1996; Cruz-Castillo et al., 2002). The size response has been attributed to increased cell number in the outer pericarp (Cruz-Castillo et al., 2002), or to increased cell size with no increase in cell number (Patterson et al., 1993).

In the current experiment, increasing early-season leaf area available to developing fruit around 2 to 3 weeks after flowering did not affect FW for any of the N inputs. The most likely reason for the lack of FW response to the pruning treatment is that Benefit® application increased fresh weight and any additional effect of additional leaf area would be masked. Benefit® application can increase FW by around 20 to 30 g, whereas retaining an extra 7 m^2 of LA per vine (~ 36 cm^2 per fruit) might be expected to make a small difference to FW (see calculations in Appendix 6.7.3). For example, 'Hayward' vines required a leaf area of 100 cm^2 per fruit to generate an increase of 6 g FW (Snelgar and Thorp, 1988).

Although the additional LA retained by the minimal pruning was relatively small, it occurred during early spring when the canopy was still growing so the relative contribution of the extra LA to total LA could be quite significant. To determine the

critical time when additional LA is most beneficial to fruit development would require minimal pruning experiments to be carried out using vines where Benefit® had not been applied.

As previously noted, treatments applied during cell division usually increase FW, but DW can also increase. Usually FW accumulation is larger than DW accumulation, for example, Antognozzi et al. (1996) found that FW, DW and non-structural carbohydrate accumulation were all significantly increased in 'Hayward' kiwifruit treated with CPPU 2 weeks after full bloom, yet mature CPPU-treated fruit had lower DMC than mature untreated fruit. If Benefit® application to all vines had already increased FW then the increase in DW would result in the observed increase in DMC.

6.4.1.2 Return bloom. As discussed in Chapter 4, both too much and too little leaf area can adversely affect return bloom in 'Hayward' vines. Canes that were shaded in the previous season produced fewer flowers per floral shoot than canes that were exposed (Grant and Ryugo, 1984a). Leader pruning (removal of vigorous growth from the central leader) of 'Hayward' vines in one season resulted in more flowers per winter bud in the following season than occurred in conventionally-pruned vines (Thorp et al., 2003b). The authors attributed this to more carbohydrate availability in the replacement canes under leader pruning. In addition to retaining extra leaf area in the fruiting zone, the MP treatment retained extra leaf area in the leader zone, also known as the replacement cane zone (Buwalda and Smith, 1990).

In the current experiment these two opposing factors: insufficient leaf and excessive shading could interact to affect return bloom. Minimal pruning adversely affected return bloom in the vines with the most canopy growth (those receiving 105 kg N ha^{-1}), possibly as a result of extra shading. Little or no effect of pruning was seen in the vines receiving 0 and 210 kg N ha^{-1}. It is not clear why the effect of MP on return bloom was not consistent across N inputs. It is possible that in the most vigorous vines retention of extra leaf area and consequent shading of replacement canes was sufficient to reduce productivity. In 'Hayward' vines, replacement canes that were shaded in the previous season were less productive than sun-exposed canes (Grant and Ryugo, 1984a), and retaining extra leaf area in the leader zone reduced return bloom in the following season (Thorp et al., 2003b). In addition the vines receiving 105 kg N ha^{-1} were the only ones where LAI measured in April was significantly higher in

the MP than the CP vines. This finding also indicates that the largest effect of MP detected later in the season was in the vines receiving 105 kg N ha^{-1}.

6.4.2 Nitrogen management and vine productivity.

The trends observed in this experiment suggest that managing nitrogen input offers potential to alter kiwifruit fruit quality and vine productivity. However, the findings need to be interpreted with caution because of the lack of replication and the lack of crop load adjustment in years 2 and 3 when flower numbers were low.

A simplified nitrogen-yield response curve (Figure 6.16) illustrates that, at low concentrations, yield increases in response to N input, but when N is optimal, yield is unaffected, and excess N decreases yield. This latter effect could be a result of phytotoxicity, an induced deficiency of another nutrient or depression of phytohormones involved in plant developmental processes (Marschner, 1995). At the first marginal zone plant growth is often described as nitrogen-limited, this is where there is a positive relationship between plant growth rate and plant N concentration (Verkroost and Wassen, 2005). Plants typically respond to low N by increasing allocation of biomass towards root production over shoot production as a means increasing N uptake so that the balance between C and N within the plant is restored (Ågren and Franklin, 2003). As N input increases, the shoot-to-root ratio tends to increase. For example Xia and Wan (2008) carried out a meta-analysis of the responses of plant species to N addition. They found that increasing N input increased above-ground biomass in woody perennials significantly more than below-ground biomass (increases of 47.9 % and 23.0 %, respectively).

The physiological processes that regulate growth and resource allocation in N-limited plants are not well-understood. In kiwifruit low N is associated with premature senescence of older leaves (Buwalda et al., 1990), reduced vigour (Buwalda et al., 1990, Costa et al., 1997a; Mills et al., 2008), and reduced individual leaf area (Costa et al., 1997a). Low plant N is often associated with reduced leaf photosynthetic rates (Paul and Driscoll, 1997). Carbohydrates accumulate in the leaves of N-deficient plants (Hermans et al., 2006) and are believed to repress photosynthesis thus releasing the N stored in the photosynthesis enzyme Rubisco (Paul and Driscoll, 1997). Alternatively, reduced photosynthesis in N-limited plants has been directly attributed

to Rubisco breakdown rather than an indirect result of feedback inhibition caused by sugar accumulation (Chen and Cheng, 2003). Low C availability has been attributed as a reason for reduced productivity of kiwifruit vines receiving low N input and defoliation treatments (Buwalda and Meekings, 1993).

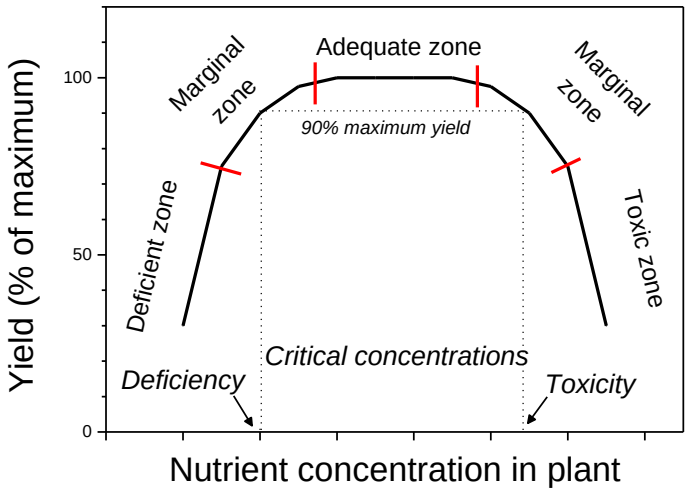


Figure 6.16 An idealised relationship between plant dry matter yield and plant nutrient concentration (redrawn from Atwell et al. 1999 and originally based on Smith and Lonergan, 1997).

6.4.2.1 Canopy health. In the current experiment, the vines receiving 0 kg N ha^{-1} tended to have reduced canopy growth, measured by DW of pruned shoots and abscised leaf, than the vines receiving 105 and 201 kg N ha^{-1}. This trend was most apparent in the second year of the experiment when overall canopy growth was the greatest in all vines. There was little or no difference in canopy growth between the vines receiving 105 and 210 kg N $ha^{-1,}$ suggesting that the vines were at or slightly beyond the adequate zone (Figure 6.16) with 105 kg N ha^{-1}.

Reduced leaf N concentrations have been associated with reduced photosynthesis in a number of crops including kiwifruit (Buwalda and Meekings, 1993), apple (Xia et al., 2009) and grape (Chen and Cheng, 2003). From December through until April in year 1 of the current experiment, leaves from the vines receiving no added N had lower N concentrations than leaves from the vines receiving 105 and 210 kg N ha^{-1}

and were at or below the minimum 'normal' range for 'Hort16A' leaves (Figure 6.11). Leaf photosynthesis rates were not measured in the current experiment, but there was no evidence that fruit quality within the 2007/2008 growing season, or return bloom in the following season was adversely affected. Typically the vines receiving the highest N input had the lowest return bloom, not the vines receiving the lowest N input.

Leaf concentrations of several other mineral nutrients appeared to be affected by different N inputs. Leaf B and Mn concentrations were highest in the vines receiving the highest N input. One of the N fertilisers used in this trial, Nitrabor™, contained B, so this could explain the increase in leaf B. Ammonium ions (NH_4^+) from ammonium nitrate fertiliser decrease soil pH, and Mn^{2+} uptake is usually increased in acidic soils (Marschner 1995). In potted apple trees, leaf N and Mn concentrations increased with increasing N supply; N supply had little effect on leaf concentrations of other nutrients (Xia et al 2009). Leaf S concentrations were highest in the vines receiving no added N. It is not clear why S accumulated in leaves of vines receiving no added N. One possibility is that sulphate and nitrate compete for uptake from the soil, and when nitrate is low, more sulphate is taken up by the plant. Sulphur is generally stored in the leaves as inorganic sulphate or in a reduced form (e.g. glutathione). It is possible that carbohydrate build-up in the leaves in low-N plants (Hermans et al., 2006) means that photosynthates are not available for S reduction and remobilisation. A second possibility is that, when nitrate levels are low, organic S compounds can be synthesised and retained in the leaves to function as osmoticants (Colmer et al., 1996).

6.4.2.2 Return bloom. In the current experiment the vines receiving 105 kg N ha^{-1} tended to produce the most flowers, with the vines receiving no added N being the same or less than the 105 or 210 kg N ha^{-1} vines. Typically vines receiving the highest N inputs produced the fewest king flowers per winter bud, and those receiving 105 kg N ha^{-1} produced ≥ KF/Bud than vines receiving no added N. In 'Hayward' vines, increased N input has sometimes been associated with increased vine productivity. In a two-year study, fruit numbers were reduced significantly in vines receiving 0 kg N ha^{-1} compared with vines receiving 250 or 750 kg N ha^{-1} (Buwalda and Meekings, 1993). After several years of treatment, vines receiving no added N

had fewer fruit per m^2 than vines receiving 200 kg N ha^{-1} (Buwalda et al., 1990). Conversely N input did not affect total bud break or floral bud break in a three-year experiment (Costa et al., 1997a), or yield at harvest in a separate three-year experiment (Johnson et al., 1997). Autumn N application had no effect on bud break or flowering in 'Hayward' vines (Boyd et al., 2007).

The results reported above make it difficult to ascertain if N input can consistently affect vine productivity. Productivity can be defined, measured and interpreted in different ways. From a commercial perspective, yield is the amount of mature fruit of the desired size profile and quality attributes. However, the benefits of increased commercial yield can be offset if costs of thinning more undersized or misshapen fruit increase. Potential productivity can be ascertained from components of yield data; and in the current experiment there was some evidence that the highest N input reduced potential productivity, particularly in 2008 when flower numbers were lowest of the three years.

One theory is that a plant with low N status reduces return bloom as a consequence of its effect on vegetative growth and photosynthesis, thereby reducing plant carbohydrate reserves. For example, low N input reduced return bloom, canopy growth and leaf photosynthetic capacity in 'Hayward' vines receiving some N treatments for two years (Buwalda and Meekings, 1993). The reduction in return bloom obtained was attributed to a reduction in vine carbohydrate reserves, although carbohydrate reserves were not measured in the experiment. These authors found reduced return bloom at the start of the second year. whilereduced LAI and whole-vine photosynthesis were detected in the second year of the experiment, but not the first year. Therefore there was no clear link between return bloom, reduced whole vine photosynthesis and reduction in carbohydrate reserves. It is possible that if sugars accumulate in leaves of low-N plants, then prior to leaf abscission, carbohydrates return to the canes and localised reserve status is actually higher in low-N vines than in high-N vines: this could be investigated in future experiments.

6.4.2.3 Individual fruit fresh weight. In this experiment any consistent affect of N input on FW accumulation could be masked by the application of Benefit® application. In addition different crop loads in years 2 and 3 would also confound results. In year 1 when crop loads were the same across the three N inputs there was a

slight decrease in FW with N input. In years 2 and 3 when crop loads were not adjusted, fruit from the vines receiving no added N tended to be smaller than fruit from the vines receiving 105 and 210 kg N ha^{-1}.

Inconsistent results were obtained on the effect of N input on FW in 'Hayward' vines. Vines receiving no added N produced more undersized fruit than vines receiving 100 and 200 kg N ha^{-1} (Tagliavini et al., 1995). Vines receiving 150, 300 and 450 kg N ha^{-1} had significantly higher FW than vines receiving 0 kg N ha^{-1} in 5 of 6 years (Vizzotto et al., 1999). No clear effect of N input on FW was found by others Buwalda et al. (1990), Costa et al. (1997a), Johnson et al. (1997). Experiments carried out on 'Hayward' vines over just one season also produced inconclusive results. Ground application of N in summer increased FW (Barnett et al., 2007), but foliar application of urea ~ 5 to 7 weeks after flowering had no effect on FW (Morton and Woolley, 2011), but increased DMC. Regular foliar sprays (which contained N, P, K, S, Mg and Ca) carried out from January until April reduced DMC but did not significantly affect FW (Mulligan, 2007).

In apple trees thinned to the same crop load, increased N input increased fruit FW by increasing fruit cell numbers without affecting cell size (Xia et al., 2009). The result was attributed to the extra leaf area per fruit providing extra resources to developing fruit during cell division phase which occurred ~ 4 to 6 weeks after mid bloom (Al-Hinai and Roper, 2004). The lack of consistent effect of N input on FW in kiwifruit might be because LA is not necessarily limiting fruit growth, therefore extra LA generated by increased N input would have no effect. In 'Hayward' vines FW increased by 5 to 6 g for every extra 100 cm^2 of LA per fruit, up till 700 cm^2 per fruit, after this, no increase in FW was detected (Snelgar and Thorp, 1988). Comparable numbers have not been calculated for 'Hort16A' kiwifruit vines, but in year 1 of this experiment LAI was approximately 5 m^2 m^{-2} and crop load 65 fruit per m^2, giving a leaf area per fruit of ~ 770 cm^2 per fruit (Appendix 6.7.3). The critical time for LA to influence FW in kiwifruit has not been determined. If the critical time was during the cell division phase of growth, it might be expected that any increase in available LA would benefit FW. In the present experiment increased LA early in the season, generated by minimal pruning, did not significantly increase FW, and any increase in early-season LAI caused by increased N input did not affect FW either.

Different crop loads or different thinning strategies may confound interpretation of FW data in long-term field trials. For example it is possible that one treatment may result in the higher production of unacceptable fruit than in other treatments. For example in 'Hayward' vines a higher proportion of unacceptably-shaped fruit were found in vines receiving higher N inputs (Costa et al., 1997a) and a greater proportion of undersized fruit (< 70 g) were found in vines receiving lower N inputs (Tagliavini et al., 1995). If unacceptable fruit were thinned off during the year then FW of the remaining fruit may be higher, even though the inverse relationship between crop load and FW is relatively weak (Richardson and McAneny, 1990; Cooper and Marshall, 1991; Lescourret et al., 1999).

6.4.2.4 Fruit dry matter concentration. In this experiment, vines receiving no added N tended to have higher DMC than vines receiving 105 and 210 kg N ha^{-1}. These results support the earlier findings of Mills et al. (2008) on the same vines. Saenz et al. (1997) found a negative relationship between N input and peach fruit DMC. Foliar sprays, which included N, applied to 'Hayward' vines reduced fruit DMC compared with unsprayed control vines (Mulligan, 2007). Conversely Morton and Woolley (2011) reported that spray application of foliar urea increased DMC in 'Hayward' vines. Low N reduced tomato fruit DMC (Huett and Dettmann, 1988).

Insufficient information is available to make any firm conclusions about the effects of N on kiwifruit DMC. Further replicated trials are required, as most of the previous work on N application and kiwifruit quality, did not report DMC. The relationship between N input and fruit DMC is likely to be influenced by where the vines are positioned on the N response curve. It is possible that high N inputs could increase shading and competition between fruit and growing shoots, thus reducing fruit DMC. It is also possible that the carbohydrate accumulation reported to occur in the leaves of N-limited plants could also occur in fruit.

6.4.2.5 Fruit maturity at harvest. High N inputs have been associated with delayed fruit maturity in a range of crops including apple (Neilsen et al., 1984; Fallahi et al., 2001), grape (Christensen et al., 1994), kiwifruit (Tagliavini et al., 1995) and peach (Saenz et al., 1997). However this relationship is not consistent and was not found for apple (Amiri et al., 2008), grape (Conradie and Saayman, 1989), kiwifruit (Johnson et al., 1997) or peach (Chatzitheodorou et al., 2004).

In the current experiment, there was a consistent trend for increased flesh hue angle with increased N input. At harvest the difference in hue angle between the high-N vines and the no-N vines was approximately 2 degrees each year. This difference would result in a delay of harvest in the high-N vines by around 8 days (calculated from Minchin et al., 2003; Appendix 4.6.1). Similar findings were obtained in Chapters 4 and 5 of this thesis where fruit with higher DMC had advanced maturity. Several factors could be involved in this DMC/flesh colour relationship with fruit maturation:

Vigour and shading. Increased vegetative vigour, such as that associated with the mid and high N inputs, would increase shading and could be responsible for delaying fruit maturity. However the relationship between maturity and sun-exposure is not consistent. 'Hayward' kiwifruit growing in vines with high LAI had lower DMC and delayed maturity in only one year of a two year project (Snelgar et al., 1998). Tombesi et al. (1993) found that fruit growing within the canopy had the same DMC, SSC and firmness at harvest as fruit growing in external positions within the canopy. In both experiments flesh chlorophyll was lower in the shaded fruit than the sun-exposed fruit, although this may not be indicative of more rapid chlorophyll breakdown, it may be that the shaded fruit had lower flesh chlorophyll throughout the season, rather than advanced chlorophyll breakdown at the end of the season.

Increased fruit dry matter concentration. Results from Chapters 4 and 5 indicate that treatments that increased fruit DMC also advanced maturity, primarily flesh degreening, although firmness and SSC were also affected. Similar results were obtained for peach where trees receiving no added N had higher DMC and reached commercial maturity approximately 10 days sooner (based on flesh firmness and background colour) than fruit from trees that received 200 kg N ha^{-1} (Saenz et al., 1997). Carbohydrates are needed to produce energy for fruit ripening (Candolfi-Vasconcelos and Koblet, 1990; Bennett et al., 2005), therefore the link between high fruit DMC and advanced maturity in not unexpected. In the current experiment, though, the variation in flesh hue angle among N input treatments appeared greater than the variation in DMC.

Nitrogen and chlorophyll degradation. The change in flesh colour from green to yellow in 'Hort16A' kiwifruit involves breakdown of chlorophylls that unmask the yellow carotenoids already present in the fruit (Montefiori et al., 2009). Nitrogen input can directly affect green coloration of fruit. For example in green apple cultivars such as 'Granny Smith' and 'Mutsu', green pigmentation is desirable to consumers both at harvest and after storage. By manipulating fruit N levels and measuring the colour response, Meheriuk et al. (1996) found a direct link between increased fruit N status and green colour. The effects of ground-applied N were compared with those of urea sprays on four cultivars of green apple. Urea sprays at 0.5 % and 1 % increased fruit N concentrations by 23 % and 47 %, respectively while ground-applied N did not affect fruit N concentrations. Foliar applications were more likely than ground application to affect skin hue angle in the apple fruit skins at harvest and after storage.

The experimental approach described above of comparing the effect of ground-applied N with foliar urea sprays on fruit chlorophyll status, could be used to test whether fruit N status affects colour change in 'Hort16A'. An advantage of applying sprays directly onto the canopy is that fruit N concentrations can be increased relatively quickly and presumably without the increased vegetative vigour that can occur with ground-applied N. This approach would enable the two effects to be examined independently. It might also be possible to alter 'Hort16A' fruit flesh colour using foliar N application without necessarily affecting firmness and brix. This approach could help determine if there is a more direct causal link between colour change and fruit N that is independent of changes in SSC and firmness. A second approach could be to determine if the relationship between degreening and softening is affected by DMC to the same extent, regardless of how DMC is changed.

6.4.2.6 Fruit storage performance. In the experiment reported in this chapter there was no evidence of N input affecting firmness after storage. There was a slight tendency for fruit from the vines receiving the highest N input to have more rapid initial softening, but this was based on only one set of measurements and the difference was relatively small.

As already discussed, both fruit composition and maturity at harvest affect fruit performance during storage. Generally earlier harvest, lower maturity at harvest, low Ca and/or high N are associated with poor storage performance (Crisosto et al., 1984; Mitchell et al., 1991; Prasad and Spiers, 1991; Tagliavini et al., 1995; Costa et al., 1997a,b; Johnson et al., 1997; Mowat, 2003; Clark et al., 2004; Maguire et al., 2005; Feng et al., 2006; Boyd and Barnett, 2011).

In the current experiment, fruit from the vines receiving no added N tended to have higher Ca/N ratios than fruit from the vines receiving 105 and 210 kg N ha^{-1} (0.154 ± 0.020, 0.113 ± 0.023 and 0.115 ± 0.012, respectively). The differences were attributed mainly to the fruit from the no-N vines containing more Ca than fruit from the vines receiving added N. The differences in Ca/N ratio were not reflected in differences in softening behaviour or firmness after a set time in cool store.

In year 2, when LTB was more prevalent than in year 1, fruit from the high N treatments were most severely affected than fruit from low N treatments. These fruit were also the least mature at harvest and it is possible that fruit from these vines harvested later would have had a lower incidence of LTB, as was the case for the less mature fruit in Chapter 4. Unfortunately in a commercial orchard all fruit were harvested as soon as they reached the commercial threshold and multiple harvests were not possible.

6.5 CONCLUSIONS

Retaining extra leaf area in the developing canopy may improve 'Hort16A' fruit quality under certain circumstances. In the current experiment two pruning techniques were used to minimise competition between rapid fruit growth and shoot growth in spring. Minimal pruning increased fruit DMC, but mainly in the vines where canopy growth had been reduced by reduced N fertiliser inputs. Minimal pruning retained extra leaf area in the fruiting zone by using tip-squeezing to halt growth of vigorous shoots relative to conventional pruning where vigorous shoots were cut back to the fruit so that no regrowth was possible. The experiment was carried out on a commercial orchard where Benefit® was applied to increase fruit FW, and the increased fruit quality manifested itself as increased DMC. The increased DMC was accompanied by advanced fruit maturation and no reduction in return bloom in the following season.

Nitrogen input appeared to affect several aspects of fruit quality and return bloom. Low N input was associated with increased fruit DMC and advanced maturation. High N input was associated with lower DMC, delayed flesh degreening and reduced return bloom. These results do not support the theory that reduced productivity with low N input is associated with reduced assimilate production.

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6.7 APPENDICES

Appendix 6.7.1 Effect of pruning on leaf mineral nutrient concentrations

Effect of pruning treatments on leaf N, P, K, Ca and Mg concentrations sampled across the 2007/2008 season from 'Hort16A' kiwifruit vines receiving different N inputs

Inorganic nutrient	Month	*P*-value		
		Nitrogen input (kg ha^{-1})		
		0	105	210
N	Nov	0.508	1.000	0.827
	Dec	0.109	0.218	0.237
	Feb	**0.033**	**0.037**	0.803
	Apr	**0.010**	0.522	0.796
	May	**0.002**	0.589	0.563
P	Nov	0.309	0.933	0.209
	Dec	0.620	0.906	0.492
	Feb	0.377	0.675	0.390
	Apr	0.586	**0.046**	**0.010**
	May	**0.054**	0.785	0.252
K	Nov	0.679	0.933	0.209
	Dec	0.614	0.763	0.766
	Feb	0.861	**0.067**	0.851
	Apr	0.211	**0.037**	0.465
	May	0.136	**0.016**	**0.061**
Ca	Nov	0.340	0.795	0.316
	Dec	0.306	0.452	0.698
	Feb	0.417	0.783	0.932
	Apr	0.679	0.216	0.191
	May	0.683	**0.031**	**0.044**
Mg	Nov	0.127	0.579	0.164
	Dec	0.449	0.283	0.589
	Feb	0.631	0.401	0.435
	Apr	0.628	**0.070**	0.175
	May	0.769	**0.055**	**0.072**

P-values < 0.100 are highlighted in bold for clarity

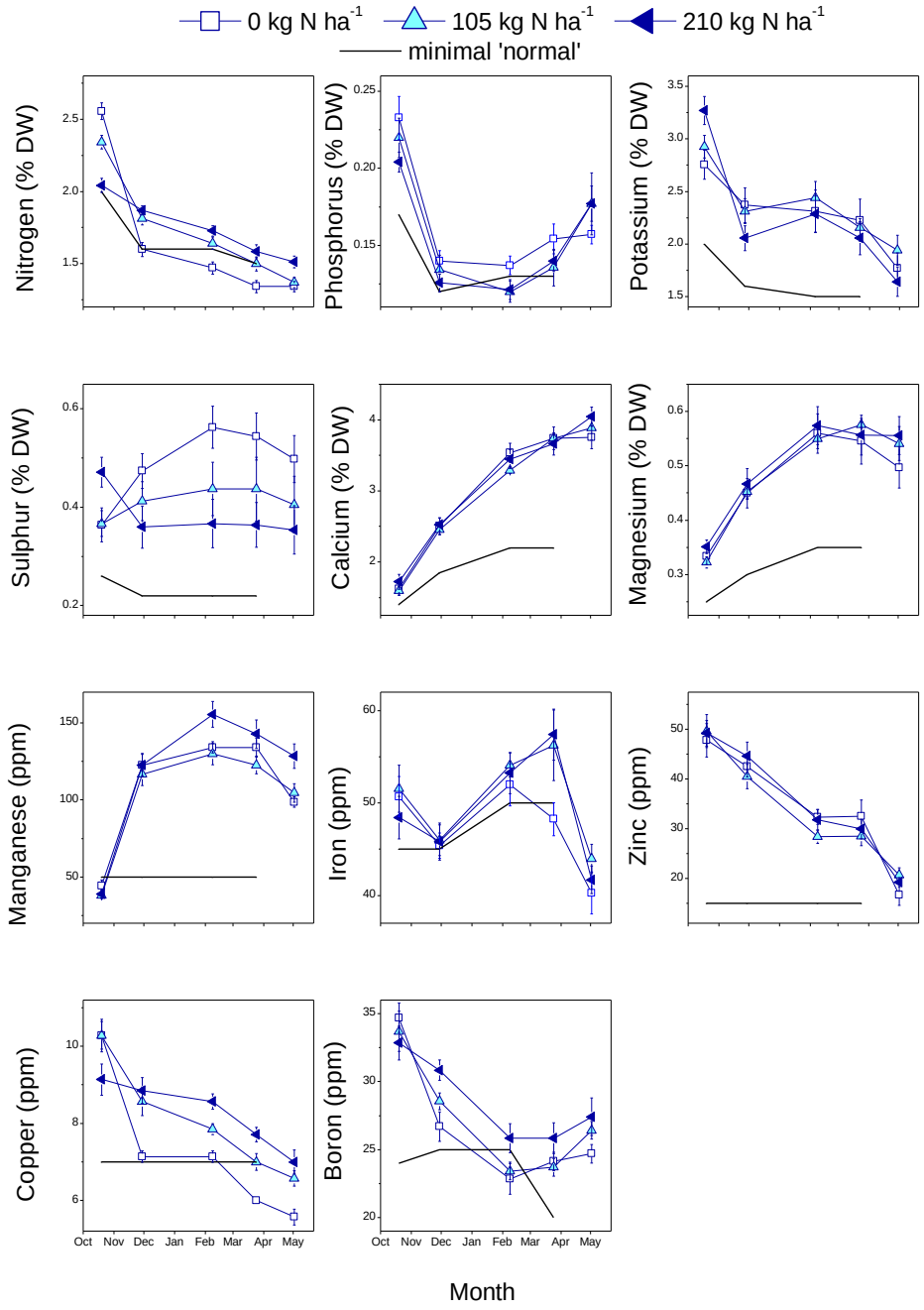
Effect of pruning treatments on leaf S, Mn, Zn, Fe, Cu and B concentrations sampled across the 2007/2008 season from 'Hort16A' kiwifruit vines receiving different N inputs

Inorganic nutrient	Month	*P*-value		
		Nitrogen input (kg ha^{-1})		
		0	105	210
S	Nov	0.908	0.140	0.876
	Dec	0.673	0.253	0.825
	Feb	0.920	0.249	0.829
	Apr	0.709	0.321	0.577
	May	0.693	0.185	0.721
Mn	Nov	0.796	0.963	0.665
	Dec	0.488	0.173	0.933
	Feb	0.715	0.138	**0.096**
	Apr	0.476	0.285	0.284
	May	0.822	**0.006**	**0.054**
Zn	Nov	0.499	0.358	0.140
	Dec	0.809	0.778	0.225
	Feb	0.503	0.200	0.465
	Apr	0.851	0.827	0.742
	May	0.592	0.123	0.368
Fe	Nov	1.000	0.813	0.961
	Dec	0.436	0.800	0.667
	Feb	0.323	0.464	0.437
	Apr	0.820	0.658	1.000
	May	0.665	0.328	0.193
Cu	Nov	0.403	0.619	0.449
	Dec	0.271	1.000	0.761
	Feb	**0.040**	0.761	0.430
	Apr	0.125	0.730	1.000
	May	0.109	0.300	0.682
B	Nov	0.715	0.842	0.675
	Dec	0.943	0.872	0.611
	Feb	0.758	0.205	0.831
	Apr	1.000	0.738	1.000
	May	0.773	0.667	0.547

P-values < 0.100 are highlighted in bold for clarity

Appendix 6.7.2 Leaf mineral nutrient concentrations – effects of nitrogen

Leaf mineral nutrient concentrations measured across the 2007-2008 growing season in 'Hort16A' kiwifruit vines that received three N inputs $n = 7 \pm SE$; black line is the minimum 'normal' concentration for leaves for the particular date, from RJ Hill Laboratories



Appendix 6.7.3 Calculating leaf area from pruning weight

The following equation was used to estimate the total leaf area per vine (LA_{tot}) from pruning weight:

$$LA_{tot} = \frac{DW_{tot}}{DW_{leaf}} \; x \; LA_{leaf} \quad (1)$$

Where:

DW_{tot} is the total DW of leaf removed, estimated as 50 % by weight of the pruned material (from Clark and Smith, 1992; Boyd et al., 2010),

DW_{leaf} = mean leaf DW, and

LA_{leaf} = mean leaf area

1.45 g and 161 cm^2 respectively, measured in November 2007.

N input (kg N ha^{-1})	Extra DW of pruned material (CP - MP)	Leaf area (m^2 $vine^{-1}$)
0	0.91	5.05
105	1.06	5.88
210	1.31	7.27

7. GENERAL DISCUSSION

7.1 THESIS OBJECTIVES

The objectives of the work described in this thesis were to determine how different orchard management techniques affected fruit quality, vine productivity and long-term vine health in mature field-grown 'Hort16A' kiwifruit vines. The orchard management techniques were designed to alter source-sink relationships within the vines so that dry matter allocation to fruit was affected. Specific research questions were:

1. Can long-term vine productivity be sustained if high yields of high DMC fruit are produced year after year?
2. Does increasing fruit DMC affect fruit maturity attributes, and therefore harvest criteria?
3. If fruit maturity is affected by altered DMC how is fruit storage performance affected?
4. Will the vines compensate for the increased productivity by up-regulating photosynthesis, and increasing shoot growth or will carbohydrate reserves be depleted
5. If reserves are depleted will nutrient uptake be affected and will nutrient deficiencies become apparent?

In Chapter 4 whole vine reserves were depleted by removing as many resources as possible from the vines as fruit, pruning to encourage shoot re-growth which would compete with other sinks, and maintaining low leaf area (carbohydrate starvation, the famine treatment). Results were compared with those from the treatment designed to provide abundant resources, the feast treatment. In Chapter 5 extended trunk girdling (ETG) was used to isolate the canopy from the roots, crown and trunk for a large proportion of the season (autumn to spring) and results were compared with those of an ungirdled control vine carrying the same crop load. Vine responses to the feast/famine and ETG/control treatments can now be compared and contrasted and implications for kiwifruit growers can be considered in light of the less extreme pruning/nitrogen treatments carried out on the commercial orchard.

7.2 LONG-TERM VINE PRODUCTIVITY

The specific question was: *can long-term vine productivity be sustained if high yields of high DMC fruit are produced year after year?*

Only the ETG vines were able to produce high yields of high DM fruit consistently each year. In the other treatments a productive year was often followed by a year when return bloom was reduced, so that fruit numbers were reduced even if fruit DMC was not. Extended trunk girdling consistently increased individual fruit DMC by around twice that achieved using standard trunk girdling (Patterson and Currie, 2011). In the ETG treatment, the girdle was open from mid-February and remained open until after fruit were harvested in late April/early May, whereas a standard commercial girdle heals 3 to 4 weeks after the February application. It appears that keeping the girdle open for longer increases the amount of DM that accumulated in the fruit. By April, leaves from the ETG vines contained nearly double the NSC concentration of the control vines. The accumulation of NSC in the leaves suggests that fruit DMC may have reached saturation. Similar results were found in girdled apple branches where the capacity of the sinks (fruit) to accumulate assimilates was saturated, and starch accumulated in the leaves (Schecter et al., 1994). Sink removal can result in carbohydrate accumulation in leaves and may lead to feedback limitation of photosynthesis, e.g. in apple (Palmer et al., 1997), disruption of the thylakoid membranes from starch accumulation in the leaves (Schaffer et al., 1986), and advanced N remobilisation, e.g. in barley leaf (Parrott et al., 2005). The ETG leaves showed no indication of reduced photosynthesis or early leaf senescence, although only 1 or 2 measurements were made after girdling in February and before leaf fall occurred in May/June.

Fruit from the feast treatment consistently had higher DMC than fruit from the famine treatment, although crop loads and therefore total yields were lower in the feast than the famine vines. In the feast vines, there was no indication of NSC accumulation in the leaves or reduced photosynthesis at the end of the season. It is therefore likely that DM accumulation in fruit did not become saturated (as happened in the ETG vines), instead NSC were allocated to other sinks particularly roots.

Extended trunk girdling had a consistent positive effect on return bloom. The increase in fruitlet numbers was a combination of a greater total bud break, more floral and less vegetative bud break and more flowers per floral shoot. There are several factors that could be responsible for the increased return bloom in the ETG vines:

1) The girdle in the ETG prevented NSC from travelling to the roots in autumn, resulting in high concentrations of NSC in the canopy.

2) The girdle was still open during bud break and at the start of flowering, meaning that polar auxin transport from growing shoots was disrupted in the weeks preceding mid-bloom.

3) Having the girdle open during early spring could generate an unknown stress signal which advances bud break and increases flowering in a manner similar to that induced by spraying with hydrogen cyanamide (Hi-Cane™).

It might be possible to separate out the effect of 1) above, from the other two responses by carrying out girdling across a range of times, and comparing floral responses. Girdling could be carried out before and around: leaf fall (to affect canopy NSC), bud break (to possibly affect timing of bud break) and flowering (neither NSC nor bud break timing would have been affected) to determine which times are more critical to increased flowering response. In addition, xylem sap could be sampled from above and below the girdle to determine if cytokinin and gibberellin concentrations were affected.

Whole vine carbohydrate depletion using the famine treatment affected return bloom, but differences were less consistent than those caused by ETG. In years 1 and 2 the fruitlet numbers in the famine vines were lower than those of the feast treatment but no treatment differences were detected in year 3. Across the three years, productivity remained relatively consistent in the control and feast vines, whereas the famine vines had more variation in productivity. The reduction in flowering of the famine vines in year 2 may have enabled the famine vines to recover from, or compensate for, any depletion of reserves caused by the famine treatment.

If reduced N inputs ultimately reduce vine carbohydrate status by reducing total leaf area and leaf photosynthetic capacity, as suggested by Buwalda and Meekings (1993), then the vines receiving no added N might display similar responses to the famine vines, specifically lower flower numbers. This did not appear to be the case in the work described in Chapter 6: the vines receiving the highest N inputs tended to have lower bud break and flower numbers than vines receiving no added N.

Girdling appears to be the best way of maintaining or increasing flower numbers because the roots are isolated. The means by which the roots affect flowering are not clear, whether simply as a competing sink for NSC (thereby reducing NSC concentrations in the canes), or if root-derived substances exert an effect on flowering. More work is needed to determine if the flowering effect is related to due to NSC accumulation above the girdle or a more short-term response such as change in the auxin/cytokinin ratio. The mechanism by which Hi-Cane™ application enhances flowering is not known, but it has been suggested that Hi-Cane™ may produce a sub-lethal stress that triggers a floral response (Walton et al., 1991). Comparing the effects of extended trunk girdling, Hi-Cane™ application and controls receiving neither treatment on the auxin and cytokinin profiles in different tissues such as leaves, buds, xylem sap and roots might help to determine which factors affect flowering in kiwifruit vines.

Growing shoots could also compete for NSC with flower production, or could export a signal which inhibited flowering in nearby shoots (Grant and Ryugo, 1982). One theory is that a high local cytokinin/auxin ratio is needed for a bud to be reproductive rather than vegetative, and growing shoots are believed to inhibit flowering by producing high levels of auxin which is transported to the roots, reducing cytokinin production (Bangerth et al., 2000).

In Chapter 6, retaining extra leaf area in the canopy early in the season (MP; minimal pruning) resulted in increased fruit DMC with no effect on FW, but only in the vines receiving no added N. The most likely explanation for this finding was that the vines receiving no added N were less vigorous and had less leaf area, therefore retaining extra leaves produced a positive effect, whereas the vines receiving added N tended to

be more vigorous, and the added leaf area had little or effect. There was no effect on return bloom in the following season. Return bloom was reduced in the MP vines, but only in those receiving the standard N application rate of 105 kg ha^{-1} where there was no effect on fruit DMC. This flowering effect may have been caused by increased shading in the MP vines; the vines in this row were generally more vigorous than the vines receiving 210 or 0 kg N ha^{-1}. Replacement canes growing in a shaded environment produced fewer flowers than more sun-exposed canes (Grant and Ryugo 1982; Miller et al., 2001), but not always (Buxton, 2005).

Further work. Managing N input appears to offer promise as a means of influencing fruit DMC and return bloom. Peach fruit from trees receiving no N had higher DMC than fruit from trees receiving added N (Saenz et al., 1997). It is possible that additional increments of N increase vigorous growth at the expense of DM allocation to developing fruit (Piller and Meekings, 1997; Minchin et al., 2010). Conversely insufficient N could reduce shoot growth and individual leaf area and leaf photosynthesis (Buwalda and Meekings, 1993; Costa et al., 1997) possibly limiting resources available to fruit and return bloom. It would be worthwhile to carry out replicated trails over a range of orchards to determine if adjustments to N input can have consistent effects on fruit DMC, FW and return bloom.

The work presented above raises an interesting question about the limit for DM accumulation into fruit. The highest individual fruit DMC obtained in this work was around 20.0 % as a result of ETG. The late-season increase in leaf NSC in the ETG vines suggests that supply was not the limiting factor in fruit DMC accumulation. It is likely that sugar transport to the fruit becomes the limiting factor in DMC accumulation when there is no source limitation. Sugars are transported to the fruit from the leaves via the phloem. Sugars can be unloaded from the phloem between cells via the plasmodesmata (symplastic) or via the extracellular space (apoplastic). Symplastic unloading is a passive process, whereas apoplastic unloading requires energy for assimilates to move into the extracellular fluid either via transport proteins or by being cleaved into smaller molecules. Phloem unloading is believed to change from symplastic to apoplastic in developing fruit, e.g. apple (Zhang et al., 2004), citrus (Koch and Avigne, 1990), grape (Zhang et al., 2006) and tomato (Patrick and

Offler, 1996). The change from symplastic to apoplastic unloading in grape berries coincides with the beginning of stage III growth, when the berries ripen and soluble sugars concentration increases (Zhang et al., 2006). It would be interesting to determine what factors cause phloem unloading to cease, and DM accumulation to become saturated, in fruit from the ETG treatment. Extended girdling could be a valuable tool for determining the upper DMC limit for new selections that are being bred.

Across all the treatments carried out in chapters 4 and 5, there was a general trend for vines that flowered earlier to be more productive (Figure 7.1). In 'Hayward' vines, earlier-opening buds are more likely to be floral than those that open later (McPherson et al., 1992), so this flowering pattern may just be a reflection of the timing of bud break. Timing of bud break was not routinely monitored in the current work, except in the final season when the ETG vines broke bud around 8 days earlier than the control vines. The control vines broke bud at about the same time as the feast and famine vines. Much of the previous work on factors affecting bud break and flowering in 'Hayward' kiwifruit focussed on naturally-occurring variability among vines, regions and seasons. The type of approach used in this thesis, where variability is generated in a replicated trial could help understand some of the factors affecting vine productivity and how these factors could be manipulated to increase productivity.

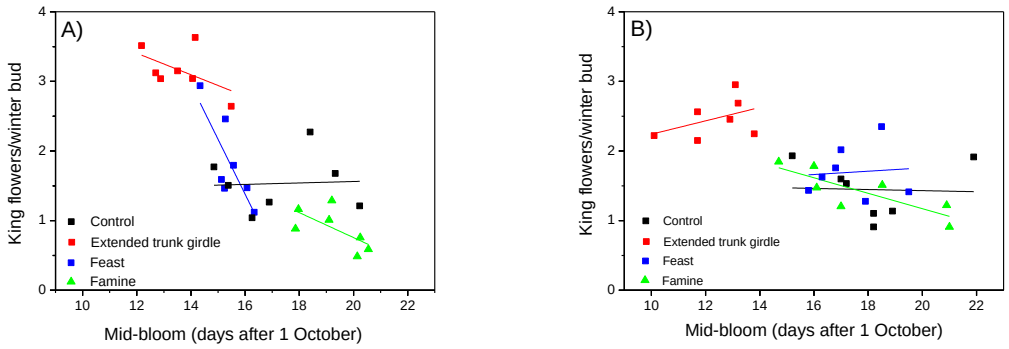


Figure 7.1 Relationship between date of mid-bloom and king flowers per winter bud in 'Hort16A' kiwifruit vines receiving different source-sink manipulations in A) year 2, and B) year 3.

7.3 FRUIT MATURITY ATTRIBUTES

The specific question was: *does increasing fruit DMC affect fruit maturity attributes, and therefore harvest criteria?*

Generally speaking, increasing fruit DMC advanced maturity. If fruit from different treatments were harvested on the same day, fruit from the treatment with the highest DMC were usually the most mature, having lower flesh hue°, higher SSC and softer flesh. Fruit from the feast vines were consistently more mature than fruit from the famine vines (Chapter 4); ETG fruit were consistently more mature than the control fruit (Chapter 5). Fruit from the MP vines receiving no added N had higher DMC and advanced maturity relative to fruit from the CP vines (Chapter 6). Fruit from the vines receiving no added N tended to have advanced maturity and DMC that was as high or higher than fruit from the vines receiving added N.

If flesh colour change occurs sooner in high DMC fruit then they would reach commercial maturity sooner than fruit with lower DMC. This could be because the time from mid-bloom to degreening is condensed in high-DM fruit or because both mid-bloom and colour change were advanced by the treatment. Data summarised from Chapters 4 to 6 suggest that there is no simple relationship between fruit DMC and the time between mid-bloom and degreening (Figure 7.2). The physiological processes underlying flesh colour change could be different depending on how fruit DMC is changed. For example different N inputs appeared to affect colour change more than they affected DMC (although results would need to be confirmed in a replicated trial). It is possible that higher N inputs increased the amount of chlorophyll in the fruit, thus delaying degreening. The amount of N per fruit was relatively consistent across all three N inputs (Chapter 6), so it seems unlikely that there was a relationship between degreening and fruit N status. Further work to examine relationships among DMC, rate of degreening and the N-to-chlorophyll ratio in fruit flesh during maturation could help to determine this. A second possibility is that when N status is high, C is used to form amino acids rather than sugars, and sugars may be involved in chlorophyll breakdown. In grapes, for example increasing N fertiliser increased the concentration of free amino acids in juice at the same soluble solids concentration (Spayd et al., 1994).

The time between mid-bloom and degreening was about the same in fruit from the feast and ETG vines (Chapters 4 and 5, respectively; Figure 7.2), despite the ETG fruit having the higher DMC. Overall fruit development time appeared to be condensed in the feast fruit whereas both mid-bloom and degreening were advanced in the ETG vines, mid-bloom being more advanced that degreening.

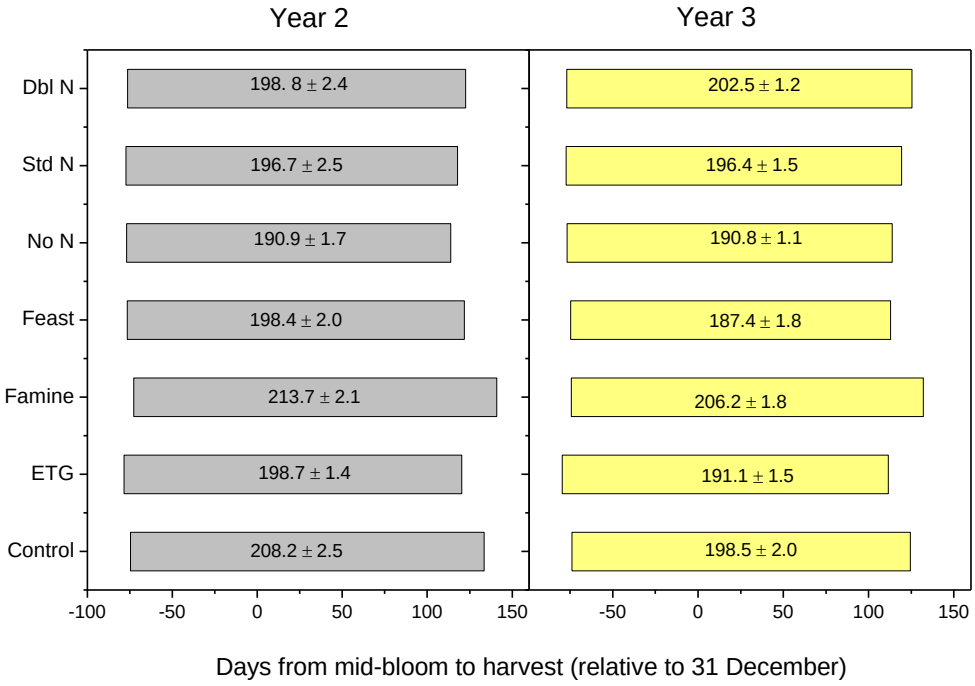


Figure 7.2 Summary of relationship between date of mid-bloom and commercial maturity (mean hue angle = 103^{o}) in fruit from vines receiving different treatments described in this thesis; day 0 = 31 December; *n* = 7 ± *SE*.

If fruit from different treatments were harvested as they reached commercial maturity, rather than on the same day, then other quality attributes could be affected. For example, fruit from the famine vines took longer for flesh hue angle to reach 103^{o} than the feast fruit (Chapter 4). During this extra time on the vine the famine fruit started to soften on the vine. It is therefore possible that 'less mature', low DMC fruit could be softer at commercial harvest than high DMC fruit that reached commercial maturity sooner and are typically considered 'more mature'. The differences in the timing of degreening and softening were most apparent in the feast/famine vines (Chapter 4) where fruit hue and firmness were changing on the vine at the same time. On the commercial orchard fruit softening had barely begun as hue angle was changing rapidly (Chapter 6) and all fruit were harvested as soon as they reached

commercial maturity, so the relationship between colour change and softening could not be determined. In year 1, for example fruit firmness was still around 65 to 70 N when mean hue angle ranged from 100° to 102°.

The uncoupling of colour change and softening on the vine was also observed by Loeffen and Jordan, (unpublished results) who noted large between-orchard variability in the window when 'Hort16A' fruit were suitable for main commercial harvest. They called this window, the N to S window, based on the date the fruit degreened (time N) to the date they became excessively soft (time S). The N to S window ranged from +26 days to -13 days, and a population of fruit with higher DMC was likely to have a larger N to S window than a population of fruit with lower DMC. The results from this thesis support Loeffen and Jordan's findings. Fruit from the low DMC famine vines had already started to soften on the vine when they degreened so would have a smaller N to S window than fruit from the higher DMC control or feast vines (Chapter 4). Unfortunately it was not possible to make accurate estimates of the N to S window from the experiments in this thesis as this would have required regular fruit sampling starting before the most mature fruit had degreened and continuing until the fruit had softened on the vine. In the commercial orchard (Chapter 6) all the fruit were harvested when fruit in the remainder of the block reached commercial maturity, so late-season measurements could not be made. In the research orchard (Chapters 4 and 5) limited fruit numbers and missing the start of degreening meant that this was not possible.

In peach fruit, the relationship between flesh firmness and background colour was uncoupled in shading/girdling experiments (Marini et al., 1991). Shading limbs during stage III growth reduced FW and SSC accumulation, delayed softening and delayed ground colour development (the change in background skin colour from green to yellow) relative to fruit from unshaded limbs. Limb-girdling did not affect fruit firmness and FW, but SSC accumulation and background colour change were delayed relative to fruit from intact limbs. In the peach experiment described above (Marini et al., 1991) girdling was used to *stop* import of assimilates from neighbouring limbs and illustrated that colour change and SSC accumulation were more dependent on assimilates than FW accumulation and firmness increase were.

The ETG treatment described in Chapter 5 of this thesis made assimilates *more* available to the fruit by stopping transport to the roots, but the results also suggested that increased assimilate availability affected flesh degreening more than softening or FW accumulation.

Further work. Source-sink manipulation can affect fruit DMC and maturity attributes for 'Hort16A' kiwifruit, most notably how firm the fruit are or how advanced the softening process is when fruit are commercially harvested. To optimise harvest and help with postharvest handling or storage it would be valuable to understand more about the relationship between fruit DMC, colour change and softening. The work described in this thesis raised several further questions that could help with our understanding:

Is localised assimilate availability rather than fruit DMC responsible for advanced degreening? By eliminating assimilate import from surrounding leaves, Marini et al. (1991) demonstrated that colour change was more dependent on localised assimilate supply than was softening. It is possible that if high DMC is caused by increased localised assimilate supply e.g. by trunk girdling, then degreening would also be advanced. Conversely if increased N input delays degreening without reducing DMC is may be that the increased vigour has affected localised assimilate supply. Replicated trials where treatments included girdling, leaf to fruit ratio and N input could be used to determine if the DMC/degreening relationship is affected by the type of source-sink manipulation. It is possible that the DMC/degreening relationship is changed when N input is varied, so localised shading/girdling experiments on vines receiving different N inputs could be carried out to determine if localised assimilate affects degreening to the same extent in high N and low N vines.

Are there better ways to measure fruit maturity attributes? The fruit subsampling issues raised in chapter 3 should also be considered when measuring maturity. The industry standard methods might be valuable for comparing batches of fruit, but might not be the best for determining mechanistic relationships among different attributes such as colour, firmness and DMC. There are longitudinal differences in not only DMC but also firmness and SSC (Thorp et al., 2007). It is also possible that a fruit

from the ETG treatment, average FW 90 g and 20.0 % DMC could have a different physical characteristics from a fruit from the high N treatment (average FW 123 g and 17.6 % DMC). Treatment-induced changes in cell size, cell wall structure and turgour might affect how certain measurements are made and interpreted. For example, McGlone et al. (1997) found that fruit softening behaviour in two populations of 'Hayward' fruit was the same when measured using a penetrometer, but different when measured using impact force, a measure of whole fruit stiffness. The physiological basis for differences in fruit stiffness are not clearly understood, but it is possible that measurement of flesh Lightness (a measure of how the applied light is reflected or scattered) could also be affected by differences in stiffness. Recent work on the expression of different genes, such as chlorophyll binding protein and β-amylase, during 'Hort16A' fruit development may help explain some of the treatment-induced differences in fruit maturation (Richardson et al., 2011).

7.4 FRUIT STORAGE PERFORMANCE

The specific question was: *if fruit maturity is affected by altered DMC how is fruit storage performance affected?*

Both LTB incidence and initial softening behaviour are stongly affected by harvest date. If fruit from different treatments within a replicated experiment are harvested on the same day then treatment-induced differences in maturity can reflect the incidence of LTB. For example when harvested on 6 May 2008, fruit from the famine treatment had a higher incidence of LTB than fruit from the control and feast treatments (around 45 %, 10 % and 4 % respectively); delaying harvest by 10 and 20 days reduced LTB incidence to 10 % and 1 % in the famine fruit. It is not known if fruit from all treatments are equally susceptible to LTB if harvested too early, and if hue angle is the best attribute to define what "too early" means. The results from this thesis suggest that there are populations of fruit that are already starting to soften at 103° hue, and other populations of fruit where softening is less advanced at 103°. Clark et al. (2004) found that there was no clear threshold maturity for when a fruit will or will not develop LTB. Pairwise comparison of sound and disordered 'Hort16A' fruit confirmed that disordered fruit had lower DMC and SSC and higher flesh hue angle than sound fruit, when harvested on the same date. However, sound fruit from the

earliest harvest had the same hue angle as disordered fruit from a later harvest (Clark et al., 2004).

This finding highlights some of the main issues surrounding preharvest/postharvest relationships in kiwifruit. Which fruit attributes need to be measured to determine the optimum harvest time that will maximise storage potential? Will harvest criteria be the same for fruit from high DM and low DM environments? And, will the criteria vary depending on what factors caused the fruit to have high or low DMC?

The results from this thesis suggest that the timeframe to harvest some populations of low DMC fruit may be very short. If the famine fruit are harvested too early they develop LTB, but are also likely to be softening as they reach 103°. Interestingly, fruit from the famine vines had high Ca/N ratios, an attribute more typically associated with good storage performance in 'Hayward' kiwifruit. The high Ca/N ratios in the famine fruit might be because they were sun-exposed, having little leaf area to provide shade, thereby they transpired more and accumulated more Ca. In 'Hayward' fruit sun-exposure is associated with higher fruit Ca concentrations (Biasi and Altamura, 1996; Xiloyannis et al., 2001). Alternatively the smaller famine fruit had a higher proportion of skin to flesh - which contains high concentrations of Ca (Clark and Smith, 1988). Previous work, however, shows that the famine fruit had higher Ca/N ratios than the feast fruit when flesh plugs were used as samples (estimated from Boyd and Barnett, 2011). In Chapter 6, fruit from the no-N vines tended to have high Ca/N ratios than fruit from the vines receiving added N. This might be because fruit from the no-N vines were more sun-exposed. The no-N vines tended to have advanced maturity and might therefore be expected to have good storage performance: they had the least LTB incidence and softened to 20N marginally later than fruit from vines receiving higher N input (Table 7.1).

This finding suggests that there might be populations of fruit with low Ca/N ratios that are also poor-storing, but the relationship is not causal, rather the Ca/N ratio is a reflection of how DMC, and therefore fruit maturation, is altered (Table 7.1).

Table 7.1 Effect of source-sink manipulation on potential relationships among fruit dry matter concentration (DMC), ratio of calcium to nitrogen, softening and LTB incidence during storage.

Source-sink manipulation	DMC	Ca/N	Days to 20 N (harvested at 103°)	LTB incidence (same harvest date)
Famine/feast	increase	decrease	decrease	decrease
Control/ETG	increase	unchanged	decrease	decrease
High-N/no-N	increase	increase	decrease	decrease

Further work. To determine if there are different populations of fruit with different DMC, hue angle, softening and LTB relationships, a well replicated experiment could be established where trunk girdling, N input and leaf to fruit ratios were altered on whole vines with sufficient fruit numbers to sample and store fruit across a greater range of maturities than was possible in this thesis. Unfortunately it was not possible to determine if fruit from treatments that degreened earlier (such as the feast and ETG fruit) would also develop LTB if harvested earlier, but there are indications that maturity relationships vary among batches of fruit and that delaying harvest by several days can strongly affect LTB incidence.

7.5 VINE CANOPY RESPONSES

The specific question was: *will the vines compensate for the increased productivity by up-regulating photosynthesis and increasing shoot growth or will carbohydrate reserves be depleted?*

Leaf area index (LAI) was reduced in the ETG vines relative to the control vines by a combination of reduced individual area per leaf and fewer leaves (less vegetative bud break, and a greater proportion of short shoots than long or medium shoots). There was no evidence of increased photosynthesis in the leaves of the ETG vines to compensate for the reduced LAI.

The famine vines showed little indication of a vegetative response to whole vine carbohydrate depletion. Responses typically associated with increased C capture include increased individual LA, reduced SLW, increased petiole length and increased vegetative growth. Individual are per leaf , petiole length and the proportion of VBB

were unaffected by the famine treatment, except there was a slight increase in VBB in year 2 relative to the feast vines. In year 2 when flower numbers were reduced in the famine vines total BB, floral BB and the number of flowers per floral shoot were reduced. In addition there was no increase in the proportion of long and medium shoots and no increase in leaf photosynthesis rates. In year 3, when flower numbers increased in the famine vines, there was a higher proportion of short shoots in the canopy compared with the canopies of the control and feast vines. This increase in the numbers of short shoots in year 3 may be a reflection of the higher crop load retained on the famine vines competing with shoot elongation for resources.

In addition to the famine vines in year 3, the ETG vines also had a higher proportion of short shoots than the control in both years. There were, however, several key differences between the ETG and famine shoots. Area per leaf, petiole length and internode length were all reduced by the ETG treatment but not the famine treatment. This suggests that the vigour reduction in the ETG vines involves a different process to that of the famine vines. In the ETG vines the girdle was still open at bud break in August and did not heal until flowering in October. Polar auxin transport from shoot to the roots is believed to play a role in canopy growth. Application of the auxin transport inhibitor 1-N-naphthylphthalamic acid (NPA) reduced shoot extension in potted apple and kiwifruit plants (van Hooijdonk et al., 2010 Vattiprolu et al., 2011). During the initial two months of canopy development of the ETG vines, auxin transport from the growing shoots to the roots was interrupted because the girdle was still open. Stopping auxin transport to the roots is believed to reduce the production of root-derived cytokinins and gibberellins (Lockard and Schneider, 1981). Cutting and Lyne (1993) found that girdling reduced the concentration of cytokinins in the sap of girdled peach branches when compared with sap from intact branches in the same tree, and this was associated with reduced shoot growth. Conversely Honda et al. (2011) linked shortened internodes and reduced leaf area to overproduction of cytokinins.

Further work. Microscopic analysis could be used to determine if cell division or elongation was reduced in the leaves, petioles and internodes in the ETG vines. If the reduced leaf and internode expansion were a result of the girdle still being open in

spring, then it is unlikely that a similar response would occur in the growing fruit, as the girdle healed during flowering. Microscopy could also be used to determine if fruit from the ETG vines had smaller, denser cells than fruit from the control vines. Different cell structures might affect fruit performance during coolstore including firmness measurement and cell integrity.

Leaves from the famine vines sometimes had higher SLW than leaves from the feast and control vines. This finding was not consistent across the season, but occurred in both years. High SLW is usually associated with NSC accumulation and low sink numbers, and would not be expected to occur in leaves from a vine subjected to low leaf to fruit ratios. Further investigation would be worthwhile to determine if this result is related to leaf water relations, cell structure or leaf composition.

7.6 RESERVE DEPLETION

The specific question was: *if reserves are depleted will nutrient uptake be affected and will nutrient deficiencies become apparent?*

Total reserve biomass was reduced by the ETG and famine treatments. In the ETG vines, treatment differences were detected in the plant parts below ground (Figure 7.3A). In comparison, biomass reduction in the famine vines (relative to the feast vines) was detected in the tissues above and below ground (Figure 7.3B). This result highlights how interrupting the signalling between roots and shoots affects plant growth. In the feast/famine vines the balance between root and shoot growth was maintained (Lockard and Schneider, 1981), whereas the interruption of phloem-mediated transport of plant growth regulators and carbohydrates in the ETG vines has altered this balance.

It is not clear how much of the reduction in root biomass in the feast/famine and control/ETG vines could be attributed to slower growth rate or advanced root death. Vines would need to be excavated at regular intervals to determine growth rates. The annual increment in biomass increase of the trunk, crown and roots of mature 'Hort16A' kiwifruit vines was estimated at ~ 0.8 kg DW (Boyd et al., 2010). If this value was applied to the current experiments, then the combined trunk, crown and

roots of a control would be expected to gain ~ 5 kg DW in six years. The values summarised in Table 7.2 suggest that a) DW allocation in the famine vines could have ceased, whilst the control vines continued as normal, b) the rate of root growth increased in the feast vines relative to the control vines, and c) the ETG vines showed signs of root death in addition to reduced rate of root growth.

Table 7.2 Effects of source-sink manipulation on change in total dry weight of trunk, crown and roots of 'Hort16A' kiwifruit vines.

Source-sink manipulation		
Control - Famine	Feast - Famine	Control - ETG
Change in DW (trunk, crown and roots) after 6 years (kg vine^{-1})		
6.6 ± 3.3	18.1 ± 2.8	20.7 ± 1.7

n = 3 ± SE.

The ETG vines contained around half the NSC reserves of the control vines. Within-vine allocation suggested some accumulation above the girdle and reduction below the girdle (Figure 7.3C). There was no significant difference in the total NSC reserves of the feast and famine vines, and concentrations of NSC in any one vine component were not affected (Figure 7.3D). This finding again highlights how severing connection between the roots and canopy affects the ability of the vine to adjust growth to match resources. In the feast/famine vines growth patterns were affected, and NSC concentrations were maintained.

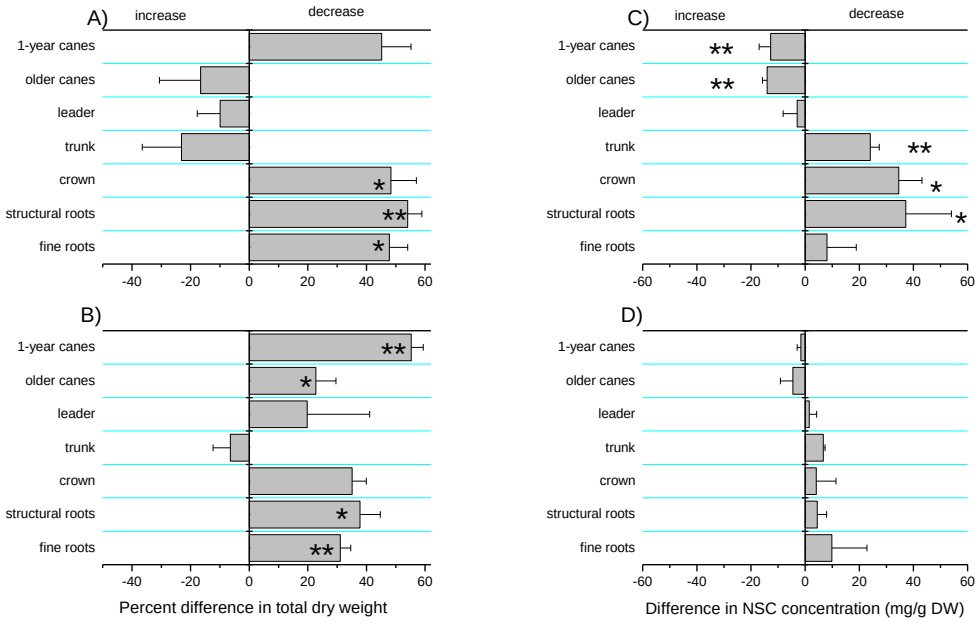


Figure 7.3 Change in biomass of different vine components as affected by A) extended trunk girdling (control - ETG), and B) whole vine carbohydrate depletion (feast - famine), and change in total non-structural carbohydrate concentration as affected by C) ETG (control - ETG) and D) whole vine carbohydrate depletion (feast - famine); values accompanied by ** and * are significantly different $P \leq 0.05$ and $P \leq 0.10$. Difference = (control – ETG)/control as a percentage.

Leaves. Leaves from the ETG vines had consistently lower concentrations of P, K, S, Ca, Mg, Mn and Zn across the season, and concentrations of N, Cu and B were lower at some, but not all, parts of the season. For many mineral nutrients the concentrations for the ETG vines were lower than the industry 'normal' values especially early in the season. When values were calculated on a per cm^2 basis on the leaf blade there were no differences for N, Zn, Fe, Cu and B, whilst the amount of the remaining minerals was lower in leaves from the ETG vines than those form the control vines. There are several possible explanations for this finding, including:

- Uptake of N, Zn, Fe, Cu and B from the soil was not affected by ETG, whilst uptake of P, K, S, Ca, Mg and Mn was.
- Reserves were sufficient to provide N, Zn, Fe, Cu and B, but not P, K, S, Ca, Mg and Mn to the leaves.
- Leaves competed successfully with fruit for N, Zn, Fe, Cu and B.

These possibilities will be discussed further in the following sections.

The famine treatment did not affect spring accumulation of mineral nutrients in the leaves, despite a reduction in fine root biomass. This finding will be discussed after the effect of the famine treatment on fruit and perennial reserves has been considered.

Fruit. Overall, mineral nutrient allocation to mature fruit was unaffected by the ETG treatment. This is based on measuring nutrients on a per fruit basis and comparing with control vines where crop load and FW were the same in both treatments. If any treatment differences were detected they were not consistent across seasons and the fruit from the ETG vines contained more of the particular mineral than the control vine, this occurred with Ca, Mg, Fe and Zn.

A direct comparison could not be made between feast and famine fruit as the feast fruit had lower crop loads and larger FW. It might be expected that the larger individual feast fruit would contain more of most minerals than the famine fruit. This was the case for N, P, K, S, Mg and Cu, but not for Ca, Mn, Zn, Fe and B where results were less consistent and typically no treatment differences were detected. When calculated on a concentration DW basis fruit from the famine vines had higher concentrations of Ca, Mn and Zn. These three minerals are primarily mobile in the xylem (Clark and Smith, 1988), and it is possible that the famine fruit, growing with less leaf area transpired more than the feast fruit, therefore accumulating more of these three nutrients.

Perennial reserves of mineral nutrients. Total perennial reserve status of most mineral nutrients was reduced in the ETG vines when compared with the control vines, and the famine vines when compared with the feast vines (Figure 7.4). Differences were most apparent for the macronutrients (N, P, K, S, Ca and Mg). Typically the micronutrient measurements had large standard errors, making it difficult to draw any firm conclusions. Generally micronutrient reserve status was also lower in the ETG and Famine vines than their respective counterparts. The exception was Zn in the ETG vines, where no treatment difference was detected and standard errors were relatively low.

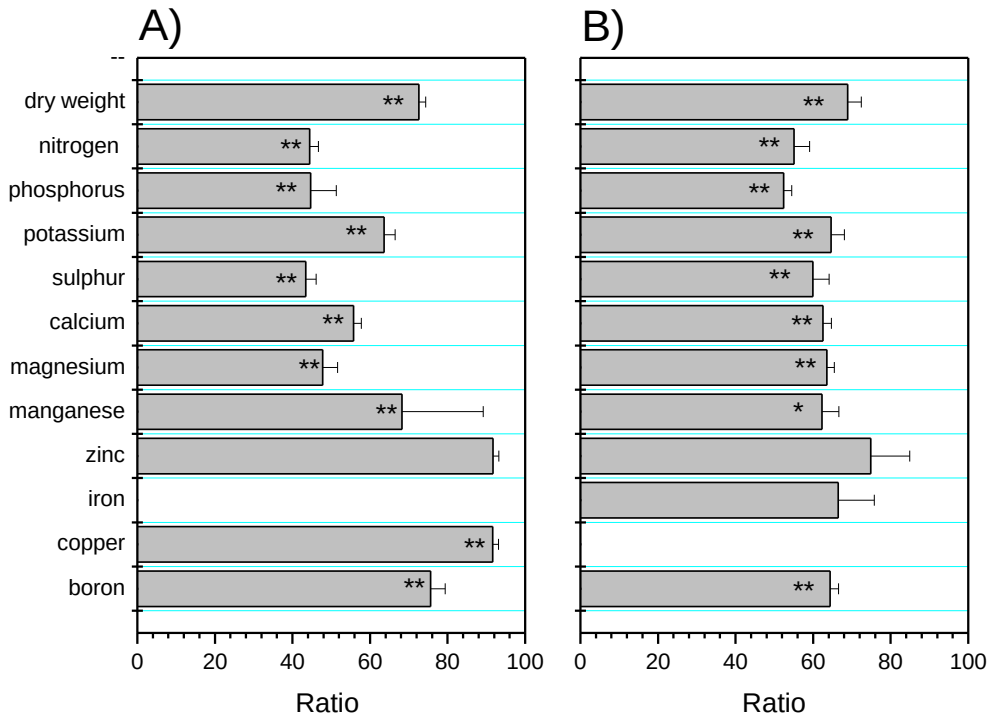


Figure 7.4 Perennial reserve biomass and contents of mineral nutrients of A) ETG as a percentage of control vines, B) famine as a percentage of feast vines; *n* = 3 ± SE; values accompanied by ** and * are significantly different *P* ≤ 0.05 and *P* ≤ 0.10; missing values because *SE* large.

Reserves are defined as food substances that are not used directly in assimilation and respiration but are stored in the tree until needed (Glerum, 1980). Both ETG and famine treatments reduced vine macronutrient reserves. The consequences of reducing vine reserves appear to vary depending on which mineral nutrient is affected, and whether this is a result of ETG or the famine treatment. For example:

- Nitrogen - reserves were reduced by both treatments, early-season leaf N (per cm^2) was unaffected in either treatment, individual fruit N contents were reduced in the famine but not the ETG vines. When the higher crop loads from the famine vines were taken into account, the amount of N allocated to fruit was less in the famine than the feast vines.
- Phosphorus - reserves reduced in both treatments, leaf P reduced in ETG but not famine vines, individual fruit P contents reduced in famine but not ETG fruit. When the higher crop loads from the famine vines were taken into account, the amount of P allocated to fruit was unaffected.

- Calcium - reserves reduced in both treatments, leaf Ca reduced in ETG but not famine vines. Individual fruit Ca contents unaffected in ETG and increased in famine treatment.

Interpretation of these results is difficult. In the ETG vines, leaf nutrient status was more adversely affected than fruit nutrient status. This may be in part because the girdle was still open whilst the canopy developed, and the girdle was closed for early fruit development. Girdling reduced xylem concentration of Ca, P, Mg and K, but not B and Zn relative to sap from intact apple trees (Bangerth, 2008). Sampling sap from girdled and intact kiwifruit vines and comparing the relative amounts of all mineral nutrients might help to explain some of the findings. On a leaf area basis the mineral nutrients unaffected by girdling were N, Zn, Fe, Cu and B, the same five that were higher on a leaf area basis in leaves from girdled apple limbs than leaves from ungirdled limbs (Schecter et al., 1994).

7.7 CONCLUSIONS

The work carried out in this thesis generated several new questions and enabled some of the initial questions to be answered. This allows growers and scientists to have a better understanding of source-sink relationships in mature field-grown kiwifruit vines.

Will long-term vine productivity be adversely affected if high yields of high DMC fruit are produced year after year? In treatments where ETG was not used, return bloom was reduced followed by recovery in the following year. This finding suggests that there will be no long-term decline in productivity if vines are over-cropped as return bloom will be reduced in the following season to compensate. The exception was if ETG is used. Isolating the canopy from the roots enabled return bloom to remain high even after several years of treatment. Commercially, there is a large cost associated with having to thin off a high proportion of fruitlets each season and there is probably no justification for using ETG but the technique could be of value to help understand the mechanism behind the floral response in kiwifruit.

Does increasing fruit DMC affect fruit maturity attributes, and therefore harvest date? An increase in DMC was typically associated with advanced degreening and therefore earlier commercial harvest. The relationship between fruit DMC and colour change varied among treatments, and advanced colour change does not necessarily mean that other maturation responses such as softening will also be advanced.

If fruit maturity is affected by altering DMC how is fruit storage performance affected? Fruit from treatments with lower DMC have delayed degreening and need to spend longer time on the vine before being reaching commercial maturity. During this time fruit can begin to soften on the vine. This can affect initial softening in storage and may result in fruit softening to certain thresholds such as 20 N sooner after storage than fruit that degreened and were picked earlier.

Will the vines compensate for increased productivity by upregulating photosynthesis, delaying leaf senescence and increasing shoot growth or will carbohydrate reserves be depleted? Whole vine starvation did not result in compensatory responses such as increased vegetative growth, larger leaves and increased leaf photosynthesis. Instead it appears that canopy growth was reduced by high crop loads, probably because shoot growth and fruit growth competed with each other for resources. The vines responded by producing fewer fruit, then return bloom recovered in the following season.

If reserves are depleted will nutrient uptake be affected and will nutrient deficiencies become apparent? The total reserve status of most mineral nutrients was reduced by both the ETG and famine treatments. Leaf nutrient deficiencies were not detected in the famine vines, apart from low K levels late in the season and K was remobilised from the leaves. It is not known if K application as soil or foliar sprays in autumn would have any tangible effect on vine productivity. Leaves from the ETG vines had lower contents of several mineral nutrients (P, K, S, Ca, Mg and Mn) than leaves from ungirdled control vines. Further work on leaf photosynthesis is needed to determine if any of these are limiting leaf performance. There was no evidence from the work in this thesis that leaf health and photosynthesis was adversely affected by ETG.

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