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**The development and taste of fruit of gold kiwifruit (*Actinidia chinensis* Planch. Var. *chinensis* “Gold3”)**

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of the requirements for the degree

of

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## Abstract

The “Gold3” cultivar of kiwifruit (*Actinidia chinensis* Planch. var. *chinensis* “Gold3”) has proven to be capable of producing high yields, however, growers have also noted an increased risk of small fruit that have high acidity, low dry matter and poor flavour. This thesis investigated how fruit composition and flavour components develop in “Gold3” kiwifruit, and whether altering the carbohydrate supply using common orchard practises would influence the accumulation and partitioning of the flavour components (starch, sugars and acids) in fruit.

Fruit from an organically managed “Gold3” orchard were sampled fortnightly, from anthesis through to harvest, from canes receiving five treatment combinations of leaf or fruit thinning, and girdling. These treatments increased or decreased carbohydrate supply, either early or late in fruit development.

Overall, the “Gold3” fruit demonstrated similar patterns of starch, sugar and acid accumulation to other *A. chinensis* cultivars, in particular the other gold kiwifruit cultivar “Hort16A”, with slight differences in timings and peak concentrations. An altered carbohydrate supply to developing fruit strongly influenced fruit composition in unique ways. As expected fresh weight growth and starch accumulation responded positively to a period of high carbohydrate supply. The regulation of organic acids were shown to be more complex, with the concentrations of some acids responding inversely to increased carbohydrate supply. At eating ripe the fruit from lower carbohydrate supply had altered sugar: acid ratios, with increased total acid concentrations, as well as decreased sugar concentrations.

To identify how these compositional changes affected the taste of fruit at eating ripeness, a controlled consumer sensory experiment was carried out with 78 inexperienced consumers. Fruit from the different treatments were all perceived as having acceptable flavours, despite the fruit having significant differences in the standard flavour determinants (DMC, rSSC and TA). Consumers were able to detect differences in sugar and acid concentrations between treatments. Low carbohydrate supply treatments had significantly higher TA, citric acid and quinic acid concentrations, combined with lower °Brix, DMC and sucrose concentrations

at eating ripe. Consumers more closely associated these fruit with being more acidic, and having more sour and under-ripe flavours, compared to the treatments that received increased carbohydrate supply.

Overall the results of the research support the hypothesis that “Gold3” kiwifruit are vulnerable to changes in composition due to changes in growing conditions, and that these changes can influence flavour as perceived by consumers. These effects may be more pronounced in orchards where high crop loads, shading, or variation in leaf to fruit ratio between shoots create populations of even more carbon deficient fruit.

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## List of abbreviations and terms used

|           |   |
|-----------|---|
| 1 High    | Treatment with six leaves per fruit and received an early season girdle (17/12/2015)        |
| 1 Low     | Treatment with one to two leaves per fruit and received an early season girdle (17/12/2015) |
| 2 High    | Treatment with six leaves per fruit and received an late season girdle (3/02/2016)          |
| 2 Low     | Treatment with one to two leaves per fruit and received an late season girdle (3/02/2016)   |
| ANOVA     | Analysis of variance  |
| Control   | Treatment which had two to three leaves per fruit and no girdle                             |
| DAA       | Days after anthesis / flowering   |
| DM        | Dry matter  |
| DMC       | Dry matter concentration  |
| DW        | Dry weight  |
| FW        | Fresh weight  |
| L/F ratio | Ratio of leaf area to fruit per cane  |
| Psa       | <i>Pseudomonas syringae</i> pv <i>actinidiae</i>  |
| rSSC      | Ripe soluble solids concentration   |
| SE        | Standard error of the mean  |
| SSC       | Soluble solids concentration  |

# Chapter One: Literature Review

## 1.1 Introduction

The kiwifruit (*Actinidia* sp.) industry is a highly successful and vital component of horticulture in New Zealand, second only to the wine industry in terms of export value (Ferguson, 1984, 1991; Garcia, 2012). In 2015, kiwifruit exports exceeded \$1 billion for the first time. This value is predicted to continue increasing, but success in this highly competitive global market relies on the continued production of high quality fruit.

A large majority of New Zealand's kiwifruit production comes from one cultivar, the green kiwifruit "Hayward" (*Actinidia deliciosa* (A. Chev.) C.F. Liang *et* A.R. Ferguson var. *deliciosa* "Hayward") (Ferguson, 1991; Ferguson & Seal, 2008; Garcia, 2012). The "Hayward" cultivar has been the backbone of the kiwifruit industry in New Zealand since it was first grown here in the 1920's (Ferguson & Seal, 2008; Garcia, 2012; Jaeger, Rossiter, Wismer, & Harker, 2003). Global competition increased during the 1990's as other countries, including Italy, Chile and China, began growing and exporting more kiwifruit (Ferguson & Seal, 2008; Huang & Ferguson, 2003). This increased the need for better quality fruit, as well as a wider variety of cultivars in order for New Zealand to keep its competitive edge as the industry is largely export based. Of these new varieties the most successful was the gold-fleshed "Hort16A" cultivar (*Actinidia chinensis* Planch. var. *chinensis* "Hort16A") with its novel colour and sweeter, tropical flavouring (Ferguson & Seal, 2008; Jaeger & Harker, 2005). The cultivar, marketed as ZESPRI® Gold Kiwifruit, along with the "Hayward" cultivar were highly successful with increasing export values throughout the 2000's until the introduction of the bacterial canker *Pseudomonas syringae* pv *actinidiae* (Psa), in late 2010 (Butler *et al.*, 2013; Ferguson & Seal, 2008; Greer, 2012). The "Hort16A" cultivar proved to be highly susceptible to the disease with many vines being devastated around New Zealand (Butler *et al.*, 2013).

Following the Psa devastation a new cultivar, "Gold3" kiwifruit (*Actinidia chinensis* Planch. var. *chinensis* "Gold3"), was introduced as a possible replacement for the "Hort16A" cultivar (Li, 2017). The "Gold3" cultivar was in

the pre-commercial trial stage of development when the Psa outbreak occurred, and it was noted by growers that the “Gold3” vines appeared to be more tolerant to the bacteria. In an attempt to fast-track the establishment process, and to minimise the damage to the industry, the “Gold3” cultivar was grafted onto many of the “Hort16A” vines around the country (Greer, 2012). As of the end of 2016 very little of the “Hort16A” cultivar remains and the “Gold3” cultivar makes up most of the gold kiwifruit grown in New Zealand (Greer, 2012).

The “Gold3” cultivar has shown to be capable of producing high yields. Because of this it is seen as a vital part of Psa recovery and the long-term sustainability of the industry. It has been noted with “Gold3” fruit that although capable of producing high yields of good quality fruit, with these high yields there is an increased risk of fruit with high acidity (measured as titratable acidity) and low dry matter concentration (DMC: % of fresh weight that is dry weight) (Thorp, 2012). This low DMC and high acidity combination results in poor flavour and a negative consumer response (Harker et al., 2009; Jaeger & Harker, 2005; Thorp, 2012).

The predicted long term plans for the kiwifruit industry are greatly reliant on the continued success of the “Gold3” variety. This continued success and ability to sell fruit in key premium markets for premium prices is dependent on producing consistently high yields of good quality fruit.

The review aims to look at what factors are influencing the quality and flavour of “Gold3” kiwifruit, when and how these factors are occurring, and what can influence the sugar/ acid ratio in fruit. Most of the information currently available comes from the “Hayward” and “Hort16A” cultivars, which have had considerably more research done about them compared to the still relatively new “Gold3” fruit. This literature review will also examine several other economically important fruit, including tomatoes and grapes, to provide insight into what may occur during “Gold3” fruit development.

## **1.2 Flavour requirements in kiwifruit**

Many factors have been identified as important in influencing the overall liking of fruit, such as the ripeness, taste, texture, health benefits, price and convenience (Harker et al., 2009; Jaeger & Harker, 2005; Jaeger et al., 2003; Rossiter, Young,

Walker, Miller, & Dawson, 2000). In kiwifruit flavour is described as the key attribute that determines what consumers are willing to pay (Garcia, 2012; Jaeger & Harker, 2005; Jaeger et al., 2003). Achieving this good flavour is dependent on several factors including the DMC, mineral composition, sugar-acid ratio, volatile content, and water content (Famiani et al., 2012). Previous consumer liking studies carried out for both the *A. deliciosa* (“Hayward”) and *A. chinensis* (“Hort16A”) cultivars, have shown that, generally, a majority of consumers respond positively to high soluble sugar content (Crisosto & Garner, 2001; Harker et al., 2009; Jaeger & Harker, 2005; Jaeger et al., 2003; Nardozza et al., 2011; Rossiter et al., 2000). This makes sweetness one of the most important quality traits for kiwifruit as it impacts the overall flavour, consumer acceptability and grower returns. Because of this importance the ability to identify the factors which influence flavour, and also being able to predict potential flavour, is vital for the industry.

The sugar content of ripe kiwifruit is measured as soluble solids content (rSSC) or as °Brix (measured with a refractometer). The °Brix measurements comprises soluble solids (mostly sugars), while the SSC measurements comprise of soluble sugars, acids and minerals (Burdon et al., 2004). In many fruit, including apples, pears, peaches, nectarines, apricots and plums, monitoring of the SSC is used as a method of quality control of fruit at harvest (Harker et al., 2009). A draw back for this method is that during the development of many fruit, nutrients are stored as carbohydrates, such as starch, which is later converted into sugars (Harker et al., 2009; Kwack et al., 2014; Minchin, Richardson, Patterson, & Martin, 2003; Simona Nardozza et al., 2010). This means that SSC measurements are only measurements of the sugar that has currently been converted (Harker et al., 2009). As the starch to sugar conversion in kiwifruit takes place over a long period of time, beginning when ripening is initiated and continuing throughout storage, the method has limited predictive power (Harker et al., 2009).

Due to this draw back, the DMC of kiwifruit at harvest is often used to predict the potential sugar content of the same fruit when it is ripe, and in turn predict the potential consumer liking (Harker et al., 2009; Jordan & Seelye, 2009; Minchin et al., 2003; Nardozza et al., 2011). Fruit DMC is primarily the result of carbohydrate accumulation, predominantly in the form of starch, and as this is

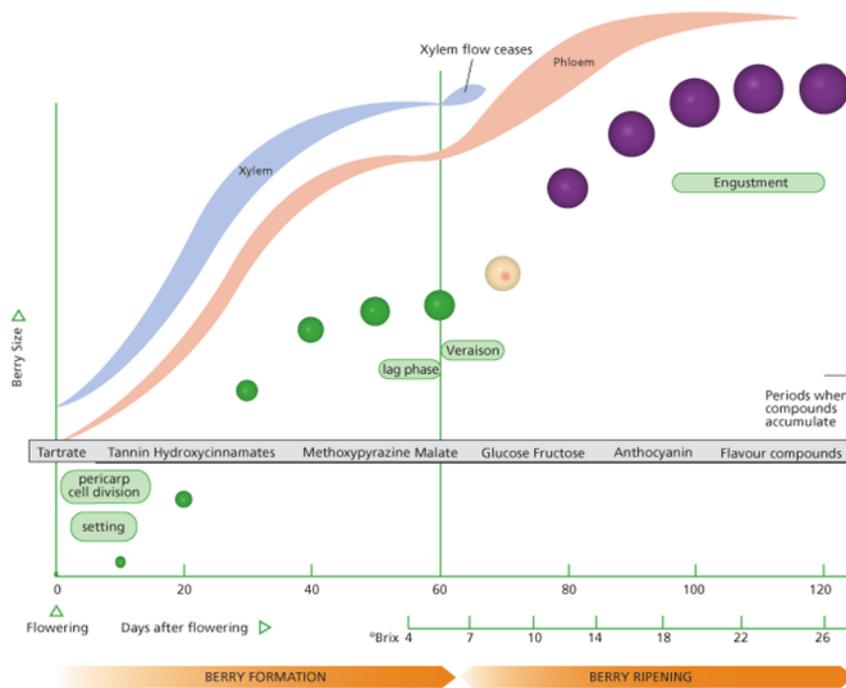
later converted into sugars it makes a dependable predictor of potential sugar content in the ripe fruit (Harker et al., 2009; Minchin et al., 2003; Simona Nardozza et al., 2010). It has been observed that measuring DMC is advantageous as the measurements can be carried out prior to, or at, harvest, and used for predicting consumer responses making DMC a useful decision making instrument.

DMC as a predictor of potential flavour has been used in a number of fruit, including mango and avocado (Gamble et al., 2010; Harker et al., 2009), but has been particularly well documented in “Hort16A” and “Hayward” kiwifruit. Consumer studies have shown the ability of the consumers to discriminate between differences as low as 1-2 % in DMC. They also show increased consumer liking and willingness to buy fruit with increased DMC (Burdon et al., 2004; Harker et al., 2009; Jordan & Seelye, 2009). These studies have also shown that, at harvest, fruit DMC was strongly correlated with, and as a result is a good predictor of the rSSC (ripe soluble sugar concentration) (Burdon et al., 2004; Jordan & Seelye, 2009; Richardson, McAneney, & Dawson, 1997). However, this relationship between sugar content and DMC at harvest has been shown to be less consistent in the “Gold3” cultivar compared to the other common cultivars “Hayward” and “Hort16A” (Thorp, 2012). This means it is harder to predict how fruit will taste when ripe, potentially allowing more bad tasting fruit in to the market.

### **1.3 Kiwifruit growth and development**

Flesh fruits undergo a wide variety of developmental patterns. A common one is the single sigmoidal curve where the growth increase follows a sigmoidal pattern. This growth pattern is seen in tomatoes, apples, pears, dates, pineapples, bananas, avocados and oranges (Coombe, 1976). Other fruit, including grape berries, olives, stone fruits, black berries and raspberries show a double sigmoidal curve pattern of development (Figure 1.1), (Coombe, 1976; Davies & Robinson, 1996). This involves an initial increase in size followed by a lag period with no volume increase. This is then followed by a second phase of growth during ripening, often associated with an increase in softness, accumulation of hexoses, decrease in malic and tartaric acids, and colour changes, (Davies & Robinson, 1996). “Hayward” kiwifruit are commonly described as showing a double sigmoidal

pattern of growth, while the “Hort16A” cultivar has been described as a single sigmoidal growth curve (Cieslak, Seleznyova, & Hanan, 2011; Hopping, 1976). There is some debate around these classifications, however, as both cultivars have been described as single, double or even triple (Coombe, 1976; Minchin et al., 2003; Pratt, 1974; Reid, 1982; Richardson et al., 2011; Walton & Dejong, 1990). Possible reasons for this may be that in the kiwifruit berry that lag phase between the first and second phases of growth is not as pronounced as it is in some other fruits, such as grape, and may therefore only be detected with more frequent and precise measurements of fruit size. Environmental factors may also cause irregularities in results from different seasons, or in different growing regions.



**Figure 1.1** Double sigmoidal growth curve of grape berries, showing the 2 main phases of growth. The initial period of growth increase of berry formation, followed by a lag period of no growth, before a second period of increase during berry ripening. Source (Kennedy, 2002).

Kiwifruit have a long period of fruit development, from flowering in the spring, to ripening in autumn, compared to tomatoes which develop in only 1.5 - 2 months (Bertin, Causse, Brunel, Tricon, & Genard, 2009; Giovannoni, 2004; Salinero, Vela, & Sainz, 2009). The main stages of fruit growth are fruit set (when the initial fruit growth begins), rapid early fruit growth, slower growth as the fruit begins to ‘mature’, and fruit senescence (Cieslak et al., 2011; Richardson et al., 2011; Salinero et al., 2009; Walton & Dejong, 1990). The initial exponential

period of growth (from fertilization to around 60 DAA in “Hort16A” fruit) involves growth increases to approximately 50% of the final fruit weight (McPherson, Richardson, Snelgar, Patterson, & Currie, 2001; Richardson et al., 2011). This period, similar in tomatoes and “Hayward” kiwifruit, is characterised by cell division and rapid growth in the pericarp tissue, along with a large amounts of water entering the fruit, compared to carbon (Gillaspy, Bendavid, & Gruissem, 1993; Hopping, 1976; Richardson et al., 2011). This initial period of growth also determines the sink strength (the potential for fruit to import dry matter from the source (the leaves)), and ultimately the final size of the fruit (Richardson et al., 2011).

The second major period of growth is considerably slower, with the fruit accumulating up to 90 % of its final weight by around 150 DAA in “Hort16A” kiwifruit (Richardson et al., 2011). This stage, again similar in tomatoes and “Hayward” fruit, is driven by a change from cell division to cell expansion (Nardozza et al., 2013; Richardson et al., 2011; Salinero et al., 2009). This is followed by more incremental, or no, increases in the weight as the fruit begins to ripen during the final stage. Fruit maturity is a commercial term and is usually defined as the ability of the fruit to ripen normally if harvested. This period is characterised by changes in flesh colour, softening of the fruit, and starch being broken down and metabolized into soluble sugars (glucose, sucrose and fructose). This ripening begins around 160 DAA and goes until around 235 DAA in “Hort16A” kiwifruit, if the fruit is left attached to the vine (Richardson et al., 2011). Following this natural ripening period the fruit is at eating ripe from around 235 to 275 DAA, after which fruit senescence begins (Richardson et al., 2011). During commercial fruit production the fruit is normally harvested during the early ripening period, once it has reached a defined level of maturity, and placed in low temperature storage which slows the ripening process. Maturity thresholds are usually defined based on a combination of: SSC, DMC, flesh colour or flesh firmness thresholds.

The general development of kiwifruit appears similar to that of other fruit except for several differences in the ripening process. Unlike other fruit such as tomato, melon, grape and peach which accumulate soluble sugars throughout fruit development, kiwifruit accumulate starch which is later converted to sugar

(similar to bananas) (Nardozza et al., 2013). The starch is broken down and metabolised into sugars (similar levels of glucose, sucrose and fructose) around 190 DAA in “Hort16A” fruit (Figure 1.2). These soluble sugars continue to increase in the pericarp until ripeness is reached (Richardson et al., 2011).

#### **1.4 Effects on kiwifruit dry matter concentration and size**

Many factors can influence the final size and DMC of fruit at harvest, which in turn can influence the flavour of fruit. In kiwifruit it has been shown that by 50 DAA the size of fruit at harvest has already been largely determined (McPherson et al., 2001). Flower numbers and quality (Burge, Spence, & Marshall, 1987), growing temperatures (Snelgar, Hall, Ferguson, & Blattmann, 2005), water availability (Miller, Smith, Boldingh, & Johansson, 1998), light conditions (Tombesi, Antognozzi, & Palliotti, 1993b) and pollination have also been shown to be important in influencing the final size of kiwifruit. Other important factors include, whether flowers are early or late (Cruz-Castillo, Woolley, & Lawes, 2002), seed size (Lawes, Woolley, & Lai, 1990), fruit number or position (Snelgar, Minchin, Blattmann, & Hall, 2012), pedicel length and vascular development (McPherson et al., 2001). These factors can influence aspects such as ovary size (early vs late flowers), competition for resources (position) or cell numbers (pollination), (McPherson et al., 2001).

These factors can also influence the fruit DMC, and likely in turn the flavour of the kiwifruit. Specifically the harvest timing, crop load, position of the fruit within the canopy and the shoot type play important roles in the development of DMC (Famiani et al., 2012; Snelgar et al., 2012). Light levels are also important as fruit growing in shaded parts of the canopy have been shown to have DMC compared to fruit in exposed parts of the canopy, likely due to early leaf senescence and shoot dieback (Thorp, 2012).

Kiwifruit are considered a fast growing vine and growth features strong competition for carbohydrates between the vegetative and reproductive components of the plant (Cieslak et al., 2011; Ferguson & Seal, 2008). To help control where carbohydrates are being distributed within the vine, kiwifruit growers typically use different training and pruning strategies (Cieslak et al., 2011). When vines have a higher crop load there is less carbon available for each

individual kiwifruit which typically results in smaller fruit and lower DMC (Famiani, 1997; Minchin, Snelgar, Blattmann, & Hall, 2010; Snelgar et al., 2012).

The timing of the harvest can also be important determinants on the DMC. At harvest “Gold3” fruit tend to have DMC ranging from 15-19 % and titratable acidity, on average, between 1 - 1.4 % (Thorp, 2012). Harvesting at later dates was shown to produce lower acidity levels, up to 0.2 % lower, in the fruit when ripened (Thorp, 2012). Harvesting earlier may also alter flavour as in “Gold3” early harvest produced fruit with lower DMC and higher titratable acidity.

### **1.5 Development of flavour components (sugars and acids)**

As introduced above, in kiwifruit dry matter accumulates primarily in the form of starch (a storage carbohydrate). In “Hort16A” kiwifruit the DMC accumulates, in a linear growth pattern between 28 and 140 DAA, followed by slower increases up to around 240 DAA (Richardson et al., 2011). The majority of this DMC is starch, which begins accumulating around 40 DAA, and reaches a maximum at 190 DAA. At this point the ripening process begins and the accumulated starch is metabolized into soluble sugars (Hopping, 1976; Pratt & Reid, 1974; Richardson et al., 2011). As this starch is broken down the soluble sugars accumulate up until fruit are at eating ripeness (Richardson et al., 2011). Although most sugars are stored as starch, small amounts of several soluble sugars (sucrose and planteose) are transported to the fruit throughout development (Klages, Boldingh, Cooney, & MacRae, 2004).

The major sugars in *Actinidia* fruit are glucose, fructose and sucrose (Nishiyama, Fukuda, Shimohashi, & Oota, 2008). The ratio of these sugars has been shown to vary between cultivars. Nishiyama et al. (2008) showed glucose and fructose were present in higher concentrations than sucrose in “Hayward” cultivars, while in *Actinidia arguta* (Sieb. et Zucc.) Planch. ex Miq. var. “Arguta” (“Arguta”) cultivar sucrose was predominant. Another sugar which is also important but present in smaller amounts, is the sugar alcohol, *myo*-inositol. This is present in relatively high levels in the “Arguta” cultivar, and in lesser concentrations in “Hayward” and “Hort16A” (Cheng et al., 2004; Nardozza et al., 2013; Nishiyama et al., 2008; Paterson, Macrae, & Young, 1991). Other sugars present in kiwifruit,

but typically in smaller quantities include, planteose, raffinose, xylose, melibiose, fucose, galactose, rhamnise, trehalose, and stachyose (Nardozza et al., 2013).

Kiwifruit have high concentrations of organic acids compared to many fruit, contributing 1 % to 3 % of fresh weight (Marsh & Harker, 2016). Of this the major acids are citric (around 40-60 % of total acids present), quinic (40-60 %), and malic (10 %), with ascorbic and oxalic in smaller quantities (Etienne, Génard, Lobit, Mbeguié-A-Mbéguié, & Bugaud, 2013; Marsh, Boldingh, Shilton, & Laing, 2009; Marsh & Harker, 2016; Nishiyama et al., 2008). The accumulation of acids is controlled by a number of agro-environmental and genetic factors, such as irrigation, source to sink ratio, fertilization, or temperature (Etienne et al., 2013).

In “Hort16A” kiwifruit acid accumulation, primarily in the form of quinic acid, was observed during the early period of rapid fruit growth (up to 100 DAA), (Figure 1.2) (Marsh, Boldingh, & Cheng, 2007; Richardson et al., 2011). This acid accumulation pattern is also seen in other kiwifruit cultivars (“Hayward” and “Arguta”) however, in the “Hayward” cultivar the quinic acid peak is earlier, between 28 and 42 DAA (Marsh et al., 2007; Marsh et al., 2009). While the “Arguta” cultivar shows a lower quinic acid peak compared to other cultivars (Marsh et al., 2009). It has been suggested that the reason for the accumulation of quinic acid in young fruit may be related to the maintenance of osmotic potential (which aids fruit growth), or due to the role quinic acid or its precursors have in other aspects of fruit development and secondary metabolite accumulation (Marsh et al., 2007; Marsh et al., 2009; Marsh & Harker, 2016; Richardson et al., 2011).

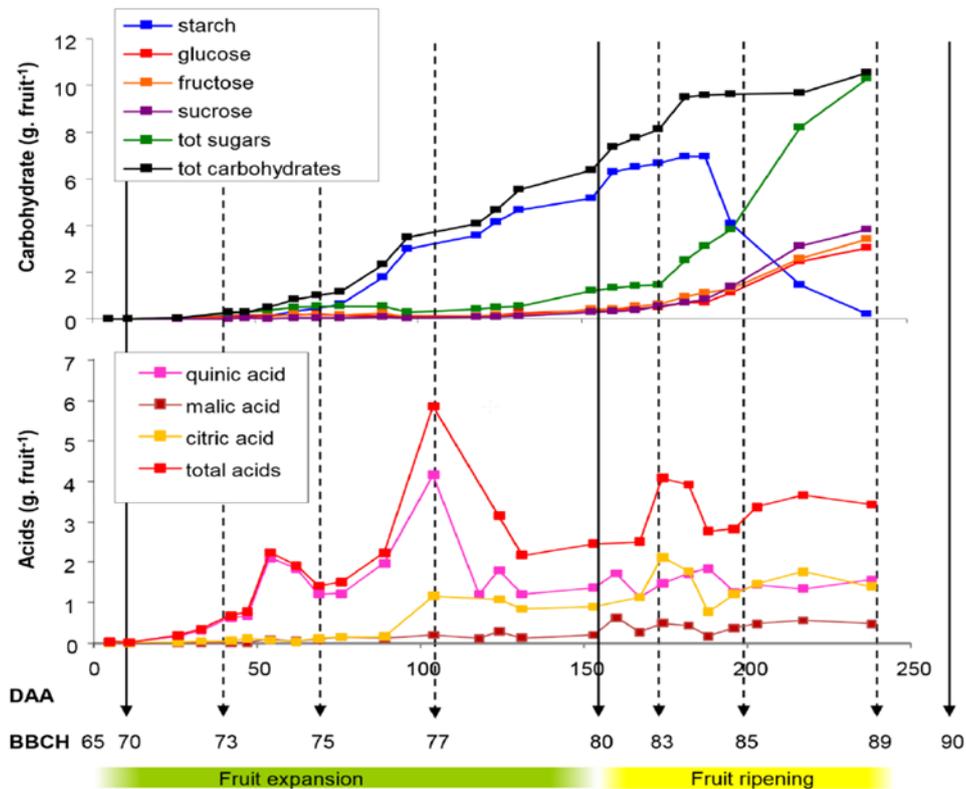
After the initial accumulation, the amount of quinic acid decreases up to 125 DAA, after which it remains relatively steady until harvest. Citric acid accumulates slowly throughout development, peaking close to harvest (Marsh et al., 2007; Reid, 1982; Richardson et al., 2011). Citric acid tends to reach a similar concentration as that of quinic acid, as the concentration of quinic acid declines following its peak, and the two acids remain similar in the total amount per fruit for the rest of development (Figure 1.2) (Richardson et al., 2011). The amount of each of the acids have been identified as varying significantly between different *Actinidia* species. For example, citric acid concentrations vary from 9.5-25 mg g<sup>-1</sup>

in “Hayward”, 5.6-47 mg g<sup>-1</sup> in “Hort16A” and 0.9-19 mg g<sup>-1</sup> in “Arguta” cultivars (Marsh & Harker, 2016)

These same sugars and acids are also the main ones present in “Gold3” kiwifruit, although the ratios and peak timing of each individual sugar or acid may vary between the different kiwifruit cultivars. The “Gold3” fruit will likely have similar ratios of sugars and acids to the other gold cultivar, “Hort16A”, which are shown to contain around 10 g of total sugars per fruit, and around 3.5 g of total acids per fruit (Figure 1.2) (Richardson et al., 2011). Early studies into “Gold3” kiwifruit have been shown to have high acidity along with insufficient development of sugars that fruit in low DMC fruit, creating a low ratio of sugar to acid, and poor consumer liking (Thorp, 2012).

The balance between sugars and acids are vital in achieving good flavour in kiwifruit as they play an important role in consumer acceptance and liking (Harker et al., 2009; Rossiter et al., 2000). The concentration of citric acid is a particularly important determinant on the balance between sugars and acids as it is often reported as having the largest effect on the consumer perception of the total acidity (Etienne et al., 2013; Marsh & Harker, 2016). The “tangy” acid flavour which is characteristic of “Hayward” fruit has been shown to strongly relate to the citric acid content (Paterson et al., 1991). Quinic acid however, has also been shown to be important in studies where acid perception was compared between different acids added in the same amounts (Marsh et al., 2003).

In studies using pulp or model solutions, increasing additions of sugar (in the form of sucrose) is shown to decrease sour flavour and increase consumer acceptability (Bonnans & Noble, 1993; Marsh et al., 2003; Marsh, Friel, Gunson, Lund, & MacRae, 2006; Rossiter et al., 2000). Also the total amount of sugar has been shown as what is most important in sweetness perception, with no difference perceived between samples with different ratios of the major sugar types (Marsh et al., 2003). Sweetness perception has been shown to decrease when citric acid was added at low SSC, but not at high SSC where the effects of acidity were suppressed by the sugars (Marsh et al., 2003; Rossiter et al., 2000).



**Figure 1.2** Development of the different flavour components in "Hort16A" kiwifruit shown from days after anthesis (DAA) and also on the BBCH scale. Source: Richardson et al. (2011).

## 1.6 Knowledge gaps and objectives of research

As discussed above, the current knowledge of kiwifruit primary metabolism is limited to the commercial cultivars, mainly “Hayward” and “Hort16A”, with very little known about the newer “Gold3” cultivar. It has also been noted that in “Gold3”, small or low DMC fruit can produce insufficient sugar concentrations to balance the acid concentrations, resulting in poor tasting fruit and low consumer acceptability. This project aims to gain a better understanding of how flavour and its components develop in “Gold3” kiwifruit as well as identify when the components that contribute to final flavour accumulate. Understanding how flavour develops and when the critical points in the fruits development are is essential in order to minimise the production of small, acidic flavoured kiwifruit.

There are two main gaps in “Gold3” kiwifruit knowledge that aim to be filled by this research and will be examined in the second chapter of this thesis. The first is how “Gold3” fruit composition develops throughout the season. As carbohydrates arrive in the fruit, they have two pathways they can take: sugars or acids, and the

balance between these has been shown as one of the biggest influences on the perception of flavour and liking. This research aims to identify what influences the accumulation of both the sugars and acids, and when these occur. Currently the development of the flavour components (sugars, acids and starch) from flowering to maturity are yet to be described in detail in the “Gold3” cultivar. What little information is known about “Gold3” is based on measurements taken close to harvest.

The second gap in “Gold3” knowledge that is addressed in chapter two is how carbohydrate supply at different stages of development affects the accumulation and partitioning of the various flavour components (starch, sugars and acids), as the factors that contribute to the variable flavour in “Gold3” are poorly understood. To try and understand this several pruning and girdling treatments were applied at different points throughout the growing season with the aim to alter the carbohydrate supply and competition between fruit during early and late development to create fruit of differing size and compositions. It is expected that the general development and accumulation of starches, sugars and acids will be similar to that of “Hort16A” fruit. It is hypothesized that shoots with a lower L/F ratio will produce fruit with lower DMC, starch and sugar concentrations at harvest compared to fruit growing on shoots with a higher L/F ratio. A girdle applied early in the season and allowed to close will increase fruit size, whereas a girdle applied later in development will have a greater influence on starch and sugar accumulation, and in turn the DMC.

The third chapter looks at composition and perception of flavour in “Gold3” fruit at the eating ripe stage. A consumer preference test was carried out on fruit from the different treatments created in chapter 2, to test whether the differences in carbohydrate supply affected the partitioning of sugars and acids enough for inexperienced panellists to taste the difference. Increased carbohydrate supply is hypothesized to have higher consumer liking compared to the fruit which had decreased carbohydrate supply. It is also expected that the reduced carbohydrate supply will produce lower DMC and make the fruit taste blander, but also alter the acid to sugar ratio which will create poor tasting fruit.

The final chapter will summarise the results of the research, drawing conclusions of how “Gold3” fruit develop, how the development can be affected by altering carbohydrate supply, and whether these differences affect consumer preference. This information will assist growers in minimising the production of small, poor flavoured “Gold3” kiwifruit.

# Chapter Two: Pre-harvest development of fruit

## 2.1 Introduction

Current knowledge of kiwifruit primary metabolism is mainly limited to the “Hayward” and “Hort16A” cultivars, with less known about the newer “Gold3” cultivar. Development of the flavour components (sugars, acids and starch) from flowering to maturity are yet to be described in detail in the “Gold3” cultivar. It has also been noted that in “Gold3”, small or low DMC fruit can produce insufficient sugar concentrations to balance the acid concentrations, resulting in poor tasting fruit, and low consumer acceptability (Thorp, 2012). These poor flavoured fruit result in a negative consumer response as studies have shown a strong consumer preference for high DMC and sugar content (Harker et al., 2009; Jaeger & Harker, 2005).

Fruit development can be effected by a number of factors including, environmental effects, crop load, fruit position, flower numbers and pollination. One commonly applied treatment that has been found to significantly alter kiwifruit development is changing the leaf to fruit ratio (L/F ratio). This practise alters the supply of carbohydrates going to each individual fruit by increasing or decreasing resource competition between fruit. A high L/F ratio tends to produce larger fruit, with higher DW as more carbohydrates are being supplied to each fruit. Defoliation, however, limits carbohydrate supply and results in reduced fruit weights (Burge et al., 1987; Cruz-Castillo, Woolley, & Famiani, 2010; Kwack et al., 2014; Minchin et al., 2010). Canes with low L/F ratio also tend to produce fruit with significantly reduced starch and sugar concentrations (Cruz-Castillo et al., 2010; Hopkirk, Beever, & Triggs, 1986; Miller, Broom, Thorp, & Barnett, 2001; Tombesi, Antognozzi, & Palliotti, 1993a). Shoots with low L/F ratio have also been shown to produce fruit with more variable fresh and dry weights (Minchin et al., 2010). It is suggested that to produce normal development and fruit quality, between two and four leaves per fruit are required (Lai, Woolley, & Lawes, 1989; Minchin et al., 2010).

Another common practise used to influence development is girdling. This practise involves cutting into the phloem and altering the source-to-sink movement of carbohydrates by directing resources to the fruit, rather than the competing sinks

such as roots (Noel, 1970). An increase in fruit DMC has been shown in response to trunk girdling in some studies (Black, Patterson, Gould, & Clearwater, 2012), while other studies have shown an increase in fruit size (Woolley & Cruz-Castillo, 2006). The changes observed in fruit depend on which stage of growth the girdle is applied during (Snelgar et al., 2012). These changes in carbohydrate supply affect not only fruit growth, size and DMC, but also the partitioning of acids and sugars in fruit (Snelgar et al., 2012).

The aim of this project was to gain a better understanding of how flavour and its components develop in “Gold3” kiwifruit, so growers can avoid producing fruit with undesirable flavour profiles. Specifically, the objectives are to describe the development of “Gold3” fruit size, DMC and composition in detail from flowering to harvest, and identify when the components that contribute to final flavour accumulate.

Several treatments were chosen to try and produce fruit of differing size and compositions. Two common orchard practises (described above) were used, pruning to alter the leaf to fruit ratio, and girdling. These practises were applied at different points of the growing season to try and affect different aspects of growth and development. It was hypothesised that shoots with a lower L/F ratio would produce fruit with lower DMC, starch, acid and sugar concentrations at harvest compared to fruit growing on shoots with a higher L/F ratio. A girdle applied early in the season and allowed to close would affect fruit size and acid concentrations more than starch, because acids accumulate throughout development. A girdle applied later in development would have a greater influence on starch and sugar accumulation, and in turn the DMC.

## **2.2 Methods**

### **2.2.1 Study site**

Field work was carried out on mature, three year old “Gold3” kiwifruit vines growing in Karapiro, Waikato (37°56'13"S, 175°32'40"E) during the 2015/2016 growing season. The orchard was managed organically. Vines were planted in 2012, with “Gold3” grafted onto “Bruno” seedling rootstocks. The study area contained two rows of vines and opposing male vines.

### **2.2.2 Selecting fruit buds**

Leading up to flowering in late October, 2015, 10 canes were randomly selected and the number of flower buds counted. These vines were monitored at three day intervals for two weeks. On each date the number of flowers that had opened were counted to determine the date of 50 % anthesis. On this date (9/11/2015), eight flowers of approximately the same age (up to one day after opening) were marked per cane using twist ties, for subsequent sampling.

### **2.2.3 Applying treatments**

In total 27 vines were selected across two rows (excluding vines at the end of the rows that may be influenced by edge effects). Two canes from each vine were randomly assigned one of five treatments.

Prior to the application of treatments, the average L/F ratio was recorded for 15 randomly selected canes after commercial thinning. This average was used as a basis for adjusting the L/F ratio on all canes involved in the experiment, to ensure all canes started with similar ratios at the beginning of the experiment, and before treatments were applied. While determining L/F ratios, leaves were included in the count if they were larger than 4 x 4 cm in size.

Five treatments were created and applied at the cane level. The treatments were high or low L/F ratios with a girdle applied either early or later during fruit development. The girdles were applied using a hand held girdling tool which removed a 5 mm strip of bark and phloem. This was applied between the start of each cane and the first shoot (Figure 2.1). The girdle was applied once and allowed to heal (Figure 2.2) and the leaf to fruit ratio adjusted on the same day, with the goal of causing an abrupt increase or decrease in carbohydrate supply

from the date of girdling until the girdle had healed. With all treatment canes, any neighbouring, shading shoots were trimmed. The treatments were:

“Control”: Two to three leaves per fruit and received no girdle

“1 High”: Early season (17/12/2015) with six leaves per and with a girdle applied

“1 Low”: Early season (17/12/2015) with one to two leaves per fruit and a girdle

“2 High”: Late season (3/02/2016) with six leaves per fruit and a girdle

“2 Low”: Late season (3/02/2016) with one to two leaves per fruit and a girdle



**Figure 2.1.** Recently applied girdle (2 weeks after application) on a 2 high treatment cane. Source: Le Lievre, D. 16/02/2016



**Figure 2.2.** Healed girdle approximately 10 weeks after girdle was applied (17/12/2015) to the 1 high treatment cane. Source: Le Lievre, D. 16/02/2016

#### **2.2.4 Fruit sampling**

Fruit were sampled at fortnightly intervals beginning two weeks after flowers were marked. To begin with, only the control treatment was sampled, until the other treatments had been applied.

On each sampling date a cane of each treatment was randomly selected from each of five randomly selected vines. Each cane was only sampled once during the experiment, and replicate samples were taken across randomly selected canes and vines, so that sampling did not affect the development of the remaining fruit.

From each of these canes a random sample of five fruit were harvested. While still in the field the fresh weight and dimensions (length and maximum and minimum diameters) of each whole fruit were measured. The length and diameters were measured using callipers, while the fresh weight was measured with a portable field balance. This was done as quickly as possible to minimize water loss, before each fruit was cut into longitudinal slices. The size of the fruit slices varied as the fruit grew. During the first several sample periods when fruit were small fruit halves were used to ensure there was enough tissue available for laboratory analysis. When the fruit were larger they were cut, longitudinally, into quarters.

A longitudinal slice from one fruit from each of the 5 canes of the same treatment, was chopped and combined into a bulk sample. This combined bulk sample was mixed and subdivided into two separate vials, half for compositional analysis and the other for dry matter content analysis. The fresh weight of the dry matter sample was weighed before subsamples were immediately frozen in liquid nitrogen.

This procedure was repeated five times for each of the different treatments, providing five replicate samples per treatment, per date. All the samples were then transferred to dry ice, transported to the laboratory and stored in a -80°C freezer.

#### **2.2.5 Dry matter**

The samples for dry matter analysis were freeze dried and the dry sample weighed. The dry matter concentration was calculated as the *dry weight/fresh weight\*100*.

### **2.2.6 Fruit harvest**

As the fruit approached the commercial harvest period (April – May) 20 fruit were sampled from each treatment, at weekly intervals for maturity testing. The fruit maturity was assessed by measuring the DMC, flesh firmness, flesh hue angle, and °Brix.

The DMC was measured on a 3 mm equatorial slice from each fruit, weighing the fresh weight, and then oven drying at 65°C for 24 hours and reweighing. The flesh firmness was measured using a penetrometer (7.9 mm probe, trigger threshold 50 g, forward speed 20 mm/s, reverse speed 30 mm/s, distance measured 7.9 mm) after removing 1 mm of skin and flesh. Flesh hue angle was measured with the Minolta chromameter CR2000 (Minolta, Ramsey, NJ, USA) using a C65 light source and the LCH colour system after 2 mm of skin and flesh had been removed from the fruit. Both the flesh firmness and hue angle were measured on two sides of each fruit, at 90° to the equator, and the results averaged. °Brix measurements were measured with a hand held refractometer, using several drops of juice from the stem and blossom end of the fruit, separately, and averaging the results to give an estimate of the fruits soluble solids concentration.

The industry maturity clearance standard for gold kiwifruit was used as a base for when fruit had reached harvest maturity. For this the average °Brix was greater than 7.5°, and the green fractile less than 111.1° for all of the 20 sampled fruit. °Brix and DMC were also used as indicators of readiness of the fruit for harvest.

### **2.2.7 Compositional analyses**

All samples were ground, staying frozen throughout the grinding process, then stored in a -80°C freezer until further analysis.

#### **Carbohydrate analysis**

Sub-samples, approximately 0.2 g ( $\pm 0.05$  g), of ground, frozen tissue were weighed out and internal standard fucose (20  $\mu$ l of 10mg/ml (10% iso-propanol)) added to each. Samples were extracted using 5 ml of 80% ethanol for an hour at 60°C, then centrifuged and the supernatant decanted. The pellet was washed again with 5 ml of 80 % ethanol and 2.5 ml 80% ethanol with the supernatant being

decanted between each. The supernatants were combined and used for sugar analysis, described below.

The remaining insoluble residue was used for the starch analysis. The residue was washed into an Erlenmeyer flask and autoclaved for 1 hour. Samples were then incubated in 5 ml of Amyloglucosidase in acetate buffer, at 60 °C for one hour. Samples, typically run at 5x dilution (50 µl sample and 200 µl 20% acetate buffer) and in duplicates, in disposable microCuvettes, along with 25 µl phenol solution and 750 µl of Trinders reagent. Colour blanks (same as samples but omitting the phenol and using 25 µl water instead) were also run for each sample. The mixtures were incubated at 37°C for 10 minute then left to stand at room temperature for 1 hour. The starch concentrations were then calculated by reading the samples at 510nm on the UV-visible Spectrophotometer UV-1650PC (Shimadzu Corp., Kyoto, Japan).

### **Soluble sugar analysis**

The sugar analysis was carried out on the 80 % ethanol supernatant obtained during the starch extraction described above. Appropriate subsamples to contain 3µg of internal standard were placed into QB well plates and dried in a centrifugal evaporator. These were then redissolved in 300µl of ultrapure water when ready to be run. The sugars were analysed using Thermofisher Dionex ICS 5000 with electrochemical detection, column PA20 with isocratic elution (Dionex Corp., Sunnyvale, CA, USA). Chromatographic peaks were used to identify sugars, using known retention times from standards of the sugars being analysed – galactose, fructose, glucose, sucrose, *myo*-inositol, raffinose and stachyose.

### **Organic acid analysis**

Sub-samples, approximately 1 g ( $\pm 0.2$  g) of ground, frozen tissue were weighed. Cold metaphosphoric acid (3 ml of 0.5%) was added to each sample. Samples were vortexed, sonicated for 20 minutes, vortexed again, and then shaken in a chiller, for 20 minutes. During these steps samples were kept cold and in low light levels. Samples were centrifuged at 3200 rpm at 4 °C for 15 minutes then the supernatant decanted off. The remaining residue was re-extracted with 2 ml of cold 0.5 % metaphosphoric acid and vortexed. Samples were again sonicated for 20 minutes, vortexed and shaken for 20 minutes. Samples were centrifuged and

decanted, with the supernatants being combined. Aliquots of the combined supernatants were centrifuged and transferred to vials for analysis by the Dionex HPLC.

### **Titrateable Acidity**

A 50  $\mu$ l sample of juice supernatant was obtained by centrifuging thawed juice from each ground tissue sample for 3 minutes at 13,400 rpm. Each sample was placed, in lots of four repeats into the centres of wells in a well plate using a positive displacement pipette. 20  $\mu$ l of 0.1% m-cresol purple dissolved in 20% ethanol was added to each well. The plate was then shaken on a vibrating mixer (IKA Vibrax-VXR with a Janke and Kunkle Typ VX1 plate holding attachment) and vibrated on the 600 dial. Titration of 0.01 M NaOH (using a Rainin EDP Plus electronic pipette) was carried out to the point at which a stable purple colour formed. Citric acid standards were also titrated to produce calibration factors used to convert titration volumes to citric acid equivalents.

### **2.3 Data analysis**

All results were examined by an analysis of variance (ANOVA) using R version 3.3.2, in order to determine if differences between treatments were present on each sampling date. All analyses were done at the 5 % level, and data was checked for normality and log transformed if necessary. All results were displayed as average  $\pm$  standard error.

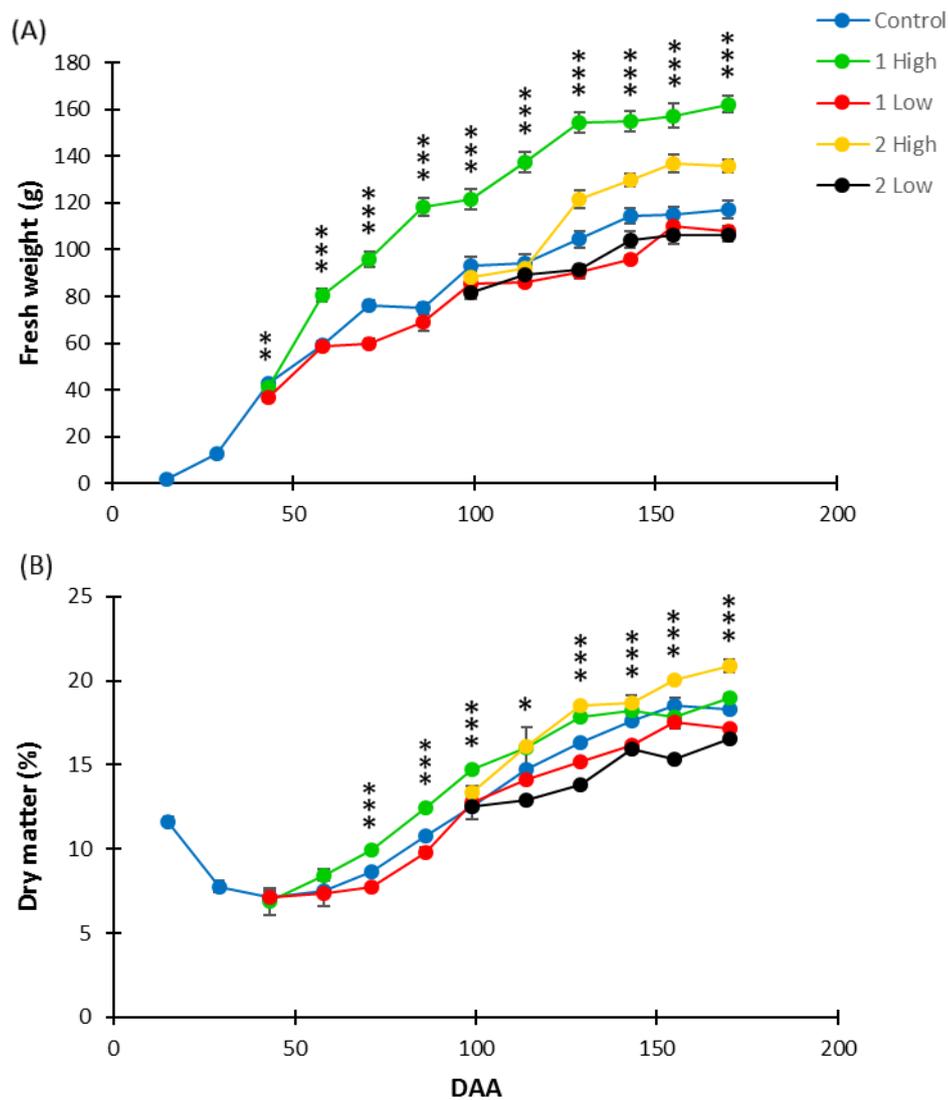
## **2.4 Results**

### **2.4.1 Fresh weight and dry matter concentration**

Fresh weight (FW) showed a period of rapid growth up to around 65-80 DAA, after which the growth rate slowed up until harvest. This general pattern was observed across all treatments.

Significant differences in FW were observed between treatments at all sample dates after the initial treatments had been applied (Figure 2.3a). The early leaf to fruit ratio treatments appeared to influence fruit FW growth more than DMC, whereas the later treatment caused stronger differences in DMC (Figures 2.3a,b). The 1 high treatment caused a strong and immediate influence on fruit size, remaining larger than all other treatments until final harvest (Figure 2.3a). However, the DMC in this treatment was only highest up until the second half of fruit development. The 2 high treatment also caused an initial increase in fresh weight following treatment application, but had a stronger effect on the DMC, which increased quickly to become the highest relative to the other treatments at harvest.

As expected, the low L/F ratio treatments decreased fruit fresh weight and DMC growth compared to control canes. The overall effects were not as pronounced as they were in the high L/F ratio treatments, however, as at harvest both low L/F ratio treatments had only slightly lower FW and DMC compared to the control treatment.

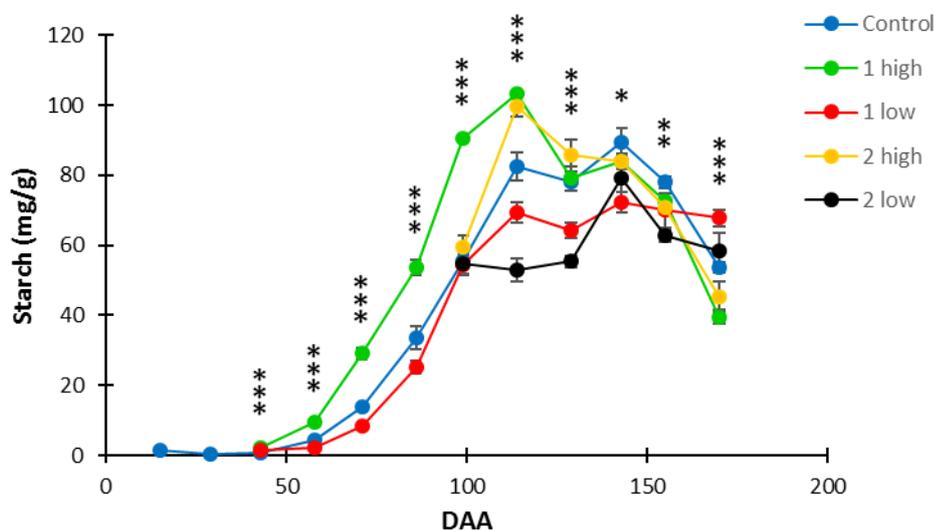


**Figure 2.3.** Changes in (A) fruit fresh weight and (B) dry matter concentration in “Gold3” kiwifruit (*Actinidia chinensis* Planch. var. *chinensis* “Gold 3”) sampled fortnightly from anthesis (November 2015) through to harvest (April 2016), from canes receiving five different pruning and girdling treatments (treatments described in methods above). Results are averages  $\pm$  standard error (SE); \* =  $P \leq 0.05$ , \*\* =  $P \leq 0.01$ , \*\*\* =  $P \leq 0.001$ . DAA= Days after anthesis.

## 2.4.2 Starch

During early development starch fell very slightly up to around 40 DAA, after which it increased rapidly for a period, before decreasing later in development (from around 115 DAA) (Figure 2.4). Starch concentrations differed significantly between treatments across all sample dates beginning from 43 DAA. Significance levels dropped later in development however, as several treatments were decreasing, while the others were still accumulating.

The girdling treatments had a strong impact on starch accumulation, with large differences seen between treatments immediately following application. The high L/F ratio treatment increased quickly while the low treatment decreased, relative to the control treatment. These effects appeared to decrease after several weeks. When the second girdle was applied the 2 high treatment was able to immediately accumulate to a similar peak starch concentrations as the earlier girdled 1 high treatment (Figure 2.4). Following the second treatment application the 2 low fruit appear to stop accumulating starch for four weeks, before recovering to higher concentrations than 1 low (Figure 2.4).



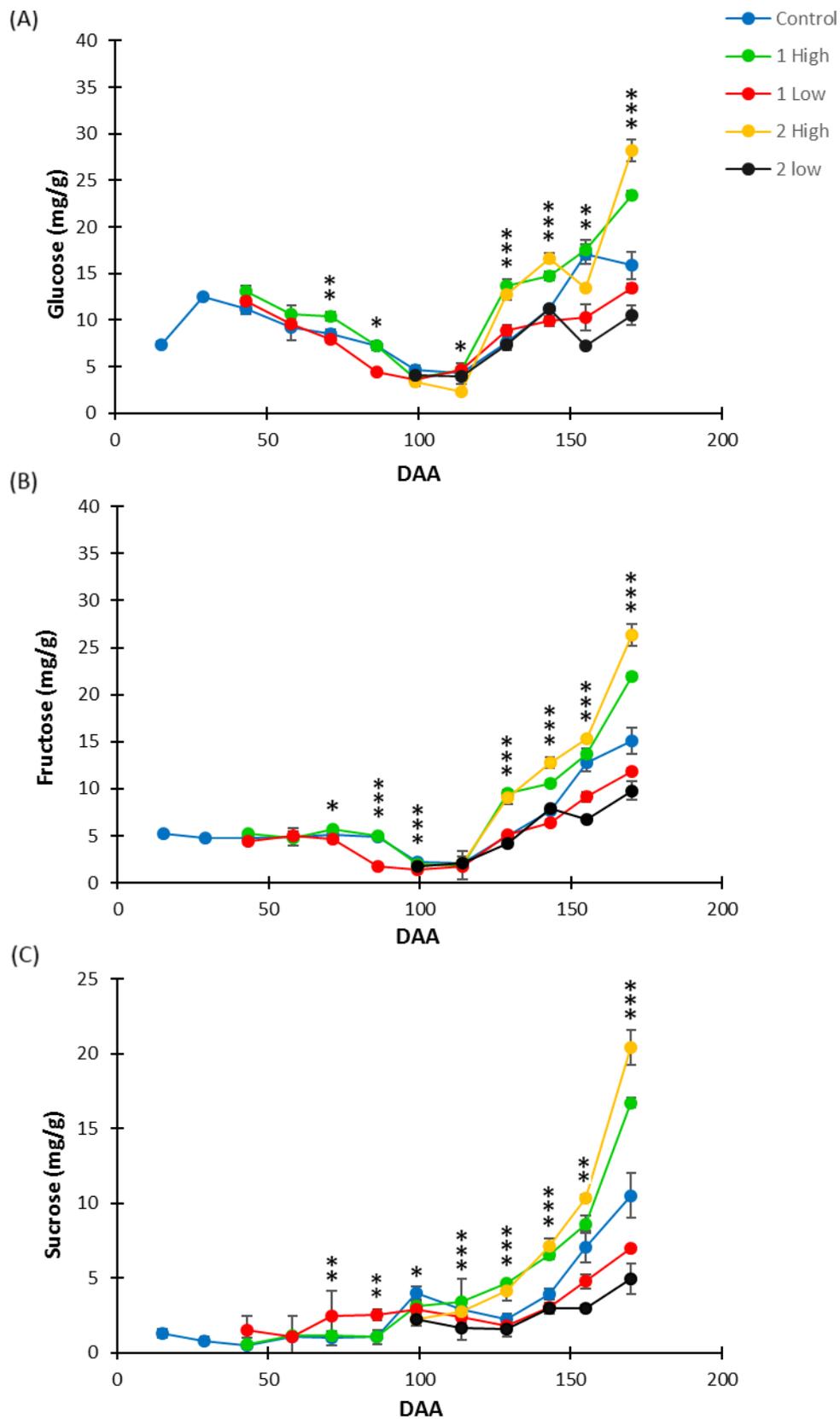
**Figure 2.4.** Changes in total starch concentrations in “Gold3” fruit sampled fortnightly from anthesis (November 2015) through to harvest (April 2016), from canes receiving five different pruning and girdling treatments (treatments described in methods above). Results are averages  $\pm$  SE; \* =  $P \leq 0.05$ , \*\* =  $P \leq 0.01$ , \*\*\* =  $P \leq 0.001$ ). DAA= Days after anthesis.

### **2.4.3 Sugars**

During early stages of development, glucose concentrations fell slowly from 30 DAA to around 115 DAA, while fructose concentrations remained low from 15 DAA before also dropping slightly around 115 DAA (Figure 2.5a,b). Sucrose also remained low and relatively constant up to around 75 DAA.

In general all three of these major sugars (glucose, sucrose and fructose) began to accumulate from around 115 DAA (Figure 2.5a,b,c), coinciding with the point at which starch began to decrease (Figure 2.5). At harvest the hexose sugars (glucose and fructose) varied between 10 and 30 mg/g of each, while sucrose ranged between 5 to 20 mg/g.

The leaf to fruit ratio did not appear to have a strong effect on sugar concentration during early phases of development, with only small differences seen between treatments up to around 100 DAA (Figure 2.5a,b,c). After this point sugars accumulated significantly faster in the high leaf to fruit ratio treatments.



**Figure 2.5.** Changes in concentrations of (A) Glucose, (B) Fructose and (C) Sucrose, in “Gold3” fruit sampled fortnightly from anthesis (November 2015) through to harvest (April 2016), from canes receiving five different pruning and girdling treatments (described in methods above). Results are averages  $\pm$  SE; \* =  $P \leq 0.05$ , \*\* =  $P \leq 0.01$ , \*\*\* =  $P \leq 0.001$ ). DAA= Days after anthesis.

#### 2.4.4 Organic acids

Overall quinic, oxalic and malic acid concentrations fell throughout development, and responded inversely to carbohydrate supply tending to fall when carbohydrate supply was increased and rise when carbohydrate supply was reduced. Citric acid showed a different pattern, increasing in concentration in a similar pattern to starch. Citric acid responded directly to carbohydrate supply, increasing in high L/F ratio treatments, and falling in low L/F ratio treatments

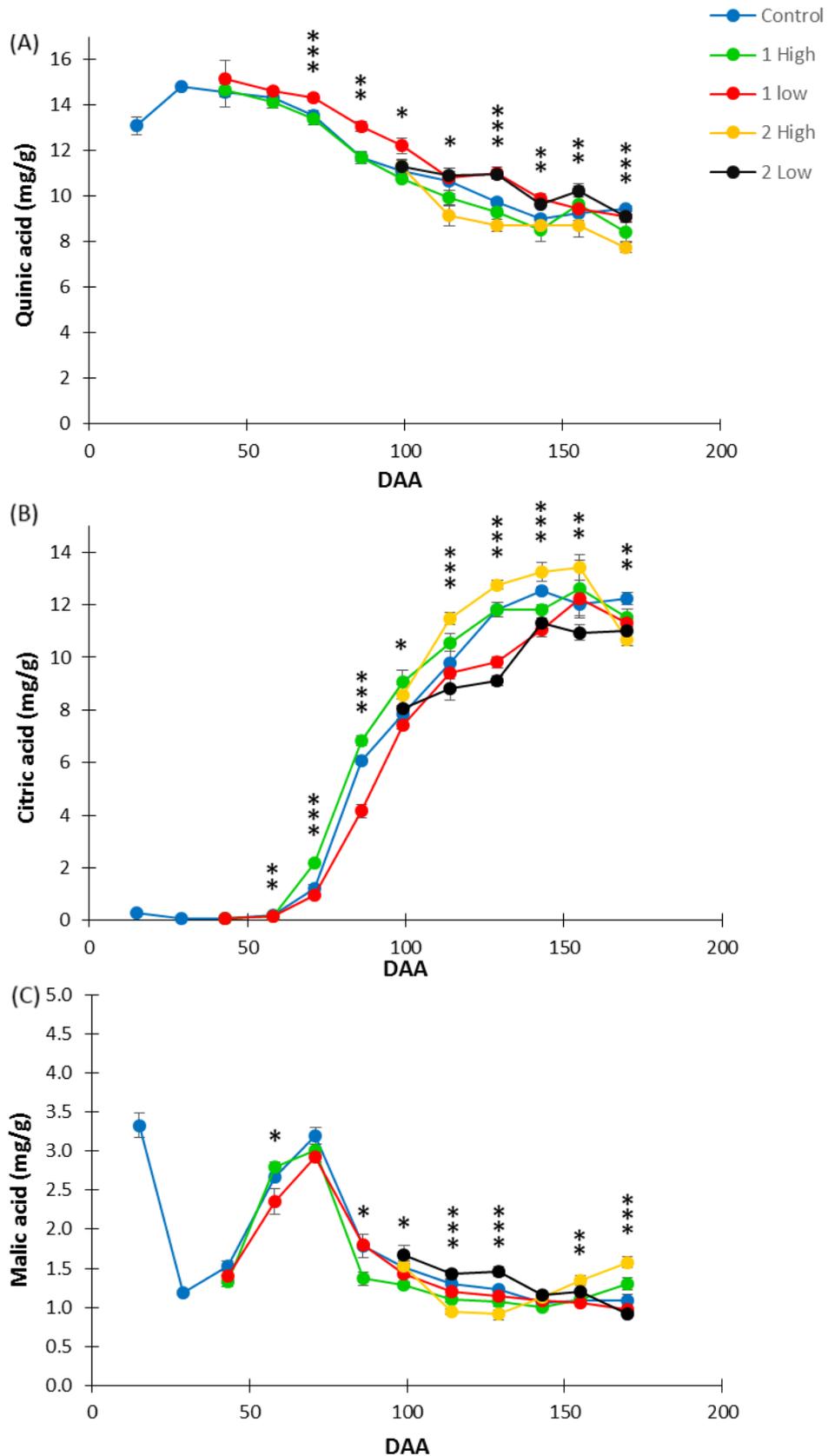
The five organic acids had distinct patterns of accumulation during fruit development. Quinic acid concentrations decreased steadily throughout the growing season, while citric acid concentrations remain low until around 60 DAA after which concentrations increase quickly before falling around 155 DAA (Figure 2.6a,b). Concentrations of malic acid rose rapidly from 30 DAA, followed by a quick fall around 70 DAA, around the same point that citric acid began accumulating (Figure 2.6b,c). In general the ascorbic acid concentrations rose from to 43 DAA before slowly dropping through to harvest while oxalic acid concentrations increased initially before falling slowly through to harvest (Figure 2.7a, b). The pattern in oxalic was very similar to the pattern seen in quinic acid accumulation, and also malic acid although the peak timings differ (Figure 2.6a,c and 2.7b).

Most of the acid concentrations appeared to be affected by treatments from early in development, as soon as the girdles and leaf to fruit ratio adjustments were applied. Quinic acid concentration was affected by L/F ratios, with both low L/F ratio treatments having accumulated more quinic acid compared to the high L/F ratio treatments (Figure 2.6a). Citric acid shows the opposite effect of altered L/F ratios, with higher concentrations in the high ratios during development (Figure 2.6b). Malic appears to be similar to quinic showing an inverse response to leaf to fruit ratio, however differences between treatment were less pronounced. The differences between treatments in acid concentrations tended to reduce by the final harvest date.

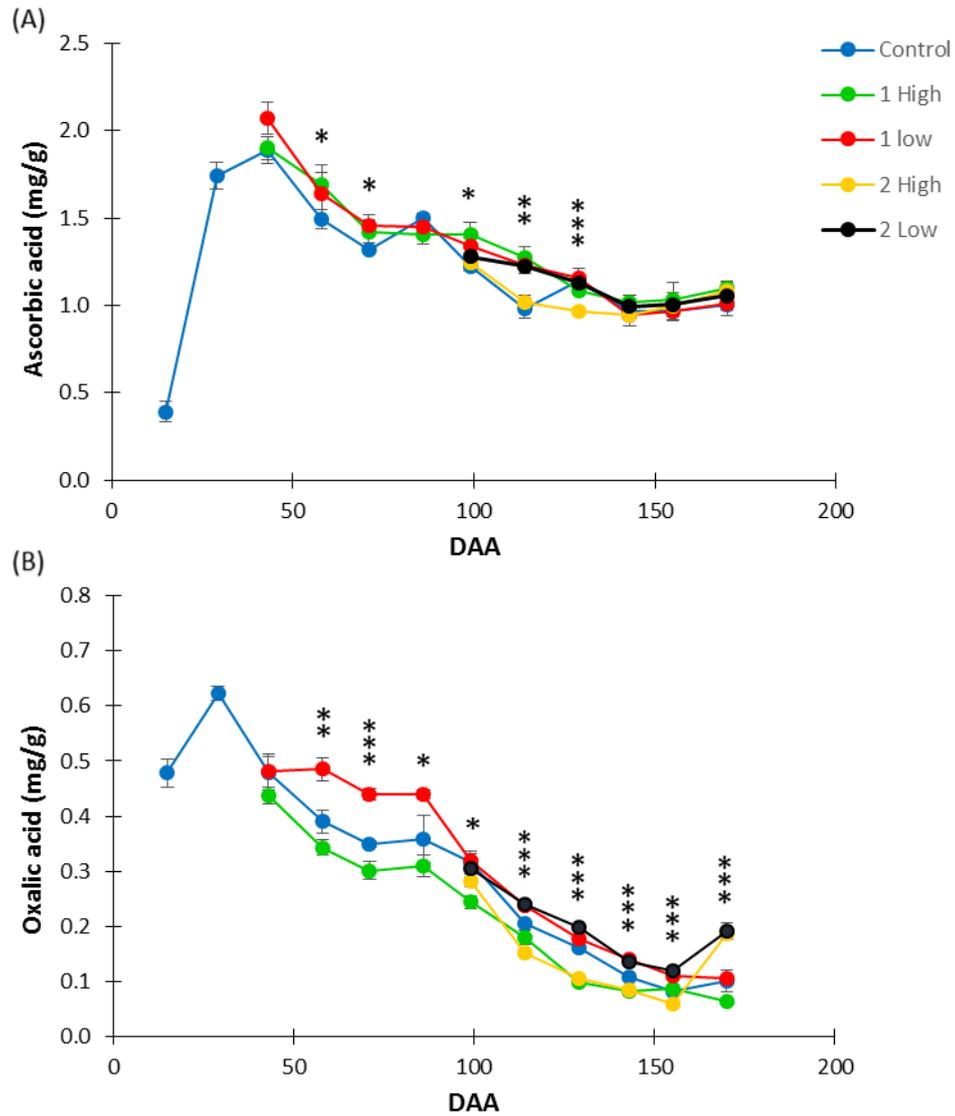
Leaf to fruit ratio had little constant effect on the accumulation of ascorbic acid, with smaller and more variable differences between the five treatments during

development and at harvest (Figure 2.7a). Highly significant differences between treatments were seen only at 114 and 129 DAA (Figure 2.7a).

Responses to leaf to fruit ratio changes in oxalic acid were similar to that of quinic and malic acids, with low L/F ratio treatments accumulating higher oxalic acid immediately following treatment application, and lower concentrations in the high L/F ratio treatments (Figure 2.7b).



**Figure 2.6.** Changes in (A) quinic acid, (B) citric acid, (C) malic acid in “Gold3” fruit sampled fortnightly from anthesis through to harvest, from canes receiving five different pruning and girdling treatments. Results are averages  $\pm$  SE. \* =  $P \leq 0.05$ , \*\* =  $P \leq 0.01$ , \*\*\* =  $P \leq 0.001$ ). DAA= Days after anthesis.

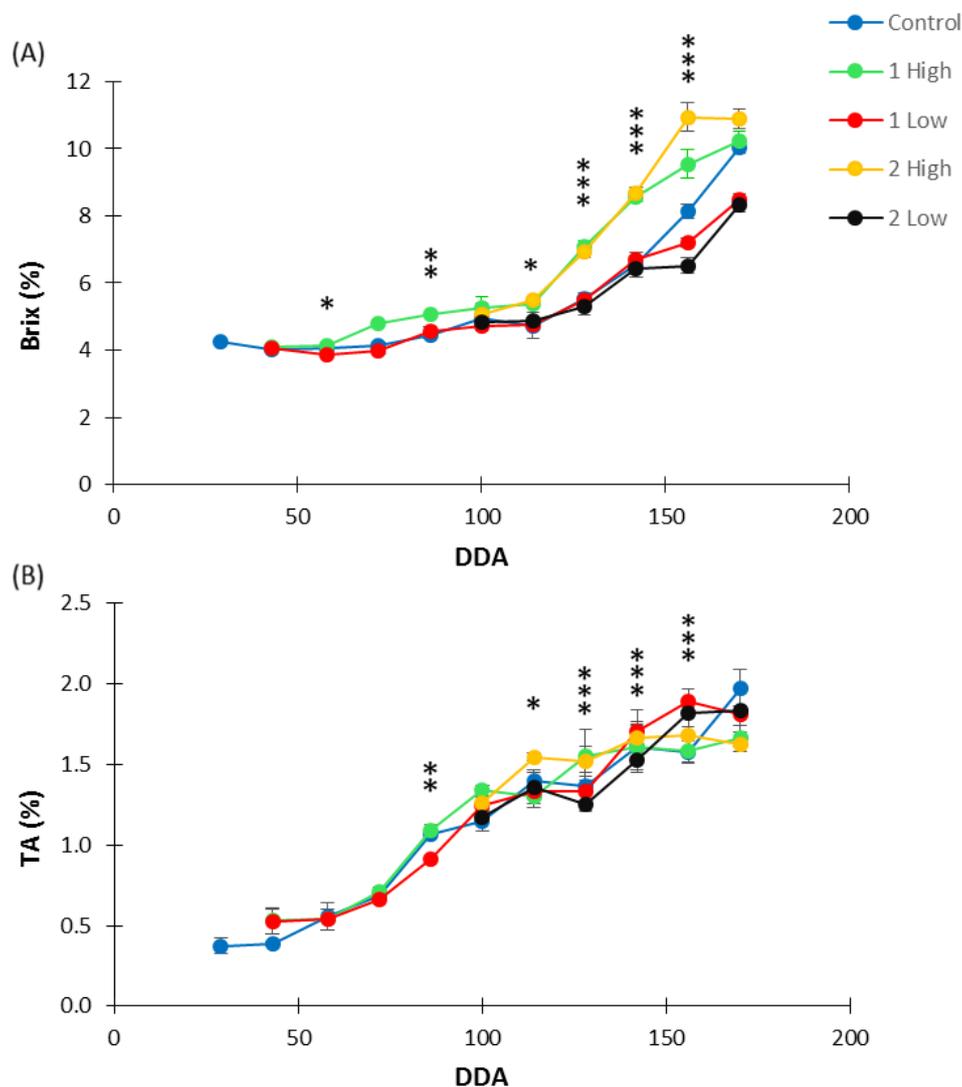


**Figure 2.7.** Changes in organic acid concentrations, (A) ascorbic acid, (B) oxalic, in “Gold3” fruit sampled fortnightly from anthesis (November 2015) through to harvest (April 2016), from canes receiving five different pruning and girdling treatments (treatments described in methods above). Results are averages  $\pm$  SE; \* =  $P \leq 0.05$ , \*\* =  $P \leq 0.01$ , \*\*\* =  $P \leq 0.001$ ). DAA= Days after anthesis.

## 2.4.5 Brix and Titratable acidity

Fruit °Brix remained relatively steady from 15 DAA through to 115 DAA (Figure 2.8a). After 115 DAA °Brix began to rise. The °Brix did not appear to differ significantly following L/F ratio changes, but did clearly differ later in development at the same time that starch loss began (Figures 2.4 and 2.8a).

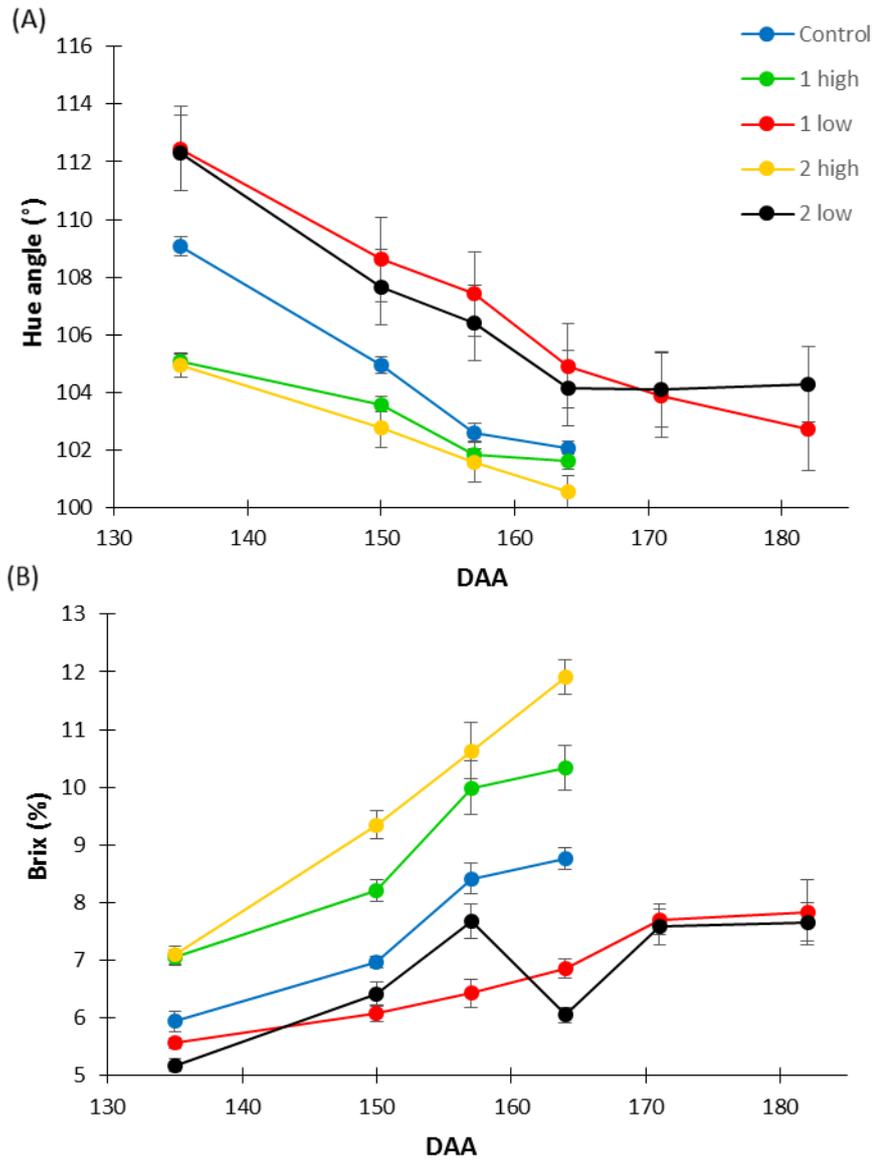
The titratable acidity (TA) began increasing slowly from 15 DAA through to harvest (Figure 2.8b). The L/F ratio did not have consistent effects on TA, with significantly higher TA on some dates with high L/F ratio, but TA then rose to higher levels in low L/F ratio treatments prior to harvest (Figure 2.8b).



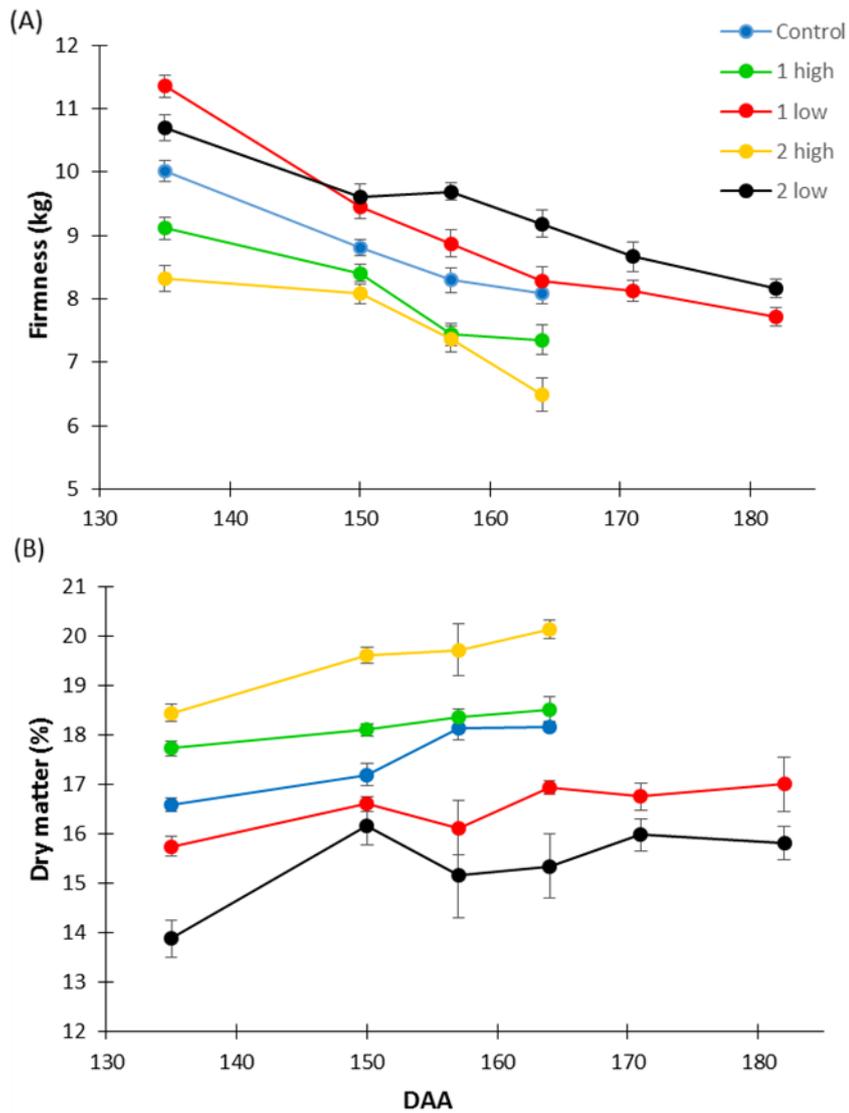
**Figure 2.8.** Changes in (A) °Brix and (B) titratable acidity in 3 fruit sampled fortnightly from anthesis (November 2015) through to harvest (April 2016), from canes receiving five different pruning and girdling treatments (treatments described in methods above). Results are averages  $\pm$  SE; \* =  $P \leq 0.05$ , \*\* =  $P \leq 0.01$ , \*\*\* =  $P \leq 0.001$ ). DAA= Days after anthesis.

#### **2.4.6 Fruit harvest**

All treatments exhibited similar general patterns of maturity leading up to harvest as the fruit firmness and hue angle decreased, while °Brix and DMC increased. A high leaf to fruit ratio advanced harvest maturity in the 1 and 2 high treatments, resulting in significantly lower firmness and hue angle, and higher DMC and °Brix compared to the control and low leaf to fruit ratio treatments (Figures 10a,b and 11a,b). The strongest effect was seen in treatments where the leaf to fruit ratio and girdle were applied later in development (2 high and 2 low), which had the biggest differences across all four measurements (Figures 2.9,b and 2.10,b). Based on these measurements the final harvest for fruit to be stored and used in the sensory testing was taken on 27/04/2016 for the control, 1 high and 2 high treatments, while the 1 low and 2 low treatments were harvested on the 09/05/2016.



**Figure 2.9.** Changes in (A) hue angle and (B) °Brix in “Gold3” kiwifruit leading up to harvest from canes receiving five different pruning and girdling treatments (treatments described in methods above). Results are averages  $\pm$  standard error (SE); \* =  $P \leq 0.05$ , \*\* =  $P \leq 0.01$ , \*\*\* =  $P \leq 0.001$ . DAA= Days after anthesis.



**Figure 2.10.** Changes in (A) firmness and (B) dry matter concentration in “Gold3” kiwifruit leading up to harvest from canes receiving five different pruning and girdling treatments (treatments described in methods above). Results are averages  $\pm$  standard error (SE); \* =  $P \leq 0.05$ , \*\* =  $P \leq 0.01$ , \*\*\* =  $P \leq 0.001$ . DAA= Days after anthesis.

## 2.5 Discussion

The results of this research show that changes in carbohydrate supply to developing fruit strongly influences fruit composition in unique ways that depend on the timing they occur during development. Overall “Gold3” fruit had similar patterns of starch, sugar and acid accumulation to other *A. chinensis* cultivars, with slight differences in timings and peak concentrations (Richardson et al., 2011). The fresh weight and starch accumulation responded positively to a period of high carbohydrate supply, as expected. However, it was shown for the first time that the response in organic acid metabolism is more complex, with some acid concentrations responding inversely to carbohydrate supply.

### 2.5.1 “Gold3” fruit development

Fruit growth during the season had a biphasic pattern of growth with an initial period of rapid growth from anthesis to around 70-80 DAA, followed by a period of slower growth up to harvest. This is similar to “Hort16A” kiwifruit where the initial period of fast growth continues to around 60 DAA, and is then followed by incremental growth during the second phase, which continues to 140 DAA (Richardson et al., 2011). This pattern is also seen in other species of *Actinidia*, with variations in the timing of the phases of growth (Boldingh, Smith, & Klages, 2000; McPherson et al., 2001; Nardozza et al., 2013; Richardson et al., 1997). During this initial increase in fruit size, growth is largely dominated by cell division. It is during this period that fruit size and sink strength is determined, and more water is coming into the fruit, relative to carbon inputs (McPherson et al., 2001; Minchin et al., 2003; Richardson et al., 2011).

Sucrose and starch concentrations decreased slightly during the first growth phase. This may have been to support the increase in glucose observed around the same time, which is in turn associated with cell division occurring (Nardozza et al., 2013). The glucose peak observed at 30 DAA during cell division is typical of kiwifruit (Klages, Donnison, Boldingh, & MacRae, 1998; Moscatello, Famiani, Proietti, Farinelli, & Battistelli, 2011; Nardozza et al., 2013; S. Nardozza et al., 2010). It has been suggested that this early glucose peak may be partly due to an increase in neutral invertase activity, likely to cleave the sucrose unloaded during this phase (Nardozza et al., 2013). Glucose concentrations begin to fall following

its peak, and as cell expansion takes over they continue decreasing up until late in the second growth phase. This drop in glucose is seen in other *A. chinensis* cultivars but not in other fruit, and may part of a signal for cell expansion to begin (Nardozza et al., 2013).

The second phase of growth, beginning around 70 DAA through to harvest, is characterised by incremental fruit growth as cell division has finished and cell expansion has begun (Nardozza et al., 2013; Richardson et al., 2004). A major component of this phase is that the starch began accumulating rapidly as this change from cell division to expansion began (Boldingh et al., 2000; Moscatello et al., 2011).

Malic and citric acid concentrations both increased significantly at different times during development (malic peaking much earlier than citric), and therefore do not appear to be diluted by growth. This malic acid peak is similar to what is seen in other *Actinidia* species, including “Hort16A”, “Hayward” and “Arguta” cultivars, which show accumulation of malic acid in young fruit (Marsh et al., 2007; Marsh & Harker, 2016; Richardson et al., 2011). This accumulation has been suggested to have an important role in the osmoregulation of fruit during the expansion stage (Nardozza et al., 2013; S. Nardozza et al., 2010). Quinic acid, however, decreased in concentration from 40 DDA through to harvest. The decrease appears to be caused by quinic acid production not keeping up with growth and being diluted as water and carbohydrates enter the fruit. When quinic acid concentrations were expressed as grams per fruit (not shown), quinic acid per fruit increased up to around 75 DAA, before plateauing until harvest, similar the pattern seen in other cultivars (Richardson et al., 2011).

Late during the second phase of growth typical kiwifruit ripening processes began to occur, including: flesh colour changing to lower hue angles, firmness dropping, fruit size plateaued, and the starch accumulation slowed and then dropped. These changes were expected and the same as is seen in other cultivars leading up to harvest (Richardson et al., 1997). As the starch was metabolized and concentrations began to decrease, this caused the soluble sugar concentrations (sucrose, fructose and glucose) to increased exponentially from this point through to harvest (Macrae, Lallu, Searle, & Bowen, 1989). This occurred slightly earlier,

but with a similar pattern, to that seen in “Hort16A” fruit, in which total sugars begin increasing from 130 DAA and increased more rapidly from 160 DAA (Richardson et al., 2011). Typically sugar concentrations continue to increase, past harvest up until all starch has been converted into soluble solids by the eating ripe stage (Macrae & Redgwell, 1992; Richardson et al., 2011).

### **2.5.2 Effects of carbon supply on development**

As expected a large difference in both fresh weight and DMC was observed between the treatments due to the varying carbon supplies produced by the different leaf to fruit ratios applied, as seen in other studies (Boyd & Barnett, 2011; Buwalda & Smith, 1990; Cooper & Marshall, 1992; Famiani, 1997; Tombesi et al., 1993a).

Fruit FW has been shown to be largely determined during early development, while DMC is strongly affected later, during the second phase of development in kiwifruit (Currie, 1997; Currie, Jackson, Max, Blattmann, & Seymour, 2008; Snelgar et al., 2012). Similar patterns are seen in the developmentally similar grape berries (Carreño, Faraj, & Martinez, 1998). The early girdled, high L/F ratio fruit were considerably larger than fruit from all other treatments due to the treatment being applied early in fruit development when fruit are rapidly increasing in size. During this phase rapid growth is seen as cell division occurs and water being imported into the cells (Coombe, 1976). Fruit size is determined largely by cell number (and also size), so the early treatments which were applied during these vital stages of cell division affected cell number and in turn growth (Coombe, 1976; McPherson et al., 2001). At this point DMC accumulation is low compared to FW increase (Carreño et al., 1998; Currie, 1997). Later in development when growth has slowed however, carbohydrates being imported into the fruit contribute more to fruit DMC as these carbohydrates are being stored as starch (which along with water content contributes considerably to DMC) (Currie, 1997; Lai et al., 1989). It is likely because of this that L/F ratio treatments applied later in development had greater effect on DMC content than growth (Carreño et al., 1998).

The high L/F ratio treatments appeared to have a greater effect than the low L/F ratio which appear to produce fruit with only slightly lower FW and DMC

compared to the control treatment. This may be due to the control treatment having a similar L/F ratio (2 to 3), to the low treatment (1 to 2), and both relatively low compared to the minimum range required (2 to 4 leaves) to support normal fruit development (Famiani, 1997; Woolley & Cruz-Castillo, 2006).

Alterations to carbohydrate supply also had a strong, direct effect on starch accumulation (Richardson et al., 1997). However, the effects of the girdle appeared to decrease after four to six weeks, likely as the girdles were allowed to heal and carbohydrates were again able to travel to or from other parts of the plant. Starch accumulation appeared to be labile and able to recover and accumulate similar starch concentrations to the early girdled high L/F ratio treatments, immediately in the second high L/F ratio, and after 4 weeks in the low L/F ratio (likely after the girdle had healed). This ability to recover is important as the starch peak concentration influences the final fruit composition after starch is hydrolysed into the soluble sugars.

Sugar concentrations appeared largely unaffected by the altered carbohydrate supply during early stages of development. Previous research has also shown that on orchard practises appear to be constrained in their ability to change soluble sugar concentrations significantly (Richardson et al., 1997; Thorp, Barnett, & Miller, 2003). The sugars may be highly regulated during this period of growth because they have an important role in primary metabolism, so concentrations cannot be too variable. Sucrose is particularly important because of its role in both cell division and the early accumulation of glucose (Nardozza et al., 2013). The differences in sugar concentrations observed between treatments later in development are likely due to differences in starch peak accumulation, which was significantly affected by changes in carbohydrate supply (Richardson et al., 1997). This is similar to other studies of fruit with different dry matters, which typically had lower sugar concentrations and lower perceived sweetness in low DMC fruit (Nardozza et al., 2011). The differences created will likely influence the final fruit taste and consumer liking as it is widely recognized that consumers respond positively to increased sweetness (Harker et al., 2009; Marsh et al., 2006; Wismer et al., 2005).

Unlike the sugars, organic acids were shown to be strongly affected by differences in carbohydrate supply to the developing fruit throughout development. It also appeared that organic acids may be able to be separated into two groups depending on their responses to increased carbohydrate supply. When fruit were starved of carbohydrates the quinic, oxalic, and to a lesser extent malic acid concentrations were inversely affected. These same acids also decline during development while citric acid responded positively to the high carbohydrate supply, and increases throughout development until decreasing post-harvest (Marsh & Harker, 2016).

Leading up to harvest some cross over was observed between treatments for the different organic acids. In citric acid this is explained by concentrations decreasing post-harvest as fruit ripen, making concentrations appear similar between treatments around harvest as concentrations in earlier maturing fruit have already begun to decrease (Marsh et al., 2004). Another possible reason for why concentrations appear more similar at harvest is that the girdles were allowed to heal resulting in compensatory responses as carbohydrate supplies to the fruit returned to normal.

Although the TA was not shown to be consistently affected by treatments, the ratios of individual organic acids were, particularly citric acid increasing in response to higher carbohydrate treatments and quinic decreasing. Quinic and citric acid are the major acids present in kiwifruit (each between 40-50 % of total acidity), so altered ratios of these acids may effect flavour perception in the final fruit (Marsh et al., 2004). Several studies have shown that an increase in quinic acid lowers the perceived sweetness in “Hort16A” fruit pulp, as well as increasing perceived acidity slightly relative to citric or malic acid, and decreasing the characteristic “Hort16A” and “Hayward” flavours (Marsh et al., 2003; Marsh et al., 2006). However, other studies have suggested that citric or malic acid are the most important influence on acidity, or that the ratio of quinic to citric acid has very little impact on consumer preference (Marsh et al., 2004; Marsh & Harker, 2016). Increased quinic acid is perceived differently than the addition of malic or citric acid, with consumers describing the flavour as chalky, aspirin like, fizzy or bitter (Marsh et al., 2003; Marsh et al., 2006). This is different to citric acid which is described as sharp, fresh and lemony, while malic acid is lemony, tangy, bitter,

sharp and green apple like (Marsh et al., 2003). The different influences of these on perception of fruit flavour and acidity have been suggested to be partly due to the different chemical characteristics, as quinic is a monocarboxylic acid, while citric and malic are tricarboxylic and dicarboxylic acids respectively (Marsh et al., 2003; Marsh & Harker, 2016).

Although there have been contrasting findings on which acid contributes most significantly to the perception of flavour, clear differences in the perception of citric and quinic acid and their associated tastes are recognized. This suggests that the changes to the ratio of acids observed by decreasing carbohydrate supply is likely to create different tastes in the final ripe fruit. This may explain why poor flavour has been observed in low DMC “Gold3” fruit. In a normal orchard which has variable crop loads or shoots that are starving at important points throughout the season, this may promote quinic and oxalic acid accumulation while decreasing citric acid concentrations, altering the ratios of acids and the final flavour of fruit. Along with this change in acid ratios, starch (and in turn sugars) responded positively to increased carbohydrates which resulted in the low DMC fruit having much lower concentrations of sugars. As described earlier kiwifruit flavour is largely determined by the balance between sugars and acids and it is widely recognised that higher sweetness is a key driver of consumer acceptance. So this change in these acid ratios combined with the decrease in starch, and once metabolized, sugar concentrations, would likely adversely affect taste in fruit growing with limited carbohydrate supply (Jaeger et al., 2003; Marsh et al., 2006).

### **2.5.3 Fruit harvest**

Pre-harvest measurements (hue, firmness, °Brix and DMC) were measured weekly until they reached the industry maturity standards specified by ZESPRI®. The criteria used as the primary determinant of readiness of the fruit for harvest was the green fractile being below 111.1, and °Brix above 7.5. Meeting the green fractile requires 90% of all fruit sampled to have hue angles below the 111.1°. The °Brix reaching 7.5, for all of the 20 sampled fruit. The control, 1 high and 2 high treatments all reached this at roughly the same time and as a result were harvested together on the 27<sup>th</sup> April 2016, 170 DAA. The 1 low and 2 low treatments appeared to be slowed by the treatments placed on them so it was anticipated that the fruit may not reach the necessary maturity levels for harvest.

Because of this these low leaf to fruit ratio treatments were harvested 12 days after the other three treatments (184 DAA), when the hue, firmness, °Brix and DMC changes appeared to be plateauing. Harvesting fruit at similar levels of maturity is considered a critical factor in their behaviour during post-harvest storage. Our goal was to present fruit to consumers for sensory evaluation at similar levels of ripeness (Chapter 3), independent of any treatment effects on post-harvest storage performance. Hence the fruit were harvested on different dates but at similar maturities.

#### **2.5.4 Limitations**

As the study was only carried out over one growing season and on relatively young “Gold3” vines (only 3 years old) there are limitations to the quality and quantity of data. Typically growth and development data is carried out over multiple seasons on the same vines to ensure reliable data and patterns. If the 2015/2016 season was particularly affected by temperature, drought or any other unique pressures the fruit development would have been affected.

#### **2.6 Conclusions**

This study had two main objectives. The first was to describe the growth and development of “Gold3” fruit from flowering to harvest, and the second was to identify what influence changing carbohydrate supply had on the accumulation of both the sugars and acids, and when these changes occurred.

Overall “Gold3” fruit was shown to have similar patterns of starch, sugar and acid accumulation to other *A. chinensis* cultivars, particular the other gold kiwifruit cultivar “Hort16A”. The results demonstrated that altered carbohydrate supply during fruit development influences composition of fruit in different ways, when applied at different stages of development. The fresh weight and starch accumulation were shown to respond positively to a period of high carbohydrate supply, as expected based on past research. However, it was shown for the first time that starch and acid metabolism respond in different ways. There is also evidence that the acids could be separated into two groups depending on their responses to increased carbohydrate supply, as quinic, oxalic and malic acid were inversely affected by carbohydrate supply while citric showed the opposite response.

This may be the reason why poor flavour is observed in “Gold3” fruit with variable crop loads as a high crop load, or shoots that are starving throughout the season, may promote quinic and oxalic acid accumulation while decreases citric acid concentrations, altering the ratios of acids and the final flavour of fruit.

Further research into acid metabolism, and the possibility that acids can be grouped based on their responses to carbohydrate supply, is needed to fully understand acid accumulation and its contribution to poor flavour in small “Gold3” fruit. Future studies could also keep girdles open to observe what effect this has on the fruit composition at harvest, and whether the differences created by the changes in carbohydrate supply were lasting.

## **Chapter Three: Post-harvest fruit composition and sensory experiment**

### **3.1 Introduction**

Flavour is commonly described as the key driver of consumer liking in most fruit. Producing fruit with consistently good flavour is therefore vital for the success of any fruit or vegetable industry. Kiwifruit flavour is largely determined by the dry matter concentration (DMC), mineral composition, sugar-acid ratio, volatile content, and water content. Few consumer studies have been carried out for “Gold3” fruit but it is assumed consumer’s preferences will be similar to that in other kiwifruit cultivars, particularly the other gold cultivar “Hort16A”.

It is widely recognised in kiwifruit that consumers respond positively to increased sugar content, with all three major sugars (sucrose, glucose and fructose) being described as having similar effects on sweetness (Harker et al., 2009; Marsh et al., 2003; Marsh et al., 2006). In kiwifruit the organic acids (primarily citric, quinic and malic acids) are found in relatively high concentrations (1-3 % of fresh weight), and this strongly contributes to the characteristic kiwifruit flavour (Marsh et al., 2004; Marsh et al., 2006; Rossiter et al., 2000). However, increased acidity can decrease the perception of sweetness and result in decreased consumer liking (Marsh et al., 2006). The balance between these sugars and organic acids is therefore a key determinant on final flavour and consumer perception of fruit quality.

Understanding what flavours consumers prefer and what differences they are able to perceive is important for growers to understand when applying different on orchard practises such as thinning, girdling and pruning. Particularly important is how the timing and intensity of these applications during the growing season affects fruit growth. These practises are known to affect fruit DMC and the accumulation of flavour components by changing the amount of carbohydrates going to each ‘sink’ (fruit), or by increasing or decreasing resource competition between fruit (Cruz-Castillo et al., 2010; Snelgar et al., 2012). Understanding of how variation in carbohydrate supply during development can affect the ratios of flavour components, and in turn consumer liking in fruit at eating ripe, is limited.

As described in chapter two, the treatments in this study altered carbohydrate supply and had similar effects to those seen in other kiwifruit cultivars (Burge et al., 1987; Cruz-Castillo et al., 2010; Minchin et al., 2010). A decreased carbohydrate supply resulted in smaller fruit with lower DM and starch concentrations (and in turn lower soluble sugar concentrations), as well as increased acid concentrations. The decreased carbohydrate supply also affected the ratio of the individual organic acids, with quinic, oxalic and malic acids responding positively, while citric responded negatively. It has been noted that in “Gold3” kiwifruit that low DMC fruit often have poor flavour mainly due to lower sugar concentrations. This poor flavour may be particularly bad due to not only the low DMC, but also the higher acidity combined with lower sugar concentration.

The aim of this research was to carry out a consumer sensory experiment on untrained panellists to see if they were able to perceive differences between the treatments created, and if these changes influenced consumers liking. Using consumers is useful as these are the preferences and opinions that drive decisions on whether people buy kiwifruit at the supermarket. However, trained panellists are able to identify smaller differences in flavour components and provide more robust results. The experiment used a range of scales and approaches, including hedonic scales and check-all-that-apply questions with randomised order to reduce the influence of misinterpreted results or subconscious biases possible in each approach (Harker et al., 2009; Meyners & Castura, 2014). For example, end-of-scale avoidance in hedonic scales, or attributes consumers consider appropriate for fruit not indicating intensity differences (Meyners & Castura, 2014).

As the flavour of fruit, particularly the sugar: acid ratio was the focus of this study, the fruit firmness and ripeness were controlled as much as possible due to their strong influence on flavour and consumer liking (Stec, Hodgson, Macrae, & Triggs, 1989; Wang, MacRae, Wohlers, & Marsh, 2011). This was controlled by ripening fruit to similar levels and then only using fruit with firmness levels between 0.6 and 0.9 kgf.

Based on past sensory studies it is expected that the differences in composition between treatments are likely to alter the flavour perception of fruit at eating ripe

(Marsh et al., 2006; Nardoza et al., 2011; Rossiter et al., 2000). It is expected that the treatments receiving increased carbohydrate supply will have higher consumer liking compared to the fruit which receive decreased carbohydrate supply. It is also hypothesized that the reduced carbohydrate supply will cause lower DMC, and will make the fruit taste blander, and also alter the acid to sugar ratio which will cause the fruit to taste bad.

## **3.2 Methods**

### **3.2.1 Fruit harvest and storage**

Fruit from each treatment were harvested on their respective harvest dates. This was the 27<sup>th</sup> April 2016 for the 1 high, 2 high and control treatments, while the 1 low and 2 low treatments were harvested on the 12<sup>th</sup> May 2016. On their specific harvest dates approximately 150 fruit were randomly picked of each treatment. These fruit were packed into single layer trays with polyliners, and stored in a cool store with temperatures maintained at 1 - 2 °C until use in the consumer preference experiment. During storage fruit were assessed every 2 - 3 weeks for the presence of rots or storage disorders on fruit.

Leading up to the consumer taste test, fruit had been stored for approximately 10 week and were assessed to see whether treatment applications had affected the ripening process of the fruit. For this the flesh firmness, DMC, flesh hue angle and °Brix were measured. The DMC was measured on a 3 mm equatorial slice from each fruit, weighing the fresh weight, and then oven drying at 65°C for 24 hours and reweighing. The flesh firmness was measured using a penetrometer (7.9 mm probe, trigger threshold 50 g, forward speed 20 mm/s, reverse speed 30 mm/s, distance measured 7.9 mm) after removing 1 mm of skin and flesh. Flesh hue angle was measured with the Minolta chromameter (Minolta, Ramsey, NJ, USA) using a C65 light source and the LCH colour system after 2 mm of skin and flesh had been removed from the fruit. Both the flesh firmness and hue angle were measured on two sides of each fruit at 90° to the equator, and the results averaged. °Brix measurements were measured with a hand held refractometer, using several drops of juice from the stem and blossom end of the fruit, separately, and averaging the results to give an estimate of the fruits soluble solids concentration.

The treatments (1 and 2 low) whose ripening appeared to be slower were removed from the cool store and stored at room temperature for 7 days to allow them to catch up to the other treatments. Over this time 20 randomly selected fruit from each treatment were monitored at 2 day intervals to ensure they did not over ripen. This is important to ensure that treatments flavour perception by consumers was not confounded by different firmness or ripeness levels. When all treatments were

at similar levels of ripeness they were stored together at room temperature for 5 days, up until the consumer sensory test.

### **3.2.2 Consumer sensory test**

The consumer preference experiment was carried out once fruit had ripened to similar levels and the dry matter percentage, flesh firmness and °Brix concentration had been measured. Only fruit with a flesh firmness between 0.6-0.9 kgf were used in the taste test experiment.

The experiment was carried out over four days (28<sup>th</sup>, 29<sup>th</sup> June, 1<sup>st</sup> 2<sup>nd</sup> July 2016) approximately 12 weeks after harvest. The selected fruit were prepared each morning with half of each fruit being used for the sensory experiment, while the second half was used to measure the soluble solids, °Brix, firmness, colour and dry matter (Figure 12). These measurements were carried out immediately and a combined juice sample from both the stem and blossom ends of each fruit, was frozen for later analysis using Fourier Transform Infrared Spectroscopy (FTIR), analysis described below.

The second half of each fruit was washed and sliced into wedges (Figure 12). The fruit was presented at room temperature and in plastic containers with random three-digit codes corresponding to the different treatments. These were served, one at a time, to volunteer participants to taste and fill out a short questionnaire (appendix 1) about fruit flavour. Each participant repeated this for fruit from each of the five treatments, given to participants in a randomised order. Participants were provided water and a plain water cracker between each sample to cleanse their pallet.

The questionnaires used both “Just About Right” (JAR) and “Check-All-That-Apply” (CATA) question formats (Questionnaire used attached as appendix 1). The overall liking was measured on a nine point hedonic scale ranging from 1= “Dislike extremely” to 9=“Like extremely”. The acidity, sweetness, flavour intensity and ripeness were measured using a JAR scales ranging from 1=“Much too low”, 3= “just about right” and 5= “Much too high”.

The terms used in the CATA sensory descriptors included a range of odour (tropical, lemon/lime, grassy/green), flavour (tropical, metallic, lemon/lime,

grassy/green, bland), taste (sour/acidic, sweet) and texture (juicy, fresh, crunchy, soft, mushy, under-ripe). The order of these attributes were randomised between surveys as to avoid response bias.

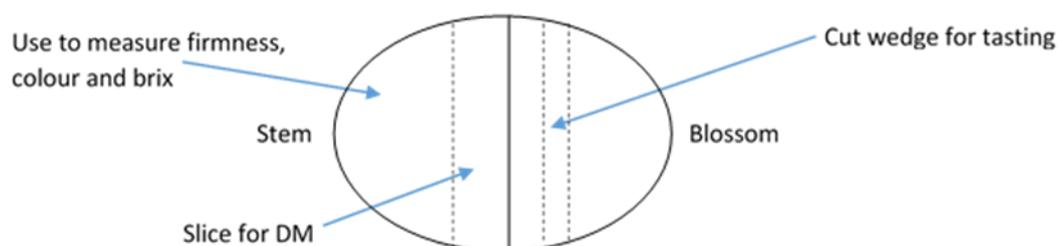
In total 78 untrained participants were surveyed, 18-20 people per day. Response of panellists were discarded due to incomplete responses in several of the survey sections. The participants of the sensory trial composed of panellists aged 18-84 years (59 % aged 18-30 years, 16% in the 31-45 year age group and 24 % 46 years or older), with an approximately even ratio of males and females (53% and 47% respectively). Of these 28 % reported eating kiwifruit commonly (at least once a week when in season), 32 % ate kiwifruit occasionally (one to three times a month), and 40 % reported eating kiwifruit rarely (once every few months to never). Of the participants 48 % reported to really like gold kiwifruit, 32 % moderately like gold kiwifruit, 15 % neither like nor dislike gold kiwifruit, and 5 % did not like gold kiwifruit.

Ethical approval was gained from the University of Waikato Human Research Ethics Committee, and the project adhered to the University of Waikato Human Research Ethics Regulations 2008 and the ethical guidelines of the NZARE.

FTIR analysis was used on the juice of each fruit used in the taste test to give estimates of the soluble sugar concentration, individual concentration of glucose fructose and sucrose, TA, and the individual acids, malic, quinic and citric acid. Several drops of thawed juice was centrifuged (3 minutes at 13,400 rpm) to separate insoluble material from juice. This juice was placed on the heated stage (fixed at 31 of a Bruker Alpha spectrometer, (Burker Corporation). Each sample took 52 seconds to run (20 seconds for sample warming time and 32 seconds for data collection). Samples were run in batches of 12, with a water sample being run in between batches to remove any spectral interferences such as temperature change (Clark, 2016). The models used for prediction of juice composition (SSC, TA, sugars and acids) were calibrated against analytical chemistry measurements of juice composition for ripe “Gold3” juice (Clark, C., Plant and Food Research).

### 3.2.3 Data analysis

An analysis of variance (ANOVA) was carried out using R version 3.3.1 on the compositional data. Where significant differences were found Tukey's test was carried out to identify where these differences were.



**Figure 3.1.** Fruit distribution plan for sensory experiment. Blossom end was used for tasting and stem end for measuring the firmness, colour, °Brix and DMC. Combined juice samples from both ends were frozen for FTIR analysis.

A linear mixed effects model was fitted to the overall liking scores, with a random effect for subject. An ANOVA was carried out using R version 3.3.1 on the overall liking. A correspondent analysis was carried out on the CATA data, with Cochran's q test used to identify whether each individual descriptor was chosen differently between treatments. JAR responses were merged to a three point scale and analysed using frequency tables and a penalty analysis. Scores that included less than 20 % of consumers were disregarded.

### 3.3 Results

#### 3.3.1 Compositional data for fruit used in sensory test

Increased carbohydrate supply early and late in fruit development both resulted in lower titratable acidity (TA) in fruit at eating ripeness (Table 3.1). The low L/F ratio treatments had similar TA percentages to the control treatment (Table 3.1). Significant differences were also observed between treatments in the firmness and hue angle of fruit at eating ripe (Table 3.1). However, there were no consistent differences in ripeness between the control, high and low leaf to fruit ratio treatments, except that the hue angle of control fruit was higher (greener) than the other four treatments (Table 3.1). Overall the differences in instrumental measurements of fruit ripeness were small and unlikely to affect sensory responses (Harker et al., 2009; Stec et al., 1989).

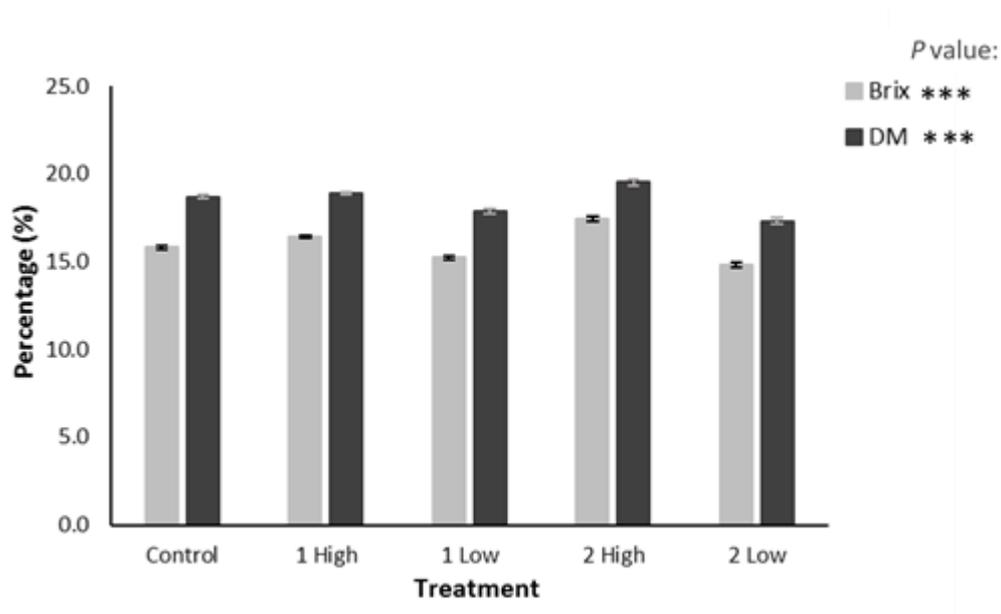
As expected °Brix and soluble sugar content (SSC) responded positively to increased carbohydrate supply, with higher °Brix and SSC in high L/F ratio treatment fruit at eating ripe, compared to the control treatments (Figure 3.2). The low L/F ratio treatment showed decreased °Brix and SSC in response to a decreased carbohydrate supply. The individual sugar concentrations also showed significant responses to the altered carbohydrate supply (Figure 3.3). The glucose concentrations showed less significant response to the treatments, with slightly lower final concentrations in the low L/F ratio treatment compared to the control and high L/F ratios (Figure 3.3). Sucrose responded positively to increased carbohydrate supply, with highly significant differences between the 1 and 2 high treatments and the rest of the treatments. The sucrose concentrations were similar between the control and low L/F ratio treatments. Fructose concentration in fruit at eating ripe was significantly higher in the control treatment compared to both the high and low L/F ratio treatments (Figure 3.3).

The three major organic acids also differed significantly in concentration between fruit with altered carbohydrate supply (Figure 3.4). Small but still significant differences were observed between the malic acid concentrations of treatments in fruit at eating ripe, with concentrations increasing in response to increased carbohydrate supply. Both quinic and citric acid concentrations were decreased in

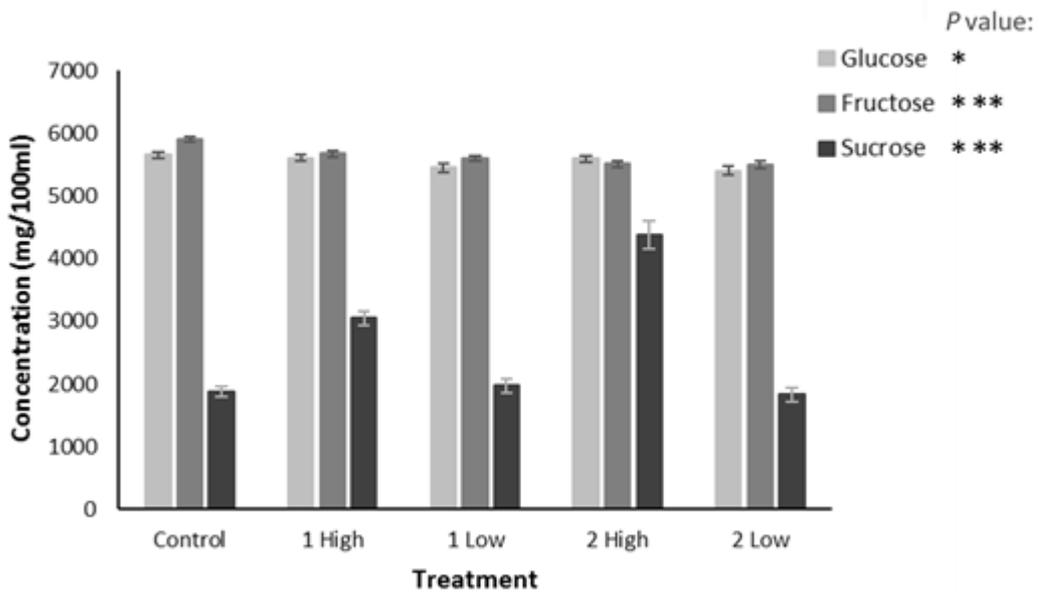
response to increased carbohydrate supply early or late in development (Figure 3.4).

**Table 3.1.** Differences in titratable acidity, firmness and hue angle in fruit at eating ripe from treatments receiving varying amounts of carbohydrates throughout the season (treatments described in chapter 2 methods). Results are averages  $\pm$  SE. *P*-values are as follow: \* =  $P \leq 0.05$ , \*\* =  $P \leq 0.01$ , \*\*\* =  $P \leq 0.001$ . Tukey's test was carried out when significant differences were between treatments. Superscript letters correspond to the results of the pair-wise analysis, with no significant difference detected between the consumer responses for treatment sharing the same letter.

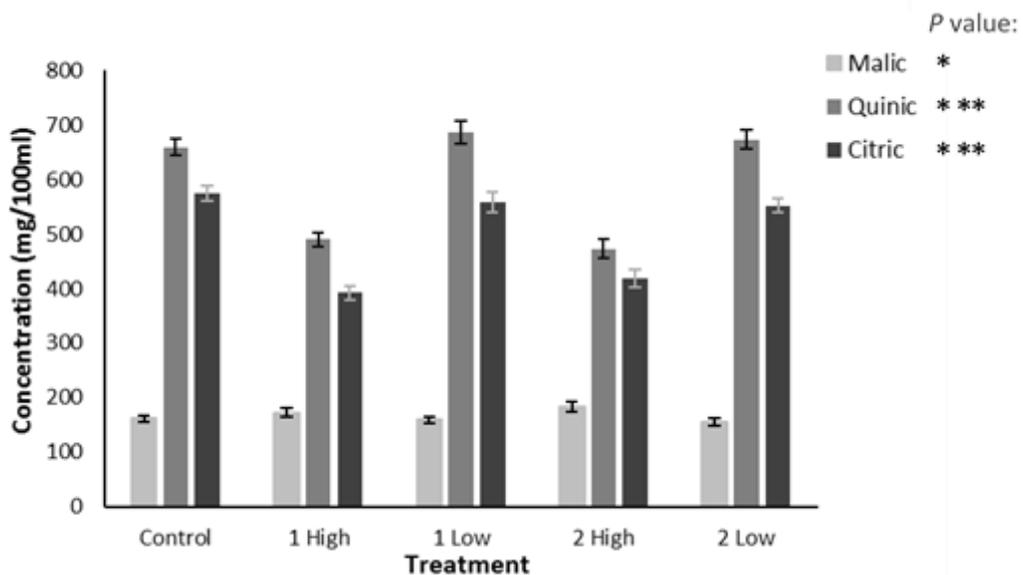
|                       | TA (%)                       | Firmness (kgf)                | Hue angle                      |
|-----------------------|------------------------------|-------------------------------|--------------------------------|
| <b>Control</b>        | 0.93 $\pm$ 0.02 <sup>A</sup> | 0.82 $\pm$ 0.01 <sup>A</sup>  | 101.44 $\pm$ 0.10              |
| <b>1 High</b>         | 0.68 $\pm$ 0.01 <sup>B</sup> | 0.78 $\pm$ 0.01 <sup>B</sup>  | 100.87 $\pm$ 0.10 <sup>A</sup> |
| <b>1 Low</b>          | 0.90 $\pm$ 0.03 <sup>A</sup> | 0.82 $\pm$ 0.01 <sup>AB</sup> | 100.76 $\pm$ 0.11 <sup>A</sup> |
| <b>2 High</b>         | 0.69 $\pm$ 0.02 <sup>B</sup> | 0.83 $\pm$ 0.01 <sup>A</sup>  | 100.23 $\pm$ 0.12              |
| <b>2 Low</b>          | 0.88 $\pm$ 0.02 <sup>A</sup> | 0.85 $\pm$ 0.01 <sup>A</sup>  | 100.92 $\pm$ 0.09 <sup>A</sup> |
| <b><i>P</i>-value</b> | ***                          | ***                           | ***                            |



**Figure 3.2.** Comparison of °Brix and DMC percentages in fruit at eating ripe between treatments receiving varying amounts of carbohydrates throughout the season (treatments described in chapter 2 methods). Results are averages  $\pm$  SE. \* =  $P \leq 0.05$ , \*\* =  $P \leq 0.01$ , \*\*\* =  $P \leq 0.001$ .



**Figure 3.3.** Differences in concentration of the three major kiwifruit organic acids in fruit at eating ripe from treatments receiving varying amounts of carbohydrates throughout the season (treatments described in chapter 2 methods). Results are averages  $\pm$  SE. \* =  $P \leq 0.05$ , \*\* =  $P \leq 0.01$ , \*\*\* =  $P \leq 0.001$ .



**Figure 3.4.** Differences in concentration of the three major kiwifruit sugars in fruit at eating ripe from treatments receiving varying amounts of carbohydrates throughout the season (treatments described in chapter 2 methods). Results are averages  $\pm$  SE. \* =  $P \leq 0.05$ , \*\* =  $P \leq 0.01$ , \*\*\* =  $P \leq 0.001$ .

### 3.3.2 Consumer sensory test

Varying carbohydrate supply to fruit during development did not significantly influence overall consumers' liking of fruit ( $P=0.1274$ ). Consumers were able to perceive significant differences in both acidity/sourness and sweetness between the different treatments.

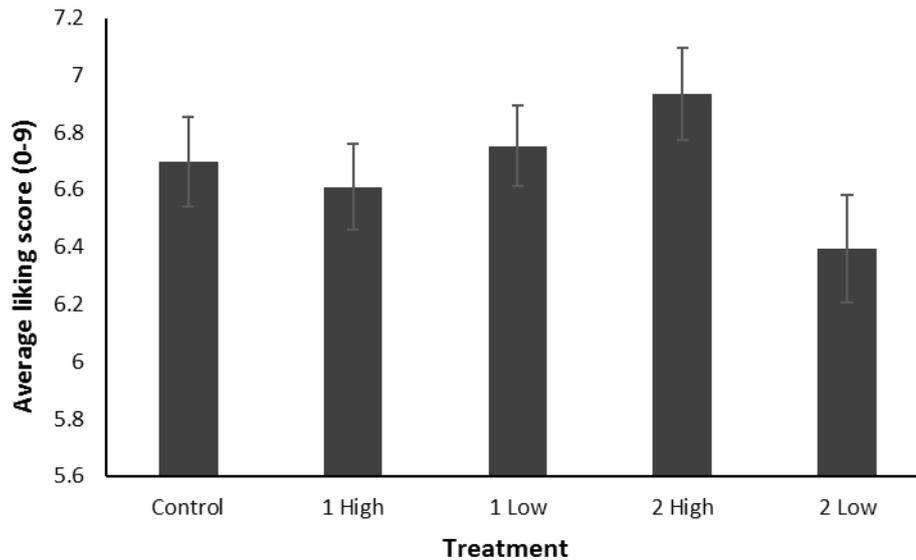
Acidic taste was more closely associated with the lower DMC and rSSC fruit (1 and 2 low). These treatments were identified by significantly more consumers as being "acidic/sour" compared to the high L/F ratio treatments (1 high and 2 high) (Figure 3.6 and Table 3.2). The high L/F ratio treatments, which had lower concentrations of quinic and citric acid, higher sucrose concentrations, °Brix and DMC, tended to be more highly associated with sweetness. The term "sweet" was used to describe the high L/F ratio and control treatments significantly more often than the low treatments (Figure 3.6 and Table 3.2).

Despite differences in the proportion of consumers using the term "acidic/sour" to the treatments, majority of consumers found acidity levels to be "just about right" (which corresponded with three on a five point scale from 'much too much' to 'much too little') for all treatments (Figure 3.7a). Consumers varied in their responses to acid intensity in the low L/F ratio treatments, with more consumers thinking acidity was too high compared to the other treatments, though many consumers also thought the acidity was too low (Figure 3.7a). The high L/F ratio fruit were thought to "just about right", although 37 consumers also described the 1 high fruit acidity as too low.

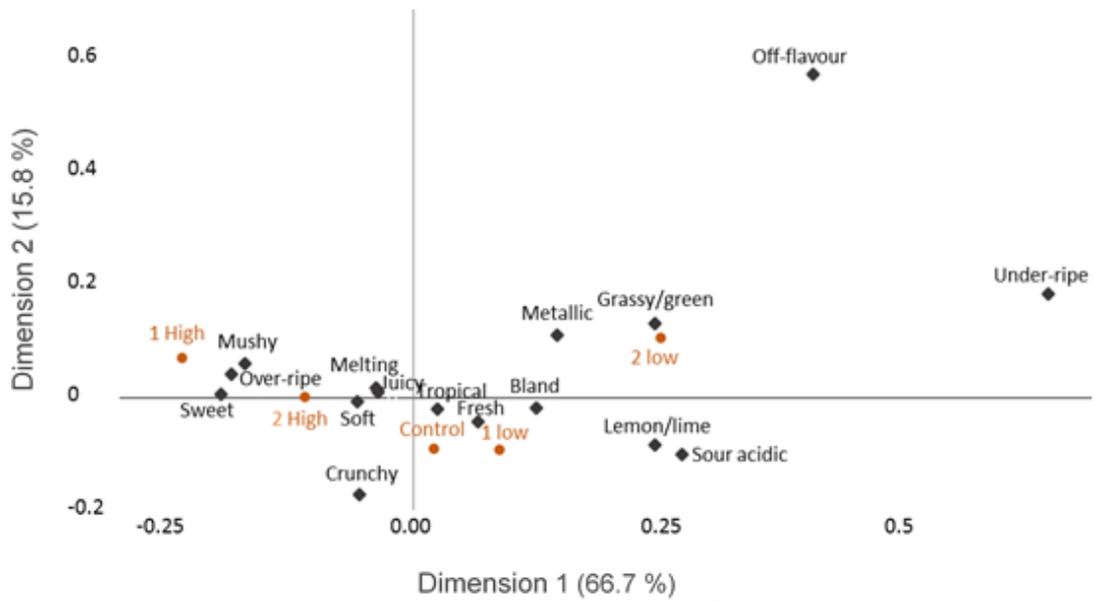
When addressing fruit sweetness "just about right" was also the most common responses for all treatments. The low L/F ratio and control treatments, showed higher responses of sweetness being too low compared the high L/F ratio treatments (Figure 3.7b). The overall flavour intensity was perceived as being too low by the majority of consumers in all but the 2 high treatment which the majority thought was "just about right" (Figure 3.7c). However, only a slightly lower percent of consumers found the control and two low L/F ratio fruit to have "just about right" flavour intensity.

There was also a significant difference in perception of ripeness, with "under-ripe" being used more in the 2 low treatment compared to the 1 high (Table 3.2).

However this relationship should be viewed with caution as the number of consumers who used this term was small. All other terms showed no significant difference between being chosen to describe each of the different treatments. When consumers were asked about the ripeness levels directly the highest percentage of consumers described ripeness as being “just about right” in all treatments (Figure 3.8).



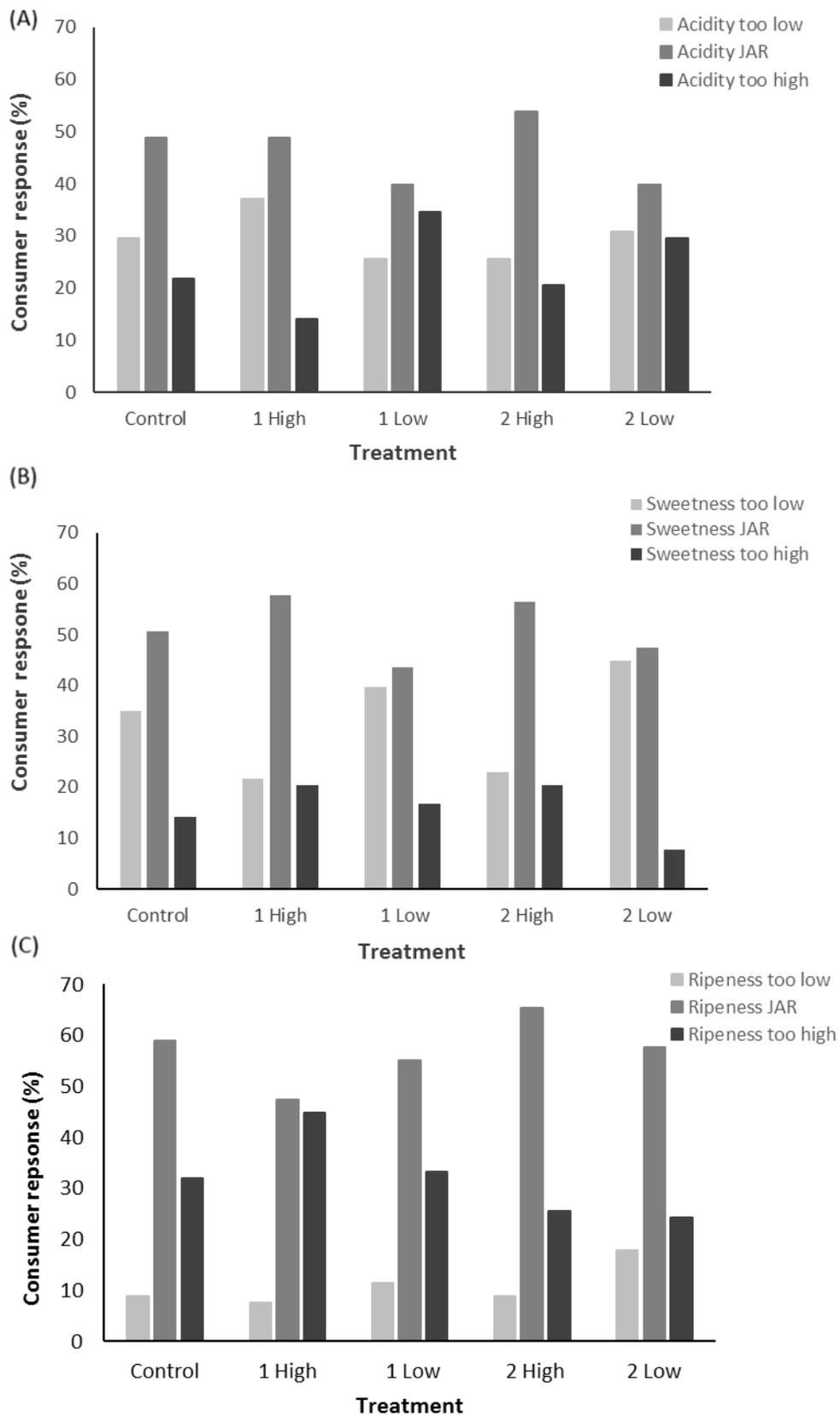
**Figure 3.5.** Overall liking scores from 78 consumers (hedonic 1-9 scale) on fruit from canes receiving five different pruning and girdling treatments (treatments described in chapter 2 methods). The scale ran from 1= dislike extremely, 5= neither like nor dislike, to 9=like extremely. Results are averages  $\pm$  SE.



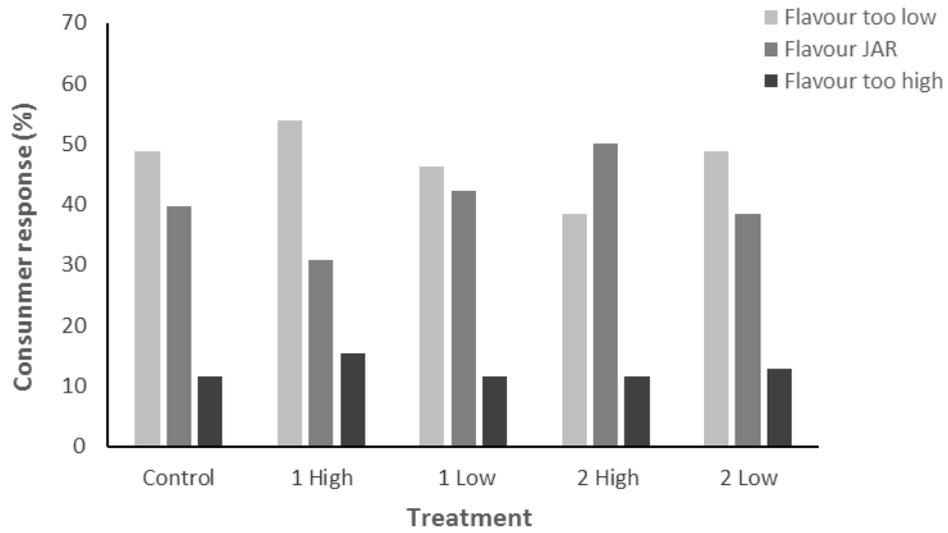
**Figure 3.6.** Plot of correspondence analysis of fruit from canes receiving five different pruning and girdling treatments (treatments described in chapter 2 methods). Consumers (n=75) profiled fruit from each treatment by selecting sensory attributes that were present in each sample. Treatments: ● ; Descriptor: ◆

**Table 3.2.** Percentage of responses for sensory attributes from 75 consumers for fruit from five treatments receiving varying carbohydrate supplies (Treatments described in chapter 2 methods above). Bolded variables had significant P values of less than 0.05. Pair-wise comparison analysis was carried out for these attributes. Superscript letters correspond to the results of the pair-wise analysis, with no significant difference detected between the consumer responses for treatment sharing the same letter.

| Descriptors        | Treatments               |                         |                         |                          |                         | No. of responses |
|--------------------|--------------------------|-------------------------|-------------------------|--------------------------|-------------------------|------------------|
|                    | Control                  | 1 High                  | 1 Low                   | 2 High                   | 2 Low                   |                  |
| Bland              | 25.5                     | 18.2                    | 18.2                    | 14.5                     | 23.6                    | 55               |
| Crunchy            | 20                       | 20                      | 26.7                    | 20                       | 13.3                    | 15               |
| Fresh              | 20.8                     | 16.9                    | 20.8                    | 20.8                     | 20.8                    | 130              |
| Grassy/green       | 10                       | 15                      | 25                      | 20                       | 30                      | 20               |
| Juicy              | 18.7                     | 20.7                    | 18.7                    | 22.7                     | 19.2                    | 198              |
| Lemon/lime         | 20.8                     | 9.7                     | 23.6                    | 20.8                     | 25                      | 72               |
| Melting/smooth     | 20.5                     | 20.5                    | 17.4                    | 22.4                     | 19.3                    | 161              |
| Metallic           | 18.2                     | 18.2                    | 18.2                    | 18.2                     | 27.3                    | 11               |
| Mushy              | 16.1                     | 28                      | 19.4                    | 20.4                     | 16.1                    | 93               |
| Off-flavour        | 5.9                      | 17.6                    | 11.8                    | 17.6                     | 47.1                    | 17               |
| Over-ripe          | 19.2                     | 30.8                    | 19.2                    | 15.4                     | 15.4                    | 52               |
| Soft               | 20.5                     | 21.7                    | 18.5                    | 21.3                     | 18.1                    | 254              |
| <b>Sour/acidic</b> | <b>22.3<sup>AC</sup></b> | <b>11.5<sup>B</sup></b> | <b>25.4<sup>C</sup></b> | <b>15.4<sup>AB</sup></b> | <b>25.4<sup>C</sup></b> | <b>130</b>       |
| <b>Sweet</b>       | <b>20.7<sup>A</sup></b>  | <b>24<sup>A</sup></b>   | <b>14.9<sup>B</sup></b> | <b>26<sup>A</sup></b>    | <b>14.4<sup>B</sup></b> | <b>208</b>       |
| Tropical           | 21.6                     | 18.9                    | 18.9                    | 20.3                     | 20.3                    | 74               |
| <b>Under-ripe</b>  | <b>25<sup>AB</sup></b>   | <b>5<sup>A</sup></b>    | <b>15<sup>AB</sup></b>  | <b>10<sup>AB</sup></b>   | <b>45<sup>B</sup></b>   | <b>20</b>        |



**Figure 3.7.** Percentage of responses from 78 consumers to the (A) acidity, (B) sweetness and (C) ripeness of fruit from five treatments receiving varying carbohydrate supply throughout the season (treatments described in chapter 2 methods). Consumer described whether each factor was too high, just about right (JAR) or too low in fruit.



**Figure 3.8.** Percentage of consumer responses to flavour intensity levels in fruit from treatments receiving varying amounts of carbohydrates throughout the season (treatments described in chapter 2 methods). Consumer responses described whether each factor was too high, just about right (JAR) or too low in fruit from each treatment.

### **3.4 Discussion**

The results of this experiment support the hypothesis that altered carbohydrate supply to fruit during development not only altered the dry matter content and sweetness of the ripe fruit as perceived by the consumer, but also the relative proportions of the major acids and sugars that contribute to fruit flavour. This resulted in increases in the proportion of consumers that detected less desirable flavours in fruit grown with a low leaf to fruit ratio. The results confirm that fruit of this cultivar are at risk of developing a poor flavour profile if grown under conditions of low or variable crop load. When combined with the developmental changes in acid composition described in Chapter 2, this study demonstrates that low leaf to fruit ratio fruit do not just become more bland when ripe, they develop an altered flavour profile, with higher concentrations of acids and lower concentrations of sugars.

Despite generally liking all of the fruit tested, consumers were able to detect particular flavour differences between treatments. Consumers showed similar liking of fruit from different treatments despite the treatments creating significant differences in the typical determinants of flavour (DMC, rSSC and TA) in kiwifruit. Treatments that received limited carbohydrate supply during the season resulted in fruit with significantly higher TA, citric acid and quinic acid concentrations, combined with lower °Brix, DMC and sucrose concentrations at eating ripe. Consumers more closely associated these fruit with being acidic and having sour or under-ripe flavours compared to the treatments that received increased carbohydrate supply.

#### **3.4.1 Overall consumer liking of fruit**

In fruit the overall eating experience and consumer liking is driven by a combination of the fruit taste, smell and texture, which are in turn influenced by the flavour, DMC, firmness, volatile content and juiciness (Gilbert, Young, Ball, & Murray, 1996; Harker et al., 2009; Walsh, 2006). Flavour, particularly the sugar to acid ratio is commonly described as the most important of these determinants, and was the focus of this sensory study. (Harker et al., 2009; Jaeger & Harker, 2005; Rossiter et al., 2000). Fruit firmness influences the perception of flavour as when fruit soften their juice levels increase as well as concentrations of

important volatiles. This means in firmer fruit the effect of odour and volatile content is less, so consumer preference is driven primarily by increasing sugar content (Harker et al., 2009). It also means soft and firm fruit may have similar TA and rSSC but the perception of acidity and sweetness differs with firmer fruit being perceived as more acidic and less sweet (Esti et al., 1998; McMath, Paterson, Young, & Ball, 1992; Paterson et al., 1991). The effects of fruit firmness were controlled between treatments in this experiment as much as possible by using only fruit with firmness between 0.6-0.9 kgf on the day of the sensory experiment to ensure consumers concentrated on the fruit flavour. The fruit appeared to have significant differences in firmness between treatments despite individual fruit all being within 0.3 kgf of one another. It is presumed that as these differences are very small they would not have had large effects on consumer perception.

This research showed consumers generally liked the overall flavour of all treatments. Fruit DMC at eating ripe is commonly used as predictor of potential flavour due to DMC being positively correlated with fruit rSSC (Jordan, Walton, Klages, & Seelye, 2000; McGlone, Jordan, Seelye, & Martinsen, 2002; Richardson et al., 1997). Consumer studies generally show that increasing DMC and rSSC positively influences consumer preference (Harker et al., 2009; Jaeger et al., 2003; Rossiter et al., 2000; Walsh, 2006). It is also commonly observed that increasing fruit TA concentrations tends to reduce consumer liking. These factors do not always alter consumer preference however. For example, a study of consumer preference to a range of kiwifruit DMC showed no significant difference in overall liking of fruit at very similar DMC levels to this study (Burdon et al., 2004). This may explain the similar liking scores in this study, as different acid and sugar ratios are perceived similarly until a threshold for sweetness or acidity is reached (Marsh et al., 2003). Despite consumers liking all treatments, it is also likely that the different ratios of individual organic acids and soluble sugars with their different tastes and strengths influenced consumer liking.

### **3.4.2 Acid and sugar perception**

Fruit TA and the individual acids at eating ripe generally responded negatively to increased carbohydrate supply during the season, while the °Brix and soluble sugars responded positively. Overall consumers did perceive differences in these

different sweetness and acidity levels between the high and low L/F ratio treatments. This is similar to the study by Burdon et al. (2004) which, at similar DMC levels to this study, showed difference in sweetness and acidity intensities.

It has been shown in apple sensory studies that trained panellists are able to detect rSSC differences of 1 °Brix, while untrained consumers are less sensitive to differences and are typically able to perceive differences of 1.5 °Brix or higher (Harker et al., 2002). Based on the °Brix in fruit at harvest, treatment differences between 2 low and both high L/F ratio treatments were above this threshold, as well as 1 low and 2 high °Brix differences, so it was expected consumers would be able to perceive the differences in the sweetness between these treatments (Harker et al., 2002). The consumers were able to perceive these differences, along with tasting significantly higher sweetness between the control and both 1 and 2 low treatments, as well as the 1 high and 1 low treatments. Interestingly the °Brix differences between these latter pairs of treatments were below the threshold consumers can typically detect (the respective differences were 0.56, 0.98 and 1.18 °Brix). However, differences in °Brix between control and 2 high treatment were just above the level consumers can perceive differences at, but in this study no significant differences were perceived.

The high L/F ratio treatments had higher total sugars and sucrose concentrations compared to the low L/F ratio treatments, while the glucose and fructose concentrations did not vary between treatments as much. This is similar to other studies which altered DMC, in which high DMC fruit tended to have higher sugar content and sweetness intensities (Harker et al., 2009; Wang et al., 2011). The significantly higher sucrose levels are likely to have contributed largely to the higher sweetness perceived in both the high L/F ratio treatments (Marsh et al., 2003; Rossiter et al., 2000). At high sweetness levels, like the 1 and 2 high treatments had, sugars have been shown to be able to suppress the influence of varying acid concentrations on flavour perception (Burdon et al., 2004; Rossiter et al., 2000). This would have likely made these fruit taste sweeter compared to the low L/F ratio treatments. Different acid amounts can also alter the perception of sweetness, along with sourness, bitterness, astringency and general acidity in fruit (Rubico & McDaniel, 1992). Increased sucrose levels have been shown to cause the release of more hydrophobic volatile components, and due to volatile

components being a key part of flavour, this would likely effect the level of sweetness perceived also (Friel, Linforth, & Taylor, 2000).

In the gold cultivar “Hort16A” increased quinic and ascorbic acid concentrations have been shown to decrease perceived sweetness (Marsh et al., 2006). It is therefore likely that the higher levels of both quinic and citric acid observed in the low L/F ratio and control treatment fruit will have decreased the perception of sweetness even more in these treatments.

The differences perceived between the control treatment and the rest of the fruit may be due the slightly higher fructose concentration in this treatment. Fructose was highest in the control treatment at harvest compared to all other treatments. In most fruit fructose has been shown to be less sweet than sucrose at the same concentration, however, in kiwifruit the individual sugar types have been shown to be similar in their relative sweetness (Harker et al., 2002; Pangborn, 1963). The difference in sugar ratio may therefore have altered the consumer’s perception of sweetness in this treatment. Increasing the overall sugar concentration does not directly result in increased consumer liking. Sometimes increased sugar concentration can influence or block physiological processes or reactions in kiwifruit. For example important enzymes involved in metabolism of volatiles can be better protected in fruit with higher sucrose concentrations stored at cooler temperatures (Strauss & Hauser, 1986).

Sugar concentrations are essential for consumer liking, but equally important is the acid concentrations, and the balance between the sugars and acids (Marsh et al., 2004; Rossiter et al., 2000). Simply having high concentrations of sugar and sweet tastes does not result automatically in a good taste, however, the addition of a small amount of acid can boost this flavour and alter the perception of sweetness (Marsh & Harker, 2016). However, at high concentrations the organic acids have the ability suppress the perception of sweetness in “Hayward” kiwifruit (Marsh et al., 2006).

Differences in the TA between the high L/F ratio treatment and both the low L/F ratio and control treatments, (0.2-0.25 % differences) were over double what trained panellists have been identified as being able to perceive in apples (0.08 % TA). (Harker et al., 2002). As expected, based on these large differences in TA

between treatments, the consumers in this study did perceive differences in the acidity levels between the two high L/F ratio and the low L/F ratio fruit, as well as between 1 high and control treatments. They did not perceive differences in acidity levels between the control and 2 high treatment however. Other sensory studies in “Hayward” and “Hort16A” fruit have found little difference in acidity intensity perception between high and low DMC fruit (Wang et al., 2011).

The differences in acid levels perceived by consumers is likely largely determined by the total acids being higher in concentration in the low L/F and control treatments as the ratios of the individual acids did not appear to vary much between the different treatments.

These changes in both acid and sugar concentrations across treatments resulted in large differences in the sugar: acid ratios the fruit at eating ripe. The treatments created opposite changes in the sugars and acids, with lower carbohydrate supply resulting in increased acid concentrations as well as decreased sugar concentrations. The high L/F ratio treatments showed the opposite effect, decreased acid concentrations as well as increased sugar concentrations. This combination of opposite changes in both sugars and acids would have resulted in the more sour/acidic flavours being perceived in these treatments, as well as the lower sweetness levels being perceived in the low L/F ratio treatments. It is also likely responsible for the higher perception of under-ripe flavours in the 2 low treatment. These changes are stronger in the 2 low treatment due to this treatment being applied later in fruit growth, during starch accumulation, causing higher acid accumulation.

Overall, the majority of consumers found the flavour intensity too low in all treatments except for the 2 high treatment in which 50 % of consumers found the intensity of flavour to be “just about right”. This response will likely be due to the ripeness, sweetness and acidity levels all being perceived by a large majority of consumers as being “just about right”. This treatment had the highest DMC, °Brix and sucrose levels, and lowest TA. The amounts of these sugars and acids, and the balance between them, created a flavour that was perceived by many consumers as a good flavour intensity.

### **3.4.3 Differences between consumers**

Along with the differences in composition, other possible reasons for there being no significant differences in consumer overall liking of fruit between most of the treatments may be related to the individual preferences of participants involved in the study. Consumer preference for optimum rSSC often shows several main groupings in responses. Between these main preferences groups is rSSC which is less preferred (Harker et al., 2009). This shows there is significant variability in consumer preference with some people preferring simply the highest sugar concentrations, while others prefer lower sugars concentrations where the acids have more influence on the flavour, and while the fruit in-between these two extremes do not appeal to either group of consumers (Harker et al., 2009).

Several studies have also identified groups of people who regularly eat kiwifruit but may prefer blander, lower sweetness tastes in kiwifruit, as they are shown to prefer the lower rSSC fruit (Harker et al., 2009; Wismer et al., 2005). This consumer group may not be eating kiwifruit for pleasure but rather for the health benefits.

Perception and liking has also been shown to vary with age and sex. It has been shown that females tend to have more consistent preference of rSSC, and these preferences tend to be for higher sugar levels compared to the males who demonstrated consistent flavour preferences (Harker et al., 2009). Harker et al. (2009) also showed that the most consistent consumer group was the older category (>46 years), while the youngest category (18-30 years) showed the least consistent preferences. In this experiment a majority of consumers were in the youngest category (59 % aged 18-30 years, 16% in the 31-45 year age group and 24 % 46 years or older). This may partly explain there were low overall differences in liking observed between treatments, if these younger consumers have inconsistent preferences.

In this study 28 % of consumers reported eating kiwifruit commonly (at least once a week when in season) and 32 % ate kiwifruit occasionally (one to three times a month). These consumers are likely to have more consistent preferences for kiwifruit taste or flavour, however, the 40 % of consumers that ate kiwifruit rarely (once every few months to never) would have variable preferences (Harker et al.,

2009). These consumers may not be used to the slightly acidic taste of kiwifruit, causing their liking scores to be different to regular consumer's likings.

#### **3.4.4 Conclusion**

The aim of this research was to describe the composition and perception of flavour in "Gold3" fruit at eating ripe from canes receiving varying carbohydrate supplies throughout the season. A consumer preference test was carried out on fruit from the different treatments. This was to test whether the different carbohydrate supplies affected sugar and acid partitioning significantly enough for inexperienced panellists to taste the difference. When combined with the developmental changes in acid composition described in Chapter 2, this study demonstrates that low leaf to fruit ratio fruit do not just become more bland when ripe, they develop an altered flavour profile, with higher concentrations of some acids and lower concentrations of sugars.

Consumers generally showed similar overall liking for fruit from different treatments, despite the fruit having significant differences in the typical flavour determinants (DMC, rSSC and TA). These differences likely gave similar results until a threshold was passed at which sweetness and acidity perception changed.

Although overall liking was similar between treatments, consumers were able to detect differences in sugar and acid concentrations. Treatments that received limited carbohydrate supply during the season resulted in fruit with significantly higher TA, citric acid and quinic acid concentrations, combined with lower °Brix, DMC and sucrose concentrations at eating ripe. Consumers more closely associated these fruit with being more acidic compared to the treatments that received increased carbohydrate supply.

The results show altered carbohydrate supply to fruit during the season causes a change in the ratio of sugars to acids in "Gold3" fruit. Not only did sugar concentrations increase in fruit from canes receiving low carbohydrate supply, but the acid concentrations rose. The altered compositions and sugar:acid ratios were significant enough for consumers to taste the difference between treatments. However, there are significant restrictions to the strength of this data due to the relatively small sample size for a consumer sensory experiment.

## Chapter 4: Synthesis

Due to “Gold3” kiwifruit being a relatively new cultivar there is limited research looking at the development of “Gold3” fruit, and the factors that influence quality and flavour. Growers have noted that with “Gold3” small or low DMC fruit have insufficient sugar concentrations to balance the high acid concentrations, resulting in poor tasting fruit and low consumer acceptability. The overall objective of this research was to gain a better understanding of how flavour and its components develop in “Gold3” kiwifruit, as well as identify when the components that contribute to final flavour accumulate. These developmental changes were then linked to changes in the fruit flavour and consumer perception. Overall the results of the research support the idea that “Gold3” kiwifruit are vulnerable to changes in composition due to changes in growing conditions, and these changes influence flavour as perceived by consumers.

Altered carbohydrate supply to kiwifruit vines throughout fruit growth was shown to have significant effect on the growth and development of “Gold3” kiwifruit. The positive response of DMC, fruit size and starch accumulation to increased carbohydrate supply was as expected and showed similar patterns of accumulation to other *A. chinensis* cultivars, particular the other gold kiwifruit cultivar “Hort16A”.

The results suggest that the regulation of acid metabolism in response to an altered carbohydrate supply differs from starch and sugars. The overall acid concentrations rose in fruit receiving lower carbohydrate supply. Decreased carbohydrate supply also resulted in lower starch concentrations, and in turn lower soluble sugars in fruit both at harvest and through to fruit at eating ripe. These difference in composition and the sugar: acid ratios between the treatments were expected to alter the taste of the fruit at eating ripe. This was supported by the consumer test, where the low L/F ratio treatments, which had higher total acidity and lower sugar concentrations, were perceived by consumers as having more acidic tastes and lower sweetness compared to the high L/F ratio treatments.

The different carbohydrate supplies were also shown to have opposing effects on the development of individual organic acids, with quinic and citric acid concentrations exhibiting opposite reactions. Quinic, and to a lesser extent oxalic

and malic acid, responded negatively to increased carbohydrate supply while citric responded positively. When carbohydrate supply was decreased early or late in development, quinic, oxalic and malic concentrations increased, while citric concentrations fell, compared to the control treatment. The resulting differences in fruit at harvest maturity were further altered during storage and ripening. At eating ripeness the low L/F ratio treatments had higher concentrations of both citric and quinic acid compared to the high L/F treatments.

The results of this experiment showed that decreased carbohydrate supply resulted in not only lower DMC in fruit at eating ripe, but also altered sugar: acid ratios (increased acid concentrations and lowered sugar concentrations). These fruit were perceived as more acidic and less sweet by consumers, however, all fruit from this experiment were perceived as having acceptable flavours. In commercial orchards crop loading and factors influencing carbohydrate supply to individual fruit can vary extremely within a single orchard. There can be individual canes that may have been missed during pruning or thinning, or be in a particularly shaded by other shoots or canes. These fruit would be at risk of low carbohydrate supply, and may be even more deficient than the low leaf to fruit ratio treatments in this experiment. This may explain why poor tasting fruit are sometimes observed within “Gold3” crops. The research demonstrates the importance of maintaining careful pruning and thinning practices, of appropriate carbohydrate supply for producing good flavoured fruit, and may assist growers in minimising the production of small, poor flavoured “Gold3” kiwifruit.

#### **4.1 Future research**

As with all new fruit cultivars there are many gaps in knowledge and limitations around the quantity and quality of data. It would be valuable to repeat aspects of this research over several seasons and on multiple orchards to identify whether variable carbohydrate supply consistently affects sugar and acid contents in the way it did in this experiment. Much of the knowledge and current understanding of “Gold3” fruit are based off other cultivars, however it is also necessary to learn more how “Gold3” differs from other cultivars.

Further research into “Gold3” growth and development is needed as starch and acid metabolism are both complicated, dynamic processes, and there are still

knowledge gaps in the understanding of primary metabolism in the most commonly researched cultivars (“Hayward” and “Hort16A”). To do this more treatments could be created, keeping the girdles open until harvest, creating more extreme treatments or more variation in the timing of altering carbohydrate supply. These more extreme treatments might be able to replicate the even more poorly flavoured fruit that are thought to exist within some “Gold3” crops, and could also provide insight into what consumers perceive as the limits for acceptable fruit flavours in “Gold3” to be. These experiments could also be used as the basis for studying the molecular controls and enzymes that influence starch, acid and sugar metabolism, and to seek an explanation as to why there are contrasting responses to altered fruit carbohydrate supply amongst the major organic acids.

Along with the general development being described from flowering through to harvest in this study, the changes in fruit composition occurring during storage and ripening should also be described in more detail. This may identify why the citric acid concentration of the high L/F ratio treatment initially responded positively to increased carbohydrate supply but at eating ripe had lower concentrations compared to the low L/F ratio.

Finally, the aroma volatiles are also important influences on chemical composition and consumer flavour perception of kiwifruit that may be affected by altered carbohydrate supply during fruit development

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# Appendix

**Appendix 1.** Participant questionnaire for the consumer sensory experiment. A questionnaire was filled out by participant for each of the five fruit tasted.

*Panellist ID: 1*

*Code: 701*

*Sample number: 1*

## Consumer questionnaire

1. Overall, how much do you like/dislike this kiwifruit?

|                                       |                                       |                                       |                                       |                                       |                                       |                                       |                                       |                                       |
|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|
| <input type="checkbox"/> <sub>1</sub> | <input type="checkbox"/> <sub>2</sub> | <input type="checkbox"/> <sub>3</sub> | <input type="checkbox"/> <sub>4</sub> | <input type="checkbox"/> <sub>5</sub> | <input type="checkbox"/> <sub>6</sub> | <input type="checkbox"/> <sub>7</sub> | <input type="checkbox"/> <sub>8</sub> | <input type="checkbox"/> <sub>9</sub> |
| Dislike extremely                     | Dislike very much                     | Dislike moderately                    | Dislike slightly                      | Neither like nor dislike              | Like slightly                         | Like moderately                       | Like very much                        | Like extremely                        |

2. Which of the following words would you use to describe this kiwifruit? *Tick all that apply*

|             |                          |                |                          |
|-------------|--------------------------|----------------|--------------------------|
| Tropical    | <input type="checkbox"/> | Lemon/lime     | <input type="checkbox"/> |
| Sour/acidic | <input type="checkbox"/> | Mushy          | <input type="checkbox"/> |
| Juicy       | <input type="checkbox"/> | Over-ripe      | <input type="checkbox"/> |
| Fresh       | <input type="checkbox"/> | Off-flavour    | <input type="checkbox"/> |
| Metallic    | <input type="checkbox"/> | Grassy/green   | <input type="checkbox"/> |
| Sweet       | <input type="checkbox"/> | Melting/smooth | <input type="checkbox"/> |
| Crunchy     | <input type="checkbox"/> | Bland          | <input type="checkbox"/> |
| Soft        | <input type="checkbox"/> | Under-ripe     | <input type="checkbox"/> |

3. What do you think about the:

|                         |                                       |                                       |                                       |                                       |                                       |
|-------------------------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|
| a) Acidity              | <input type="checkbox"/> <sub>1</sub> | <input type="checkbox"/> <sub>2</sub> | <input type="checkbox"/> <sub>3</sub> | <input type="checkbox"/> <sub>4</sub> | <input type="checkbox"/> <sub>5</sub> |
|                         | Not sour enough                       |                                       | Just about right                      |                                       | Much too sour                         |
| b) Sweetness            | <input type="checkbox"/> <sub>1</sub> | <input type="checkbox"/> <sub>2</sub> | <input type="checkbox"/> <sub>3</sub> | <input type="checkbox"/> <sub>4</sub> | <input type="checkbox"/> <sub>5</sub> |
|                         | Not sweet enough                      |                                       | Just about right                      |                                       | Much too sweet                        |
| b) Intensity of flavour | <input type="checkbox"/> <sub>1</sub> | <input type="checkbox"/> <sub>2</sub> | <input type="checkbox"/> <sub>3</sub> | <input type="checkbox"/> <sub>4</sub> | <input type="checkbox"/> <sub>5</sub> |
|                         | Much too weak                         |                                       | Just about right                      |                                       | Much too strong                       |
| c) Ripeness             | <input type="checkbox"/> <sub>1</sub> | <input type="checkbox"/> <sub>2</sub> | <input type="checkbox"/> <sub>3</sub> | <input type="checkbox"/> <sub>4</sub> | <input type="checkbox"/> <sub>5</sub> |
|                         | Much too unripe                       |                                       | Just about right                      |                                       | Much too over-ripe                    |