‘Hass’ avocado tree water use
and
the effects of water stress on fruit development

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Abstract

‘Hass’ avocado tree water use and the effects of water deficits on fruit development were assessed in New Zealand from 2017 to 2019.

Avocado tree water use was quantified by sap flow measurement and the soil water balance method at three sites in the main avocado growing regions of the Bay of Plenty, Whangarei, and the Far North, New Zealand, with consideration of local differences in climate and soil type, and variation in tree fruit load, leaf area, crown size and orchard shaded area. The results showed that all three sites had similar patterns of tree water use relative to reference evapotranspiration (ET_o), regardless of differences in soil type, showing the highest tree water use in January at 2.7 mm d^{-1} and the lowest in June at 1.2 to 1.4 mm d^{-1}. Crop coefficients (K_c) were provided for each site, and the three sites had similar K_c values throughout the year, as all three orchards were considered mature, with >75% orchard shaded area. However, fruit load influenced tree water use. At all three sites, the K_c values increased from light to heavy fruit load, therefore, K_c values should be adjusted for variation in fruit load. The relationship between orchard shaded area and K_c was also considered, to provide a method for estimating K_c for orchards differing in tree size and spacing.

Irrigation requirements were assessed in more detail at the avocado orchard in the Bay of Plenty, investigating the effects of any water deficits resulting from a lack of irrigation on tree physiology and fruit growth. Rainfall at the study site was variable over the three years, but each summer there was a dry period without any rainfall for more than two weeks that resulted in a significant decrease in soil water content if avocado trees were not irrigated. Tree fruit load was highly variable between years and trees within years, and increasing fruit load caused decreasing individual fruit weight and dry matter content. However, in the year with the highest average fruit load, a dry period occurred during early fruit development, causing fruit weight at harvest in the non-irrigated treatment to decrease by 26.4 g per fruit, independently of the effects of fruit load on fruit weight. The trees responded to dry conditions by reducing stomatal conductance (g_s) by 20%, resulting in a constant leaf water potential (Ψ_{leaf}) above -0.25 MPa. This finding suggests that irrigation is important for New Zealand avocado production because photosynthetic activity and fruit growth can be reduced by even mild water stress during a dry period. In the Bay of Plenty with allophanic soil, irrigation of avocado trees is recommended when soil water content decreases below 0.40 m^3 m^{-3} (-30 kPa of soil tension) at a depth of 30 cm. Adverse effects on g_s and fruit growth caused by water stress may appear when soil water content decreases below 0.35 m^3 m^{-3} (-50 kPa of soil tension).

To gain a better understanding of how water stress affects fruit development, an experiment was conducted on potted avocado trees in a glasshouse over the summer of 2018-19. Vascular water flows were monitored in the shoot stems and fruit pedicels with external sap flow gauges, while fruit growth was monitored continuously using linear transducers (LTs). The fruit water balance was modelled as the sum of fruit transpiration and inward and outward vascular flows, and compared with actual fruit diameter growth over time while a period of water stress followed by re-watering was imposed. The results showed that water flows in one direction through shoot stems from the parent stem to leaves, while flow occurred in both directions at different times of day through the fruit pedicels. Fruit size fluctuated in a response to water movement, but overall, avocado fruit increased in size by 1.4 (±0.09 S.E.) cm^3 d^{-1} under well-watered conditions. However, under water-stressed conditions, the plants showed a clear response to water stress with a reduction in g_s, photosynthetic net assimilation (A), and sap flow within shoot stems of 60-70%. Fruit water inflow was also decreased by 31% and the outflow from fruit to the parent stem was increased by 65% under water stressed conditions. As a result, the fruit growth rate decreased to 0.4 (±0.06) cm^3 d^{-1}. After the period of water stress was ended by re-watering, the fruit growth rate of the water-stressed treatment recovered quickly, and vascular flows and fruit volume growth exceeded that of the non-stressed treatment. Final fruit size did not differ between the two treatments in this experiment. Avocado fruit growth is sensitive to water stress because of direct effects of stress on vascular flows of water to the developing fruit, and potentially indirect effects of stress on photosynthesis and phloem flows, mediated by an isohydric stomatal response to water potential. Further research to investigate the interacting contributions of phloem and xylem flows to fruit growth in avocado is recommended.

Key words: Hass, avocado, water, stress, deficits, fruit, crop, load, crop coefficient, shaded area, water balance, irrigation, isohydric, New Zealand.

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List of abbreviations

A	Photosynthetic net assimilation
CHPM	The compensation heat-pulse method
E	Soil evaporation
ET_c	Crop evapotranspiration ($E + T$)
ET_o	Reference evapotranspiration
ET_{wb}	Crop evapotranspiration calculated from the soil water balance method
g_s	Stomatal conductance
K_c	Crop coefficient
K_{cb}	Basal crop coefficient
$L_1D_1D_2$	Fruit growth measurement - fruit length (L_1), and two diameters (D_1 and D_2)
LTs	Linear transducers
T	Plant transpiration
VPD	Vapour pressure deficit
Ψ_{fruit}	Fruit water potential
Ψ_{leaf}	Leaf water potential
Ψ_{pd}	Pre-dawn leaf water potential
Ψ_{stem}	Stem water potential
Ψ_{xylem}	Xylem water potential

Chapter 1

Introduction

1.1 Introduction

Avocado *(Persea americana* Mill.) is a perennial evergreen plant indigenous to Central and Northern South America humid subtropical and tropical areas. In recent years, following successful commercialisation as a horticultural crop, its distribution has become widespread throughout many countries from the tropics to Mediterranean climates (Carr, 2013; Lahav *et al.*, 2013). In New Zealand, avocado production for export started in the 1980s, and since then, the avocado industry has been expanding, with avocado becoming the third largest fresh fruit export by value (White, 2001; The New Zealand Horticultural Export Authority, 2014) (Figure 1.1).

Avocado evolved in a climate where rainfall is evenly distributed throughout the year, so it has poor drought tolerance and is known to be very sensitive to water status (Carr, 2013). For horticultural production in drier climates, irrigation is an important part of avocado orchard management. However, understanding plant-water relations is complex. Fruit crop species generally consume more water during the fruit developmental stage. This is because fruit require water and carbohydrate to grow, and carbohydrate is derived from photosynthesis that uses a large amount of water (Matthews & Shackel, 2005). The energy (and thus water) requirements of avocado fruit may be especially high, compared to other fleshy fruits, due to high contents of protein, oil, and fat-soluble vitamins (Duarte *et al.*, 2016).

The most appropriate timing or amount of irrigation depends on crop species, soil type, weather conditions, and plant physiological stage. Low water supply can decrease crop yield. Hence, over the past few decades, irrigation management has been studied in detail for many fleshy fruit, such as grape (Acevedo-Opazo *et al.*, 2010), peach (Crisosto *et al.*, 1994), apple (Naor & Cohen, 2003), olive (Moriana *et al.*, 2003), and avocado (Lahav *et al.*, 2013; Pleguezuelo *et al.*, 2018).

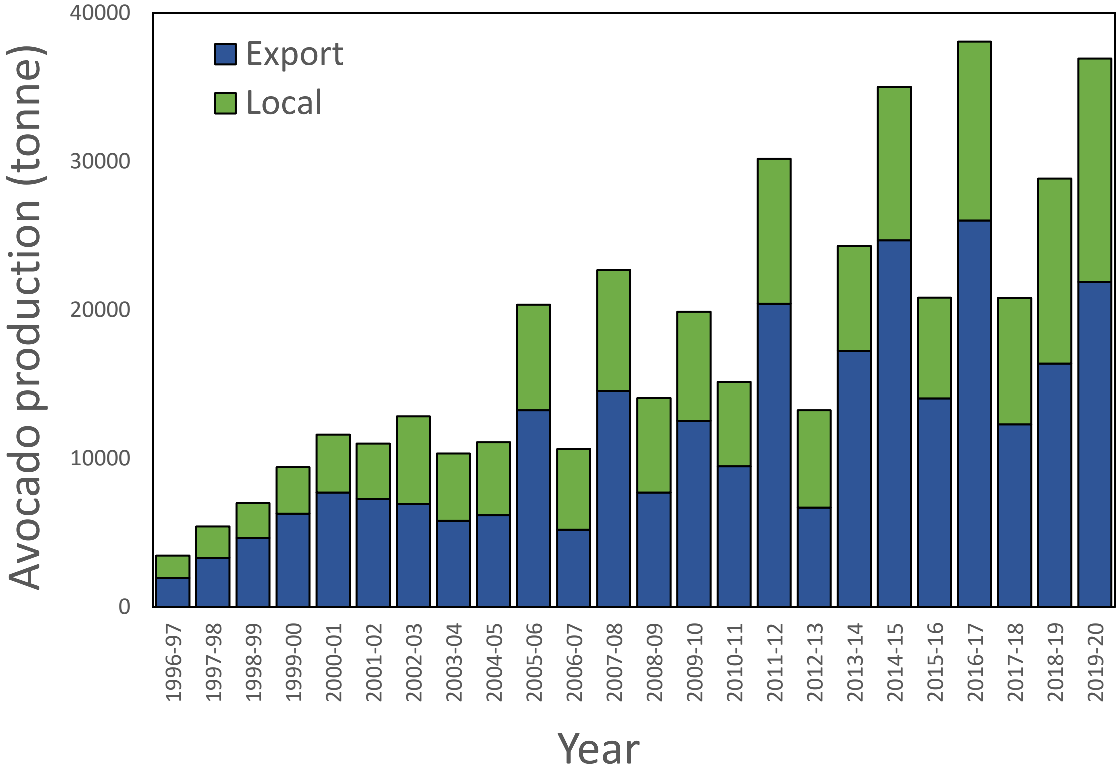

Figure 1.1: New Zealand avocado production from 1996-97 to 2019-20 (tonne) (data sourced from New Zealand Avocado, 2008, 2018, 2020b), separated into export and local.

A number of irrigation studies on avocado plants have been carried out in dry regions. For example, Lahav and Kalmar (1977) investigated four irrigation intervals (7, 14, 21, and 28 days with average annual water application of 8890, 7450, 6680 and 5940 m^3/ha, respectively) on avocado cv. Hass, Ettinger and Fuerte over a six-year period in Israel, and found that shorter irrigation intervals led to larger fruit size with higher oil content. Similarly, Michelakis *et al.* (1993) examined the level of irrigation at 30, 60 and 90% of reference evapotranspiration (ET_o) on 'Fuerte' avocado for five years in Greece, and concluded that the driest treatment had less fruit and consequently had the lowest fruit yield. More recently, Holzapfel *et al.* (2017) conducted a four-year study on 'Hass' avocado in Chile to evaluate four irrigation regimes (25, 50, 75, and 100% of ET_o), and their results showed fruit size and fruit yield increased with increasing water application. Fruit size is an important parameter for avocado crop production so that inadequate soil water supply may result in a large negative economic impact on the avocado industry.

Water deficit reduces not only fruit size and fruit yield, but also post-harvest fruit quality and plant health. Bower *et al.* (1989) identified that pre-harvest water stress on avocado plants during early fruit development caused a high browning potential after harvest. Chartzoulakis *et al.* (2002) observed 'Hass' and 'Fuerte' avocados under prolonged water-stressed conditions, and found ‘Hass’ avocado had significant damage to the root system, therefore, they concluded that the ‘Hass’ avocado plant is more vulnerable to drought than ‘Fuerte’.

Conversely, excess water application can also cause adverse effects in avocado. One major problem is *Phytophthora cinnamonmi* infection, that causes wilting, leaf discolouration, dieback, lower xylem water potential, and reduction of new vegetative and reproductive growth in avocado plants (Sterne *et al.,* 1978; Zentmyer, 1984). Another problem is a reduction of nutrient absorption due to lower soil aeration (Levinson & Adato, 1991; Gil *et al.*, 2009). Avocado is sensitive to the soil aeration condition, and low soil oxygen results in physiological problems in avocado plants, such as a decrease in nutrient uptake from the soil, stomatal closure, and reduction of root growth (Labanauskas *et al.*, 1978; Ferreyra *et al.*, 2014).

This chapter is a brief literature review. Section 1.2 will firstly describe general information for the avocado plant. Section 1.3 will address water use of the avocado plant relative to weather conditions and fruit load. Section 1.4 will present irrigation requirements over summer, explaining possible impacts of water stress on fruit size and quality. Finally, Section 1.5 will focus on avocado fruit growth from the plant physiological perspective, including vascular functioning during drought. Additional detail for a number of these topics is provided within the introductions to the three experimental chapters that follow.

1.2 General information about avocado production in New Zealand

‘Hass’ is the most popular avocado variety in New Zealand, accounting for 95% of avocado fruit production, and is the only variety used to produced fruit for export (New Zealand Avocado, 2020a).

Under New Zealand conditions, the major avocado flowering season is in spring from early October to middle November, although flowers may also be produced in autumn from April to June (Figure 1.2). Avocado produces thousands of small flowers during the flowering season, but final fruit set measured at harvest is low, typically less than 0.1% due to high rates of flower and immature fruit abscission (Garner & Lovatt, 2008). Avocado flowering behaviour is unusual and is described as synchronous protogynous dichogamy (Carr, 2013; Salazar-Garcia *et al.*, 2013). The avocado flower opens twice over a two day period. On the first day, the avocado flower opens in the female phase with a receptive stigma. Then it closes at night and opens again on the following day in the male phase with a non-receptive stigma and dehisced anthers. It then closes again that night (Salazar-Garcia *et al.*, 2013). This unusual flowering behaviour is believed to promote cross-pollination between the two flowering genotypes, referred to as type A (e.g. Hass), and type B (e.g. Fuerte, Bacon, Zutano), since these two types have different flower opening times during the day. Type A opens as female in the morning and male in the afternoon, and type B as female in the afternoon and male in the morning (Salazar-Garcia et al., 2013). Avocado fruit set occurs approximately two weeks after flowering in the female phase, but flower abscission is a common occurrence for avocado, with approximately 80% of flowers abscising before fruit set (Kaneko, 2016).

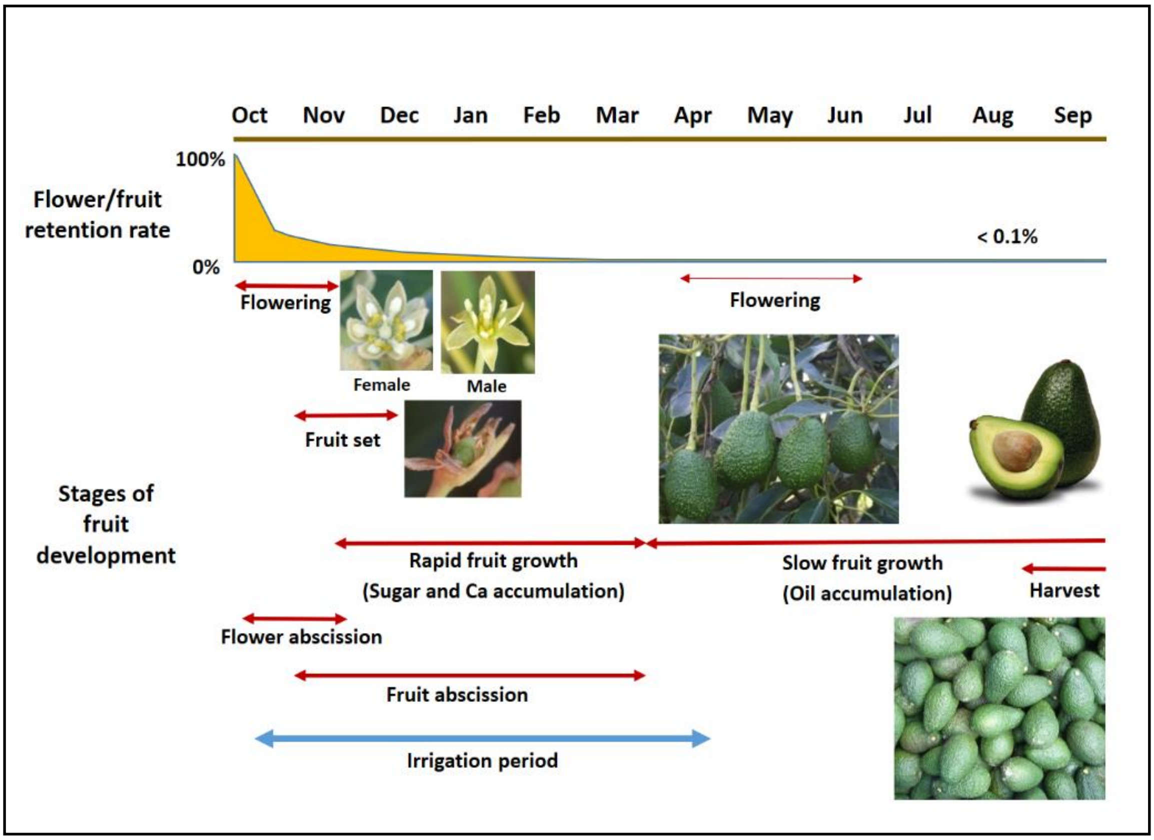

Figure 1.2: Diagram showing stages of fruit development of ‘Hass’ avocado flowering and fruit growth under New Zealand conditions. Where used, irrigation is applied between October and April (blue arrow).

Avocado fruit growth shows a single sigmoid curve with two different phases; a rapid growth period (Stage I) after fruit set with high sugar accumulation, and then a slow growth period (Stage II) with increasing oil accumulation (Liu *et al.*, 1999b) (Figure

1.3). The immature fruit abscission rate is very high for avocado, and more than 50% of immature fruit are lost during rapid fruit growth (Carr, 2013; Kaneko, 2016). Under New Zealand conditions, rapid fruit growth occurs from November to March, and then slow fruit growth continues until harvest.

In New Zealand, harvest generally starts from August to September when the fruit are approximately 10 months old, but may continue through to March the following year. In terms of fruit yield, year-to-year variation is high for avocado, due to alternate bearing when a low (‘off’) crop year is followed by a high (‘on’) crop year (Garner *et al.*, 2008; Salazar-Garcia *et al.*, 2013). An off-cropping year can be initiated by environmental events (e.g. cold temperatures and drought causing a loss of flowers or fruit), and lower fruit yield will create a heavy cropping year in the following season, and then a heavy cropping year will reduce fruit yield in the next season due to limitation of resources for flowers (Gould *et al.*, 2019). Alternate bearing is a common feature of many tree crops, but is particularly acute in avocado (Mickelbart *et al.*, 2012; Kaneko, 2016). In particular, the New Zealand maritime climate directly influences physiological conditions of avocado, resulting in irregular fruit yield that brings economic problems to growers and the avocado industry (Figure 1.1), such as a price fluctuation and difficulty to retain market share (Paz-Vega, 1997).

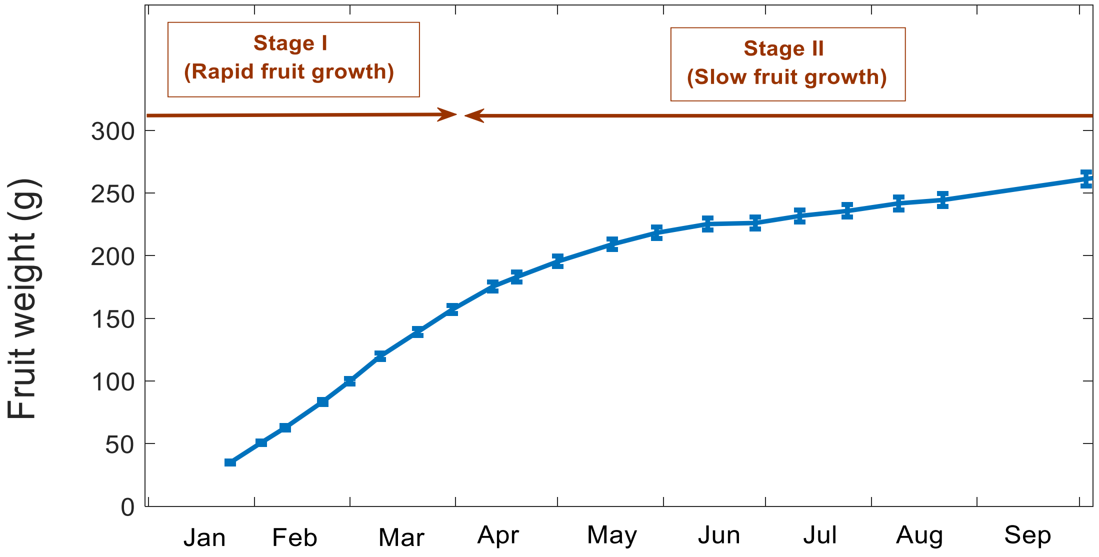

Figure 1.3: ‘Hass’ avocado fruit growth in weight (g) on the tree under the New Zealand conditions (Kaneko, 2016).

1.3 Avocado plant water use

Knowledge of plant water use is essential for appropriate crop water management. The most common method to estimate plant water use is the $K_c \cdot ET_o$ approach (Allen *et al.*, 1998; Allen *et al.*, 2006). This approach is derived from the observation that plant water use is related to the weather conditions, and the crop coefficient (K_c) is the ratio of crop evapotranspiration (ET_c) to reference evapotranspiration (ET_o). Therefore, the crop water requirement can be estimated if K_c and ET_o have been determined.

The K_c value depends on crop species, and the water requirements for a crop depend on the local weather conditions, orchard conditions (e.g. landscape and soil characteristics) and plant conditions (e.g. fruit load, total leaf area relative to the ground area, and plant age). However, water use of fruiting trees generally varies, depending on their physiological stages. Many fruit crop species, such as peach and apple increase tree water use from bloom to harvest, but the magnitude of K_c depends on species (Marsal *et al.*, 2014). Therefore, in the case of fruiting plants, ET_c can be split into two factors; plant transpiration (T) and soil evaporation (E), and the basal crop coefficient (K_{cb}) can be determined as a ratio of T to ET_o, while K_c values can be identified as a ratio of ET_c to ET_o.

To obtain reliable K_{cb} and K_c values for avocado, monitoring local weather conditions and quantifying crop water use are necessary. The ET_o calculation, measurement of crop water use (T) and crop evapotranspiration calculated from the soil water balance (ET_{wb}), and the relationship between plant water use and fruit load are briefly reviewed below.

1.3.1 Reference evapotranspiration

ET_o is a sum of water movement from the Earth’s surface to the atmosphere, including from the ground surface by evaporation and from leaves by plant transpiration (Allen *et al.*, 1998). Evaporation is largely influenced by weather conditions, and plant transpiration depends on the weather, the type of plant and its physiological and morphological characteristics.

In recent years, a parameterised version of the FAO-56 Penman-Montieth equation recommended by Food and Agriculture Organization of the United Nations (Allen *et al.*, 1998; Allen *et al.*, 2006) has been widely adopted to calculate ET_o. The FAO-56 Penman-Montieth equation requires basic meteorological data, such as air temperature, vapour pressure deficit, solar radiation, and wind speed, and ET_o is an estimate of water use for a well-watered short grass of uniform height (Allen *et al.*, 1998). In some cases such as an area of patchy vegetation, the FAO-56 Penman-Monteith equation becomes less reliable (Moran *et al.*, 1996), but the features of agricultural vegetation are more uniform than those of natural vegetation. Thus, the FAO-56 Penman-Monteith equation is generally appropriate to obtain ET_o in orchards.

The FAO-56 Penman-Monteith equation is a global standard (Pereira *et al.*, 2015). It has been used for many studies in numerous locations, for example, irrigation management in citrus in Italy (Consoli *et al.*, 2017), grape in Spain (Valdes *et al.*, 2019), mango in Brazil (Bezerra *et al.*, 2012) and sugarcane in South Africa (Bastidas-Obando *et al.*, 2017). The FAO-56 Penman-Monteith equation is a useful tool to estimate accurate ET_o for better crop irrigation management if local meteorological data is available. Chapter 2 of this thesis provides a more detailed description of the FAO-56 Penman-Monteith equation.

1.3.2 Plant transpiration

Quantification of plant water use is the key to determine K_{cb} values for a crop. There are a number of ways to measure plant water use. For example, a lysimeter is a device that measures actual evapotranspiration by plants (Tyagi *et al.*, 2000; Lopez-Urrea *et al.*, 2012), but it is expensive and might involve root restriction for large trees (Allen *et al.*, 1998). Stomatal conductance (g_s) measurement (Williams *et al.*, 2012) is another method to estimate plant transpiration, but it is not a direct measurement, therefore, might induce an error in calculation (Costes *et al.*, 2002). The soil water balance method involves soil moisture monitoring (Pocas *et al.*, 2015; Odi-Lara *et al.*, 2016), and is a simple method if there is little spatial variation (Bois *et al.*, 2020). Finally, in recent years, thermometric methods such as sap flow measurements have become widely accepted (Smith & Allen, 1996; Green *et al.*, 2003), as a convenient and reliable measurement to estimate water uptake by a crop (Fernandez *et al.*, 2001; Alarcon *et al.*, 2005; Nicolas *et al.*, 2005).

Sap flow measurement is a plant-based water use indicator that measures the flow of water through the sapwood by applying heat and measuring temperature upstream and downstream of the heater (Green *et al.*, 2003). Sap flow measurement with the compensation heat pulse method (CHPM) applies a few seconds of heat pulse periodically and monitors temperature changes following the pulse (Green *et al.*, 2003). It is a non-destructive measurement used in woody plants, which is easily replicated and automated, and suitable for long-term monitoring. The sap flow measurement with the compensation heat-pulse method (CHPM) has already been used in a wide range of crop species, such as apple, olive, Asian pear (Fernandez *et al.*, 2008), and avocado (Kaneko, 2016). Sap flow in the main trunk is an important indicator of plant water uptake. As a long-term monitoring technique, sap flow measurement with the CHPM is suitable to compare avocado tree water use among regions and over multiple years.

1.3.3 Crop evapotranspiration calculated from the soil water balance

Crop evapotranspiration can be calculated from the soil water balance method (ET_{wb}). Sap flow measurement can determine T, while ET_{wb} includes crop transpiration and soil evaporation because transpiration occurs within the soil-plant-atmosphere continuum. By comparing T and ET_{wb}, the water loss from the soil surface (E) can be determined.

The soil water balance method predicts water storage and water losses in the soil by estimating the flow of water in and out of a system. The inputs of water are rainfall and irrigation, and the outputs are crop transpiration, soil evaporation (including transpiration from grass), surface runoff, and drainage out of the root zone (Palomo *et al.*, 2002; Teixeira *et al.*, 2008). Therefore, water loss by a crop and the soil surface can be determined by quantifying the water inputs (rainfall or irrigation) and the output (runoff and drainage). Soil moisture measurement is the most common method to monitor changes in water storage in soil. It can also estimate how much water is available for plants, as only the soil water between field capacity and the wilting point is effectively used by plants (Myers & Talsma, 1992).

E includes soil evaporation and water loss from the grass, therefore, E is highly variable, depending on the type of the ground surface, amount of vegetation cover, and area shaded by the crop (Allen & Pereira, 2009). ET_{c} and K_{c} can be calculated more accurately when T and E are obtained separately.

1.3.4 Avocado tree water use and fruit load

Water use of fruiting trees is influenced by their physiological conditions. For deciduous trees, such as cherry (Juhasz *et al.*, 2013) and peach (Abrisqueta *et al.*, 2013), water use is very low initially, but increases rapidly during leaf development, becomes a plateau in mid-season, and then decreases at the end of the season. However, avocado is a large evergreen tree, and tree water use may be associated more with fruit development, as water demand for avocado is highest in summer during a period of early fruit growth (Kaneko, 2016).

The amount of tree water use might depend on fruit load because fruit growth requires significant amounts of carbohydrates, hence, fruiting plants need to increase photoassimilate capacity during fruit development (Berman & DeJong, 1996; Sade & Moshelion, 2014; Bustan *et al.*, 2016). Increases in g_s also have been observed during the period of fruit growth in grapevines (Downton *et al.*, 1987; Naor *et al.*, 1997), apple trees (Naor *et al.*, 1995), and olive trees (Naor *et al.*, 2013). Studies in apple (Naor *et al.*, 2008) and olives (Bustan *et al.*, 2011) have reported that Ψ_{stem} is correlated with fruit load, as Ψ_{stem} decreases with an increase in fruit load.

Avocado is no exception. Silber *et al.* (2013) investigated the relationship between irrigation application and fruit load on 'Hass' avocado trees in Israel, and they found that fruiting plants consumed 40% more water than non-fruiting plants, even though fruiting plants had less vegetative growth compared with non-fruiting plants. Avocado trees typically have a variable fruit load, and tend to have an alternate bearing cycle. This cycle may be reflected in water consumption of avocado plants over multiple years. However, there is no publication that provides K_c for avocado in New Zealand, and internationally there is no information for K_c relative to fruit load.

1.4 The effects of water stress on avocado plants and fruit development

To increase crop productivity, effective water management in Avocado is necessary in drier countries, such as Australia and Spain (Carr, 2013; Pleguezuelo *et al.*, 2018). However, plants regulate water use by physiological responses toward soil water availability and weather conditions, and they might behave differently from place to place.

Under drought conditions, plants display two different ways to regulate their water status as a continuum of responses from one extreme to the other, called isohydric and anisohydric behaviours (McDowell *et al.*, 2008; Klein, 2014; Buckley, 2019). Water enters the plant through the roots, flows up in the stem xylem driven by a water potential gradient, and then evaporates and diffuses out of stomata in the leaves when they are open for the photosynthetic net assimilation of CO_2 (A). Theoretically, isohydric species respond to physiological water stress by reducing g_s, so isohydric plants tend to avoid water loss and inhibit A to maintain a constant Ψ_{stem} and leaf water potential (Ψ_{leaf}) at midday. In contrast, anisohydric species do not exhibit stomatal closure as readily, therefore, anisohydric plants show a decline in Ψ_{stem} and Ψ_{leaf} as a response to water deficit, but maintain their photosynthetic capacity until more severe water-stressed conditions develop (Sade & Moshelion, 2014). Anisohydric species are thought to be more drought tolerant than isohydric species, but they have less stomatal regulation that leads to sudden deterioration caused by xylem cavitation (McDowell, 2011).

A study of plant behaviour is important because it helps to understand how plants regulate water status, and how water is allocated to plant organs during a period of water shortage. In this section, I will focus on the “plant response to water stress” and “impacts of water stress on avocado fruit development”.

1.4.1 Plant physiological response to water stress

Stomatal regulation is the main factor that influences water flow in a plant. In angiosperms, stomata are controlled by the movement of guard cells that react to hydraulic and chemical signals, or changes in vapour pressure deficit (VPD) in the atmosphere, and control the balance between CO_2 uptake and water loss (Lawson *et al.*, 2014). In general, there is a strong relationship between g_s and A, and between xylem sap flow and plant transpiration (Sakuratani, 1981; McAdam *et al.*, 2016). However, plants often show complex behaviours, like shifting from isohydric to aniohydric behaviours in response to environmental changes and physiological conditions (Rogiers *et al.*, 2012).

Some crop species, such as apple, olive, grape and avocado, exhibit changing stomatal behaviour during a period of fruit development, since the fruit becomes a strong reproductive sink and accumulate photoassimilates throughout fruit growth (Sade & Moshelion, 2014). Due to the high demand for carbohydrate assimilates, fruiting plants might have higher g_s and A. In particular, avocado fruit contains high oil content (typically 20-30% fresh weight) with a single large carbohydrate-rich seed (Whiley *et al.*, 1996; Liu *et al.*, 1999b). To produce such nutritious fruit, avocado fruit development requires high energy inputs from photosynthesis (Liu *et al.*, 1999a).

Sterne *et al.* (1977) and Bower *et al.* (1978) reported that stomatal closure in avocado began when Ψ_{stem} decreased to approximately -1.0 MPa. Therefore, avocado is considered to be an isohydric species, even though the stomatal behaviour of avocado plants remains unclear.

1.4.2 Impacts of water stress on avocado fruit development

Avocado is known to be sensitive to plant water status, especially during early fruit development, and it is believed that water stress during such a critical period can negatively affect final fruit yield due to a decrease in fruit size and fruit quality.

Water moves through the xylem and accumulates in the fruit during rapid fruit development, but limitation of soil water content affects plant water status and changes the pressure gradients in the xylem (Sevanto *et al.*, 2011). When that happens, fruit growth may become slower due to less water availability to fruit, resulting in smaller sized fruit at harvest (Matthews & Shackel, 2005). In addition, the fruit of water-stressed plants may have a different carbohydrate accumulation process compared with non-water stressed plants. If the avocado plant is a true isohydric species, the pressure gradient effects will be minimized, but photosynthesis will be reduced due to stomatal closure during drought conditions, and consequently, it will cause a limitation in carbon supply to the fruit, changing source-sink activity in the phloem and carbohydrate compositions in fruit (Richings *et al.*, 2000).

Northern New Zealand has a mild climate, and severe water deficit is rare in the avocado growing regions (NIWA, 2020). Therefore, there is little information regarding irrigation and the effect of water stress on avocado if avocado plants are not irrigated during summer under New Zealand conditions. Currently, only two-thirds of avocado orchards in New Zealand use irrigation, and irrigation decisions vary, depending on growers' perspectives.

1.5 Avocado fruit growth

Botanically, avocado fruit is defined as a berry which consists of three layers; the exocarp, fleshy mesocarp, and a single large seed (Chanderbali *et al.*, 2013).

Many fleshy fruit show a double sigmoid pattern of fruit growth with three stages. In general, fruit growth is enhanced by cell division during Stage I, but it slows down during Stage II. During Stage III, fruit increases in size again due to the expansion of existing cells (Matthews & Shackel, 2005). In most fruit, cell division occurs only during Stage I (McAtee *et al.*, 2013). However, unlike many other fruit, the avocado exhibits a single sigmoid pattern with two main stages of growth: a rapid growth period of Stage I, and a slow growth period of Stage II (Kaneko, 2016). Cell division continues throughout fruit development, and final fruit size is determined by cell number rather than cell size (Cowan *et al.*, 2001) (Figure 1.3).

The oil concentration of avocado fruit increases through fruit development, and is an important indicator for fruit maturity. In general, oil content is expressed by fruit dry matter content (%), while there is a strong correlation between dry matter content and oil content (Hofman *et al.*, 2013). In New Zealand, fruit dry matter content needs to be tested before harvest. The current minimum maturity standard for 'Hass" avocado fruit is 23% for local and 24% for export markets (New Zealand Avocado, 2020b).

Mature avocado fruit contains high concentrations of protein and seven-carbon (C7) sugars (Davenport & Ellis, 1959). C7 sugars are uncommon in fruit, but they have an important role in carbon allocation processes in the avocado plant (Salazar-Garcia *et al.*, 2013; Pedreschi *et al.*, 2019). Fruit growth is based on carbon metabolism and C7 sugars are thought to enhance sugar uptake and continuous fruit growth, and contribute to the carbon balance of the plant (Liu *et al.*, 2002). Avocado fruit grow more slowly than most other fruit. In particular, New Zealand has a cool climate when compared with other avocado growing countries, and 'Hass' avocado fruit reaches maturity 10–14 months after the fruit sets. Avocado fruit do not ripen while they remain attached to the tree and C7 sugar transport continues in the phloem. Ripening begins when there is a decline in C7 sugars in avocado fruit. In other words, the fruit remain on the tree without ripening if they are not harvested (Salazar-Garcia *et al.*, 2013).

Water supply is essential for avocado fruit development, hence, water deficit may directly influence avocado fruit growth. In this section, "xylem and phloem transport" and "the fruit water balance" are described.

1.5.1 Xylem and phloem transport

Water moves between the stem and fruit through the xylem and phloem of the peduncle. A diurnal pattern of water flow into and out of fruit has been observed in various crop species, such as apple, citrus, mango (Sakuratani, 1981; Turpin & Chandra, 2015), with shrinkage during the day and expansion at night. This fluctuation relates to changes in the gradient of water potential in the xylem between stem and fruit. Net water inflow to fruit occurs primarily at night, when photosynthesis stops, while outflow from the fruit occurs when Ψ_{stem} is more negative than fruit water potential (Ψ_{fruit}) (Matthews & Shackel, 2005).

However, water also moves into fruit through the phloem with photosynthetic products. Carbohydrates are produced in leaves, and carbon transport occurs by the pressure gradient in the phloem between source and sink (Savage *et al.*, 2016). The xylem and the phloem are generally close to each other, and an increasing hydraulic gradient in the xylem influences water movement in the phloem (Sevanto, 2014). Some fleshy fruit, such as grape (Greenspan *et al.*, 1994) and tomato (Ho *et al.*, 1987), show a hydraulic shift in flow from the xylem to the phloem as the fruit develops. It is a common occurrence that xylem water flow into fruit decreases, and phloem flow increases or is at least maintained during later fruit development. It has been hypothesised that hydraulic resistance in the xylem of the fruit pedicel and receptacle contributes to the shift in water flow between the two vascular tissues into the fruit (Mazzeo *et al.*, 2013). For example, in grape, it was initially proposed that the xylem through the fruit pedicel and receptacle becomes dysfunctional after the onset of ripening, whereas the phloem remains functional (Findlay *et al.*, 1987). More recently it has been shown that the reduction in water movement in the xylem may instead be caused by changes in solute distribution and the pressure gradients driving flow (Bondada et al 2005). However, the mechanism of water flow into fruit varies between species, and the patterns of xylem and phloem water transport into avocado fruit have not been studied in detail.

1.5.2 The fruit water balance

Understanding the relationship between fruit development and the water balance is particularly important, because the water balance has a huge impact on fruit growth. At a plant scale, the effect of water stress on fruit growth can be examined by testing two hypotheses that it directly influences fruit growth by altering the pressure gradients and vascular flows during fruit development, and/or that it indirectly affects fruit growth by reducing photosynthesis and assimilate supply.

Fruit gain water through the xylem and the phloem, and lose water due to outflow through the xylem and via the fruit skin as fruit transpiration (Greenspan *et al.*, 1994; Greenspan *et al.*, 1996). Fruit size increases when water input (vascular inflow) surpasses water output (vascular outflow and fruit transpiration) each day, but fruit size is possibly reduced when plants have water stress and water withdrawal from the fruit increases relative to water accumulation in the fruit. In theory, through the xylem, reverse flow increases with a decline in Ψ_{stem}, and Ψ_{stem} becomes more negative when soil water content is limited (Matthews & Shackel, 2005). The thermal sap flow technique (Cermak *et al.*, 2004; Higuchi & Sakuratani, 2006; Clearwater *et al.*, 2013) and continuous fruit diameter monitoring by linear transducers (LTs) or similar devices (Jones & Higgs, 1982; Morandi *et al.*, 2014; Keller *et al.*, 2015) have been used to investigate water movements in fruit pedicels and shoots in some fruit species. In addition, in response to drought conditions, a change in the xylem and phloem flow has been observed in some fleshy fruit species, such as peach (Morandi *et al.*, 2014), mango (Higuchi & Sakuratani, 2006), and grape (Greenspan *et al.*, 1994).

However, changes in fruit vascular flows in response to water deficits highly depends on species and the plant physiological stage, and the daily mechanisms of avocado fruit growth at a plant scale have not been reported yet. The question about how water stress reduces avocado fruit growth remains unclear. So, more information is required for its use as a water-stressed indicator, relative to fruit size and final fruit yield for avocado.

1.6 Conclusions

This literature review presented general information regarding avocado reproductive phenology, avocado plant water use, the effects of water stress on plant water status and fruit development, and avocado fruit growth. Informed decision making for irrigation management requires an understanding of a plant’s response toward water stress. A number of studies have assessed the water requirements of the avocado plant in dry countries. However, there is a lack of supporting scientific research for the best irrigation practice in the New Zealand avocado industry. This is reflected by the wide variation in irrigation regimes implemented by growers, and about one-third of growers do not use irrigation in this country. The influence of water stress on fruit growth is complex, therefore, for successful fruit production, there is a high level of need for this research to investigate how much water the avocado requires, relative to local abiotic conditions in New Zealand.

1.7 Research aim, objectives and hypotheses

The aim of this thesis was to understand ‘Hass’ avocado tree water use under New Zealand conditions and the effects of water stress on avocado fruit development and vascular functioning.

There were three main objectives:

- Objective 1: Measure ‘Hass’ avocado tree water use in New Zealand.
- Objective 2: Document the effect of water deficits on ‘Hass’ avocado fruit development in the orchard.
- Objective 3: Examine the dynamics of avocado fruit water balance and the effects of water stress on fruit growth.

Objective 1

Objective 1 was to measure ET_o, T and ET_{wb} for mature ‘Hass’ avocado trees in the three avocado growing regions of the Bay of Plenty, Whangarei, and the Far North (Figure 1.4, Appendix A). ET_o was calculated from local meteorological data with the FAO-56 Penman-Monteith equation, T was obtained from sap flow measurement, and ET_{wb} was determined from the soil water balance. Based on the ET_o, T, and ET_{wb} values, K_c values for avocado were determined. To provide reliable K_c values and an irrigation guideline to New Zealand growers, this study examines variation in avocado tree water use associated with the combination of weather conditions, soil type, crown size, and fruit load.

Figure 1.4: New Zealand map showing the three study sites

The hypotheses were that there is a strong relationship between tree water use and ET_o (T is high when ET_o is high). Also, there is a correlation between T and ET_{wb}, even though soil water capacity is associated with soil types. In addition, fruit load influences tree water consumption, as heavy crop plants consume more water and vice versa.

Objective 2

The aim of Objective 2 was to assess the irrigation requirements of 'Hass' avocado in the Bay of Plenty, New Zealand, and to investigate the effects of water deficit on fruit growth and fruit yield, by comparing between irrigated and non-irrigated trees. During the experiment, soil water content was monitored continuously, and fruit growth and fruit abscission were monitored periodically in the on-cropping year. Also, plant water status was examined by measurement of plant water potential (Ψ_{plant}) and g_s.

The hypothesis was that a decrease in soil water content during the early fruit development stage from November to February would reduce fruit size, hence, fruit weight at harvest. Isohydric stomatal closure in response to decreased soil water content was expected to cause a reduction in fruit dry matter content. It was also hypothesized that avocado has highly variable fruit load between trees, and that fruit load would interact with the effect of water stress on fruit size and dry matter content.

Objective 3

Objective 3 included monitoring water flow through shoot stems and fruit pedicels, assessing changes in water flow in response to water stress, and quantifying the effect of water stress on avocado fruit growth. This was a glasshouse experiment because the plant water status of potted plants was more controllable than that of the field. Water movement was observed by installing heat ratio sap flow gauges on shoot and fruit stems, and fruit growth was monitored continuously using LTs. Also, plant water potential and g_s were measured periodically.

The hypothesis was that fruit size is reduced by drought because of less fruit water inflow and more outflow to the parent plant via the xylem. It was also hypothesized that photosynthesis is reduced by water stress, and this will lead to a reduction in phloem flow and, consequently reduced fruit dry matter content.

1.8 Thesis structure

There are five chapters in this thesis. The details of each chapter are outlined below.

Chapter 1: Introduction

This chapter is an introduction to the thesis. It contains a literature review that focuses on a general information of avocado plant physiology, fruit growth and avocado response to water stress. It also provides research objectives.

Chapter 2: 'Hass' avocado tree water use in New Zealand (Objective 1)

This chapter provides a study of avocado tree water use and estimates of ET_c in the Bay of Plenty, Whangarei, and the Far North. At each site, the ET_o, T, and ET_{wb} values are obtained from meteorological monitoring, sap flow measurements, and the soil water balance method, respectively. From these ET_o, T and ET_{wb} values, the K_c values in the three different regions are provided in order to standardise irrigation practices in New Zealand. Tree water use and crop coefficients are similar between growing regions, regardless of soil type, and are influenced by tree size and fruit load.

At the time of the final thesis submission, this chapter had been submitted to the journal of Agricultural Water Management.

Chapter 3: Effect of water deficit on 'Hass' avocado fruit development in the orchard (Objective 2)

This chapter evaluates the effect of water stress on mature avocado plants and fruit growth in an orchard in the Bay of Plenty, New Zealand. During an irrigation period, soil water content and plant water status are monitored, and the impact of water stress on fruit development is assessed at harvest. Plant water status is also monitored by Ψ_{stem}, Ψ_{leaf}, and g_s measurements. Fruit weight reduction is observed in an on-cropping year. From the results, irrigation requirements are assessed.

Chapter 4: Dynamics of avocado whole plant water balance and the effects of water stress on fruit growth (Objective 3)

This chapter examines the dynamics of avocado fruit growth, focusing on vascular transport and the effect of water stress on fruit growth. Water movement on fruiting and vegetative branches are measured using external sap flow probes, fruit growth is monitored continuously by LTs, and fruit quality is analysed at the end of this experiment. This research provides more detail regarding vascular functioning in developing avocado fruit. Fruit growth slows down under water-stressed conditions, because there is less water inflow to fruit and more outflow from fruit under water-stressed conditions.

Chapter 5: Summary and Conclusion

This chapter provides a summary including the research findings of each objective, overall conclusion, and suggestions for further research on the avocado plant, including further assessment of water requirement in response to climate change, deficit irrigation and xylem-phloem transport.

Appendix

The appendices provide additional site information, soil-type specific calibration information for the soil moisture probes used for Objectives 1 and 2, and regression relationships used for non-destructive estimation of fruit fresh weight, volume and surface area.

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Chapter 2

Water use of 'Hass' avocado trees in New Zealand

2.1 Introduction

Planting of horticultural crops across the world is increasing, putting pressure on the amount of water available for crop production. This is true for the New Zealand avocado industry, where orchard area has increased over the past 20 years. Avocado is an important crop in New Zealand, for both export and local market. To improve fruit production, avocado trees should be irrigated if there is a prolonged dry period during fruit development (Carr, 2013; Pleguezuelo *et al.*, 2018). However, developing an irrigation strategy for a novel crop requires research that incorporates the effects of climate, soils and crop management.

Plant water use is largely influenced by weather conditions, such as air temperature, humidity, solar radiation, and wind speed (Allen *et al.*, 1998a; Alves *et al.*, 2007). The $K_c \cdot ET_o$ approach (the FAO56 method) may be the simplest method to calculate crop water use, if weather data are available. The crop coefficient (K_c) is defined as the ratio of crop evapotranspiration (ET_c) to reference evapotranspiration (ET_o) (Doorenbos & Pruitt, 1977; Wright, 1982; Allen, 1986). ET_o can be calculated from weather data using the FAO-56 Penman-Monteith equation (Allen *et al.*, 1998a; Allen *et al.*, 2006). Thus, from a known K_c for a specific crop and measurements of local weather conditions, ET_c can be estimated.

However, unlike herbaceous crops, the K_c values of fruiting trees can be variable, depending on plant physiological conditions, such as leaf area, tree density, pruning operation, fruit load and ground cover (Juhasz *et al.*, 2013). Avocado in particular has unique characteristics compared to other tree crops, including a long fruit development period and a strong tendency towards alternate bearing (on and off crops across two years), that creates variation in fruit yield between on and off cropping years (Lovatt, 2010). To understand the water use of fruiting trees independently of evaporation from other surfaces, ET_c can be divided into two components; plant transpiration (T) and water loss from the ground surface or ground cover crop (E) (Wright, 1982; Allen *et al.*, 1998b). In this case, the basal crop coefficient (K_{cb}) is determined as the ratio of T to ET_o, whereas K_c is defined as a ratio of ET_c to ET_o, and ET_c is the sum of T and E (Goodwin *et al.*, 2006; Paco *et al.*, 2006; Flumignan *et al.*, 2011).

The K_{cb} values represent plant (crop) water use itself, regardless of soil evaporation and transpiration from ground any cover such as grass. For accurate estimation of the K_{cb} values (T/ET_o), the T values can be obtained by sap flow measurement that involves the installation of needle-like probes in the main trunk of woody species (Green *et al.*, 2003). Sap flow measurement with the compensation heat pulse method (CHPM) has been widely used to quantify the water use of tree crops, such as lemon (Alarcon *et al.*, 2005) apricot (Nicolas *et al.*, 2005), olive (Fernandez *et al.*, 2001), almond (Nortes *et al.*, 2009), orange (Fernandez *et al.*, 2006), and avocado (Kaneko, 2016). In contrast, the K_c values include both plant water use and the water loss from the soil, and the soil evaporation can be influenced by the type of ground cover and the proportion of ground area shaded by the crop (Allen & Pereira, 2009). Both T and E are required for calculation of K_c (ET_c/ET_o). E can be obtained from the difference between T and crop evapotranspiration calculated from the soil water balance method (ET_{wb}). Estimation of ET_{wb} is based on continuous soil moisture measurements, knowledge of drainage and surface flows, and the concept of water fluxes from the soil to plant to atmosphere, or from the soil to atmosphere, as a continuum (Bonan *et al.*, 2014).

Avocado is a large evergreen tree that can grow over 10-15 m in height (Chanderbali *et al.*, 2013). For large trees, T and E are strongly influenced by tree size, leaf area, and planting density, due to solar energy partitioning (Wang *et al.*, 2007). When trees are young, there is generally a linear relationship between the K_c values of an orchard and the area of soil surface shaded by the tree canopy, as has been observed in peach (Johnson *et al.*, 2002) and pecan trees (Winer, 2007). However, this linear relationship usually reaches a plateau or maximum K_c when the proportion of area shaded reaches a threshold value (typically around 0.8) as the tree canopy develops (Andales *et al.*, 2006). Thus, adjustment of K_c values may be needed for avocado, to account for tree spacing and age, crown size, leaf area, and consequent variation in shaded area.

For fruiting trees, fruit load also has an important role. Many studies have reported that plant water use increases from light to heavy fruit load, for example, in apple (Naor *et al.*, 2008), olive (Gucci *et al.*, 2007), peach (Berman & DeJong, 1996), grapevine (Naor *et al.*, 1997), and avocado (Silber *et al.*, 2013). There is some literature (Du Plessis, 1991; Carr, 2013; Lahav *et al.*, 2013b; Kaneko, 2016) that provides K_c values for avocado orchards, as a basic irrigation guideline. However, plants behave differently from place to place, and these K_c values were determined without consideration of fruit load.

In New Zealand, avocado is grown in the warmer regions of the North Island, and the major avocado growing areas are the Western Bay of Plenty district, rural land near the city of Whangarei in the Whangarei district, and the Aupori peninsula (North of Kaitaia) in the Far North district (Thorp *et al.*, 1997). These areas have different characteristic soil types: allophanic soil, clay soil, and sandy soil, respectively (Hewitt, 2010). Soil properties influence both plant performance and the way irrigation should be managed. Allophanic soil has a high water-holding capacity and lower soil bulk density that offers less resistance to root growth (Environment Bay of Plenty, 2010). Compared to allophanic soil, clay soils consist of smaller particles, less pore spaces and are poorly drained; therefore, a longer irrigation interval may be adequate. In contrast, sandy soil has larger particles and poor water retention properties and requires a shorter irrigation interval (Molloy, 1988). Avocado is known to be sensitive to water status, as water deficits might impact fruit size and fruit yield, while excessive water causes root disease and tree decline. In New Zealand, to avoid adverse effects of water stress on fruit, avocado trees are generally irrigated in spring and summer, during the periods of the early fruit growth and intensive shoot growth. However, avocado tree water requirements under variable New Zealand soil and climatic conditions have not been quantified.

The aim of this research was to measure ET_o, T and ET_{wb} for mature 'Hass' avocado trees in the three avocado growing regions of the Bay of Plenty, Whangarei, and the Far North. ET_o was calculated from local meteorological data with the FAO-56 Penman-Monteith equation, T was obtained from sap flow measurement, and ET_{wb} was determined from the soil water balance. Based on the ET_o, T, and ET_{wb} values, K_c values for avocado were determined. To provide reliable K_c values and an irrigation guideline to New Zealand growers, this study examines variation in avocado tree water use associated with the combination of the weather conditions, soil type, crown size, and fruit load.

2.2 Methods

Study sites and the experiment

This research was conducted at orchards in three avocado growing regions, here referred to as the Bay of Plenty (the Western Bay of Plenty district), Whangarei (near the city of Whangarei), and the Far North (the Aupori peninsula in the Far North district). The orchards were located at 62 Prole Road, Omokoroa, Bay of Plenty (37°39’17.964’’S, 176°1’12.936’’E), 326 Whatitiri Road, Whangarei (35°46’41.376’’E, 174°7’56.9712’’E), and 97b Burnage Road, Pukenui, Far North (34°48’54’’S, 173°7’4.8’’E). According to their 30 year climate normals from 1981 to 2010, the mean annual average temperatures were 14.9 °C in the Bay of Plenty, 15.8 °C in Whangarei and 15.7 °C in the Far North (NIWA, 2020). The normal annual total rainfall was approximately 1190 mm in the Bay of Plenty, 1300 mm in Whangarei, and 1250 mm in the Far North (NIWA, 2020). Soil types were sandy loam (Typic Orthic Allophanic soil) (Hewitt, 2010) in the Bay of Plenty, Whatitiri clay loam (Typic Oxidic Granular soil) (Hewitt, 2010) in Whangarei, and Houhora sand (Typic Sandy Brown soil) (Hewitt, 2010) in the Far North.

The experiment was conducted from January 2017 in the Bay of Plenty, and from April 2017 in Whangarei and the Far North. It was conducted until November 2019 in the Bay of Plenty and Whangarei. However, in the Far North, the experimental trees began to show symptoms of disease from July 2018. All branches were pruned at this site in January 2019, and all sensors were removed in April 2019.

Plant materials

At each site, five mature trees of *Persea americana* ‘Hass’ avocado grafted on ‘Zutano’ seedling rootstock were selected. Basic information about tree age, trunk diameter, tree height, and plant spacing is presented in Table 2.1. Experimental trees were well irrigated, with one micro-sprinkler per tree positioned under the canopy. The amount of water applied at each irrigation was 600 L in the Bay of Plenty and Whangarei, and 210 L in the Far North, equivalent to 8-12 mm and 6.6 mm (based on tree spacing, Table 2.1), respectively. Irrigation intervals were 6-8 days, 8-12 days, and 3-4 days, respectively, with irrigation decisions made by the individual growers based on measurements of soil moisture content. Within the orchards, the ground was covered by leaf litter under the tree canopy, and the grass between rows was mowed periodically by the growers.

Table 2.1: Tree age, an average of tree diameter and height (±S.E.) of the five experimental trees at the study sites in the Bay of Plenty, Whangarei, and the Far North, measured in April 2017.

	Tree age (years)	Trunk diameter (cm)	Tree height (m)	Plant spacing (m)
Bay of Plenty	10	33.9 (± 1.11)	6.7 (± 0.39)	7.5 × 9
Whangarei	10	33.5 (± 1.48)	6.8 (± 0.38)	7 × 7
Far North	6	22.5 (± 1.18)	5.3 (± 0.21)	4 × 8

Weather monitoring

Meteorological stations were installed in January 2017 in the Bay of Plenty, and April 2017 in Whangarei and the Far North. Meteorological data were collected until November 2019 in the Bay of Plenty and Whangarei, and March 2019 in the Far North. In addition, meteorological data (NIWA, 2020) at the nearest stations (Tauranga, Whangarei, and Kaitaia, respectively) were used to obtain three full years of weather information (December 2019 in the Bay of Plenty, from January to March 2017 and December 2019 in Whangarei, and from January to March 2017 and from April to December 2019 in the Far North).

At each site, rainfall (TR-525I/TR-525USW tipping bucket rain gauge, Texas Electronics Inc, Dallas, TX), solar radiation (LI200 pyranometer, LI-COR Inc, Lincoln, NE), air temperature and humidity (the Bay of Plenty, HMP50 temperature and relative humidity probe, Campbell Scientific, Logan, UT; Whangarei and the Far North, 107 and C215 temperature and relative humidity probe, Campbell Scientific, Logan, UT), and wind speed (Vector A101M anemometer, Vector Instruments, Denbighshire, UK) were monitored every minute, and the data recorded every 15 minutes by a data logger (CR1000, Campbell Scientific, Inc, Logan, UT). For access to data, a modem was connected to the logger.

Based on the meteorological data, hourly potential evapotranspiration for a short grass canopy (ET_o) was calculated continuously by the data logger using the FAO-56 Penman-Monteith equation (Allen *et al.*, 2005; Allen *et al.*, 2006) as:

$$ET_o = \frac{0.408\Delta(R_\mathrm{n} - G) + \gamma \frac{C_\mathrm{n}}{T_\mathrm{a} + 273} u_2 (e_\mathrm{s} - e_a)}{\Delta + \gamma(1 + C_\mathrm{d} u_2)} \qquad [1]$$

where R_n is net radiation (MJ m^{-2} h^{-1}), G is soil heat flux (MJ m^{-2} h^{-1}), T_a is average air temperature (˚C), C_n is the numerator constant for the reference crop type and time step (37 mm h^{-1}), C_d is the denominator constant (day-time = 0.24 MJ m^{-2} h^{-1} and night-time = 0.96 MJ m^{-2} h^{-1}), u_2 is wind speed (m s^{-1}), (e_s - e_a) is saturation water-vapour pressure deficit (kPa) as e_s is saturation vapour pressure at 1.5 to 2.5 m height, and e_a is the average of e_s at maximum and minimum air temperature, Δ is the slope of the vapour pressure-temperature curve (kPa ˚C^{-1}), and ϒ is psychometric content (kPa ˚C^{-1}). The daily ET_o was calculated from the sum of hourly ET_o over 24 h, and then the monthly mean daily ET_o at the three study sites was calculated. (Note: in the calculation, soil heat flux was ignored as the magnitude of daily soil heat flux beneath the reference grass surface is relatively small).

The meteorological data from the study sites in the Bay of Plenty, Whangarei and the Far North were compared with the 30 year historical means from 1981 to 2010 for Tauranga, Whangarei, and Kaitaia, respectively (NIWA, 2020).

Sap flow

Plant transpiration (T) was obtained from sap flow measurements. Two sap flow probe sets (model HP3TC-S, Tranzflo NZ Ltd., Palmerston North, New Zealand) per tree were installed in February 2017 in the Bay of Plenty, in April 2017 in Whangarei and the Far North, and the measurements were recorded until November 2019 in the Bay of Plenty and Whangarei, and March 2019 in the Far North.

Each sap flow probe set was composed of two temperature probes and one heater, and the temperature probes had three copper-constantan thermocouples positioned at depths of 15, 25 and 40 mm from the bark. The heater probe was 1.8 mm in diameter and 50 mm in length. The probes were installed on the main trunk around 500 mm above the soil, by using a drill with a 2.0 mm drill bit and a jig to make three vertically aligned holes. The distance from the heater to downstream and upstream temperature probes were 10 mm and 5 mm, respectively.

At each site, the sap flow probes were connected to a multiplexor (AM25T, Campbell Scientific, Inc., Logan, UT) and data logger (CR1000, Campbell Scientific, Inc., Logan, UT). Every 30 minutes, heat was applied for 3 seconds, and temperatures were measured every 1.5 seconds for 8 minutes. The logger was connected with a modem. To avoid error of the measurements, the sap flow probes were re-installed each year.

Sap flow calculation

Heat pulse velocities were calculated using the Compensation Heat Pulse method (CHPM) (Swanson & Whitfield, 1981; Green *et al.*, 2003).

Initially, the heat pulse velocity V_h (cm h^{-1}) was calculated from:

$$V_\mathrm{h} = \frac{x_\mathrm{d} + x_\mathrm{u}}{2T_\mathrm{z}} 3600 \qquad [2]$$

where x_d was the distance from the heater to the downstream temperature probe, and x_u was the distance from the heater to the upstream temperature probe. Then, V_h was corrected for probe-induced effects and wounding, by the equation:

$$V = a_0 + a_1 V_\mathrm{h} + a_2 {V_\mathrm{h}}^2 \qquad [3]$$

where V (cm h^{-1}) is corrected sap flow velocity, V_h (cm h^{-1}) is the uncorrected heat pulse velocity, and a_o, a_1, and a_2 are correction factors (Green *et al.*, 2003).

The sapwood consists of three phases (gas, liquid, and solid matrix), and sap flow velocity was calculated based on the equation of Edwards and Warwick (1984), as:

$$J_\mathrm{s} = (kV_\mathrm{wood} + V_\mathrm{water})V \qquad [4]$$

where J_s is the sap flux velocity (cm h^{-1}), V_wood refers to the volume fraction of the woody matrix in the xylem, and V_water is mean volume fraction of water, and the k factor is related to the thermal capacity of the woody matrix. Here, J_s is calculated based on $V_\mathrm{wood} = 0.45$, $V_\mathrm{water} = 0.45$ (Green *et al.*, 2003), and $k = 0.505$ (Edwards & Warwick, 1984), as the value is expected to be constant within and between species (Alarcon *et al.*, 2000).

Sap flux density in the trunk was not uniform, therefore, V_h was measured and J_s was calculated at three radial depths per probe (Cohen *et al.*, 1981; Green *et al.*, 2003). Total sap flow of each tree was calculated from the mean sap flux density as:

$$F = 2\pi \int_{H}^{R} rJ_{\mathrm{s}}(r)\mathrm{d}r \qquad [5]$$

where F is the volume sap flux (L h^{-1}) is R is a cambial radius (m) identified from the measurements of bark depths, H is heartwood radius (m), and r is the radial depth. In this experiment, based on previous dye injection tests, the full stem cross-sectional area under the bark was assumed to be hydro-active sapwood, with no heartwood. Because each tree had two probe sets, tree water use (L h^{-1} $tree^{-1}$) was taken as the average of two measurements, and daily tree water use was calculated.

T was calculated from tree water use obtained from sap flow measurements as:

$$T = \frac{S}{PA} \qquad [6]$$

where T is tree transpiration (mm d^{-1}), S is daily tree water use (L d^{-1}) and PA is projected area of the canopy (m^2). All three orchards were considered mature, therefore, PA was assumed to be equivalent to the orchard area per plant estimated from plant spacing (9 m × 7.5 m in the Bay of Plenty, 7 m × 7 m in Whangarei, and 8 m × 4 m in the Far North). To examine the relationship between tree water use and fruit load (light to heavy crop), *T* was calculated for each tree.

Leaf area

At each site, the total leaf area of five experimental trees was estimated before and after shoot flush and pruning, using a Plant Canopy Analyzer (LAI-2200C, LI-COR, Inc. Lincoln, NE). Using a 90° view cap, above- and below-canopy readings were obtained. The measurements below-canopy were taken around the trunk facing outward at a height of 1 m, and at each tree, the measurements were repeated on the North, East, South and West sides. Direct sunlight induces a probable error, therefore, the measurement was taken at either dawn or dusk. The calculation of total leaf area was accomplished using the FV-2000 software (LI-COR Biosciences, 2019).

Soil water content

Crop evapotranspiration (ET_{wb}) was obtained from the soil water balance. The soil water content below the five sap flow trees was monitored using soil moisture probes

(CS615, CS616, and CS650, Campbell Scientific Inc., Logan, UT) from January 2017 to November 2019 in the Bay of Plenty, and from April 2017 to November 2019 in Whangarei, and from April 2017 to March 2019 in the Far North. These probes were calibrated before installation, using soil samples taken from the three orchards at the depths where the probes were buried. Calibration was based on moistening dry soil to a range of water contents, packing the soil in a cylinder (24 cm in diameter × 40 cm in height) to the same bulk density as observed in the field, and obtaining the linear relationship between the probe output and the known volumetric soil water content (Appendix B, Figure 6.2A-C).

At each site, the probes were installed vertically at the halfway point between the trunk and the edge of the canopy on the east side, spanning three depth ranges 0-30 cm, 31-60 cm, and 61-90 cm. These probes were connected to a data logger (CR1000, Campbell Scientific, Inc., Logan, UT), and soil water content was recorded every hour.

Based on data obtained from volumetric soil moisture measurements, ET_{wb} was calculated using the equation:

$$ET_{\mathrm{wb}} = P + I - \Delta C - D \qquad [7]$$

where ET_{wb} is crop water consumption, P is precipitation, I is irrigation, ΔC is the change in soil water content, and D is drainage (mm). Due to unknown surface runoff and drainage, ET_{wb} was calculated for periods of 3-5 days when significant rainfall did not occur, by assuming D and I were 0. The ET_{wb} was obtained each month during the experiment.

The basal crop coefficient (K_{cb}) and crop coefficient (K_{c})

Monthly K_{cb} values were calculated as:

$$K_{cb} = \frac{T}{ET_o} \qquad [8]$$

Individual K_{cb} values were compared with fruit yield each year and then compared across the three different regions.

Based on the T values obtained from sap flow measurements and the ET_{wb} values obtained from soil water balance, soil evaporation (E) was calculated as:

$$E = ET_{wb} - T \quad [9]$$

In this study, E includes loss of water from the ground surface including soil evaporation and transpiration from grass. Then, K_c was calculated as:

$$K_c = \frac{ET_c}{ET_o} \quad [10]$$

where ET_c is the sum of T and E.

The ET_c and K_c values were calculated monthly during the experiment. Monthly mean ET_c and K_c values were calculated from the average of two years of observations, and for trees with the lowest and highest crop.

Harvest

Fruit of the experimental trees were harvested on 02 October 2017 and 16 September 2018, in the Bay of Plenty, 18 October 2017, 10 October 2018, and 07 October 2019, in Whangarei, and 12 September 2017 and 09 October 2018 in the Far North. At harvest, fruit yield per experimental tree was obtained by total fruit weight, and then 50 fruit samples were selected randomly and weighed to measure the average fruit weight (Table 2.2). There was a failure of fruit set in the Bay of Plenty in 2019, resulting in no harvest for that year.

Table 2.2: Average fruit yield of five experimental trees with S.E. at the study sites in the Bay of Plenty, Whangarei, and the Far North from 2017 to 2019.

Year	2017		2018		2019	
	Yield (kg)	Fruit weight (g)	Yield (kg)	Fruit weight (g)	Yield (kg)	Fruit weight (g)
Bay of Plenty	116 (± 4.96)	260.8 (± 6.68)	216 (± 15.0)	254.7 (± 5.57)	-	-
Whangarei	80 (± 58.3)	276.0 (± 29.0)	206 (± 17.8)	225.7 (± 15.67)	158 (± 62.9)	215.4 (± 12.9)
Far North	136 (± 21.7)	193.8 (± 12.2)	82 (± 13.6)	156.0 (± 5.23)	-	-

Shaded area

A light-bar was used to measure shaded area at the orchards in the three regions. The light bar was made using a 1 m length and 25 mm width of aluminium box section, with 20 photodiodes (G2711-01, Hamamatsu Photonics K.K., Hamamatsu, Japan) aligned on the bar. The light-bar was connected to a data logger (CR1000, Campbell Scientific, Inc., Logan, UT), and installed on a trolley. During the measurements, data were recorded every second, and output was summed from all 20 sensors. To compare solar radiation between above and below canopy, a pyranometer-type sensor (LI200, LI-COR Inc, Lincoln, NE) was installed in the open, and connected to a data logger (CR1000, Campbell Scientific, Inc., Logan, UT), with data recorded every second. Shaded area was calculated as the ratio of the light bar measurements to above canopy measurements. The area of one measurement was from the trunk to the middle of the inter-row under three to five average-sized trees, moving the light-bar at a velocity of approximately 0.3 m s^{-1}. The measurement was taken within the area occupied by three trees (9 m × 22.5 m) in the Bay of Plenty, three trees (7 m × 21 m) in Whangarei, and five trees (4 m × 20 m) in the Far North, and replicated three times in different rows.

The measurements were taken three times over a day at 10:00, 12:00 and 14:00 in mid-summer 2019, on 17 January in the Bay of Plenty, 22 January in Whangarei, and 23 January in the Far North. In addition, data for tree spacing, crown diameter and K_c for a young avocado orchard in the Bay of Plenty were obtained from Kaneko (2016) for the relationship between shaded area and K_c values.

Statistical analyses

Statistical analyses were performed using R (R Core Team, 2016). Regression analysis was used to compare between the ET_o and T values, the ET_o and ET_{wb} values, and the T and ET_{wb} values at each study site. The relationships between T and total leaf area, and between the K_{cb} values and fruit yield were also analysed. Analysis of covariance (ANCOVA) was used to test if there was a difference in the slopes of the ET_{wb} versus T relationships between the three sites, and analysis of variance (ANOVA) was used to compare the monthly K_{cb} values between the three sites. A p-value of less than 0.05 was considered to be statistically significant.

2.3 Results

Weather

Monthly mean meteorological data over three years from 2017 to 2019 were compared with the 30-year normal (1981-2010) (Figures 2.1A--2.3A). From the observations at the study sites, the annual mean air temperatures were 15.5 ˚C in the Bay of Plenty and Whangarei, and 16.4 ˚C in the Far North. The Bay of Plenty and the Far North had slightly higher annual air temperatures than the 30-year normal (NIWA, 2020), whereas temperatures at the Whangarei was similar to the normal. There was a tendency for increasing daily maximum temperatures from year to year. In particular, the summer of 2018-19 was warmer compared to the 30-year normal, and February 2019 had the highest mean daily maximum temperature for the recorded period of three years at 28.0 ˚C in the Bay of Plenty, 25.3 ˚C in Whangarei, and 26.7 ˚C in the Far North.

Rainfall was characterized by drier summers and wetter winters in all three years at all three sites (Figures 2.1B-2.3B). Over the three years from 2017 to 2019, the annual total rainfalls were 1931, 1951, and 1037 mm in the Bay of Plenty, 1479, 1708, and 826 mm in Whangarei, and 1112, 1115, and 908 mm in the Far North. In the Bay of Plenty and Whangarei, the annual rainfall was above normal in 2017 and 2018, but below normal in 2019. In the Far North, rainfall was below normal from 2017 to 2019.

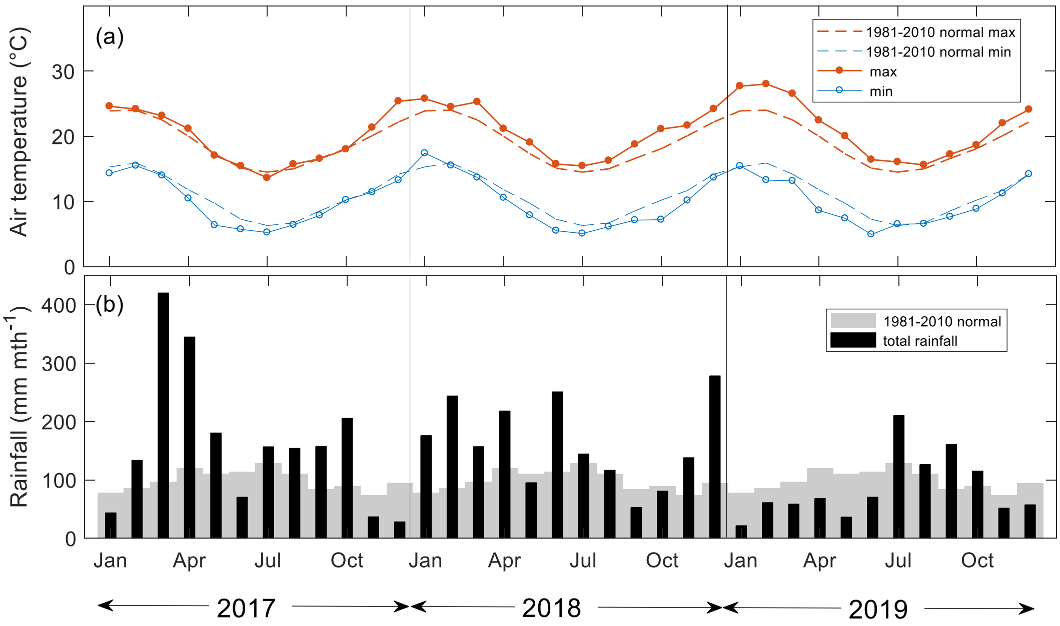

Figure 2.1: Monthly mean daily maximum and minimum air temperatures compared to the 30-year normal (a), and monthly total rainfall with the 30-year normal (b), from January 2017 to December 2019 in the Bay of Plenty.

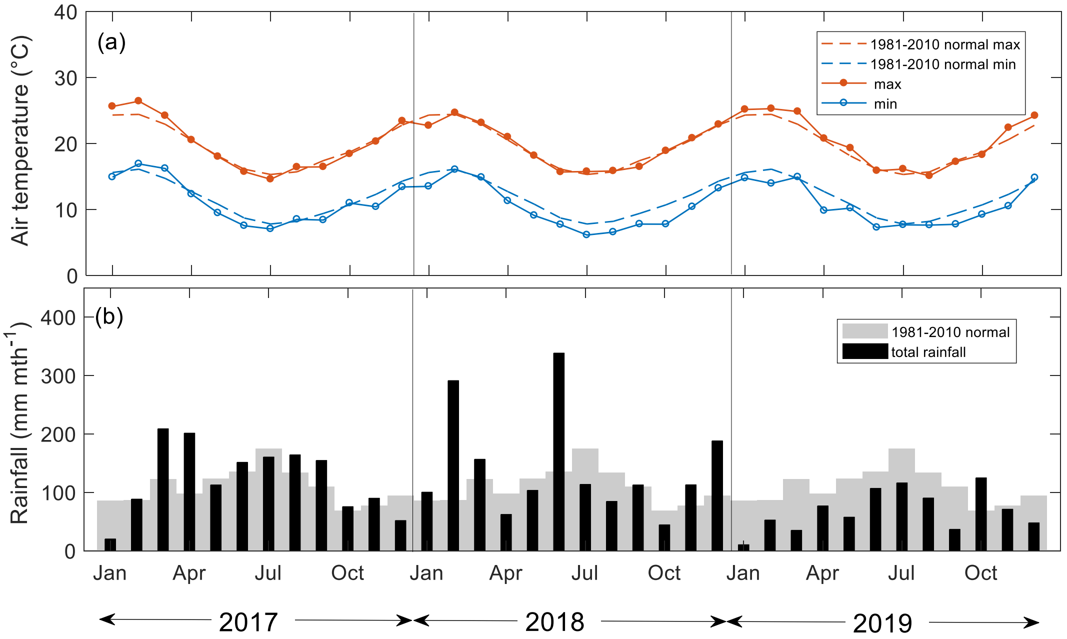

Figure 2.2: Monthly mean daily maximum and minimum air temperatures compared to the 30-year normal (a), and monthly total rainfall with the 30-year normal (b), at the orchard, from January 2017 to December 2019 in Whangarei.

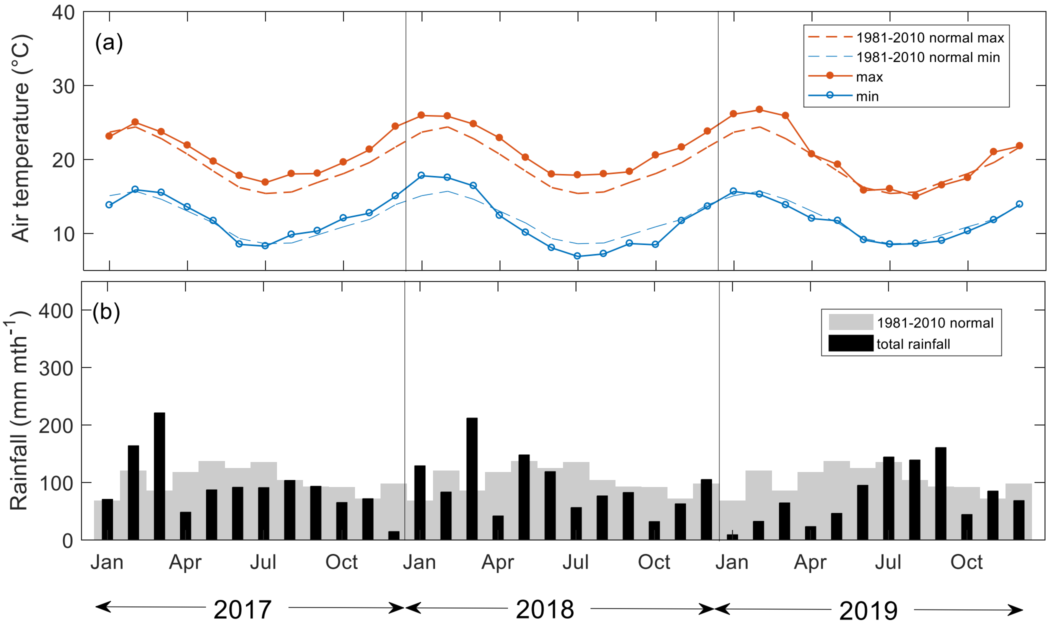

Figure 2.3: Monthly mean daily maximum and minimum air temperatures compared to the 30-year normal (a), and monthly total rainfall with the 30-year normal (b), from January 2017 to December 2019 in the Far North.

Sap flow measurement

Daily tree water use was measured over two years in the Bay of Plenty (Figure 2.4A), Whangarei (Figure 2.4B), and the Far North (Figure 2.4C), and compared with the seasonal trend of daily ET_{o} at the study sites. Overall, there was a strong relationship between the daily tree water use and ET_{o}, and this relationship changed seasonally.

Regardless of the local weather conditions and different soil types, avocado trees behaved with a similar seasonal cycle at all three sites, although the absolute amount of water required for the trees depended on tree size (Figure 2.4). Avocado tree water use corresponded with ET_{o}. In January, when ET_{o} was highest at over 4 mm d^{-1}, tree water use was also highest at approximately 150 L d^{-1} in the Bay of Plenty, 120 L d^{-1} in Whangarei, and 70 L d^{-1} in the Far North. In June, when ET_{o} was below 0.8 mm d^{-1}, avocado tree use decreased to 70 L d^{-1} in the Bay of Plenty, 60 L d^{-1} in Whangarei, and 30 L d^{-1} in the Far North. However, in relative terms, the decline in tree water use from summer to winter was less than the decline in ET_{o}. At all study sites, a brief decline or plateau in tree water use was identified after all fruit were removed in September or October, but tree water use increased again in late-November to early-December. Compared between years, average tree water use in late summer was higher relative to ET_{o} in 2018 (a high crop year) compared to 2019 (a low crop year) in the Bay of Plenty and Whangarei.

In the Far North, tree water use dropped dramatically after May 2018, in response to leaf abscission (Figure 2.4C). Due to a health issue, all branches of the experimental trees were pruned in January 2019.

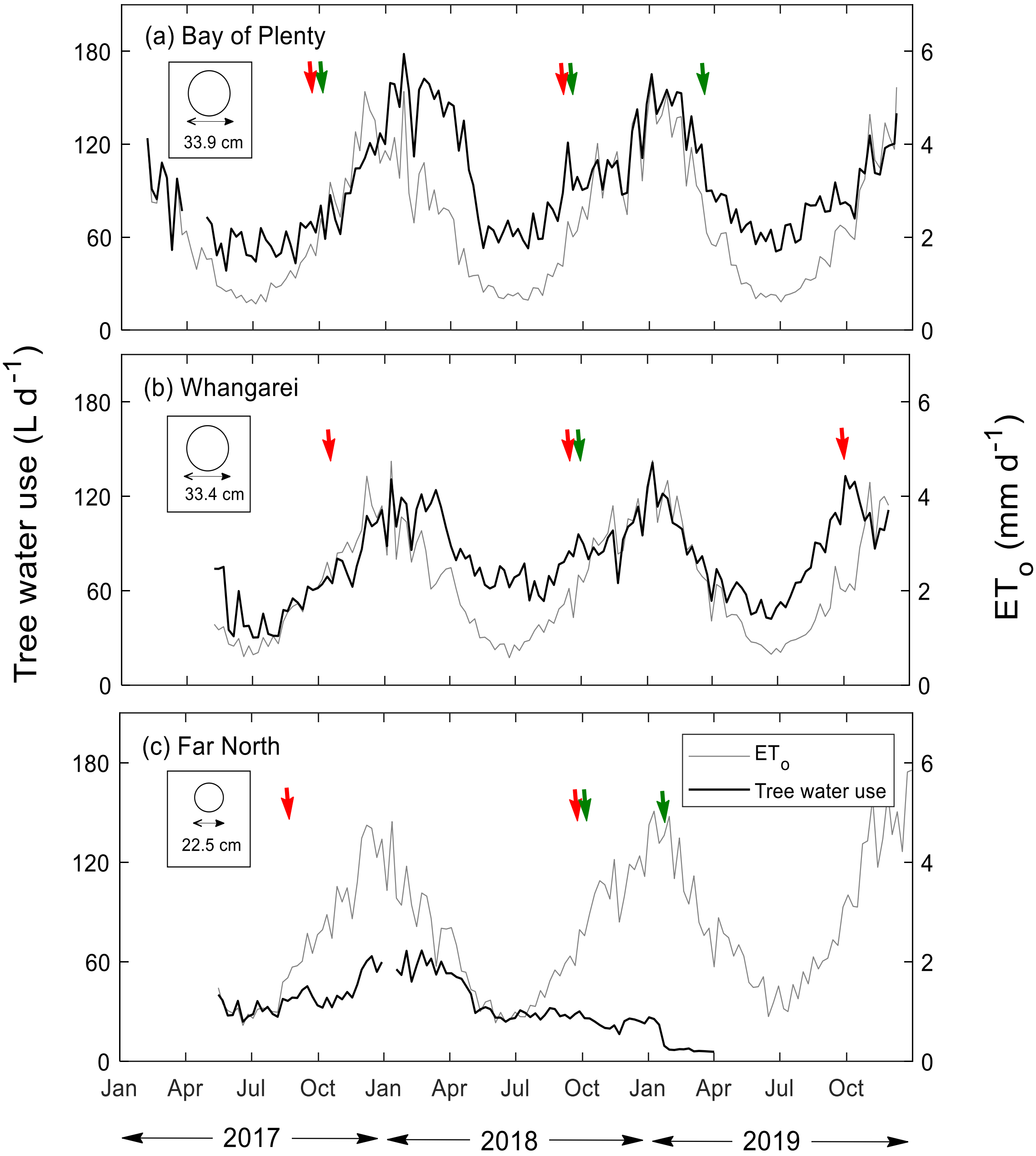

Figure 2.4: 'Hass' avocado tree water use (L d^{-1}) obtained from sap flow measurements and ET_o (mm d^{-1}) obtained from meteorological data, from February 2017 to November 2019 in the Bay of Plenty (a), from May 2017 to November 2019 in Whangarei (b), and from May 2017 to March 2019 in the Far North (c), averaged weekly. The red arrows indicate harvest, and the green arrows indicate pruning. The square windows on the top-left present the average trunk diameter. (Note: In the Far North tree health and water use began to decline in August 2018, heavy pruning was applied in January 2019, and all sensors were uninstalled in April 2019)

Leaf area

Over the experimental period, average total leaf area per tree ranged from 200 to 320 m^2 in the Bay of Plenty, from 180 to 350 m^2 in Whangarei, and 120 m^2 in the Far North (Figure 2.5A). In the Bay of Plenty and Whangarei, the trees increased in canopy size each year, as new shoots were developed. However, in the Far North, leaf area decreased caused by significant leaf abscission after May 2018.

The relationship between total leaf area and tree water use in January 2018 was analysed (Figure 2.5B). Comparing across the three sites, the tree water use in mid-summer was strongly related to total leaf area, and this relationship was statistically different ($P<0.05$).

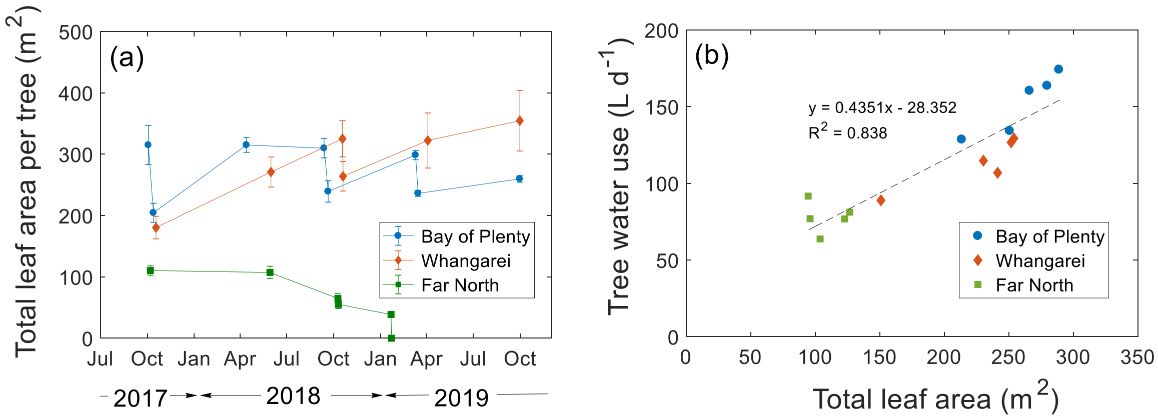

Figure 2.5: (a) Average total leaf area of five ‘Hass’ avocado experimental trees (±1S.E.) in the Bay of Plenty, Whangarei and the Far North from October 2017 to September 2019. The sharp declines in the total leaf area in Bay of Plenty and Whangarei were a result of pruning. In the Far North, the gradual decline from May 2018 was caused by a large amount of leaf abscission and the sudden decline in January was due to heavy pruning and removal of all leaves. (b) Relationship between total leaf area (m^2) and tree water use ($L\ d^{-1}$) of five experimental trees in January 2018, in the Bay of Plenty, Whangarei, and the Far North.

Soil water content

Volumetric soil water content at the three depths was monitored throughout the experimental period in the Bay of Plenty (Figure 2.6A), Whangarei (Figure 2.6B) and the Far North (Figure 2.6C). The soils of these three sites had different water holding capacities. Whangarei had higher mean soil water content due to the high clay content of the soil, while the Far North had lower mean soil water content due to the higher sand content. The allophanic soil of the Bay of Plenty also had a high soil water content at saturation, but drained more readily than a clay soil and had a steeper gradient in water content with depth than the other two sites. At all study sites, soil water content was highest in winter, and tended to decline in summer during periods of low rainfall and higher evaporation. There was significantly less rainfall in the mid-summer 2018-19 at all three sites, with decreasing soil water content even though irrigation was applied.

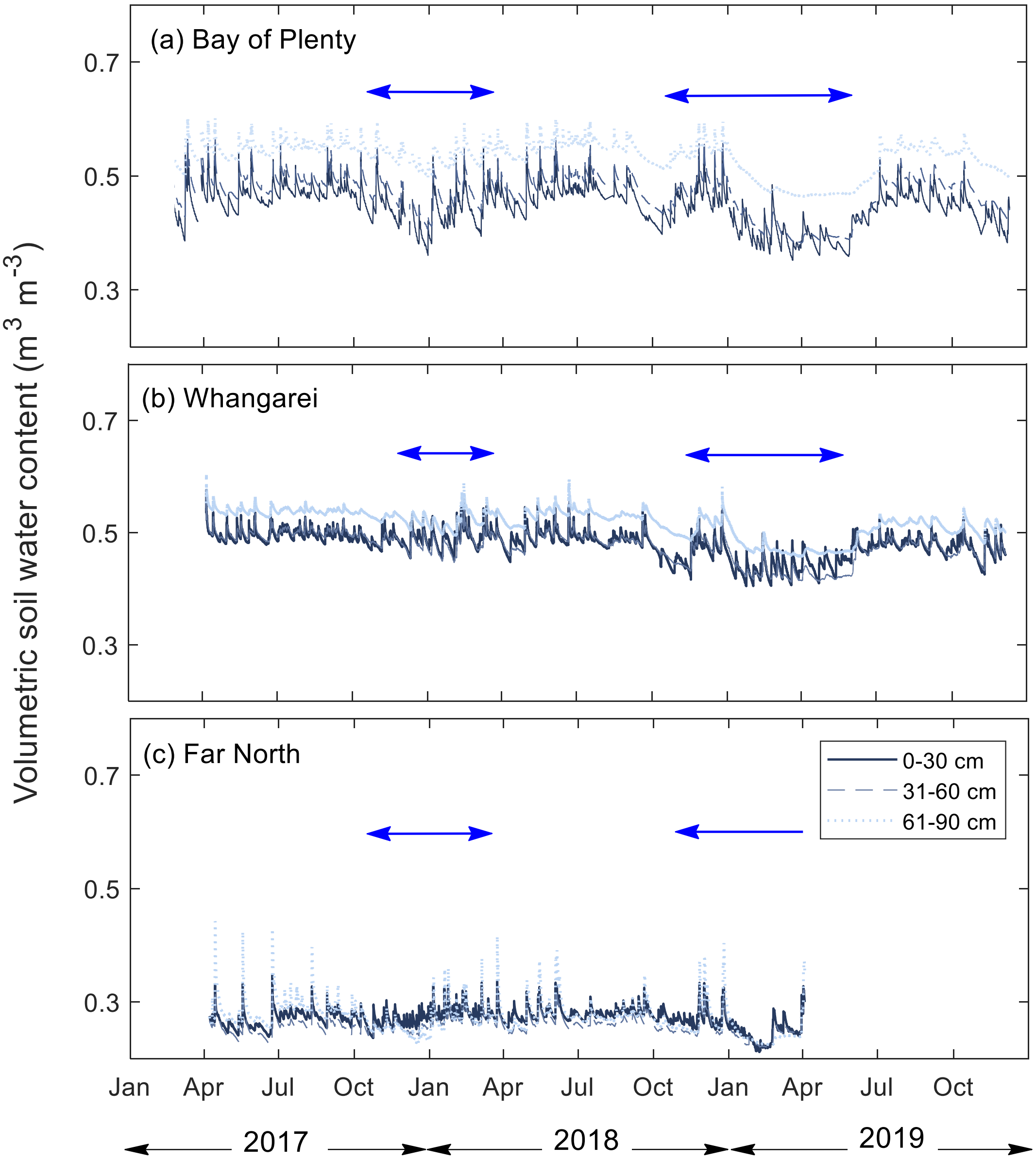

Figure 2.6: Volumetric soil water content at the three depths (0-30, 31-60, and 61-90 cm) from February 2017 to November 2019 in ‘Hass avocado orchard in the Bay of Plenty (allophanic soil) (a), from April 2017 to November 2019 in Whangarei (clay soil) (b), and from April 2017 to March 2019 in the Far North (sandy soil) (c). Blue arrows indicate periods of irrigation. (Note: Due to a tree health issue, the experiment was conducted until March 2019 in the Far North)

Sap flow compared to the soil water balance

Based on the observations over two fruit growing seasons, there was a strong linear relationship between monthly mean daily *T* values calculated from sap flow measurements and monthly mean daily ET_{wb} values calculated from the soil water balance at all three sites (Figure 2.7). According to regression analysis, there was a strong positive relationship between the two variables at each site ($P<0.05$), with the similar slope across sites ($P>0.05$; ANCOVA). ET_{wb} are higher than *T* for the same time points, because *T* indicates tree transpiration whereas ET_{wb} includes tree transpiration and evaporation from the soil and any ground cover (*E*).

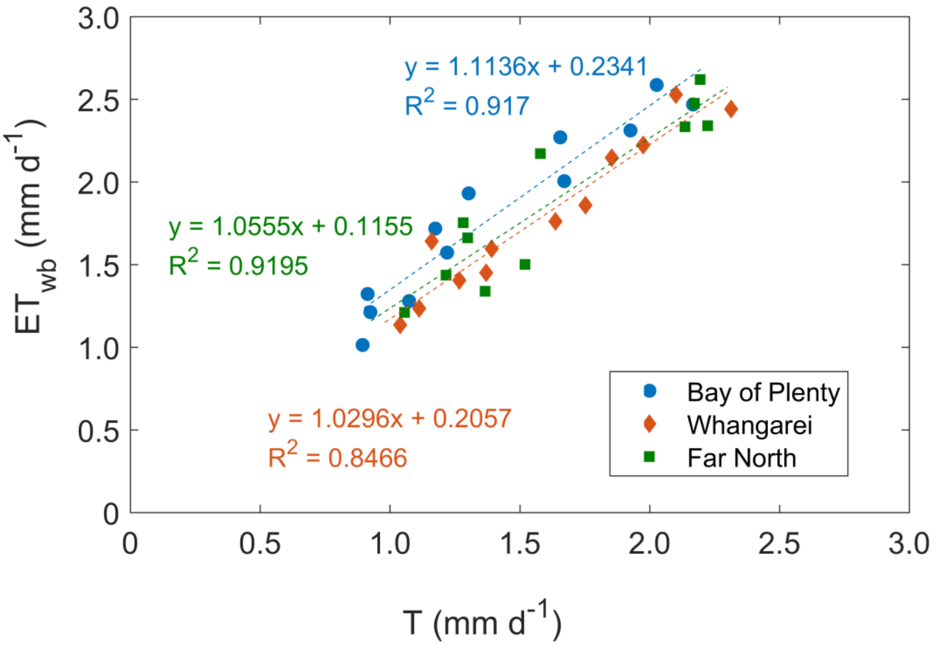

Figure 2.7: Relationship between monthly mean daily *T* values (mm d^{-1}) obtained from sap flow measurements and monthly mean daily ET_{wb} values (mm d^{-1}) obtained from soil water balance, for ‘Hass’ avocado orchareds in the Bay of Plenty, Whangarei, and the Far North ($P>0.05$: ANCOVA). Points present the average of five experimental trees at each site.

Mean monthly ET_o, *T* and *E* for avocado were calculated from October (flowering) to September (harvest) (Figure 2.8A-C). At all three study sites, the *T* values were highest in January at 2.4 mm d^{-1}, when ET_o was highest, and lowest at 1 mm d^{-1} in June and July when ET_o was lowest. The mean monthly K_{cb} was calculated based on ET_o and *T* at each site (Figure 2.8D). All three sites had the lowest K_{cb} in November at 0.45, and the highest K_{cb} in June at 1.45.

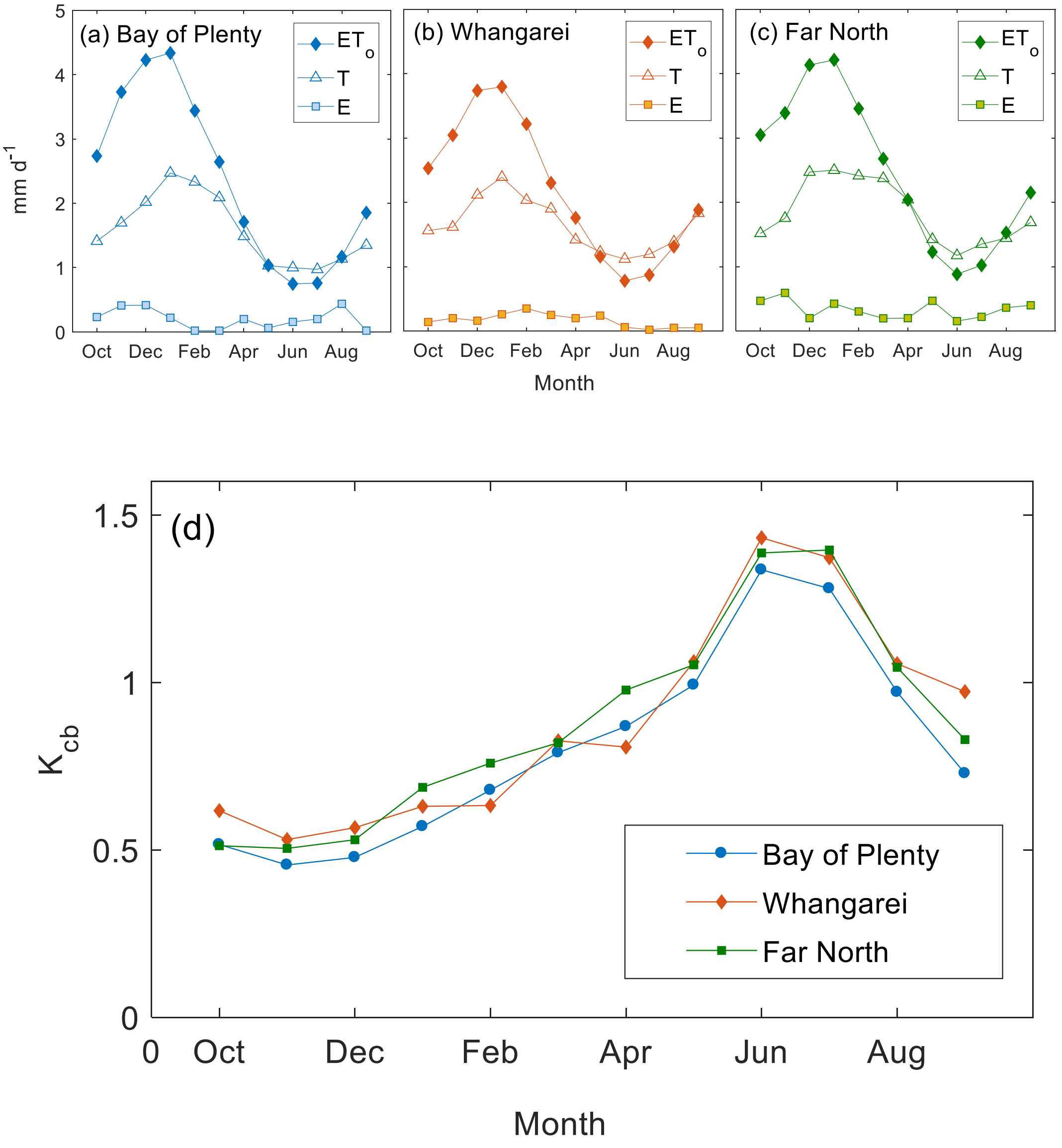

Figure 2.8: 'Hass' avocado orchard ET_o, T, and E (mm d^{-1}) from October (flowering) to September (harvest) in the Bay of Plenty (a), Whangarei (b), the Far North (c), and comparison of monthly K_{cb} values between the three study sites (d).

Harvest

Crop yield for each tree was obtained at harvest and compared with K_{cb} for the preceding January (the middle of the irrigation period). From three years observation in the Bay of Plenty (Figure 2.9A) and Whangarei (Figure 2.9B), there was a strong relationship between individual fruit yield and the K_{cb} values in January, as the higher crop trees had the higher K_{cb} values ($P<0.05$). In the Far North, the relationship between the two variables was significant in 2017 ($P<0.05$) and 2018 ($P<0.05$), but weaker when the years were combined into a single regression (Figure 2.9C) ($P>0.05$).

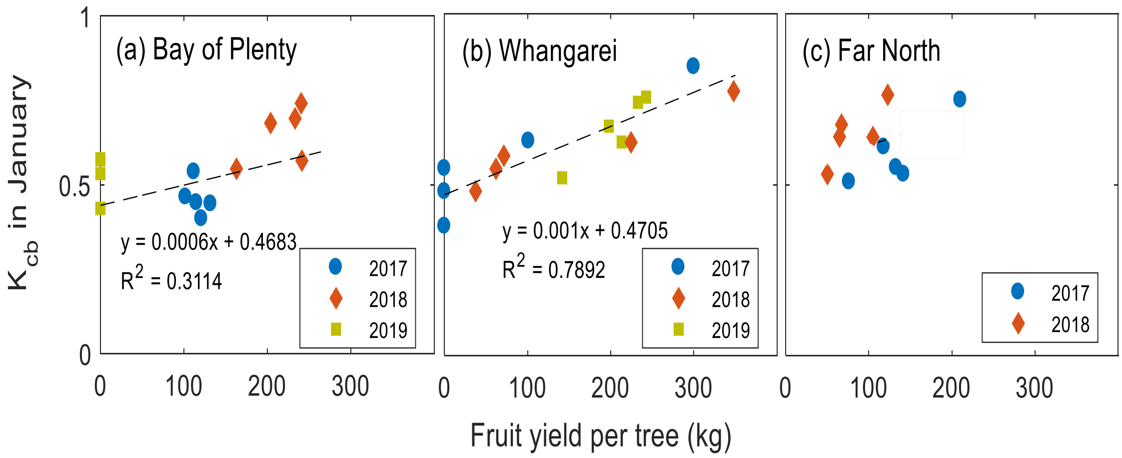

Figure 2.9: The relationship between 'Hass' avocado fruit yield and K_{cb} in January, with a trend line in the Bay of Plenty ($P<0.05$) (a), Whangarei ($P<0.05$) (b), and the Far North ($P>0.05$) (c).

Shaded area

The overall relationship between K_{cb} and orchard shaded area for the mature orchards in the present study (the Bay of Plenty, Whangarei, the Far North), and the the young orchard (3 years; Kaneko 2016) is summarized (Figure 2.10). The canopy cover of the three mature orchards was high, with 83%, 93%, and 77% of the ground surface shaded by the canopy in the Bay of Plenty, Whangarei and the Far North, respectively. Regardless of the percentage of the shaded area, these three sites had similar K_{cb} values around 0.6 in mid-summer. The young orchard had a lower % shaded area, and a correspondingly lower K_{cb}.

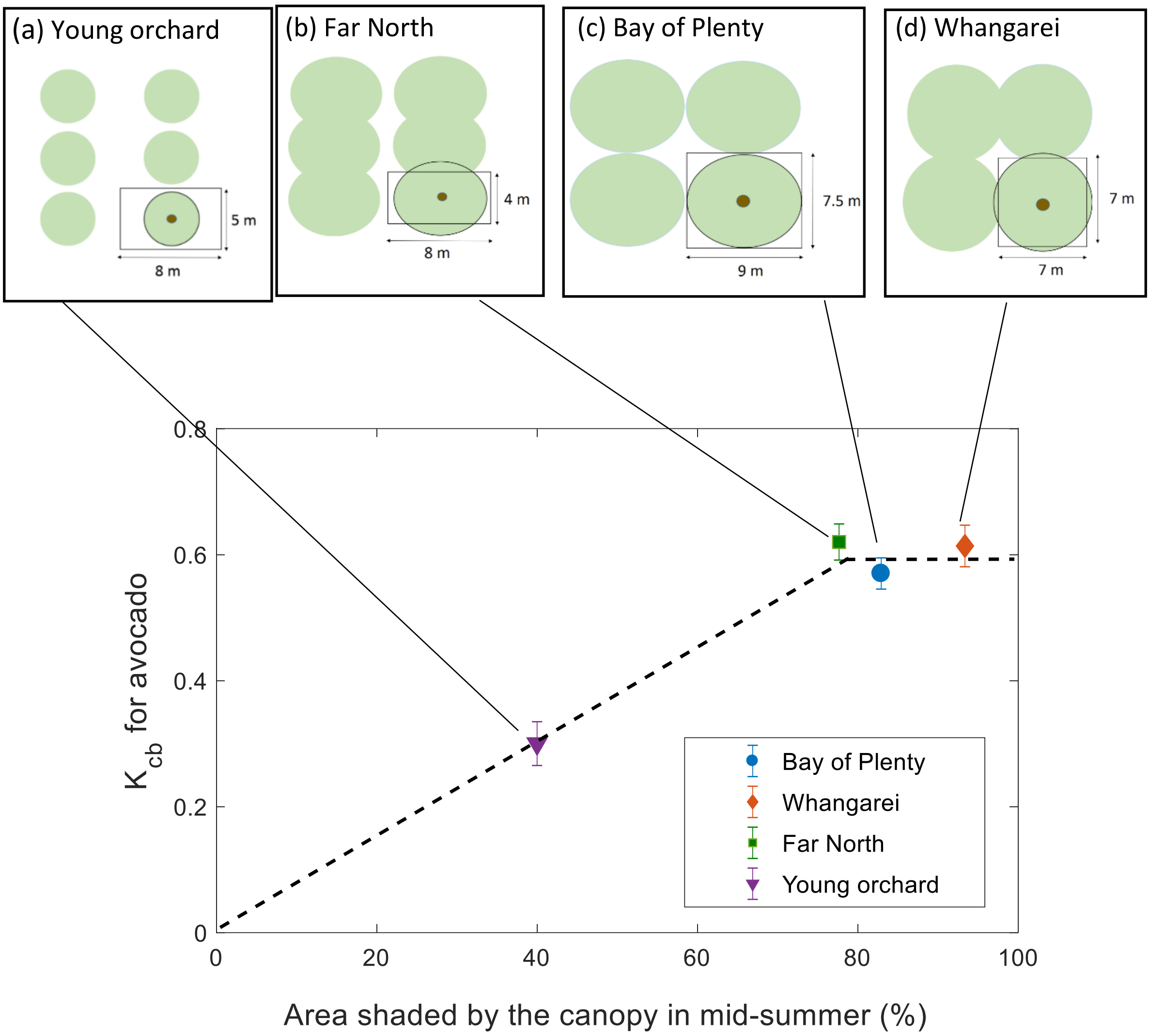

Figure 2.10: Orchard information showing plant spacing and individual tree canopy size obtained from shaded area measurements (top), and the relationship between K_{cb} and shaded area (%) in mid-summer (January) of the ‘Hass’ avocado orchards in the Bay of Plenty, Whangarei, and the Far North, with comparison to the young (three year old) orchard in the Bay of Plenty (Kaneko, 2016). The values were the average of five experimental trees for the three study sites and of three trees for the young orchard (±1S.E.). The dashed line is a hypothesized relationship between shaded area and K_{cb} (see discussion).

Crop coefficients in New Zealand

Daily water usage of the avocado plant was summarized by month for the Bay of Plenty (Table 2.3), Whangarei (Table 2.4), and the Far North (Table 2.5). Under New Zealand conditions, the average ET_o values range from 0.7-0.8 mm d^{-1} in June to 3.8-4.3 mm d^{-1} in January, and the average of ET_c values for the avocado tree range from 1.2-1.4 mm d^{-1} in June, to 2.7-2.8 mm d^{-1} in January. The monthly average K_c values ranged from 0.6-0.65 in October/November to 1.5-1.75 in June.

Table 2.3: Crop coefficient for the Bay of Plenty; ET_o (mm d^{-1}), ET_c (mm d^{-1}) and K_c values, from the avocado flowering season in October to harvest in September.

Month	Daily ET_o (mm)	Daily ETc (mm)	K_c (light to heavy crop)
October	2.7	1.6	0.60 (0.50 - 0.70)
November	3.7	2.2	0.60 (0.50 - 0.65)
December	4.2	2.4	0.60 (0.50 - 0.70)
January	4.3	2.7	0.60 (0.55 - 0.75)
February	3.4	2.3	0.65 (0.50 - 0.70)
March	2.6	2.0	0.75 (0.50 - 0.80)
April	1.7	1.7	1.00 (0.85 - 1.30)
May	1.0	1.1	1.05 (0.85 - 1.25)
June	0.7	1.2	1.55 (1.25 - 1.85)
July	0.7	1.2	1.55 (1.20 - 1.95)
August	1.1	1.6	1.35 (1.05 - 1.55)
September	1.8	1.3	0.75 (0.50 - 0.85)

Table 2.4: Crop coefficients for Whangarei; ET_o (mm d^{-1}), ET_c (mm d^{-1}) and K_c values, from the avocado flowering season in October to harvest in September.

Month	Daily ET_o (mm)	Daily ET_c (mm)	K_c (light to heavy crop)
October	2.5	1.6	0.65 (0.60 - 0.80)
November	3.1	1.8	0.60 (0.50 - 0.70)
December	3.7	2.3	0.60 (0.50 - 0.70)
January	3.8	2.7	0.70 (0.55 - 0.85)
February	3.3	2.5	0.75 (0.65 - 0.90)
March	2.3	2.2	0.95 (0.80 -1.10)
April	1.8	1.8	1.00 (0.80 - 1.15)
May	1.2	1.5	1.25 (1.05 - 1.65)
June	0.8	1.2	1.50 (1.10 - 1.80)
July	0.9	1.2	1.35 (1.00 - 1.50)
August	1.3	1.4	1.10 (0.80 - 1.20)
September	1.9	1.9	1.00 (0.70 -1.15)

Table 2.5: Crop coefficients for the Far North; ET_o (mm d^{-1}), ET_c (mm d^{-1}) and K_c values, from the avocado flowering season in October to harvest in September.

Month	Daily ET_o (mm)	Daily ET_c (mm)	K_c (light to heavy crop)
October	3.0	1.8	0.60 (0.50 - 0.65)
November	3.4	2.2	0.65 (0.55 - 0.70)
December	4.1	2.6	0.65 (0.55 -0.70)
January	4.2	2.7	0.65 (0.55 - 0.80)
February	3.5	2.5	0.70 (0.65 - 0.85)
March	2.7	2.4	0.90 (0.80 - 1.20)
April	2.0	2.1	1.05 (0.90 - 1.20)
May	1.2	1.5	1.25 (1.05 - 1.30)
June	0.8	1.4	1.75 (1.40 - 1.90)
July	1.0	1.7	1.70 (1.40 - 1.90)
August	1.5	1.5	1.00 (0.85 - 1.20)
September	2.1	2.1	1.00 (0.85 - 1.10)

2.4 Discussion

This research provided new insight into water use of ‘Hass’ avocado trees in the main avocado growing regions in New Zealand; the Bay of Plenty, Whangarei, and the Far North. There was a strong and seasonally variable relationship between ET_o and T, and between T and ET_{wb}. These three study sites had similar T and K_{cb} values, even though soil water holding capacity differs across the three regions. Avocado tree water use was more influenced by fruit load, as the heavier cropping trees used more water.

The relationship between weather and ET_o

Throughout the three years of observations from 2017 to 2019, the summer of 2018-19 was warmest at all three study sites. In particular, January 2019 had higher mean monthly daily maximum air temperatures by more than 0.5 ˚C, compared to January 2017 and 2018. Air temperature is the most sensitive variable for the FAO Penman-Montieth ET_o calculation, therefore, ET_o was slightly higher in the summer of 2018-19.

The Far North had fewer rainfall events compared to the other two regions over the three years, but a prolonged dry period occurred each summer in all three regions. In particular, the summer of 2018-19 was far drier, and rainfall events were scarce with a significant dry period from January to March.

Avocado tree water use

Sap flow measurement was used to identify estimate avocado tree water use, and the results clearly corresponded with weather conditions. From the daily observations, the T values for avocado correlated well with daily ET_o values, with seasonal changes. Regardless of different local weather conditions and different soil types, the seasonal patterns of avocado tree water use were similar at all three sites over the fruit growing season. The T values increased greatly from October, reached a peak in late-January to early-February, and then decreased from March. Interestingly, comparing the T values between the two fruit growing years in the Bay of Plenty and Whangarei, tree water use was high through mid-summer from December to February in summer 2017-18. However, tree water use started declining in February in summer 2018-19, which was one month earlier than the previous year (see Figure 2.4). Particularly, Whangarei had a sharp decline in the T values in February and March. There are some possible reasons, as discussed below.

Tree water use was shown to be influenced by physiological conditions, such as fruit load. In the Bay of Plenty, the experimental trees carried fruit in 2017-18, but they had no fruit in 2018-19, explaining why the trees used more water relative to ET_o in 2017-18. In Whangarei, the experimental trees had fruit in both years, and there might be another reason why the relationship between T and ET_o differed between the two years. In 2019, there was a significant decline in soil water content from mid-January to March due to prolonged dry conditions, and January 2019 was the second warmest January in Whangarei on record since 1967 (NIWA, 2019). Unusually warm air temperatures could increase water use of avocado trees, and the amount of irrigation water applied probably did not match tree demand. Average fruit size in 2019 was smaller than that in 2018 by 9.5%. This may be because the experimental trees experienced some degree of water stress during the summer 2018-19.

The Far North had lower plant water use per plant in the summer of 2017-18 than the other two sites, due to smaller tree size. However, a decline of tree water use after winter 2018 was due to a root disease rather than the weather or other physiological conditions. From visual observations, the major symptoms were branch dieback, leaf abscission and shoots with necrotic tips, suggesting infection by *Phytophthora cinnamomi*, a common cause of disease in New Zealand avocado orchards (Zentmyer, 1984; Dann *et al.*, 2013). After the trees were infected, tree water uptake was significantly reduced suggesting that root disease caused by *Phytophthora* infection restricted water transport from soil to leaf within the plants (Sterne *et al.*, 1978).

Tree water use and leaf area

In general, plant transpiration is directly related to tree size (Goodwin *et al.*, 2006). The results indicate that the trees in the Bay of Plenty had higher water use per tree, while the trees in the Far North had the least, because of differences in tree size. However, tree size was less variable within each orchard, and tree water use was more related to fruit load than leaf area per tree.

Tree water use depends on the net solar radiation absorbed by the leaves (Green, 1993; Johnson *et al.*, 2000; Maddonni *et al.*, 2001). In the Bay of Plenty, avocado tree water use was not significantly different before and after pruning in March 2019 when our experimental trees didn’t carry fruit. This might be because the canopy was shading itself before pruning due to high canopy density. Pruning removed approximately 25% of the total leaf area, decreased the shaded area within the orchard, and redistributed light onto previously shaded areas of canopy. Also, a sudden reduction in the canopy size usually enhances new vegetative growth, resulting in higher water use for photosynthesis and new shoot development (Heilman *et al.*, 1996).

Tree water use and fruit load

Tree water use is also affected by the physiological change from flowering to early and late fruit developmental periods, and it is known that fruit load influences tree water use, as higher fruit load trees consume more water (Naor *et al.*, 1997; Silber *et al.*, 2013). The results of this study showed there was a positive relationship between fruit yield and tree water use in the Bay of Plenty and Whangarei. This relationship was weak and not significant in the Far North, likely because of the decline in tree health in 2018. Average fruit size in 2018 decreased by 20% compared to 2017. Smaller fruit size lowered total fruit yield significantly in 2018 in the Far North, affecting the relationship between fruit yield and tree water use.

The fruit is a strong sink for carbohydrate, therefore, the presence of fruit on a tree will strongly influence the source-sink carbon relationship (Sade & Moshelion, 2014). Some studies, for example, in peach (Johnson *et al.*, 2002; Ayars *et al.*, 2003), olive (Bustan *et al.*, 2016), apple (Wibbe & Blanke, 1995), and pear (Marsal *et al.*, 2014), reported a sharp decline in daily tree water use after de-fruiting. The decline in tree water use after fruit removal is likely to be the result of a sudden decrease in sink demand and consequently, a reduction in photosynthesis (Bustan *et al.*, 2016). However, this decline is usually only temporary, because fruit growth inhibits vegetative growth, and fruit removal enhances new growth (Lavee, 2006).

The results of this study demonstrated that avocado tree water use decreased after fruit were removed, but tree water use recovered a month after harvest (see Figure 2.4). In the case of avocado under New Zealand conditions, the fruit requires 11 months or longer from flowering to maturity. At the study sites, flowering started in early-October, and fruit were picked in September and October. Soon after harvest, there is a high water demand for flowering and new fruit and shoot growth.

Crop coefficients for avocado

This study showed that the ET_{wb} was slightly higher than T, because T indicates tree water use itself whereas ET_{wb} includes T and E. T was calculated from sap flow measurement, while ET_{wb} was calculated from the soil water balance method. According to linear regression analysis, there was a strong relationship between the T and ET_{wb} values at all study sites, providing confidence in the two methods for estimating the orchard water use. Sap flow measurement was a good tool for quantifying individual avocado tree water use, independently of other evaporative losses, but monitoring soil water content was also important at an orchard scale.

The water stress level of a crop can depend on soil type, hence, soil type can affect the K_{c} values for a crop (Popova *et al.*, 2006). This study found that avocado trees behaved similarly in the Bay of Plenty, Whangarei, and the Far North, regardless of their different soil types. However, the soils of these three regions have different soil water holding capacities that require different irrigation strategies. Based on soil moisture monitoring, the volumetric soil water content of the top layer increased to 0.45 m^3 m^{-3} in the Bay of Plenty, 0.50 m^3 m^{-3} in Whangarei, and 0.28 m^3 m^{-3} in the Far North within a few days after rainfall, and decreased by approximately 0.01 m^3 m^{-3} each day in mid-summer at all sites. Based on observations in this study, irrigation should be applied to avocado when volumetric soil water content of the top layer decreases below 0.40 m^3 m^{-3} in the Bay of Plenty with allophanic soil, 0.45 m^3 m^{-3} in Whangarei with clay soil, and 0.25 m^3 m^{-3} in the Far North with sandy soil. Irrigation should be applied when there is low rainfall from November to March, and irrigation intervals should be 5-8 days in the Bay of Plenty and Whangarei, and 3-4 days in the Far North.

These three study sites had similar ground cover conditions, as weeds were controlled by the growers and a large proportion of the area of the ground was covered by leaf litter and canopy. From a comparison between the T and ET_{wb} values, water loss from the surface was generally lower than 0.5 mm d^{-1} at all three study sites. However, water loss from the ground surface depends on atmospheric demand, shaded area, type of ground cover, and the frequency of rainfall and irrigation. Bare soil and active natural vegetation increase water loss from the soil (Allen & Pereira, 2009; Shrestha & Shukla, 2014). Also, a shorter irrigation interval can increase soil evaporation, as the rate of soil evaporation is relatively high after wetting the soil (Benli *et al.*, 2006).

There are several studies investigated the relationship between K_{c} and shaded area in other orchard crops. In general, K_{c} is linearly correlated with canopy development. When plants are young, there is no restriction to grow, thus, tree water use generally increases as plants grow, and there is a positive linear relationship between the K_{c} values and shaded area (Johnson *et al.*, 2000; Williams & Ayars, 2005). However, water consumption plateaus and does not show a further increase when the shaded area is developed and leaves are overlapped greatly (Williams & Ayars, 2005; McClymont *et al.*, 2009), and this generally happens when shaded area becomes greater than 80% (Sammis *et al.*, 2004; Wang *et al.*, 2007). This is because of the amount of leaf area exposed to direct sunlight reaches a maximum, and the canopy becomes restricted in growth (Williams & Ayars, 2005). In this study, there was no difference in K_c between the three study sites, and the results of shaded area measurements showed the majority of radiation was largely intercepted by the tree canopy at all study sites (over 77%). Once this level of canopy development has been reached there can be no further increase in K_c, explaining why these three sites had similar K_c values regardless of site differences such as plant spacing and tree size. The relationship between K_c and shaded area is proposed in this study based on the addition of a single measurement of K_c from a younger orchard with a lower shaded area (Figure 2.10). Whilst further estimates of K_c from orchards with a range of planting densities and tree sizes would be appropriate, this preliminary relationship can be used to adjust K_c from young to mature avocado orchards.

It is interesting to note that the K_c for avocado increases above 1 in the late autumn and winter from April to August, indicating that crop water use is higher than the reference during this period. There is little information regarding K_c for evergreen fruit tree species during the non-irrigated season, except for citrus (Peddinti and Kambhammettu, 2019) and olive (Villalobos et al., 2000), that showed an increase in K_c during winter when the ET_o decreased. Avocado requires high energy in autumn and winter for oil accumulation in fruit and floral development (Salazar-Garcia et al., 2013). It would be no surprise if avocado consumes a significant amount of water for photosynthetic activity throughout a year.

The K_c values provided here were 0.60-0.70 in mid-summer, values that are slightly lower than those in the literature. For example, Volker (2005) provided the K_c value of 0.72 in Chile, and Allen and Pereira (2009) estimated a K_c value of 0.9 to 1.0 during the fruit growing season in California. Maturity of 'Hass' avocado fruit depends on climate. Harvest begins eight months after peak bloom in the warmer countries (Lahav *et al.*, 2013a). New Zealand has a mild climate, therefore, avocado fruit grows more slowly, and requires 11 months or longer for fruit maturity. While climate strongly affects avocado fruit growth periods, it is probably important to identify the original K_c values for each country.

2.5 Conclusions

This research quantified ‘Hass’ avocado tree water use in the three avocado growing regions of New Zealand; the Bay of Plenty, Whangarei, and the Far North, relative to weather conditions, soil type, canopy size and fruit load, and estimated K_c as a basis for irrigation guidelines within each region.

In this sense, to our knowledge, this is the first report measuring water use of ‘Hass’ avocado tree, relative to the local weather conditions, soil type, canopy size, and fruit load. In the case of avocado, consideration of fruit load is important because tree-to-tree variation and year-to-year variation in fruit load are very high. Irrigation is necessary for constant fruit growth under New Zealand conditions, but irrigation management should account for weather conditions and fruit load (Chapter 3).

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2.7 Disclosure statement

No potential conflict of interest was reported by the author.

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Chapter 3

Effect of water deficits on 'Hass' avocado fruit development in the orchard

3.1 Introduction

Avocado (*Persea americana* Mill.) originates from subtropical and tropical regions that have a wet-summer and dry-winter climate (Lahav *et al.*, 2013a), but as a crop it is now widespread from Spain to New Zealand (Schaffer *et al.*, 2013). Successful commercialisation has led to an increase in global avocado production, from approximately 3.9 million tonnes in 2009 to 6.4 million tonnes in 2018 (Food and Agriculture Organization of the United Nations, 2020). To enable the production of this valuable fruit outside of its optimal climatic range, avocado is often cultivated under irrigation. In Mediterranean climates, such as in parts of California, Chile, Israel, Peru and Australia, frequent summer irrigation is vital for avocado crop production. In contrast, New Zealand has a maritime climate characterized by moderate temperatures and intermittent summer rainfall and irrigation of avocado is often considered optional. About one-third of avocado orchards in New Zealand currently do not use irrigation (Phillip West NZ Avocado Pers. Comm.).

Avocado is known to be highly sensitive to water status, and lack of water can decrease fruit weight and therefore fruit yield (Lahav & Kalmar, 1977; Michelakis *et al.*, 1993). However, identifying an appropriate soil- or plant-based threshold for applying irrigation can be difficult because crop responses vary with weather conditions, soil type, physiological status, fruit load, and the timing or degree of water scarcity (Jones, 2007). For example, when plants experience water stress, species or cultivars can vary from relatively isohydric behaviour, with regulation of plant water status by stomatal closure, to more anisohydric behaviour and continued transpiration as plant water status declines (McDowell *et al.*, 2008; Klein, 2014). Theoretically, when severe drought occurs, isohydric species are more likely to have a negative carbohydrate balance caused by limitation of photosynthesis, whereas anisohydric species are more likely to experience hydraulic failure (McDowell, 2011). For effective irrigation management it is important to understand how a crop responds to water deficit, and how effects on fruit growth may be caused by water stress.

There are several plant-based indicators that can be used to estimate the level of water stress. These include stem water potential (Ψ_{stem}) and leaf water potential (Ψ_{leaf}) (Scholander *et al.*, 1965; McCutchan & Shackel, 1992), stomatal conductance (g_s) (Naor *et al.*, 1997; Liu *et al.*, 2008), changes in trunk diameter (Goldhamer & Fereres, 2001, 2004), leaf temperatures (Girona *et al.*, 1993; Liu *et al.*, 2011), and sap flow (Smith & Allen, 1996; Alarcon *et al.*, 2000). Some recent studies (Shackel, 2011; Spinelli *et al.*, 2017) have suggested that Ψ_{stem} and g_s are closely related to soil water availability. For avocado, Sterne *et al.* (1977) and Scholefield *et al.* (1980) reported that trees were water stressed in California when Ψ_{stem} dropped below -1.0 MPa, while Silber *et al.* (2019) stated that the water stress threshold for avocado is -0.8 MPa of Ψ_{stem} under Mediterranean conditions. Avocado is considered to be an isohydric species (Carr, 2013). A reduction in photosynthesis caused by stomatal closure in response to water stress has been observed when Ψ_{stem} decreased to -0.9 MPa in South Africa (Bower *et al.*, 1978) and -1.2 MPa in California (Sterne *et al.*, 1977). Fruit require water and carbohydrates to grow, and carbohydrates are derived from photosynthesis (Matthews & Shackel, 2005). If the avocado is isohydric, the level of stress may not be detectable as a decease in Ψ_{stem}, but both water and carbohydrate supply to fruit may be affected. Fruit weight and oil content in fruit (expressed as dry matter content - the ratio of dry weight to fresh weight) are important parameters for avocado crop production. Inadequate water supply could have a large negative impact on both of these parameters.

Understanding how water stress influences fruit yield and fruit quality in avocado is complicated by the variable nature of tree growth and reproduction from year to year. Avocado has unique characteristics, such as a protogynous flowering behaviour (flowers open as a female on the first day and as a male on the second day) (Carr, 2013), an alternate bearing habit (a light off-cropping year after a heavy on-cropping year) (Garner *et al.*, 2008; Lovatt, 2010), and high levels of flower and immature fruit abscission (Garner & Lovatt, 2008). In particular, the New Zealand maritime climate directly influences these physiological conditions, creating irregular bearing, a prolonged fruit growth period, and frequent failure of the flowering opening process (Gould *et al.*, 2019). The resulting tree-to-tree variation in fruit load, fruit weight and flowering patterns may make it more difficult to detect the effects of moderate levels of water stress on fruit development.

Avocado is successfully grown in the northern regions of New Zealand (Thorp *et al.*, 1997). In the main avocado growing region of the Bay of Plenty with allophanic soils, soil water deficit is rare during the flowering period in spring due to high soil water content caused by frequent rainfall in winter. Water management in avocado is thought to be most important during the period of rapid fruit growth in summer when water deficits are more likely to occur (Kaneko, 2016). However, there is a lack of scientific knowledge about the benefit of irrigation under New Zealand climatic conditions. Variable rainfall over the summer means there are no clear guidelines for irrigation management, and irrigation strategies vary from grower to grower. Therefore, there is a need for research investigating the role of irrigation and how water stress impacts avocado fruit growth in New Zealand avocado production in the absence of irrigation.

The aim of this research was to assess the irrigation requirements of 'Hass' avocado in the Bay of Plenty, New Zealand, and to investigate the effects of water deficit on fruit growth and fruit yield, by comparing between irrigated and non-irrigated trees. The hypothesis was that a decrease in soil water content during the early fruit development stage from November to February would reduce fruit weight at harvest. Isohydric stomatal closure in response to decreased soil water content was expected to cause a reduction in fruit dry matter content. It was also hypothesized that avocado has high variable fruit load between trees, that interact with the effect of water stress on fruit weight and dry matter content.

3.2 Methods

Study sites and plant materials

This research was conducted in a commercial avocado orchard at 62 Prole Road, Omokoroa, Bay of Plenty, New Zealand (37°39'17.964''S, 176°1'12.936''E). The region is characterized by a mild climate with rainfall throughout the year. In Tauranga (25 km from the study site), mean monthly maximum and minimum air temperatures (1981-2010) are 24 and 15 °C in January, and 14 and 6 °C in July respectively, and normal annual rainfall is 1189 mm, with mean monthly total rainfalls of 74, 95, 78, 86, and 97 mm in November, December, January, February, and March, respectively (Figure 3.1) (NIWA, 2020). The soil type of the study site was sandy loam (Typic Orthic Allophanic soil) (Hewitt, 2010).

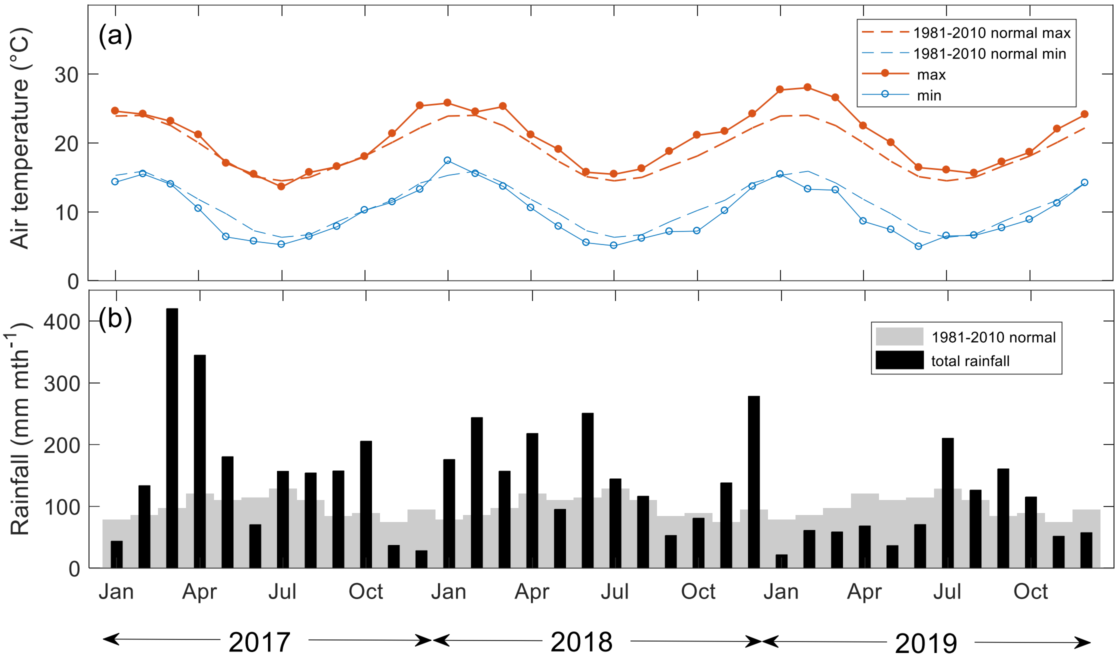


Figure 3.1: Mean monthly daily maximum and minimum air temperatures compared to normal maximum and minimum air temperatures (1981 to 2010) (a), and monthly total rainfall with normal rainfall (1981 to 2010) (b), at the orchard in the Bay of Plenty from January 2017 to December 2019.

Plant materials and experimental design

Ten year old *Persea americana* 'Hass' avocado trees were growing on 'Zutano' seedling rootstocks, with 18 trees per row, 7.5 m between trees, and 9 m between rows. Within three adjacent rows, 24 healthy and mature trees with a height of 7 m and a canopy diameter of 7.5 m were selected for this study.

A complete block design with two treatments (irrigated and non-irrigated) and three rows as blocks was established (Figure 3.2). There were four replicate trees per row, providing 12 trees in total per treatment. There were always non-irrigated buffer trees next to the non-irrigated experimental trees.

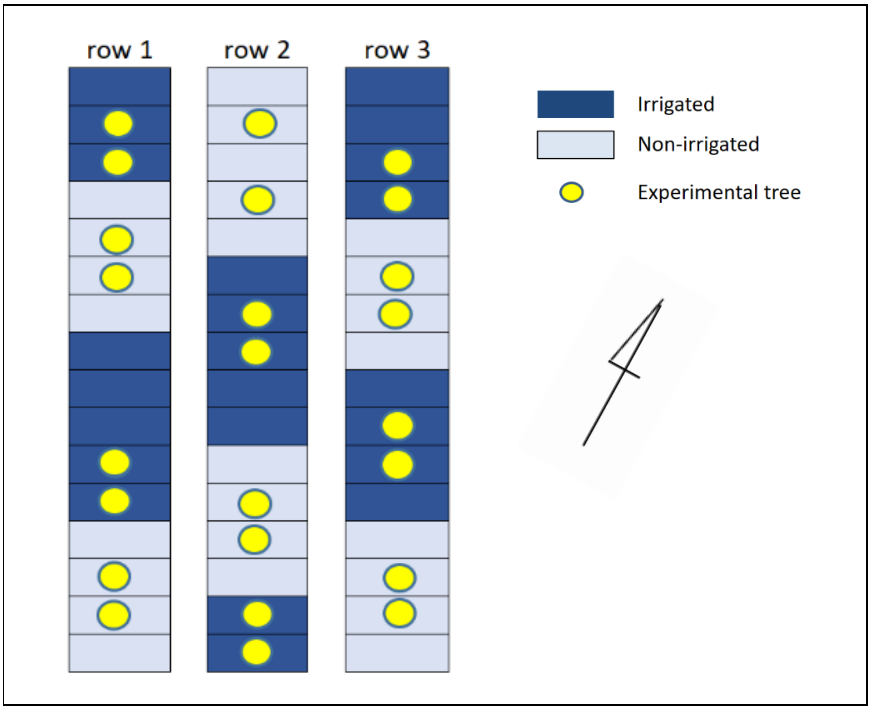


Figure 3.2: The experimental design with 12 irrigated and 12 non-irrigated trees within three rows (blocks). Non-irrigated trees were separated from the adjacent irrigated trees by non-irrigated ‘buffer’ trees.

Micro-sprinklers with a convex rotor (Rondo 75 lph, Rivulis, Migdal HaEmek, Israel) were installed below the canopy 2.5 m above the ground and irrigation applied for 6-8 hours when soil water content dropped below 0.4 $m^3\ m^{-3}$. The volume of water recommended for irrigated trees was calculated as:

$$I = ET_0\ K_c - R \tag{1}$$

where I is the depth of irrigation (mm), ET_o is the FAO-56 Penman-Montieth reference evapotranspiration (mm), K_c is a crop coefficient (Kaneko, 2016), and R is total rainfall (mm), calculated weekly. Actual irrigation was controlled by the orchard owner, and tended to be less than the recommended amount because of restrictions on water availability and requirements for other parts of the orchard (see results).

Sprinklers were disabled for the non-irrigated treatment from January to April in Year 0 (2016-17), and from November to April in Year 1 (2017-18) and Year 2 (2018-19), therefore, non-irrigated trees received only precipitation during the summer.

Experiment timeline

Under New Zealand’s conditions, the flowering season for avocado is from early-October to early-November, and harvest starts in September the following year. The irrigation period is generally from November to March (Table 3.1).

The experiment was conducted for three cropping years; ‘Year 0’ from October 2016 to September 2017, ‘Year 1’ from October 2017 to September 2018, and ‘Year 2’ from October 2018 to September 2019. Overall, there was no difference in soil water content between the two treatments in Year 0, because the experiment was established during that summer, and irrigation treatments did not start until mid-summer (Table 3.2). Differences in soil moisture appeared in Year 1 and Year 2 after irrigation treatments were applied from the beginning of the summer period. Plant water potential was measured periodically during the summer of all three years. Fruit growth and fruit abscission were monitored throughout the fruit growth period of Year 1. Fruit yield, size and dry matter content were measured at harvest in Year 0 and Year 1. Fruit set was extremely low in Year 2 despite sufficient flower numbers, resulting in a lack of fruit over the entire orchard. However, monitoring of the impact of irrigation treatments on tree water status was continued for that year.

Table 3.1: A typical timeline for flowering, rapid and slow fruit growth, harvest, and an irrigation period under New Zealand conditions.

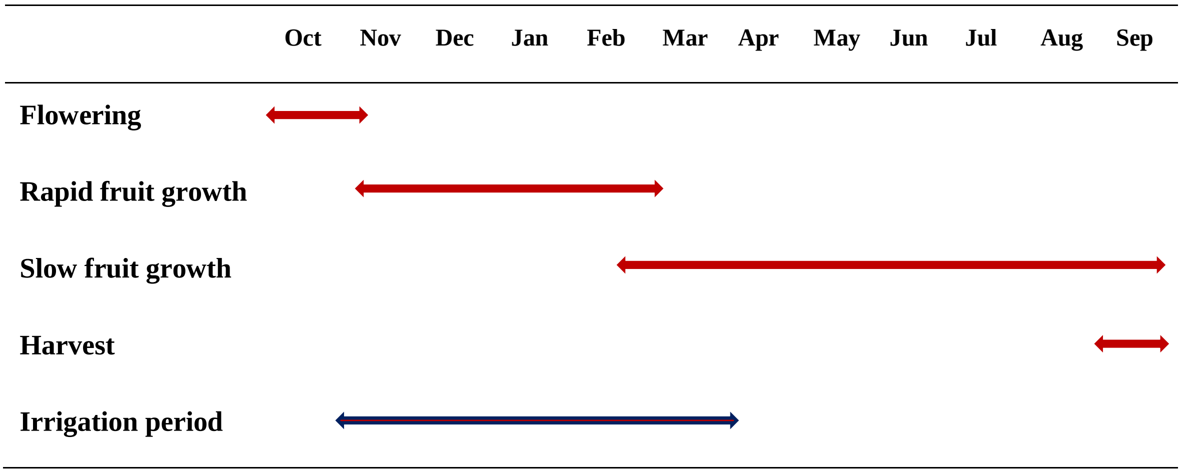


Table 3.2: Summary of the three experimental years, including the timing of periods of dry conditions, tree cropping status, and the years the irrigation treatment, weather and soil moisture monitoring, water potential and stomatal conductance measurements, fruit monitoring and fruit analysis were conducted.

	Year 0 **2016-17**	**Year 1** **2017-18**	**Year 2** **2018-19**
Dry period	mid-Jan to early-Feb	mid-Nov to late-Dec	mid-Feb to late-Mar
Cropping status of alternate bearing cycle	off	on	off
Irrigation treatment	No	Yes	Yes
Weather monitoring	Yes	Yes	Yes
Soil moisture monitoring	Yes	Yes	Yes
Water potential measurement	Predawn Stem Leaf	Predawn Stem Leaf	Stem Leaf
Stomatal conductance measurement	No	No	Yes
Fruit growth and fruit abscission monitoring	No	Yes	-
Harvest and fruit analysis	Yes	Yes	-

Weather

A meteorological station was installed in an open area within the orchard to record rainfall (TR-525I, tipping bucket rain gauge, Texas Electronics Inc, Dallas, TX), solar radiation (LI200 pyranometer, LI-COR Inc, Lincoln, NE), air temperature and humidity (HMP50 temperature and relative humidity probe, Campbell Scientific, Logan, UT), and wind speed (Vector A101M anemometer, Vector Instruments, Denbighshire, UK) from January 2016 to November 2019. Sensors were connected to a data logger (CR1000, Campbell Scientific, Inc, Logan, UT), meteorological data were recorded every 15 minutes, and daily potential evapotranspiration (ET_o) for a short vegetated surface was calculated by the data logger using the FAO-56 Penman-Monteith equation (Allen *et al.*, 2006). Meteorological data for the nearby city of Tauranga were obtained for the period October to December 2016 (NIWA, 2020).

Soil water content

To monitor volumetric soil water content, soil moisture probes (CS616 and CS650, Campbell Scientific Inc., Logan, UT) were installed in January 2017. The soil moisture probes were calibrated in the laboratory before installation using soil samples taken from the orchard at the depths where the probes were buried. Dry soil was moistened to a known water content and packed to the same bulk density as measured in the orchard to obtain the relationship between the output values of the probes and actual volumetric soil water content (Appendix B, Figure 6.2A).

The soil moisture probes were installed vertically halfway between the trunk and the edge of the canopy at the three depth ranges (0-30 cm, 31-60 cm, and 61-90 cm) on the eastern side of six irrigated trees and two non-irrigated trees. More irrigated trees were monitored because these trees were part of a separate trial monitoring tree water use at the same site. These probes were connected to a data logger (CR1000, Campbell Scientific, Inc., Logan, UT), and soil water content was recorded hourly. Tensiometers are commonly used by avocado growers in New Zealand (30 cm and 60 cm, SR Irrometer, Irrometer Company Inc., Riverside, CA) and therefore were also installed next to a set of electronic soil moisture probes to obtain a cross-reference between volumetric soil moisture and manual tensiometer measurements. A soil moisture release curve was prepared from periodic tensiometer readings and the output recorded from soil moisture probes at the same times.

Water potential

Predawn leaf water potential (Ψ_{pd}) was measured using a pressure chamber (Scholander *et al.*, 1965) (PMS Instrument Co. Ltd., Corvallis, OR) on 17 and 29 January, and 4 March in 2017 (Year 0), and 12, 21 and 31 December 2017 (Year 1). At each measurement, a leaf was placed in a plastic bag before removing from the tree using a razor blade.

Leaf water potential (Ψ_{leaf}) and stem water potential (Ψ_{stem}) were measured around midday on 17 and 28 January, and 12 February 2017 (Year 0), and 30 December 2017 (Year 1), and 12 February and 6 April 2019 (Year 2), when the soil water content of non-irrigated trees decreased below 0.35 m^3 m^{-3}. At each time, for the Ψ_{leaf} measurement, a fully sun-exposed leaf was selected, placed in a plastic bag before removing from the tree using a razor blade, and then measured. For the Ψ_{stem}

measurements, leaves were covered with aluminium foil for two to three hours before measurements.

Fruit weight, dry matter and yield

At harvest on 2 October 2017 (Year 0) and 15 September 2018 (Year 1) fruit yield was obtained for each experimental tree by weighing all fruit, then a 50 fruit sample was selected randomly and weighed as a measure of average fruit weight. Three to five fruit per tree were placed in a plastic bag and carried to the laboratory for analysis.

In the laboratory, a 10 mm corer was used to take a longitudinal core from the stem end to the stylar end. The mesocarp was separated from the skin and seed, weighed and dried at 60 ˚C in an oven for three days. Dry matter content was calculated as the ratio of dry weight to fresh weight of the mesocarp sample.

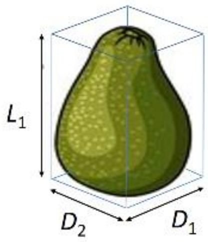
Fruit growth and abscission

Non-destructive fruit growth measurements were conducted from December 2017 until harvest in September 2018 (Year 1). For each tree, 20 fruit were labelled, and one length and two diameters of labelled fruit were measured using digital callipers every two to three weeks.

Fruit weight was estimated using the equation (Kaneko, 2016):

$$W_{\text{fruit}} = 0.4644 L_1 D_1 D_2 + 8.721 \qquad [2]$$

where W_{fruit} is fruit weight (g), L_1 (cm) is fruit length, and D_1 (cm) and D_2 (cm) are fruit diameters (Figure 3.3) (Appendix C, Figure 6.3A).



Length 1 (L_1)	Maximum fruit length
Diameter1 (D_1)	Maximum diameter perpendicular to length 1
Diameter2 (D_2)	Maximum diameter perpendicular to diameter 1

Figure 3.3: Non-destructive fruit growth measurement, fruit length (L_1), and two diameters (D_1 and D_2).

Fruit abscission was monitored from December 2018 until harvest (Year 1). To monitor 50 fruit per tree, 5-7 fruiting branches were selected and labelled, and the remaining fruit on these branches counted every 2-3 weeks.

Stomatal conductance

Stomatal conductance (g_s) was measured on six dates during the dry period of Year 2 with a portable photosynthesis system (LI-6400, LI-COR Inc. Lincoln, NE). Measurement were conducted five times a day from 11:00 to 16:00 hours on six irrigated and six non-irrigated trees. At each time, five mature and fully sun-exposed leaves per tree were selected.

Statistical analysis

All statistical analyses were carried out using the R Studio (R Core Team, 2016). Water potential, stomatal conductance and fruit weight were compared by analysis of variance (ANOVA), and Tukey's HSD test was performed for post-hoc comparison. Fruit growth rates were compared by analysis of variance (ANOVA) of the slopes of linear regressions fitted to the first four dates of non-destructive fruit size measurements, with fruit nested within tree. Analysis of covariance (ANCOVA) was used to test for the effect of irrigation on fruit weight and dry matter content, with yield per tree (fruit load) as a covariate and row as a blocking factor. A p-value of less than 0.05 was considered to be statistically significant.

3.3 Results

Weather

Over the three summers, January was consistently the warmest month with a monthly mean daily maximum air temperature of 29.9 °C in Year 0, 28.6 °C in Year 1, and 31 °C in Year 2 (Figure 3.4A-C). An extended dry period occurred in all three summers, although its timing and length was variable: January in Year 0, December in Year 1, and February in Year 2, with a total rainfall of 43.6, 28.2, and 61.1 mm for each of these months, respectively (Figure 3.4D-F).

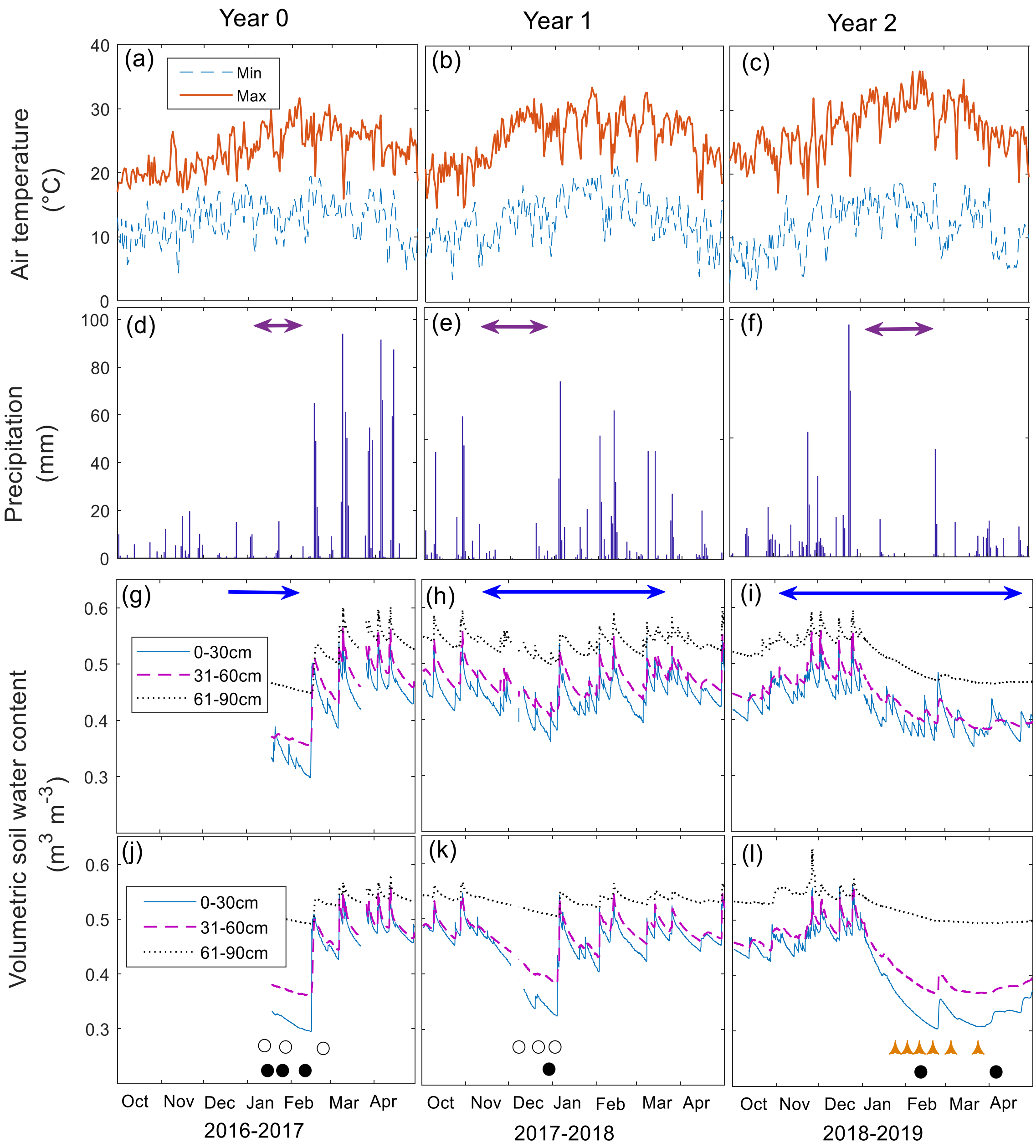


Figure 3.4: Daily maximum and minimum temperatures (°C) (a-c) and daily total rainfall (d-f), soil water content for irrigated trees (g-i) and non-irrigated trees (j-l) at three depths (0-30, 31-60, and 61-90 cm) for the summer period from October to April in three years; Year 0 (2016-17), Year 1 (2017-18) and Year 2 (2018-19). The purple horizontal arrows indicate low rainfall periods, the blue arrows the irrigation seasons, white circles (○) indicate dates of Ψ_{pd} measurement (17 and 29 January, and 4 March in 2017, Year 0, and 12, 21 and 31 December 2017, Year 1), black circles (●) date of Ψ_{stem} and Ψ_{leaf} measurements (17 and 28 January, and 12 February 2017, Year 0, and 30 December 2017, Year 1, and 12 February and 6 April 2019, Year 2, and orange triangles (▲) dates of g_s measurements.

Soil water content

In Year 0, the irrigation treatments did not start until partway through the summer, therefore, soil water content was similar between the two treatments for the remainder of the summer period. In this year, soil water in the top layer (0-30 cm) decreased to 0.3 m^3 m^{-3} until a significant rain event occurred on 16 February (Figure 3.4G, J). In Year 1 and Year 2 the soil water content of the top layer remained above 0.35 m^3 m^{-3} for the irrigated trees (Figure 3.4H, I). In contrast, the soil water content for the non-irrigated trees declined below this level during the dry periods. The driest measurement of the top layer was 0.32 m^3 m^{-3} (-80 kPa) on 3 January in Year 1, and 0.30 m^3 m^{-3} (-110 kPa) on 22 February in Year 2 (see Figure 3.4K, L, and Figure 3.5 for conversion from volumetric soil water content to soil water tension).

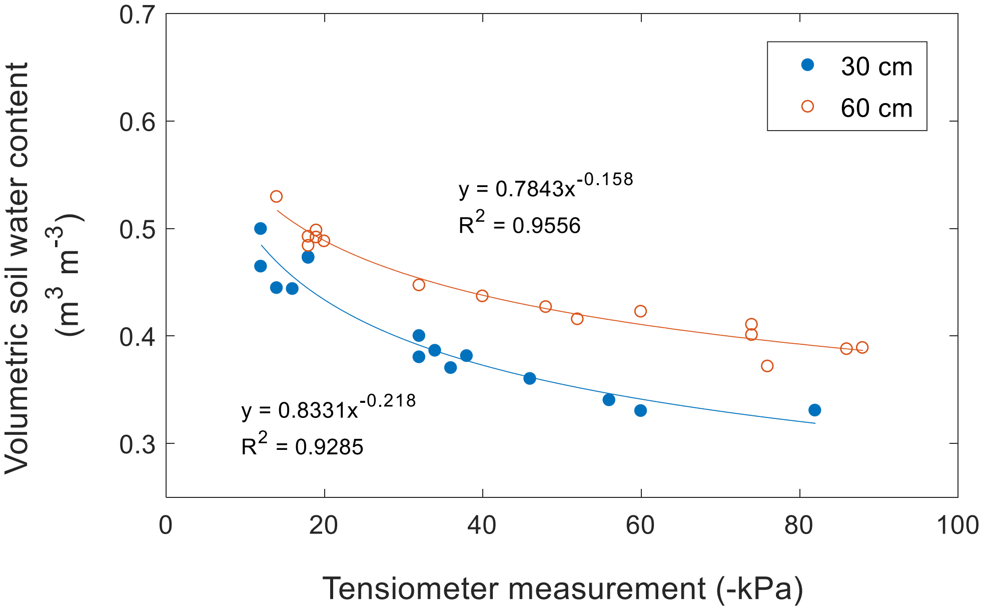


Figure 3.5: Soil moisture release curves for depths of 30 cm and 60 cm at the orchard in the Bay of Plenty. The values were obtained from tensiometer readings and output values of soil moisture probes recorded at the same time.

Water potential

Ψ_{pd} was measured in Year 0 and Year 1 (Figure 3.6A, B). Both treatments were above -0.06 MPa, and no difference was detected between the two treatments in either year ($P>0.05$). Ψ_{stem} and Ψ_{leaf} were also measured during the dry periods in Year 0 and Year 1 (Figure 3.6C, D). Overall, there was no difference in Ψ_{stem} and Ψ_{leaf} in Year 0. In Year 1, non-irrigated trees had a more negative Ψ_{stem} than the irrigated treatment ($P<0.05$), but there was no difference in Ψ_{leaf} ($P>0.05$).

Diurnal variation in Ψ_{stem} and Ψ_{leaf} was observed during the dry period in Year 2 (Figure 3.6E, F). Ψ_{stem} and Ψ_{leaf} was lowest in the morning and increased in the afternoon. On 12 February, the two treatments had similar values of Ψ_{stem} and Ψ_{leaf} ($P>0.05$). However, by 6 April, at 1100h, the non-irrigated treatment had lower Ψ_{stem} and Ψ_{leaf} values, relative to the irrigated treatment, and the difference in Ψ_{leaf} between treatments was significant ($P<0.05$).

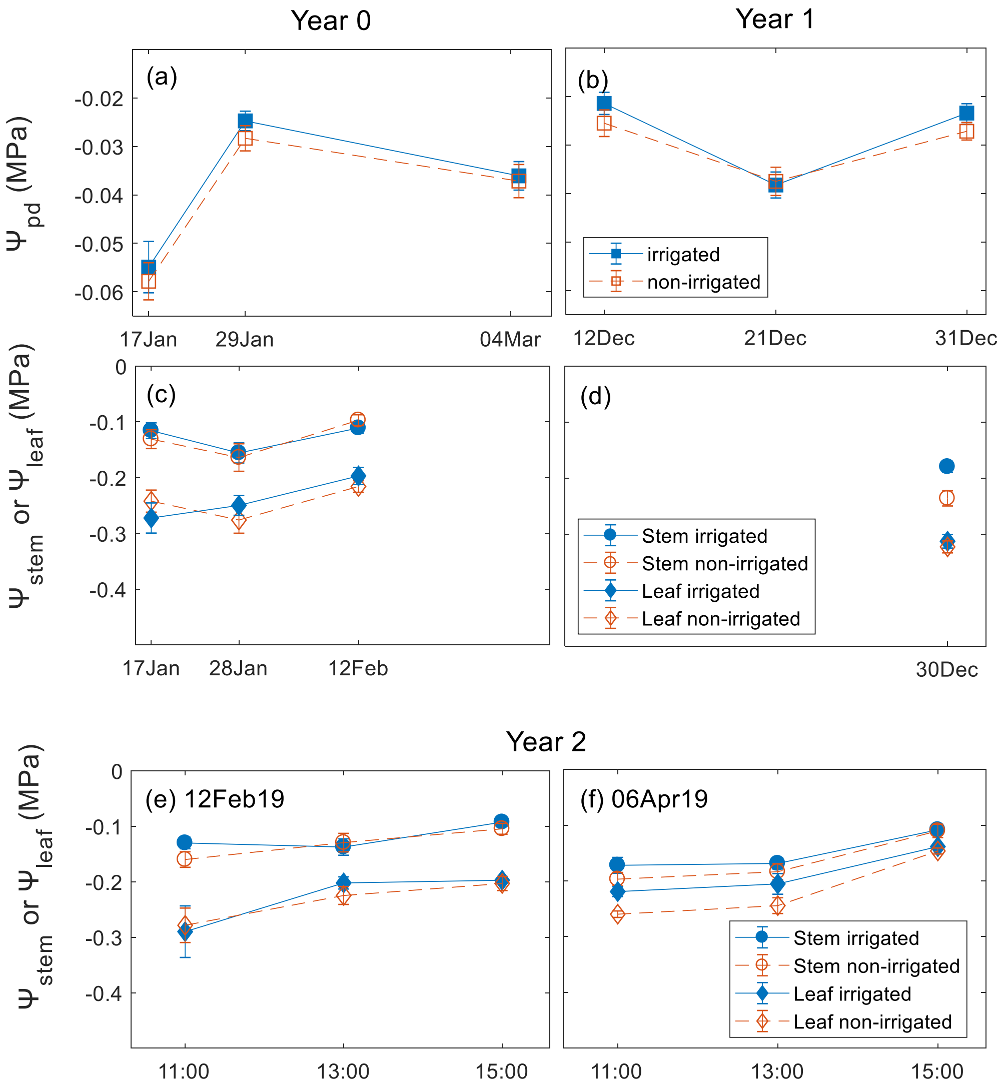


Figure 3.6: Predawn leaf water potential (Ψ_{pd}) in Year 0 (a) and Year 1 (b), stem water potential (Ψ_{stem}) and leaf water potential (Ψ_{leaf}) in Year 0 (c) and Year 1 (d), and a diurnal pattern of Ψ_{stem} and Ψ_{leaf} in Year 2 on 12 February and 6th April 2019 (f) for irrigated and non-irrigated treatments. Values are means ± S.E. (n=12).

Stomatal conductance

Stomatal conductance varied diurnally from late-January to late-March in Year 2 (Figure 3.7). Stomatal conductance of the irrigated trees was usually above 0.3 mol m^{-2} s^{-1}, whereas for non-irrigated trees g_s was slightly lower, around 0.25 mol m^{-2} s^{-1}, and the difference between the two treatments became more significant when the vapour pressure deficit (VPD) increased above 2.0 kPa (P<0.05).

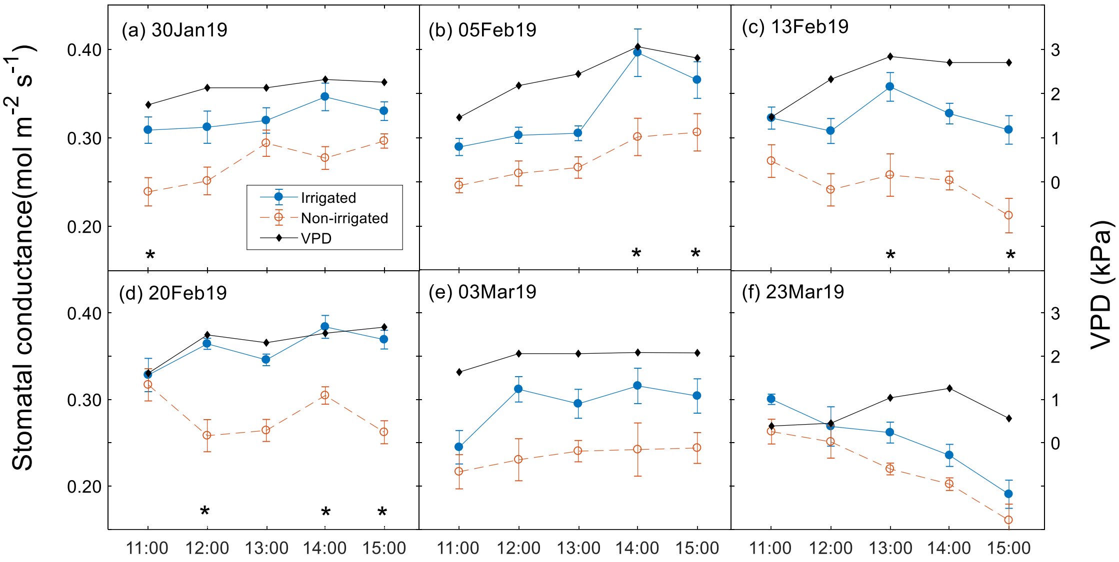


Figure 3.7: Diurnal stomatal conductance (g_s) (±1S.E.) of the irrigated and non-irrigated 'Hass' avocado trees, and VPD obtained from meteorological measurement at the study site. The measurements were made during a dry period between late-January and late-March, 2019 (Year 2) (▲ in Figure 3.4L). (P<0.05 *) (n=6)

Fruit growth and fruit abscission

Fruit grew rapidly until early-March in Year 1, then more slowly until harvest in September (Figure 3.8A). Overall, from February to September, fruit increased in weight from 66 (± 1.82 [S.E.]) g to 246 (± 8.63) g in the irrigated treatment and from 61 (± 1.50) g to 234 (± 3.90) g in the non-irrigated treatment. The fruit abscission rate was highest from January to early-March, and both the irrigated and non-irrigated trees lost more than one-third of their fruit in this period (Figure 3.8B). The irrigated trees had higher early fruit abscission, compared to the non-irrigated trees. However, there was a high level of variation in the proportion of fruit abscised between trees, and there were no significant differences in fruit growth or fruit abscission between the two treatments.

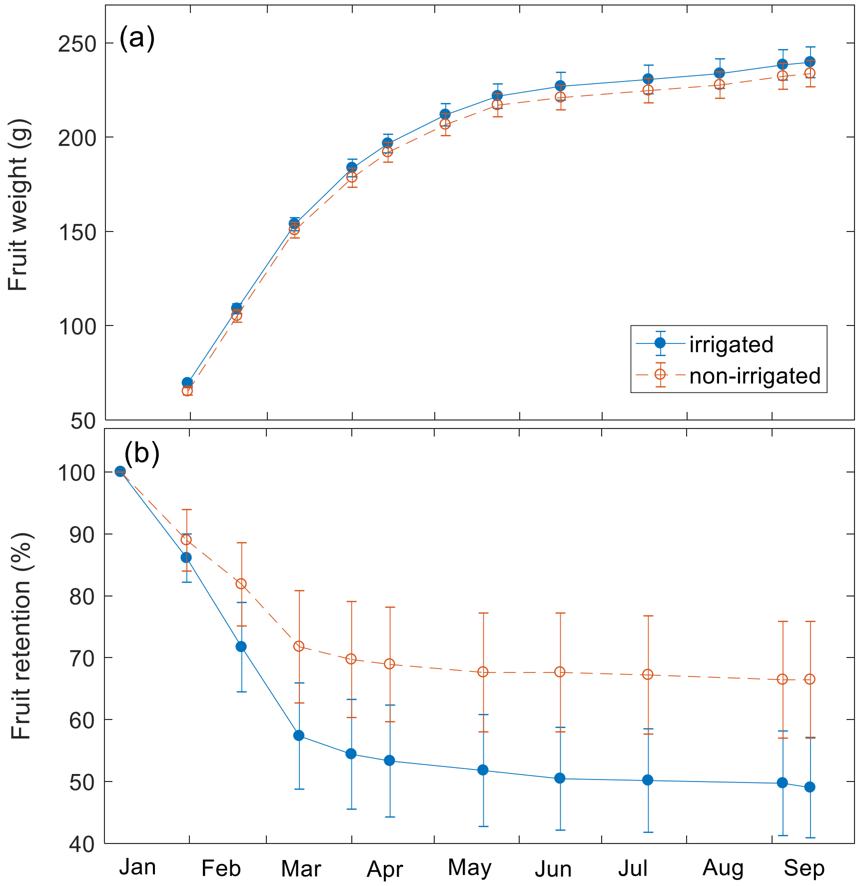


Figure 3.8 Non-destructive estimates of fruit weight (P>0.05) (a), and fruit retention (%) (P>0.05) (b) of the irrigated and non-irrigated trees, from January to harvest in September in Year 1. Values are means ± S.E. (n=12).

Fruit weight, dry matter and yield

Fruit yield varied widely between trees and years. In both Year 0 and Year 1 the lowest yields were below 30 kg per tree, whereas the highest yields were more than 200 kg per tree. Year 0 was an off-cropping year, with only one tree producing more than 200 kg, whereas Year 1 was an on-cropping year, when more than half of all trees produced more than 200 kg. Overall, non-irrigated trees had higher average yields per tree compared to irrigated trees in both Year 0 and Year 1 (Table 3.3). In Year 2 very few fruit were set and no harvest was possible.

Individual fruit weight decreased with increasing fruit yield per tree (-17.0 g per fruit with every 100 kg increase in yield per tree, ANCOVA, $p<0.001$; Figure 9A, B). The effect of yield on fruit weight did not change with year or irrigation (no year or irrigation interactions with yield, $p>0.05$; Figure 3.9A, B). Lack of irrigation in Year 1 decreased individual fruit weight by 26.4 g per fruit, regardless of yield per tree (significant interaction between irrigation and year, p<0.05; Figure 3.8B). Fruit dry matter content also decreased with increasing yield per tree in both years (-1.2% for every 100 kg increase in yield, ANCOVA, P<0.001; Figure. 3.9C, D), but there were no separate effects of irrigation or year on fruit dry matter content (no irrigation, year or interaction effects p>0.05; Figure 8C, D).

Table 3.3: Average fruit yield (kg) of 12 irrigated trees and 12 non-irrigated trees with S.E. over the three years.

	12 irrigated trees	12 non-irrigated trees
2017 (Year 0)	91 (±14.5)	130 (±10.5)
2018 (Year 1)	204 (±22.6)	220 (±22.0)
2019 (Year 2)	-	-

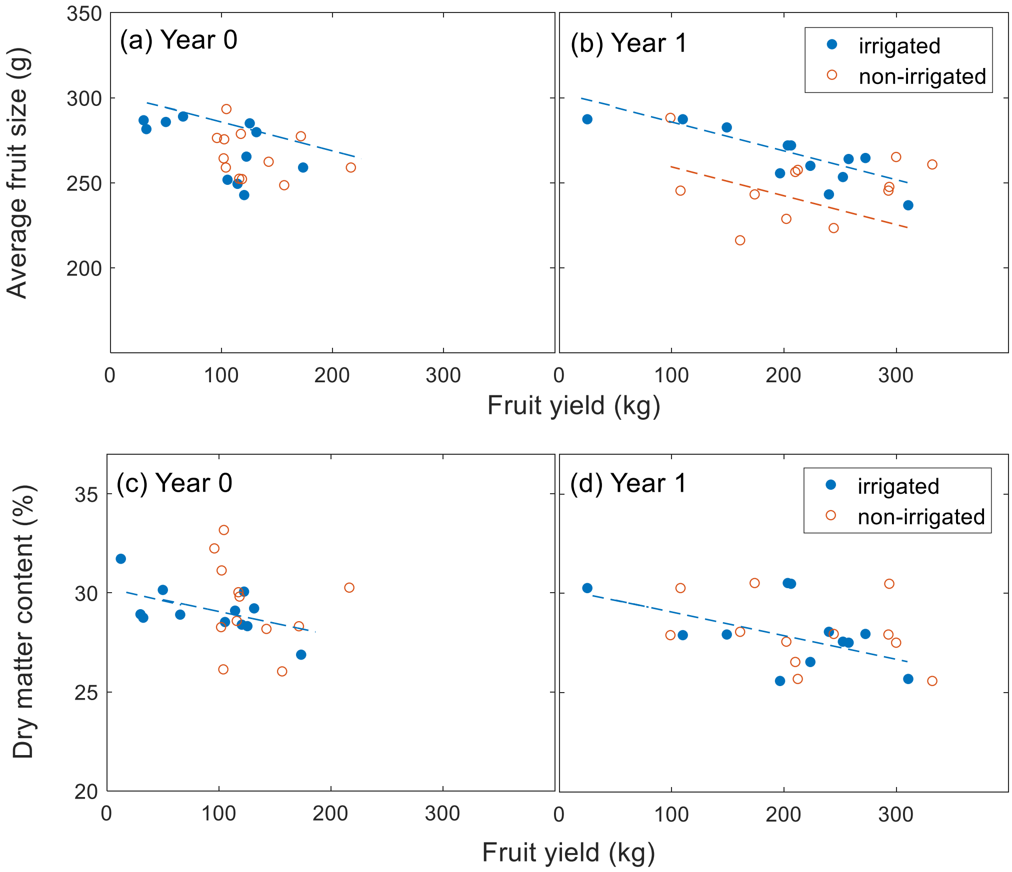


Figure 3.9: The relationship between fruit yield and average individual fruit weight for irrigated and non-irrigated trees in 2017 (a) and 2018 (b), and the relationship between average fruit yield and dry matter content for irrigated and non-irrigated trees in 2017 (c) and 2018 (d). The dotted lines presents trend lines where the correlation is significant (Note: the trend lines of the irrigated treatment were obtained from two years results).

3.4 Discussion

This study demonstrates the sensitivity of avocado fruit growth to water status. Fruit weight was reduced in the absence of irrigation, even when the level of water stress was mild compared to the levels typically seen in other avocado growing regions. Reductions in fruit weight caused by mild water stress were significant irrespective of bearing status and fruit load. Isohydric stomatal closure minimized changes in plant water status as the soil dried, but may have impacted fruit growth by reducing carbohydrate supply during early fruit development. Fruit load was highly variable between seasons, with orchard yield changing from low, to high, to no fruit at all, over the three years of this study. Fruit load also varied strongly between trees. Both forms of variation are typical of New Zealand avocado production and make it difficult to quantify the effects of irrigation management on fruit yield or quality. Despite these high levels of variation, this study demonstrates how the effect of water status on individual fruit weight can be separated from the effects of a variable climate, fruit set and fruit load.

Weather and soil water content

Soil water deficit during the flowering period (from late-September to late-October) is rare in the Bay of Plenty, indicating that there is a low probability that water stress affects the flowering process (Kaneko, 2016). Soil water content was high at all depths in early-spring in the three years of this study and no difference was detected between the two treatments. However, rainfall was irregular in summer (from December to March). Although the timing of the dry period was variable, each summer there was a period with significantly less precipitation for a month that caused the soil water content of the non-irrigated trees to decrease below 0.35 m^3 m^{-3} (-40 kPa) between the depth 0-30 cm. Water application to the irrigated treatment was slightly under the recommended amount because of constraints on irrigation management at the orchard, however, the dry periods still created clear differences between the two treatments.

Avocado is known to have a shallow root system. Michelakis *et al.* (1993) and Lahav *et al.* (2013a) stated that about two-thirds of the root system is located in the top 60 cm of soil, although the actual proportion is likely to depend on the soil type. In this study on sandy loam soil the top (0-30 cm) and middle layers (31-60 cm) dried quickly during the dry periods, and soil water content in the top 30 cm appeared to be most important.

In contrast, soil water content in the deeper layer (61-90 cm) was high (above 0.45 $m^3 m^{-3}$) throughout the year. A crop with a shallow root system is known to be highly sensitive to water scarcity, and avocado may be included.

Plant water status

Water potential is a good water stress indicator for a plant, and the level of water stress observed in this study was less than that recorded elsewhere. For avocado in Chile, Celedon *et al.* (2012) stated that Ψ_{stem} values were between -0.45 and -0.6 MPa under well-watered conditions, and dropped to -0.9 MPa during water stress with a Mediterranean marine climate, when the VPD was 3.6 kPa on sunny days. Compared to these values, the Ψ_{stem} values obtained here were higher and usually above -0.3 MPa in both treatments. Environmental conditions such as temperature and evaporative demand affect plant water potential measurements (Williams & Baeza, 2007). In New Zealand, VPD rarely exceeds 3.0 kPa possibly explaining why the Ψ_{stem} values were higher in this experiment.

Stomatal regulation is complex and involves many factors, such as plant physiological conditions, water status, nutrient availability, and weather conditions (Farquhar & Sharkey, 1982; Paul & Pellny, 2003; Buckley, 2019). The description of stomatal behaviour of avocado is somewhat controversial, with some studies (Bower, 1978; Ramadasan, 1980; Chartzoulakis *et al.*, 2002) having suggested that water stress induces stomatal closure, while others (Celedon *et al.*, 2012; Ferreyra *et al.*, 2014) have reported that water deficit did not influence g_s but caused a decrease in Ψ_{stem}. In our observations, compared to the irrigated treatment, the non-irrigated trees had approximately 20% lower g_s when soil water content was below 0.35 $m^3 m^{-3}$. This suggests isohydric behaviour and that stomatal closure in response to water stress may have caused a decrease in carbohydrate production.

Carbohydrate supply is important for fruit growth. In many crop species, such as plum (Gucci *et al.*, 1991), apple (Wibbe & Blanke, 1995), citrus (Iglesias *et al.*, 2002) and avocado (Silber *et al.*, 2013a), a higher photosynthetic rate has been observed during fruit development. This is because fruit are a strong sink for carbohydrates, and high demand for carbohydrate for fruit growth lowers carbohydrate concentration in leaves, leading to increased photosynthetic rates (Silber *et al.*, 2013a). However, in isohydric species such as avocado, photosynthetic activity decreases when soil water availability decreases, limiting carbohydrate accumulation in fruit. This limitation on fruit growth should be more significant for trees with a heavy crop.

Fruit growth and fruit abscission, alternate bearing

In year 1 the dry period occurred early, before the first period of very high rates of fruit abscission had subsided and non-destructive fruit growth measurements could begin (Kaneko, 2016). There was no difference in fruit growth rate between the two treatments after the dry period had ended. However, mean fruit weight was higher in the irrigated treatment on the first date of non-destructive fruit measurements, and on every date throughout the fruit growth period. A larger and more representative destructive sample of fruit at harvest confirmed that the non-irrigated treatment had smaller fruit. Tree-to-tree variation in fruit load and the early timing of the dry period made it difficult to detect treatment effects on fruit growth rate.

Fruit abscission caused by soil water deficit was not detected in this experiment. Some studies suggested that immature fruit abscission is increased by heat stress (Lomas, 1988; Silber *et al.*, 2012) or carbohydrate stress (Wolstenholme *et al.*, 1990; Silber *et al.*, 2019), rather than water stress (Garner & Lovatt, 2008). Severe water stress might lead to carbohydrate stress if a photosynthetic activity is reduced for an extended period, resulting in excessive fruit abscission (Silber *et al.*, 2012). However, the level of water stress imposed on the non-irrigated trees was moderate in this experiment, and the stress period occurred before monitoring of fruit abscission began. Therefore, we conclude that there was no treatment effect on fruit abscission.

Alternate bearing in avocado is typically observed as an on-cropping year followed by an off-cropping year (Lovatt, 2010). Fruit production was low in Year 0 and Year 2, and high in Year 1 across the entire orchard of this experiment. However, it appears that the exceptionally low fruit yield in Year 2 was caused by the climatic conditions, rather than just the regular alternate yield pattern. This orchard experienced an unusually cold spring in Year 2. The October monthly average daily minimum air temperature in Year 2 was 3 °C lower than that of Year 1 (15 days were recorded below 8 °C in Year 2). The reproductive process of avocado is known to be highly sensitive to temperatures (Salazar-Garcia *et al.*, 2013). Unusually low temperatures can negatively affect the synchrony of male and female flower development (Sedgley & Annells, 1981; Herrero, 2003), ovule viability (Sedgley, 1976), and pollen tube growth (Johannsmeier & Morudu, 1999). This may be an extreme example of how New Zealand's climate creates a more irregular pattern of avocado production (Gould *et al.*, 2019).

Effect of water stress on fruit yield and fruit quality

Water stress decreased avocado fruit weight by 26.4 g with no decline in dry matter content. This effect was detected independently of variation in individual fruit weight between trees in response to fruit load, with mean fruit weight decreasing as total fruit weight increased. Holzapfel *et al.* (2017) and Moreno-Ortega *et al.* (2019) also reported that water stress reduces avocado fruit weight without affecting oil content measured as dry matter content. Therefore, our finding was consistent with the previous studies.

Fruit weight in avocado may be determined by variation in the rate of cell division in the mesocarp (Bower *et al.*, 1989). Cell division continues throughout avocado fruit growth, but is most active during the first six weeks after fruit set (Barmore, 1976). This is the period from November to December under the New Zealand conditions. Water stressed conditions during this period may directly affect fruit growth by reducing the rate of early cell division. The lack of reduction in dry matter content may be because avocado fruit have a long growing period. Oil accumulates throughout fruit growth, in particular during later fruit developmental stages (Liu *et al.*, 1999). Therefore, water stress during early summer is less likely to affect oil accumulation.

How water stress affects dry matter content in fruit depends on the plant species, and the timing and the level of water stress relative to the timing of fruit developmental phases. For example, in plum, moderately water-stressed trees had smaller fruit with higher total soluble solids because of a concentration effect (Intrigliolo & Castel, 2010). In peach, water stress affected carbohydrate accumulation in fruit on trees holding a heavy crop, resulting in a significant reduction in fruit dry matter content (Berman & DeJong, 1996). Accumulation of photosynthetic products in fruit might be limited if the photosynthetic activity is inhibited by stomatal closure (Chapter 4). Irrigation regimes and fruit load can therefore have a combined effect on fruit development (Gucci *et al.*, 2007).

Irrigation requirement

The results of this study show that fruit weight is sensitive to water stress. Therefore identifying the threshold level of water stress required for this effect to occur is important. In this experiment, the amount of irrigation water applied was calculated based on ET_o and K_c. The timing of irrigation was usually determined by when soil water content dropped below 0.40 m^3 m^{-3} (-30 kPa) at a depth of 30 cm, half way between the trunk and the edge of the tree canopy. This threshold was probably appropriate, as the two treatments diverged and the effects of water stress on g_s appeared when soil water content decreased below 0.35 m^3 m^{-3} (-50 kPa).

Compared to other 'Hass' growing countries, such as California and Israel, New Zealand has a cooler maritime climate (Lahav *et al.*, 2013b), and lower air temperatures can lead to frost damage in winter and pollination failure in spring. Therefore, low temperatures might be more important than water shortage in this country. However, the greatest effect of climate change may be on water resources. Due to global warming, daily maximum air temperatures are predicted to increase in many countries, and New Zealand is no exception. In fact, over the last 10 years, the average daily temperatures in the Bay of Plenty have increased by about 0.5 °C (Niwa 2020). In particular, the last three summers from 2016-17 to 2018-19 were the warmest in the last 20 years (Niwa 2020). Drought is also predicted to occur more frequently and more intensely in eastern regions, and temperature rises will increase water demand for agricultural use (Kim, 2013). Irrigation management will become central to maintaining crop productivity, as well as a sustainable water resource.

In recent years, deficit irrigation has been used for some other crop species, such as olive (Motilva *et al.*, 2000) and grape (Santesteban *et al.*, 2011). Fruit load has an important role in irrigation management of avocado, as trees with a light crop might have a lower water demand (Silber *et al.*, 2013b) (Chapter 2). Year-to-year variation in fruit yield is large for avocado because of alternate and irregular bearing. For avocado, deficit irrigation may be acceptable for light crop trees without affecting fruit yield, if the plant's physiological response to water stress is better understood.

3.5 Conclusion

This study has assessed the impact of water stress on avocado fruit weight and yield by comparing between the irrigated and non-irrigated treatments in the Bay of Plenty, New Zealand. The results demonstrated that lack of irrigation reduced avocado fruit weight when there was a period of low precipitation during early fruit development, even though drought conditions were not prolonged and there were no changes in leaf water potential. However, the benefits of irrigation can be hidden by high levels of variation in fruit weight caused by tree-to-tree variation in fruit load.

Water is a limiting resource, with decreasing amounts of freshwater availability for agricultural use, and an urgent need to utilize available water more efficiently. The present study revealed the importance of irrigation for avocado, and an indication of the threshold level of soil and plant water stress that causes reductions in yield. The thresholds for irrigation scheduling may be influenced by fruit load, therefore, further research is required to compare the irrigation requirement of avocado between on- and off- cropping years (Chapter 2).

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3.7 Disclosure statement

No potential conflict of interest was reported by the author.

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Chapter 4

Dynamics of avocado whole water balance and the effects of water stress on fruit growth

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4.1 Introduction

Water enters into a plant from the roots, moves up the stem through the vasculature, and may eventually evaporate from the leaves via the stomata. During fruit growth, water also accumulates in fruit, or is lost from the fruit surface via transpiration (Greenspan *et al.*, 1994; Greenspan *et al.*, 1996; Matthews & Shackel, 2005).

Fleshy fruit contain a large amount of water when fresh, for example, by weight more than 90% in melons, 81 to 90% in citrus, various berries and most vine fruit, and over 65% in avocados (Pomeranz & Meloan, 1994). Fruit growth is strongly influenced by the net water balance of the fruit, as fruit size increases when water enters into fruit and decreases when water moves out from the fruit. Many fruits, such as kiwifruit (Clearwater *et al.*, 2009), cherry (Bruggenwirth *et al.*, 2016), tomato (Guichard *et al.*, 2005), olive (Fernandes *et al.*, 2018), apple (Lang, 1990) and avocado (Schroeder, 1958), exhibit a diurnal fluctuation in fruit size by shrinking during the day and expanding at night, and these fruit grow when expansion surpasses shrinkage. The water status of the plant may change this diurnal pattern, and hence an understanding of the overall plant water balance is important for fruit crop production management.

Water flows into fruit via the xylem and the phloem, and water loss from fruit occurs either as outflow to the parent plant through the xylem or via fruit transpiration (Matthews & Shackel, 2005). Through the xylem, water flows in and out of fruit following gradients in water potential. When leaf water potential (Ψ_{leaf}) and stem water potential (Ψ_{stem}) become more negative than fruit water potential (Ψ_{fruit}), water flows out from the fruit back into the parent stem, and vice versa. Water also flows into the fruit through the phloem, with the delivery of carbohydrates and mineral nutrients in solution. Water moves into the phloem down an osmotic potential gradient in the source tissues (photosynthetic leaves or storage organs) and flows to the sink tissues (fruit or new growth) via bulk flow down a hydrostatic pressure gradient (Savage *et al.*, 2016).

Environmental conditions, such as drought, may cause changes in these osmotic and hydrostatic pressure gradients, and consequently may alter the overall water balance within a plant (Sevanto *et al.*, 2011). Previous studies (Lahav & Kalmar, 1977; Kaneko, 2016) of avocado have shown that fruit size decreases when the tree experiences water stress, yet exactly how fruit size reduction is caused by water deficit is not known.

In response to drought conditions, plants exhibit a spectrum of stomatal responses between two extremes, from isohydric to anisohydric behaviour (Sade *et al.*, 2012). Under drought, isohydric species regulate water use by controlling stomatal conductance (g_s) to maintain a constant xylem water potential in a plant (Ψ_{xylem}), whereas anisohydric species show less stomatal regulation, allowing a decrease in Ψ_{xylem} to supply the demand for water for photosynthesis (Tardieu & Simonneau, 1998; McDowell *et al.*, 2008). Avocado is considered to be an isohydric species, because it can reduce stomatal conductance (g_s) when experiencing water stress (Carr, 2013) (Chapter 3). In general, both leaves and fruit have a high water demand. Stomatal closure for water conservation may promote water potential equilibrium throughout the plant and potentially maintain water movement to the fruit. Daily measurements of the plant water balance and its effect on fruit growth will help reveal how the avocado plant allocates limited water to its organs.

Thermal sap flow techniques have been widely used over the last few decades for the measurement of plant water uptake (Cermak *et al.*, 2004). Even though monitoring the diurnal pattern of water flow in small shoots, flowers and fruit pedicels is challenging, Higuchi and Sakuratani (2006) successfully measured water flow through intact shoots and fruit peduncles in mango, using their original stem heat balance method (Sakuratani, 1981; Baker & Vanbavel, 1987). Kiwifruit has a smaller diameter of fruit pedicels with lower sap flow, but Clearwater *et al.* (2009) quantified such low sap flow using external heat ratio sap flow gauges. Roddy and Dawson (2012) modified the design of Clearwater *et al.* (2009) to quantify the water requirements of leaves and flowers of four tropical species. The heat ratio sap flow technique can measure water flow in both directions (inflow and outflow), hence it provides useful information about the direction and magnitude of water flow within a plant.

Linear transducer (LT) measurements is a common method for fruit growth monitoring. LTs of various types have been used to monitor changes in fruit diameter continuously in apple (Jones & Higgs, 1982), grape (Keller *et al.*, 2015), cherry (Bruggenwirth *et al.*, 2016), kiwifruit (Clearwater *et al.*, 2013), olive (Fernandes *et al.*, 2018), and pear

(Morandi *et al.*, 2014). This is a useful tool because gain or loss of fruit volume or weight can be calculated from a fruit diameter measured by LT measurements.

With a combination of non-destructive external sap flow measurement and continuous fruit growth monitoring using LTs, and the addition of predawn leaf water potential (Ψ_{pd}), Ψ_{leaf}, Ψ_{stem}, g_s, and A measurements, the goal of this research was to understand the dynamics of water flow in fruiting avocado. By comparing the plant water balance between well-watered and water-stressed conditions, it provides more information about how a change in plant water status affects fruit growth.

Avocado fruit growth is characterized by two developmental stages: an initial period of rapid fruit growth (stage I) and a second period of slower growth (stage II) (Liu *et al.*, 1999; Mickelbart *et al.*, 2012; Kaneko, 2016). The water content of fruit is high, around 90%, during stage I, but tends to decrease as oil accumulates in the fruit during stage II (Figure 1.3, Chapter 1). At maturity, avocado fruit flesh contains about 70% water (Liu *et al.*, 1999). This experiment was conducted during stage I; two to three months after fruit set, when water deficit is likely to occur under New Zealand conditions.

The research objectives included monitoring water flow through shoots stems and fruit pedicels, assessing changes in water flow in response to water stress, and quantifying the effect of water stress on avocado fruit growth. The hypothesis was that fruit size is reduced by drought because of less fruit water inflow and more outflow to the parent plant via the xylem. It was also hypothesized that photosynthesis is reduced by water stress, and this will lead to a reduction in phloem flow and, consequently reduced fruit dry matter content.

4.2 Methods

Plant materials and the experiment

The experiment was conducted in a glasshouse at the University of Waikato (37.7869° S, 175.3185° E) in the summer from December 2018 to January 2019. Ten potted plants (ca. 7 years) of *Persea americana* ‘Hass’ avocado were used for this experiment (five control and five drought plants), randomly arranged amongst a similar number of trees that were not included in the experiment. These plants were grafted on Dusa rootstocks. The plants were approximately 1.5 m high and 1.5 m wide, and basal trunk diameters were between 5.5 and 7.5 cm. They were grown in 130 L pots containing soil. In the glasshouse, the flowering season was from mid-September to mid-October, and bumblebee and hand pollination were conducted during this period. Fruit set was observed from early-October. Significant fruit drop is a natural occurrence for avocado during early fruit growth, and fruit number on the experimental trees was counted on 15 December 2018, after early fruit drop had ceased (Table 4.1). Not all plants retained fruit. Plants were allocated to treatment so that three of the five plants in each treatment had fruit.

Table 4.1: Plant ID and fruit counts of the control and drought plants.

Treatment	Control					Drought				
Plant ID	C1	C2	C3	C4	C5	D1	D2	D3	D4	D5
fruit count	9	6	1	0	0	3	10	6	0	0

Experimental conditions and irrigation regime

Within the glasshouse, the air was circulated during the day by automated roof vents, end wall fans, and an evaporative cooling system. Air temperatures were regulated between 15 and 28 °C. Air temperatures and humidity were monitored independently of the glasshouse control system by a temperature and relative humidity sensor (HMP50, Campbell Scientific, Logan, UT), and vapour pressure deficit (VPD) was calculated by a data logger (CR3000, Campbell Scientific Inc., Logan, UT) that was connected to the sensor, and data was recorded every 5 minutes.

For the five control plants, irrigation was controlled automatically by the greenhouse system. Water was applied twice a day; 2.5 L of water at 0600 h and another 2.5 L of water at 1200 h. Water was applied manually to the drought plants. To decrease soil water content, no water was applied for four days from 26 to 29 December 2018 (Week 0). Then, from 30 December 2018 to 14 January 2019, 2 L of water was applied at 0700 h each day (Week 1 and 2). From 15 to 20 January 2019, water application was further reduced to 1 L per day (Week 3), then the drought plants were re-watered with the same amount as the control treatment plants from the evening on 26 January 2019 (Week 4).

Soil moisture

The soil water content of three control and three drought potted plants was monitored by soil moisture probes (EH_2O, Decagon Devices Inc., WA). These were installed vertically in the depth range 0-20 cm. Two additional soil moisture probes (CS615, Campbell Scientific Inc., Logan, UT) were installed vertically at depths of 0-30 cm in a pot of one control and one of the drought plants. These probes were connected to a multiplexer (AM16/32B, Campbell Scientific Inc., Logan, UT) and a data logger (CR1000, Campbell Scientific Inc., Logan, UT). Soil water content was recorded every hour.

Plant sap flow

Four compensation heat pulse sap flow probe sets were installed on the main trunk of two control and two drought plants in early December 2018. Each sap flow probe set (model HP4TC-S, Tranzflo NZ Ltd., Palmerston North, New Zealand) consisted of two temperature probes with two copper-constantan thermocouples each, and one heater probe (Green *et al.*, 2003). The two thermocouples were positioned at depths of 15 mm and 30 mm from the bark surface.

The sap flow probe sets were connected to a data logger (CR1000, Campbell Scientific Inc., Logan, UT). Every 30 minutes heat was applied for three seconds, and temperatures were monitored every two seconds for eight minutes after the heat pulse. Sap velocity was calculated by the compensation heat pulse method described by Green *et al.* (2003), with a wound depth correction of 2.0 mm (see Methods in Chapter 2).

Shoot and fruit external heat ratio sap flow

Twelve external heat ratio sap flow gauges (Clearwater *et al.*, 2009) were used for monitoring water movement through shoot stems and fruit pedicels (three shoot stems and three fruit pedicels per treatment). The average diameter of the shoot stems was 7.1 (± 0.56 [S.E.]) mm, with 12 (± 2.0) leaves with total leaf area of 770 (± 92.7) cm^2, and that of the fruit pedicels was 6.0 (± 0.44) mm.

Each gauge consisted of two copper-constantan thermocouples and one heater on a silicone block. The heater was a 47 **Ω** resister (3.2 x 1.6 mm). These gauges were connected to a multiplexer (AM25T, Campbell Scientific Inc., Logan, UT) and a data logger (CR3000, Campbell Scientific Inc., Logan, UT). To eliminate errors caused by external thermal changes, all probes were insulated by two layers of pipe insulation foam, and the multiplexer and the logger were placed in a polystyrene foam box. Throughout the experimental period, measurements were taken every 10 minutes, with the heat pulse applied for two seconds. The heat pulse velocity was calculated as described by Clearwater *et al.* (2009).

In order to quantify sap flow through the shoot stem and fruit pedicels, the external heat ratio sap flow measurement were needed to calibrate against actual flow through these shoot stems and fruit pedicels using a XYL'EM flow meter (Bronkhorst, Montigny les Cormeilles, France). At the end of the experiment, the shoot stems and fruit pedicels were removed with the sap flow gauge still installed, and a 10 cm length of stem material was prepared by cutting both ends using a razor blade. For the shoot stems, the proximal end was connected to the tubing of the flow meter, and water flow was measured using the flow meter while applying a range of pressures. Sap flow measurements were recorded at the same time using the sap flow gauges. For the fruit pedicels, xylem water flow was induced in both directions (basipetal and acripetal), while sap flow and hydraulic flow measurements were recorded.

Linear transducer fruit diameter

Ten potentiometric linear transducers (LTs) (MM10, Megatron Elektronik GmbH & Co., Munich, Germany) were used for the measurement of fruit diameter changes. On 20 December 2018, four LTs, one per fruit, were installed on fruit of two control plants, and six were installed on fruit of three drought plants. The LTs were connected to a data logger (CR1000, Campbell Scientific Inc., Logan, UT) and data were recorded every hour. Measurements were continued until late-January, and the positions of the LTs were adjusted each week during the period of observation.

Stomatal conductance, photosynthetic assimilation and plant water potential

During the experiment, g_s and A measurements were taken twice a week, using a portable photosynthesis system (LI-6400, LI-COR Inc., Lincoln, NE). Measurements were made every two hours in ambient light on three randomly selected sunlit mature leaves per plant, from 0800 h to 1800 h.

Ψ_{pd} was measured twice per week at 0600 h, using a pressure chamber (PMS Instrument Co. Ltd., Corvallis, OR). At each time, one healthy mature leaf was chosen, covered in a plastic bag, removed from the plant with a razor blade, and used for the measurement immediately. Ψ_{leaf} and Ψ_{stem} were measured once per week. For the Ψ_{leaf} measurements, full sun-exposed leaves were chosen. For the Ψ_{stem} measurements, selected healthy mature leaves were covered with aluminium foil at least two hours before measurements. Measurements were taken three times per day from 1000 h to 1500 h.

Weekly fruit growth

In addition to the LT's non-destructive fruit growth measurements were conducted on all fruit throughout the experiment and fruit volumes calculated from fruit dimensions. All fruit on the plants were labelled, and three dimensions of fruit ($L_1D_1D_2$; maximum fruit length (L_1) (cm), maximum diameter perpendicular to L_1 (D_1) (cm) and maximum diameter perpendicular to D_1 (D_2) (cm)) were measured once per week, using digital callipers (Figure 3.3, Chapter 3). From the $L_1D_1D_2$ measurement, fruit volume was calculated, based on the relationship between $L_1D_1D_2$ and fruit volume:

$$V_{\text{fruit}} = 0.4926L_1\,D_1D_2 + 1 \qquad [1]$$

where V_{fruit} is fruit volume (cm^3) ($R^2 = 0.9973$, $P<0.01$) (Appendix C, Figure 6.3B).

Harvest

All fruit were harvested on 2 and 3 February 2019. The fruit were placed in a plastic bag and brought to the laboratory.

In the laboratory, with constant temperature, humidity and air flow by a fan, fruit surface conductance was measured by weighing the fresh fruit every 30 minutes over 6 hours and calculating water loss based on the change in fruit weight. The relationship between $L_1D_1D_2$ and fruit surface area was obtained from actual measurements of 20 fruit by peeling the skin and measuring the surface area using a leaf area meter (LI-3100, LI-COR Inc, Lincoln, NE):

$$S_{\text{fruit}} = 0.287L_1\,D_1D_2 + 48.875 \qquad [2]$$

where S_{fruit} is the fruit surface area (cm^2) ($R^2 = 0.9871$, $P<0.01$) (Appendix C, Figure 6.3C).

Fruit weight and volume were measured, and 20-30 g of fruit flesh were taken for analysis of dry matter content, using a corer (Chapter 3). The flesh fruit samples were dried in an oven at 60 °C for three days. Dry matter content was calculated as the ratio of dry weight to fresh weight.

The fruit water balance

The fruit water balance was compared between fruit on drought plants before (Week 1) and during the water stress period (Week 3). Estimated sap flow was obtained from the average of three fruit measured by external sap flow gauges over a 24 h period, and fruit transpiration was estimated as the product of fruit surface conductance and VPD, assuming that the fruit surface conductance was the same for stressed and non-stressed plants. The fruit water balance was calculated as the sum of sap flow and transpiration, and was compared to volume changes for the same fruit measured using the LTs. The diurnal time course of stem and fruit water potential were estimated based on actual Ψ_{pd} and Ψ_{stem} measurements, and the fruit water potential time course were created based on a water potential difference that corresponds with the observed pattern of inward and outward sap flow.

Statistical analysis

All statistical analyses were carried out using R (R Core Team, 2016). Measurements of Ψ_{pd}, Ψ_{leaf}, Ψ_{stem}, g_s, and A, were compared between treatments using analysis of variance (ANOVA), and Tukey's HSD tests were performed for post-hoc comparisons. A linear mixed effects model was used to test for differences in fruit growth between the two treatments, and the final fruit size was compared using T-tests. Analysis of covariance (ANCOVA) was used for the relationship between fruit size and dry matter content to determine whether regression slopes were significantly different between the two treatments. A p-value of less than 0.05 was considered to be statistically significant.

4.3 Results

Weather, soil water content and plant water uptake

The average daily air temperature in the glasshouse was 20.6 °C with a maximum of 25.2 °C and a minimum of 16.8 °C in December 2018, and 22.1 °C with a maximum of 27.8 °C and a minimum of 17.9 °C in January 2019 (Figure 4.1A). Daily average VPD was 0.77 kPa in December and 0.91 kPa in January, with a range between 2.6 kPa during the day and 0.3 kPa at night.

Soil water content rapidly increased in response to irrigation, and decreased with plant water uptake (Figure 4.1B). Throughout the experiment, the control plants had relatively stable soil water content that oscillated between 0.22 and 0.25 $m^3 m^{-3}$. In contrast, the soil water content of the drought plants decreased below 0.2 $m^3 m^{-3}$ on 30 December 2018, and with a daily fluctuation continued a downward trend from Week 1 to Week 3 reaching a minimum of 0.16 $m^3 m^{-3}$.

Diurnal water uptake of avocado plants measured using sap flow followed the expected pattern of increasing water use in the morning, a peak around the middle of the day, and decreasing in the afternoon. Over the summer, the control plants used an average of 4.8 $L d^{-1}$. Compared to the control plants, water uptake of the drought plants decreased when soil water content dropped below 0.2 $m^3 m^{-3}$, suggesting that the drought plants started to experience water stress from 30 December 2018. Water uptake of the drought plants continued to decrease over time from Week 1 to Week 3 when the soil water content was below 0.18 $m^3 m^{-3}$. After re-watering on 19 January, soil water content recovered quickly, but the transpiration of the drought plants recovered gradually, with progressive daily increases in water uptake (Figure 4.1C).

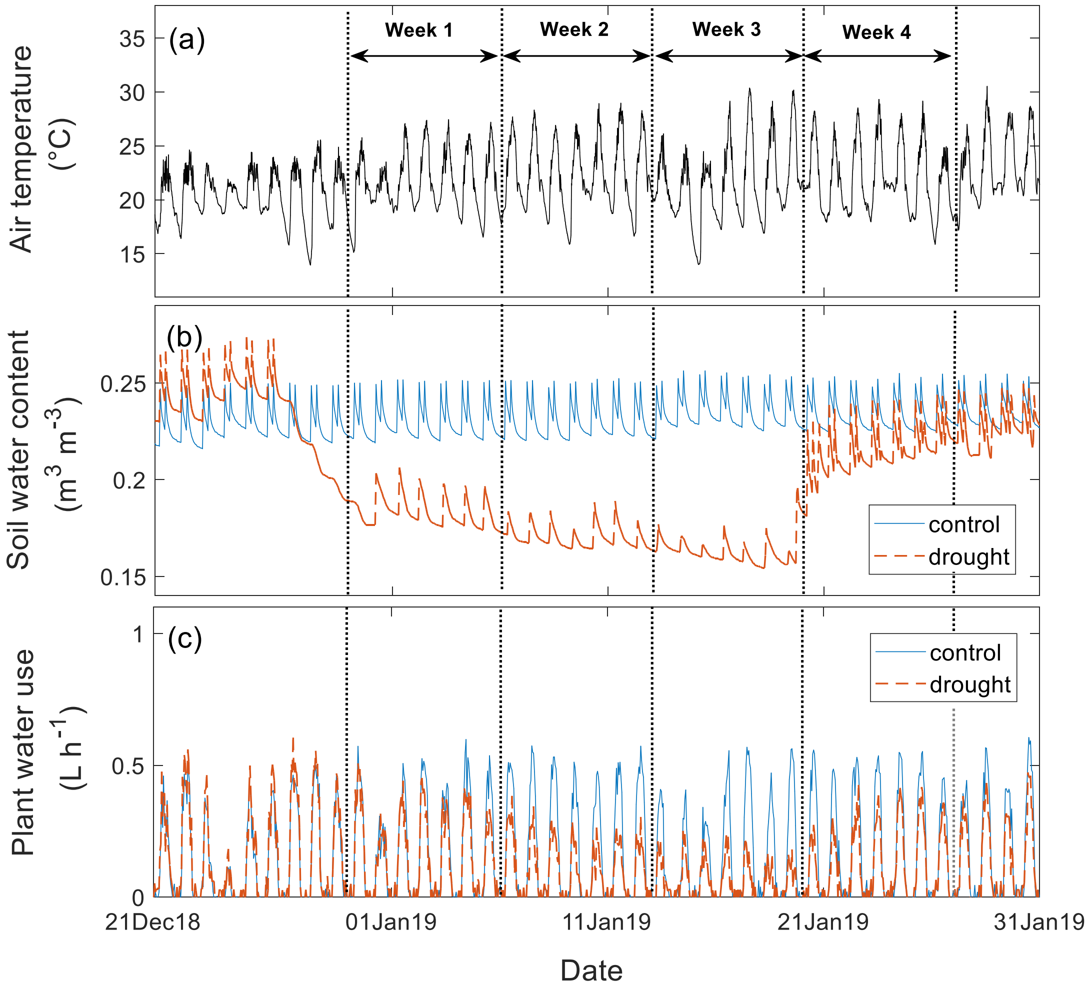

Figure 4.1: Sample caption hourly glasshouse air temperature (a), volumetric soil water content of the control and drought plants in the top 20 cm of soil (b), and plant water use of the control and drought plants, calculated from sap flow measurement on the main trunk (c), from 21 December 2018 to 31 January 2019.

Plant water potential

Ψ_{pd} of the control plants was stable around -0.04 MPa, whereas Ψ_{pd} of the drought plants became more negative during the drought experiment (Figure 4.2). The difference appeared 10 days after the drought treatment started. On 5 January, Ψ_{pd} of the control was -0.043 ± 0.004 MPa and Ψ_{pd} of the drought was -0.182 (SE±0.019) MPa ($P<0.01$). This difference continued during the drought treatment, with Ψ_{pd} of the drought plants recovering to control levels after re-watering.

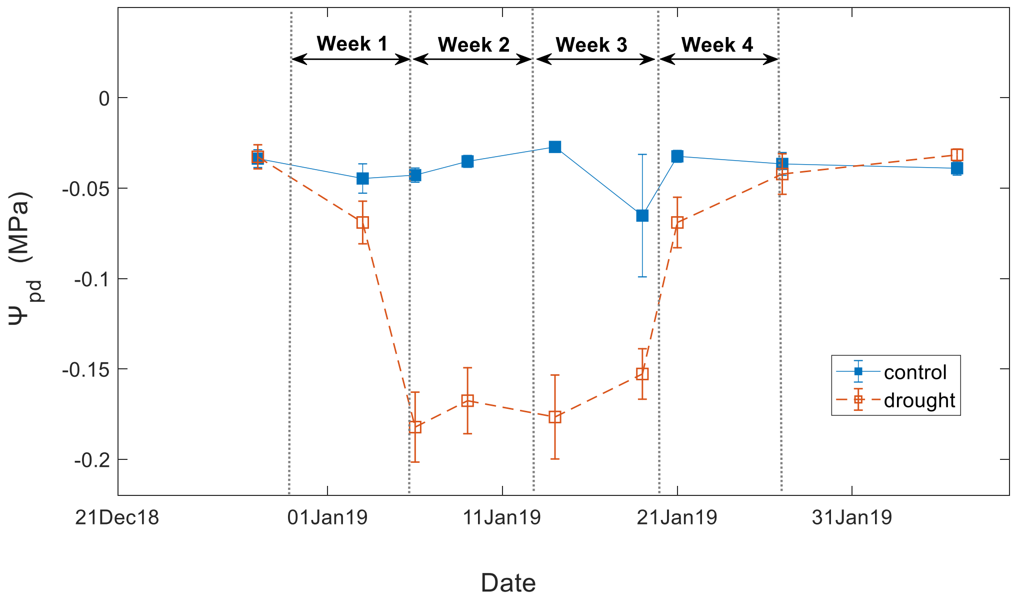

Figure 4.2: Predawn leaf water potential (Ψpd) of the five control and five drought plants (±1S.E.), measured from late-December 2018 to early February (n = 5 plants per treatment).

Both treatments had slightly more negative values of $\Psi_{leaf,}$ compared to Ψstem throughout the observation period (Figure 4.3). At the beginning of the drought period, on 2 January (Week 1), the drought plants had slightly more negative Ψ_{leaf} and Ψ_{stem} values compared to the control plants, but the differences were not statistically significant (P>0.05). On other days, the control and drought plants had similar values of Ψ_{leaf} and Ψ_{stem}, and both Ψ_{leaf} and Ψ_{stem} had a tendency to increase from the morning to the afternoon from approximately - 0.17 to -0.15 MPa, and -0.15 to -0.12 MPa respectively. Overall, there was no significant difference between the control and drought treatments in Ψ_{leaf} and Ψ_{stem} (P>0.05).

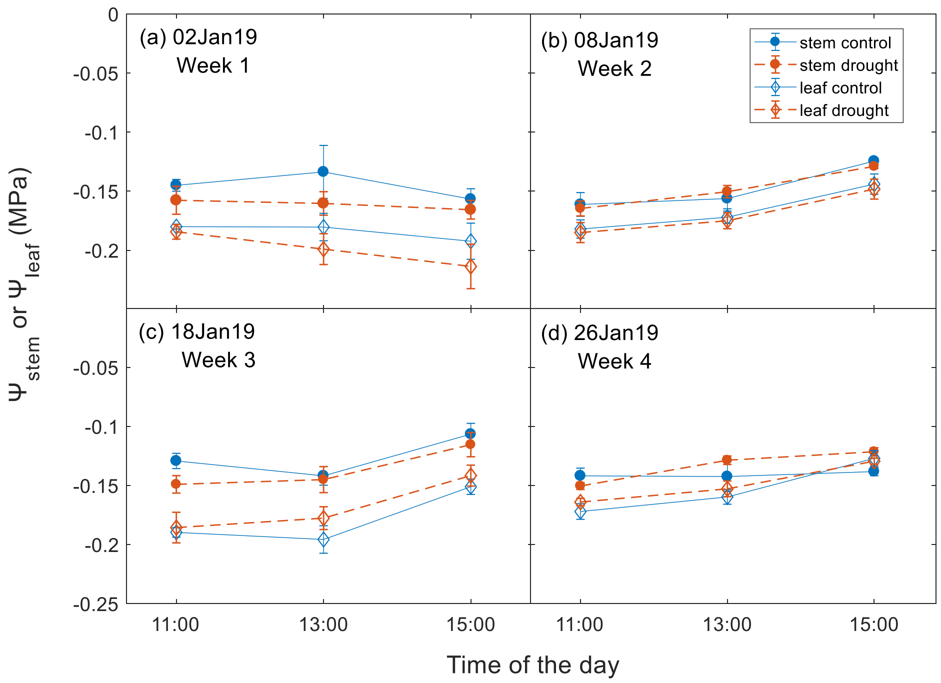

Figure 4.3: Leaf water potential (Ψ_{leaf}) and stem water potential (Ψ_{stem}) (±1S.E.) measured on 2 January (Week 1), 8 January (Week 2), 18 January (Week 3), and 27 January 2019 (Week 4) (n=5 plants per treatment) (P>0.05).

Stomatal conductance (g_s) and photosynthetic net assimilation (A)

Under the glasshouse conditions, g_s generally increased in the morning and decreased in the afternoon. The maximum value during the day was approximately 0.24 mol m^{-2} s^{-1} at 1200 h (Figure 4.4).

Clear effects of the drought treatment on g_s were observed. On 2 January, in the afternoon, g_s of the drought plants fell by 40% compared with that of the control plants ($P<0.05$). As the water deficit continued, the drought plants tended to reduce g_s, especially in the afternoon. The most apparent decline in g_s occurred on 19 January (Week 3) when the drought plants were severely water stressed and the VPD exceeded 2 kPa. On that day g_s of the drought treatment was below 0.01 mol m^{-2} s^{-1}, indicating completely closed stomata after 1400 h.

The daily maximum A values of the control plants was between 10 and 14 μ mol m^{-2} s^{-1} between 1000 h and 1400 h from Week 1 to Week 4 (Figure 4.5). Compared to the control plants, the drought plants had lower A values in Week 2 and Week 3. On 8 and 19 January, the photosynthetic activity of the drought plants was reduced by 60%.

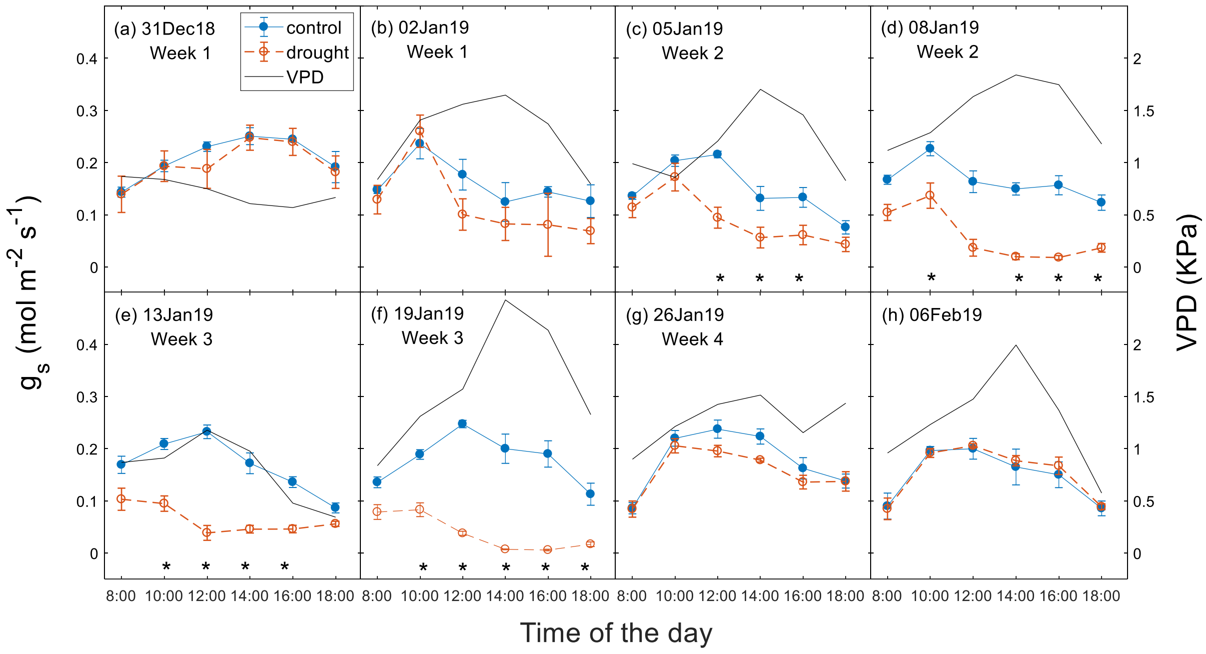

Figure 4.4: Stomatal conductance (g_s) (±1S.E.) (mol m^{-2} s^{-1}) of the control and drought plants and VPD of the ambient air within the glasshouse, measured every two hours from 8:00 to 18:00 (* $P<0.05$).

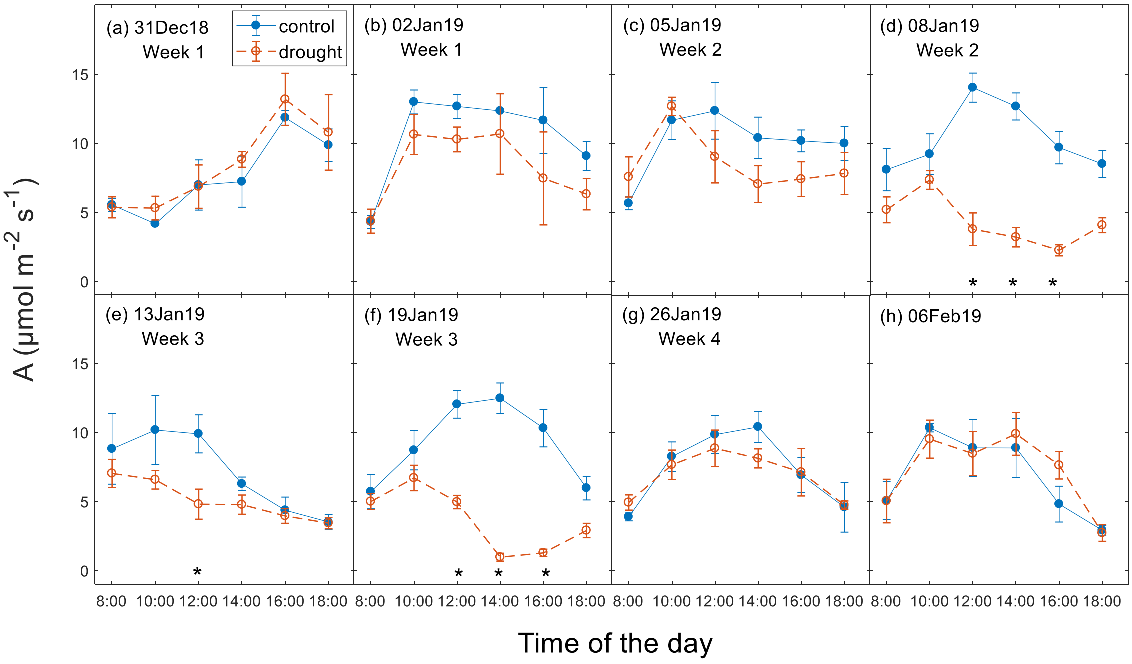

Figure 4.5: Photosynthetic assimilation (*A*) (±1S.E.) (μmol CO$_2$ m^{-2} s^{-1}) of the control and drought plants, measured every two hours from 8:00 to 18:00 (* $P<0.05$).

Non-destructive fruit growth

Growth increment was calculated based on non-destructive fruit growth measurements. Over the observation period, the control fruit grew at a near constant rate, increasing in size by an average 1.4 (± 0.09 [S.E.]) cm^3 d^{-1} over the entire seven weeks of the experiment (Figure 4.6). Compared to the control fruit, the drought fruit had a similar growth rate of 1.4 (± 0.09) cm^3 d^{-1} prior to treatment. However, after two weeks of drought treatment, the fruit growth rate decreased to 0.4 (± 0.06) cm^3 d^{-1}. After re-watering the drought fruit grew faster than control fruit, increasing in size by 2.5 (± 0.26) cm^3 d^{-1} in Week 4. The overall fruit size reduction caused by the drought treatment at harvest on 2 February was approximately 5%, but this difference was not statistically significant (P>0.05).

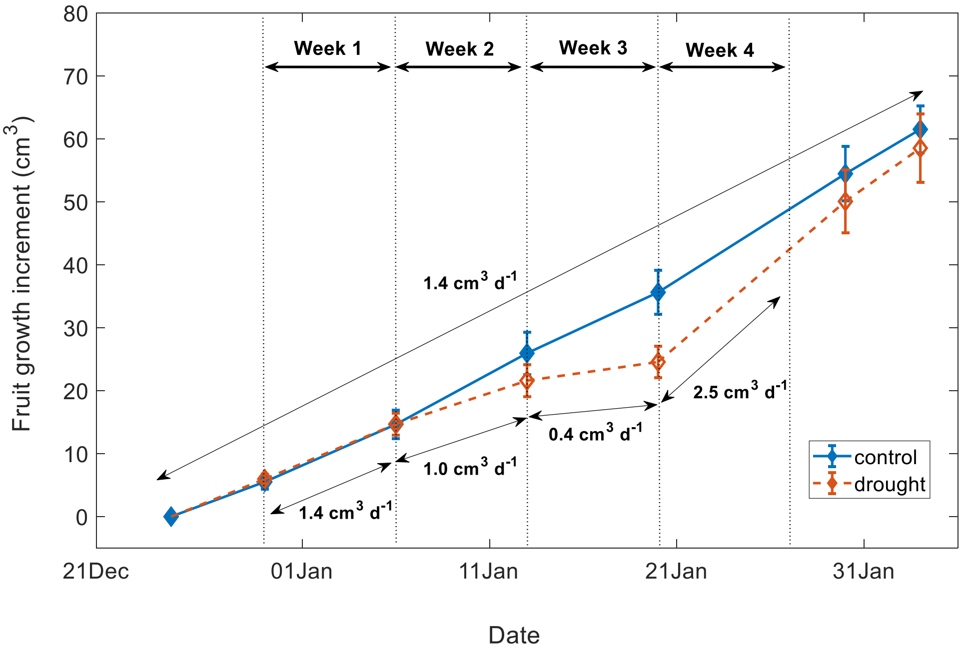

Figure 4.6: Mean fruit growth increment (±1S.E.) (cm^3) estimated from non-destructive fruit growth measurements using callipers. The first date of measurement was 24 December 2018, when mean fruit volume was 29.8 ± 8.4 cm^3, and the final date of measurement was 4 February 2019 (n = 16 control fruit on three plants, and 19 drought fruit of three plants) (P>0.05).

External heat ratio sap flow and LTs

Sap flow through the shoot stems was unidirectional. Flow started in the morning at 0700 h, reached a peak between 1000 and 1400 h, and ceased in the evening by 1900 h (Figure 4.7A-D). Over the observation period, the control had an average daily shoot water use of 72 g d^{-1} with a maximum flow of 10 g h^{-1}. Shoots reduced water use more quickly than the fruit pedicels in response to water stress, exhibiting a decreasing trend of shoot water inflow from Week 1 to Week 3. During the period of severe water stress in Week 3, shoot water flow was reduced by 60 to 70%. In Week 4, shoot water inflow increased steadily after re-watering.

Sap flow through the fruit pedicels was observed each day in both directions (Figure 4.7E-H). There was usually a period of relatively constant sap flow into the fruit prior to dawn, followed by flow reversal and a period of outflow from fruit to the stem in the morning between 0700 h and 1100 h water reaching a maximum rate between mid-afternoon and sunset, before decreasing again to predawn levels. From Week 1 to Week 4, the control fruit had constant daily total flows. The average total inflow to the fruit was 2.72 g d^{-1}, and the outflow was 0.37 g d^{-1}. Transpiration was the most significant loss of water from the fruit, accounting for 1.06 g d^{-1}. Drought caused a change in sap flow to the fruit. There was no clear effect in Week 1. Total daily inflow to the fruit was reduced by 20% and 31%, and outflow increased by 15% and 65% in Weeks 2 and 3, respectively. After re-watering, a large amount of water flowed into the fruit. On the day following re-watering, net water gain by the drought fruit was more than double that of the control fruit (Figure 4.7E-H).

LTs measurements provided a more detailed view of changes in fruit size and growth rate, revealing diurnal fluctuations in the rate and direction of growth (Figure 4.7I-L). Each day, the control fruit increased in size from the evening to the next morning, and decreased in size slightly after the middle of the day. The highest growth rate occurred in the evening, after the peak in sap flow. Overall, the control fruit exhibited a constant net daily growth from Week 1 to Week 4. For the drought fruit, similar diurnal fluctuations were observed and there was no treatment effect on fruit growth in Week 1, but daily growth slowed in Weeks 2 and 3. By week 3 the night time increase in fruit size was starting later and occurred more slowly than the control fruit, and net daily growth was approximately 25% that of control fruit. After re-watering, the growth of drought fruit accelerated rapidly, with positive growth continuing throughout the afternoon, and net growth exceeding that of the control fruit for the entire week following re-watering.

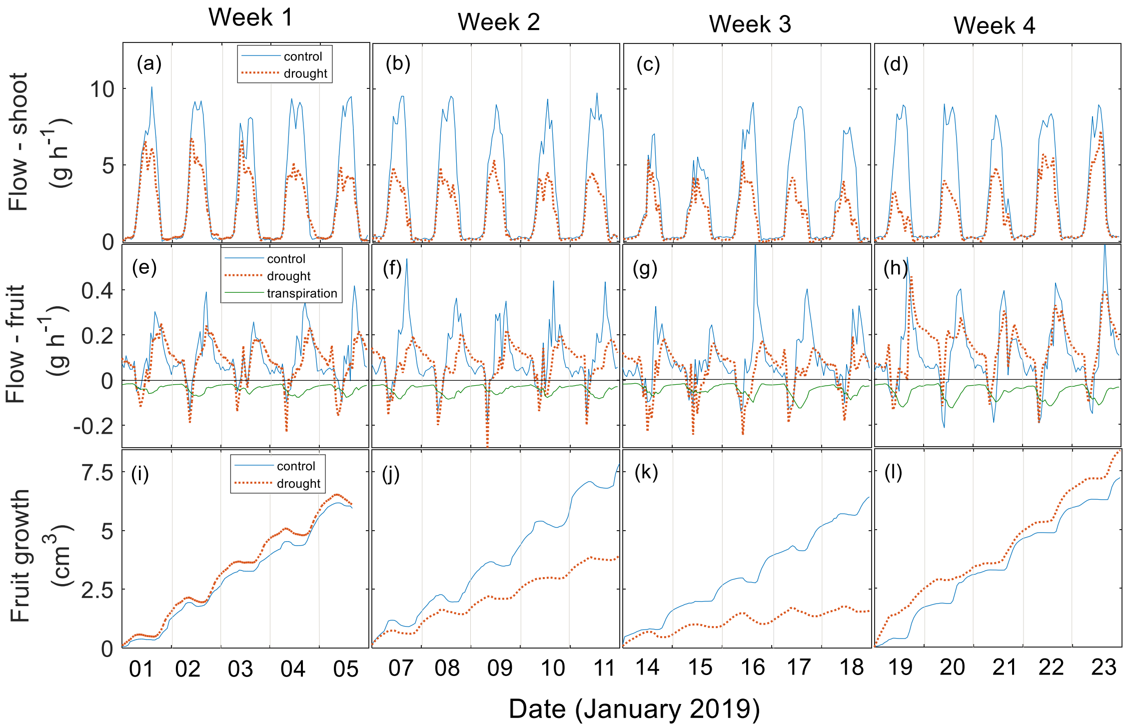

Figure 4.7: The average water flow of three shoots (top) (n=3), the average water flow of three fruit and estimated water loss by fruit transpiration (middle) (n=3), and LT measurement (bottom) (control: n=4, drought n=6) of the control and drought plants in Week 1, Week 2, Week 3, and Week 4. Water flows were obtained from external heat ratio sap flow measurements.

Final fruit size and dry matter content

There was considerable variation in the timing of flower opening among and within trees. Fruit volume at the end of experiment varied between 40 and 160 cm^3 and fruit set from early opening flowers was generally bigger. Whilst there was no difference in average final fruit size between treatments (P>0.05), the fruit from the two treatments differed in the relationship between fruit dry matter content and fruit size (Figure 4.8). There was a significant positive relationship between fruit volume and dry matter content in the control plants (P<0.05; ANCOVA), with dry matter content increasing by 0.0277% for each 1 cm^3 increase in fruit volume. However, there was no relationship between the two variables in the drought plants (P>0.05). Overall, the difference between the regression slopes for the two treatments was statistically significant (P<0.05).

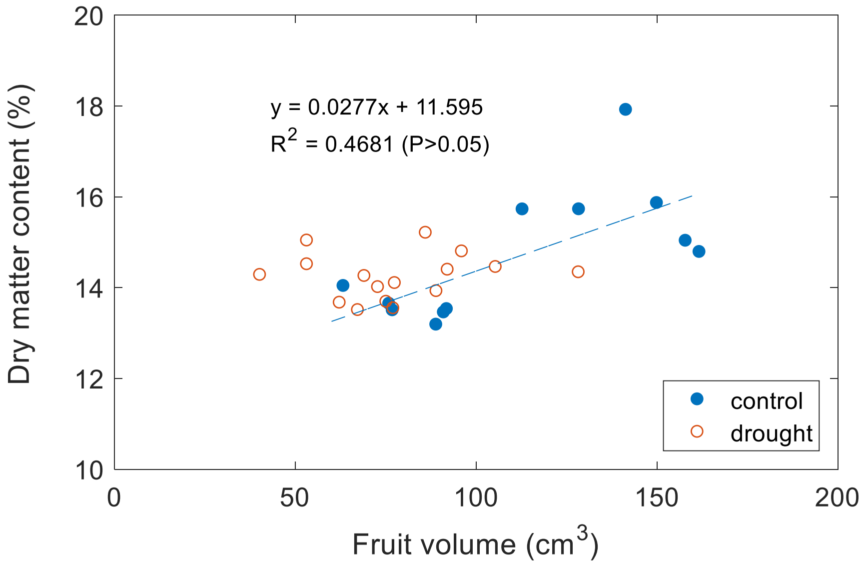

Figure 4.8: Relationship between the fruit volume (cm^3) and dry matter content (%) of individual fruit at the end of the drought experiment. (Control; R^2 = 0.4681, P<0.05.and Drought; R^2 = 0.0136, P>0.05)

The fruit water balance

Figure 9 presents a summary of the fruit water balance and the factors that influence it, comparing fruit on well-watered and water-stressed plants. Firstly, a diurnal pattern of Ψ_{stem} and Ψ_{fruit} between well-watered and water-stressed conditions were described (Figure 4.9A, B). During the day, Ψ_{stem} and Ψ_{fruit} were similar in both conditions because avocado is isohydric, but a difference in Ψ_{stem} and Ψ_{fruit} appeared at night as a result of a decline in soil water potential, therefore, the diurnal fluctuation in Ψ_{stem} and Ψ_{fruit} was reduced under the water-stressed condition. Second, the pattern of net sap flow suggests that outflow from fruit occurs in the morning because Ψ_{fruit} lag behind $\Psi_{stem.}$ A lag in simulated Ψ_{fruit} creates a diurnal pattern of Ψ_{xylem} differences that corresponds with the oscillations in sap flow. The start of the period of flow reversal corresponds with sunrise and start of shoot and fruit transpiration. The lag is longer in drought stressed fruit, creating a longer period and larger volume of outflow (Figure 4.9C, D). It is assumed that the measured sap flow represents the sum of a relatively constant inward phloem flow, and a xylem flow that oscillates between inward and outward flow.

Third, fruit growth rates were predicted as the sum of net sap flow and fruit transpiration has a similar diurnal pattern to fruit growth measured at the widest point of the fruit using LTs, with fruit size decreasing during the day and increasing at night. Daily net growth is lower under water-stressed condition because of a longer period of outflow and slower maximum growth. However, the growth curve estimated from the water balance predicts an earlier decrease and recovery of fruit volume each day compared to that measured towards the distal end of the fruit (Figure 4.9E, F).

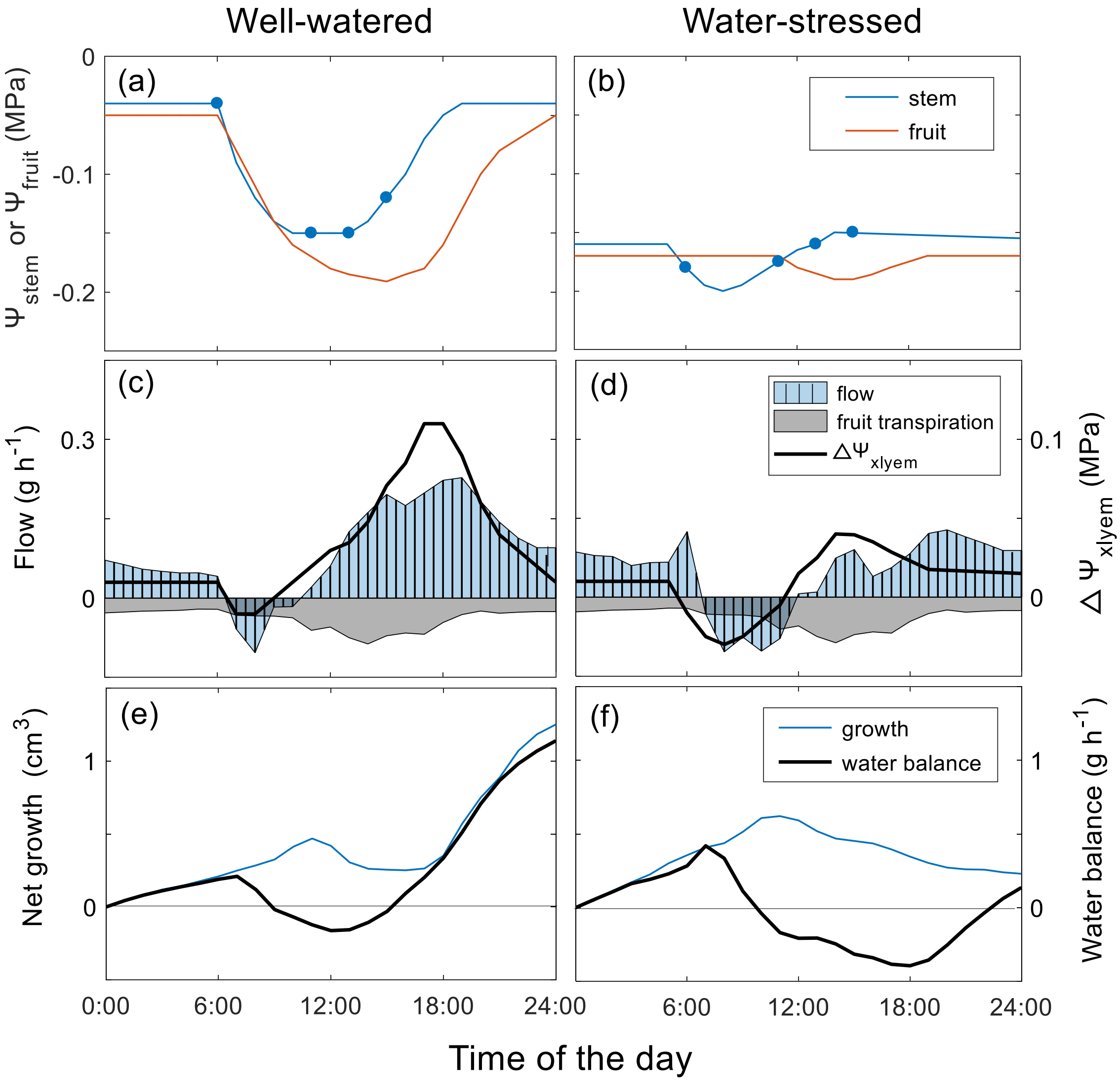

Figure 4.9: Model of the diurnal pattern of Ψ_{stem} and Ψ_{fruit} (a, b), water flow measured by external sap flow gauges, estimated fruit transpiration, the difference in water potential between stem and fruit $\Delta\ \Psi_{xylem} = \Psi_{stem}$-$\Psi_{fruit}$ (c, d), and fruit growth calculated as the sum of sap flow and fruit transpiration (the water balance), and measured using linear transducers (e, f) over a 24h period for well-watered (left) and water-stressed (right) 'Hass' avocado plants. The blue points in (a) and (b) represent actual Ψ_{stem} measurements.

4.4 Discussion

This study quantified sap flow in the small stems and fruit pedicels, and provided evidence that water stress negatively impacts on avocado fruit growth, because the pattern of water flow to the fruit was changed. The results showed fruit water inflow was decreased and outflow was increased when the avocado experienced water stress. This has an immediate effect on fruit growth, but there can be a substantial recovery in fruit size when water stress ends because xylem inflow can increase to higher than normal levels. This suggests that Ψ_{fruit} declines over time, but changes in sap flow to and from the fruit during the stress period are minimized by the avocado plant's isohydric stomatal behaviour. When the stress period ends, xylem flows increase dramatically because Ψ_{stem} rises rapidly compared to Ψ_{fruit}. Drought may also affect phloem flows of water and carbohydrates, but the mechanism may be more indirect, with the primary effect being a reduction in leaf photosynthesis caused by isohydric stomatal behaviour. The sap flow measurements and water balance model were not able to separate phloem flows, but from the changes in fruit dry matter content it is concluded that the drought may cause a decrease in the rate of fruit dry matter accumulation relative to growth in fresh weight.

Plant water status and stomatal regulation

'Hass' avocado is known to be sensitive to water availability (Chartzoulakis *et al.*, 2002; Carr, 2013). In this experiment, the drought plants exhibited isohydric behaviour, showing a clear response to water stress by stomatal closure without changing Ψ_{leaf} and Ψ_{stem} in the xylem, even during simulated severe drought condition in Week 3. At the daily scale, water flow to the shoots increased when stomata were open and decreased with a decline in g_s. At the weekly scale, as the drought condition continued, g_s decreased, especially when VPD was high, and the daily peak in g_s decreased over time. The key effect on fruit growth was a decline in soil water potential that affected Ψ_{xylem} and the gradient at night, when fruit increase in size under normal conditions. Interestingly, the drought affected plants showed quick recovery when water became available in Week 4. The avocado showed an isohydric behaviour during severe drought, and seemingly, stomatal closure and a reduction of photosynthetic activity prevented loss of functionality of the vessels (Cardoso *et al.*, 2020). This isohydric behaviour brought a lower limit on Ψ_{stem} during the day, preventing even larger outflows from the fruit to the stem than would occur if avocado had anisohydric stomatal behaviour.

Stomatal behaviour is not only related to soil water availability, but is also responsive to VPD. Many studies (Medrano *et al.*, 2002; Buckley, 2005; Lawson *et al.*, 2014; McAdam *et al.*, 2016) have suggested that stomata are open when VPD is low, and are closed when VPD increases. In the glasshouse conditions, VPD was generally higher in the afternoon, and this can explain why g_s of the control plants reduced after the middle of the day (Buckley, 2019). Stomatal behaviour is a complex mechanism that may be influenced by some other environmental factors, such as light intensity and temperatures (Tardieu & Simonneau, 1998; Hetherington & Woodward, 2003), and plant physiological factors such as fruit load (Silber *et al.*, 2013; Sade & Moshelion, 2014). Hence, avocado plants might behave differently in field conditions (Chapter 3).

Water relations and fruit growth

LT measurements showed a diurnal pattern of avocado fruit growth, with fruit expanding from the evening to the morning, and shrinking from the middle of the day to the evening. This observation was consistent with the data obtained by Schroeder (1958) on avocado. Similar patterns of fruit growth have been observed in other fruit, including apple (Lang, 1990), grape (Greenspan *et al.*, 1994), kiwifruit (Clearwater *et al.*, 2013), olive (Fernandes *et al.*, 2018), and cherry (Bruggenwirth *et al.*, 2016). In this study, with daily fluctuations, the control fruit grew constantly with a daily growth rate of 1.4 cm^3, when the fruit was 2-3 months old.

Daily fluctuations in fruit volume can be a result of water input (vascular inflows) and output (vascular outflows and fruit transpiration). Using external heat ratio sap flow gauges, we detected water flows through the fruit pedicels in both directions. Under the well-watered condition, water outflow was observed from the fruit to the stem in the morning. Water loss from the fruit to the stem is caused by the demand for water in the leaves for gas exchange and photosynthesis, and water flows in and out of the fruit in the xylem as a result of changes in the water potential gradient between the fruit and the stem (Matthews & Shackel, 2005; Clearwater *et al.*, 2009). At the early fruit developmental stage, morning outflow from the fruit to the parent plant is a common occurrence in fleshy fruit, for example, kiwifruit (Morandi *et al.*, 2010; Clearwater *et al.*, 2013) and mango (Higuchi & Sakuratani, 2006), and this is because Ψ_{leaf} and Ψ_{stem} becomes more negative than Ψ_{fruit} when leaf water demand was high. The results of this study showed that outflow from fruit started when shoot transpiration started, at the time when the leaf stomata began to open to admit CO_2.

In this study, water inflow toward fruit mainly occurred in the afternoon, when g_s decreased and Ψ_{leaf} and Ψ_{stem} increased. The peak of fruit water inflow observed here was at about 1800 h when water flow to the shoot dropped significantly, and then fruit water inflow continued steadily overnight. These results were consistent with the findings on kiwifruit by Clearwater *et al.* (2013). Nocturnal water flow to fruit is also a normal phenomenon that presumably contributes to fruit expansion at night. Regarding the comparison of the fruit water balance with the LTs measurements, these two matched in the overall pattern and daily net growth, but the timing of flow reversal and predicted diurnal fruit size reductions based on the balance between sap flow and fruit transpiration occurred earlier in the day than the changes in volume estimated from fruit diameter. This may be because the fruit has a large elastic volume, and the LTs measurements were separated from the pedicel where the sap flow was measured, suggesting there was a lag between flow in the stem and flow from the distal end of the fruit. This was also consistent with other observations of large internal hydraulic resistances in grape (Tyerman *et al.*, 2004; Choat *et al.*, 2009), and kiwifruit (Mazzeo *et al.*, 2013).

The effects of water stress on fruit growth

The first sign of water stress on the avocado plants was a decline in leaf g_s commencing in Week 1. However, the impact on fruit growth rate of drought fruit wasn't observed until Week 2. Fruit growth is the sum of water flow; water inflow to fruit, and water loss due to outflow via the xylem and/or fruit transpiration from the fruit skin (Clearwater *et al.*, 2012). So, fruit growth ceases when fruit water loss exceeds fruit water inflow.

The results showed that the morning outflow from the fruit to the stem became more significant when the drought plants had lower soil water content. However, the amount of water flow back from the fruit to the stem was relatively low. The maximum value obtained was only 0.7 g d^{-1}, and it was about half of the water loss by fruit transpiration. Therefore, during drought, fruit growth reduction was more likely to occur caused by a decrease in fruit water inflow.

Fruit water inflow was largely reduced due to water deficit. Still, water flow toward the fruit was identified even during the severe water-stressed period. Water flow to fruit can be attributed to the water potential gradient in the xylem. When the drought plants closed stomata in response to water stress, Ψ_{leaf} and Ψ_{stem} did not decrease greatly. However, unlike leaves, the fruit doesn't regulate water status, and continued water loss due to fruit transpiration may lower Ψ_{fruit}, especially under the water-stressed condition (Morandi *et al.*, 2014; Bruggenwirth *et al.*, 2016). The decreased Ψ_{fruit} allows fruit growth to resume in the afternoon or evening when Ψ_{stem} starts to rise, and inward xylem flows can resume.

The majority of water loss was fruit transpiration. In this study, fruit surface conductance was estimated by measuring the weight loss over time of detached avocado fruit, and the transpiration of intact fruit was estimated as the product of fruit surface conductance and VPD. This approach is based on the assumption that fruit transpiration is not changed by detaching the fruit (Lang, 1990; Rogiers *et al.*, 2004), is closely related to VPD (Morandi *et al.*, 2010; Zhang & Keller, 2015; Bruggenwirth *et al.*, 2016), and is not regulated by stomata (Greenspan *et al.*, 1994; Greenspan *et al.*, 1996; Fernandes *et al.*, 2018). Avocado fruit has many small stomata on the fruit surface (Blanke, 1995). Blanke and Whiley (1995) examined the functionality of stomata on avocado fruit, and found that fruit stomatal conductance tends to decrease as the fruit develops, from 0.16 mmol m^{-2} s^{-1} pre-anthesis, to 0.05 mmol m^{-2} s^{-1} at 40 g fruit weight, and to 0.01 mmol m^{-2} s^{-1} at fruit maturity. This experiment started when fruit weight was between 30 and 40 g, therefore, the fruit stomata may still have been slightly functional in the beginning. If so, the estimates of fruit transpiration by intact fruit may have been underestimated. However, fruit surface conductance at this stage of growth is already relatively low compared to leaf conductance, suggesting that water loss is primarily through the cuticle or exocarp by transpiration, and will be primarily a function of VPD.

Xylem and phloem flow

The aim of this experiment was to identify the process of water flow through the avocado plants, and to understand how water flow changes under drought and the effect of water stress on fruit growth. In this study, the external sap flow gauges were used to obtain total sap flow to the shoots stems and fruit pedicels, and the relative contributions of xylem and phloem flow to fruit pedicel sap flow were not estimated. However, water flow to the fruit occurs both through the xylem and phloem, but xylem flow is thought to be the dominant water pathway during early fruit development (Matthews & Shackel, 2005).

Many studies have attempted to estimate the separate contributions of xylem and phloem transport to fleshy fruit development, but these were usually indirect estimates based on calculating xylem transport after eliminating phloem transport by girdling (Lang & Thorpe, 1989; Lang, 1990; Guichard *et al.*, 2005; Morandi *et al.*, 2014; Hanssens *et al.*, 2015). However, the xylem and phloem tissues interact with each other by exchanging water, and water shortage caused by phloem girdling will almost certainly also affect xylem flows (Fishman *et al.*, 2001). Similarly, drought is likely to affect both phloem and xylem flows, and the way the two interact (Sevanto, 2014; Savage *et al.*, 2016). Very few studies have included direct flow measurement to examine the contribution of xylem and phloem flows, either under non-stressed conditions or during drought. With current techniques, investigating the effects of water stress on phloem transport is very difficult.

The dry matter content of fruit should be related to the rate of phloem flow. Unlike other fleshy fruit, avocado fruit continues to grow and accumulate oil content until it is removed from the tree (Lahav & Kalmar, 1977; Lahav *et al.*, 2013). This research presented a positive relationship between the fruit volume and dry matter content for the control fruit, but no correlation for the drought fruit. During drought, the major problem for a species with isohydric stomatal behaviour may be the reduction of carbohydrate production caused by reducing CO_2 uptake as a result of stomatal closure (McDowell *et al.*, 2008). In this study, the drought condition may have reduced phloem transport and therefore the transport of carbohydrates and water to the fruit. This situation has slowed the rise in dry matter content that would normally occur.

This study focused on fruit growth, and vegetative growth was therefore not monitored during the experiment. However, from visual observations, the drought plants had less vegetative growth during the drought period, and the vegetative buds and new leaves were visibly damaged by the end of stress period. Intrigliolo and Castel (2007), Hernandez-Santana *et al.* (2017), and Fernandes *et al.* (2018) have suggested that fruit are a stronger sink for water and carbohydrates than vegetative organs during drought. This may also be true for avocado, but the findings of this study suggests effects of water stress on growth in fruit fresh weight can appear very quickly, and that longer term effects on both fresh and dry weight are likely if the period of stress is prolonged.

4.5 Conclusion

This research has described the dynamics of water flow in avocado plants, including the diurnal pattern of water flow through the shoot stems and fruit pedicels under well-watered and water-stressed conditions, and details how plant water status influences fruit growth. During drought, avocado plants respond quickly to soil water status with stomatal closure, and exhibit very stringent regulation of the whole plant water balance. Fruit growth of the drought plants was reduced during the stress period by a reduction in water inflow to fruit, but recovered after re-watering. Our findings suggest that short-term drought stress did not cause either fruit size reduction or a reduction in fruit dry matter content, but negative impacts might appear if the drought treatment were applied for a longer period. To improve our knowledge of fruit growth responses to stress, an understanding of the mechanisms influencing the dynamics of xylem and phloem transport is essential. In particular, a study of phloem transport may be key for fruit crop production, as dry matter content is translocated through the phloem, and this determines the final fruit quality. Furthermore, this research was conducted when the experimental plants had relatively few fruit. Plant water status may be influenced by fruit load (Chapter 2), therefore, it would also be useful to investigate the plant and fruit water balance in plants carrying a high fruit load.

4.6 Disclosure statement

No potential conflict of interest was reported by the author.

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Chapter 5

Summary and Conclusion

5.1 Overview

Avocado is a valuable fruit with a growing market globally including within New Zealand. To increase avocado crop productivity, water management is essential because avocado is known to be sensitive to soil water deficits (Carr, 2013). However, prior to this thesis, there was a lack of scientific research to support best irrigation practice for the New Zealand avocado industry, which is reflected by the wide variation in irrigation regimes implemented by growers. In fact, about one-third of avocado orchards currently do not use irrigation in this country (Phillip West NZ Avocado Pers. Comm.). Hence, this study aimed to assess avocado tree water use and to identify the effects of water deficits on avocado fruit development.

Water is an important natural resource, but available freshwater for horticulture is decreasing, due to increased demands as a result of population growth (De Pascale *et al.*, 2011). In many countries, the limits of available water have been reached creating a water shortage for crop production (Odegard & van der Voet, 2014). In New Zealand, approximately 58% of freshwater (surface water and groundwater) has been allocated for irrigation (Ministry for the Environment & Stats NZ, 2020). In the future, irrigation for agriculture in New Zealand is likely to be regulated because of water scarcity (WWAP & UN-Water 2018).

Besides, agricultural water pollution is becoming a serious worldwide problem (Jiao *et al.*, 2004; Kiggundu *et al.*, 2012). In orchards, fertilizers are commonly applied to increase crop productivity. However, a combination of fertilizer use and over-irrigation can raise environmental concerns such as nutrient leaching and runoff, especially of, nitrogen (N) and phosphorus (P) (Kiggundu *et al.*, 2012), causing dissolved and particulate nutrients to move from agricultural fields to ground and surface waters, changes in soil characteristics, and decreases in water quality (Bouwer, 2000). Agricultural fertilizers are highly susceptible to leaching or runoff if misapplied or not targeted to the root system, because they are soluble in water.

Due to the issues of water scarcity and water pollution, all horticultural industries in New Zealand have been under pressure in recent years to increase productivity while also reducing environmental impacts (Hart *et al.*, 2004). To increase crop production and to conserve and protect this important natural resource, irrigation management and determination of crop water consumption require more attention. In particular, avocado has unique characteristics, such as high levels of flower and immature fruit abscission (Garner & Lovatt, 2008), alternate bearing (Lovatt, 2010), high oil content in fruit (Liu *et al.*, 1999), and susceptibility to root diseases associated with water logging (Sterne *et al.*, 1977). These features create year-to-year variation in both crop production and water use. Therefore, careful water management is required for avocado.

There were three objectives undertaken in this thesis:

Objective 1:	To quantify 'Hass' avocado tree water use in the three avocado growing regions of New Zealand; the Bay of Plenty, Whangarei, and the Far North, and to provide a suggested irrigation guideline, based on reliable estimates of crop coefficients (K_c) relative to local weather conditions, season and fruit load.
Objective 2:	To assess the irrigation requirements of 'Hass' avocado in the Bay of Plenty, New Zealand, and to investigate the effect of water deficits on avocado fruit growth and fruit yield, by carrying out field trials with irrigated and non-irrigated trees.
Objective 3:	To identify the patterns of water flow through the vascular tissues of shoot stems and fruit pedicels, assessing changes in water flow in response to water stress, and quantifying the effect of water stress on fruit growth.

5.2 Summaries and findings

The major results and findings of three objectives are presented below.

5.2.1 'Hass' avocado tree water use in New Zealand

Objective 1 focused on quantifying 'Hass' avocado tree water use in orchards, relative to the local weather conditions of the three main avocado growing regions (Chapter 2). Globally, the $K_c \cdot ET_o$ approach is widely used to calculate crop water use (Allen *et al.*, 1998; Allen *et al.*, 2006). However, prior to this thesis, there was no standard irrigation guideline for avocado under New Zealand conditions, because there was no publication that provided K_c values for avocado in this country. Obtaining the K_c values is important for proper irrigation management (Allen *et al.*, 1998; Allen *et al.*, 2006). Therefore, Objective 1 aimed to provide reliable measurements of crop evapotranspiration (ET_c) and K_c. Because water use depends on many factors when the crop is a large fruiting tree (Allen & Pereira, 2009), Objective 1 examined avocado tree water use in relation to the local abiotic and biotic environment (weather conditions, soil type and ground cover), and plant physiological state (tree size, total leaf area, canopy area, and fruit load).

The experiment was conducted in the three main avocado growing regions (the Bay of Plenty, Whangarei, and the Far North) over three years, as irregular bearing is common for avocado under New Zealand conditions (Gould *et al.*, 2019). The results of this study showed that avocado tree water use obtained from sap flow technique (Green *et al.*, 2003) was similar at all three sites, with the highest tree water use in January and the lowest in June and July. However, avocado tree water use was influenced by fruit load, therefore, the K_c values need to be adjusted from light to heavy fruit load. Influences of tree water use by fruit load is a common occurrence in fleshy fruit, for example, plum (Intrigliolo & Castel, 2007) and pear (Marsal *et al.*, 2008), but the variation is extremely large for avocado.

This study provided the first quantitative measurements of avocado tree water use under New Zealand conditions. It provided a sound basis for management of avocado irrigation, including a method for scaling of water requirements based on tree size and spacing or shaded area, and demonstrated that adjustments should be made for fruit load. These three study sites had similar K_c because these orchards had mature trees and had high percentages of shaded area (over 77%), indicating K_c had reached its maximum.

However, consideration of fruit load is required, as tree water use increases from light to heavy cropping trees.

There was a good correlation between sap flow measurement and soil moisture monitoring. Sap flow measurement was more accurate to measure water use at a plant level, but soil water monitoring is essential to manage water application at an orchard scale. The three study sites also had similar K_c values because they had similar weather conditions. Weather conditions greatly affect avocado fruit growth, hence, K_c values may be slightly different between countries. Each country or growing region may need original K_c values for avocado.

To obtain reliable ET_c and K_c values, the experimental trees needed to be well-watered, allowing the assumption that these trees did not have water stress. Based on meteorological observations over three years from 2017 to 2019, rainfall in summer was variable in all three regions. The avocado trees required more than 2 mm d^{-1} of water during early fruit development, but each summer there was a non-rainfall period that decreased volumetric soil water content below 0.35 m^3 m^{-3} in the Bay of Plenty, 0.40 m^3 m^{-3} in Whangarei, and 0.25 m^3 m^{-3} in the Far North. Thus, irrigation was required to fill the deficit during the drier periods in all three regions. What happens if avocado trees are not irrigated? The aim of Objective 2 was to answer this question.

5.2.2 The effect of water deficits on 'Hass' avocado fruit development

Reduction in fruit yield caused by water stress has been observed in 'Hass' avocado in some countries, for example, Israel (Silber *et al.*, 2012; Silber *et al.*, 2013b) and Chile (Holzapfel *et al.*, 2017). However, the literature regarding the effect of water deficits on avocado fruit growth is scarce. Prior to this thesis, there was no information about how soil water deficits impact on avocado fruit growth under New Zealand conditions. Therefore, Objective 2 investigated irrigation requirements for 'Hass' avocado trees in an orchard in the Bay of Plenty, New Zealand by comparing between irrigated and non-irrigated treatments. This experiment was conducted over the three summers (2016-17, 2017-18 and 2018-19) (Chapter 3).

Northern New Zealand features a maritime climate, which is characterized by mild air temperatures with intermittent summer rainfall (NIWA, 2020). However, rainfall was variable at the study site and there were dry periods each summer that decreased soil water content significantly, creating a clear difference in soil water content between the two treatments. When there was no rainfall for more than two to three weeks, volumetric soil water content of the non-irrigated treatment decreased below 0.35 $m^3 m^{-3}$. The effect of soil water deficit on fruit growth was difficult to detect during fruit development, because fruit load strongly influenced avocado fruit weight and dry matter content, increasing variation between trees and the two treatments. However, accounting for fruit load revealed that the non-irrigated treatment decreased avocado fruit weight by 26.4 g ($P<0.05$) at harvest, independently of the effects of fruit load on fruit weight. In addition, during the prolonged dry period in the summer of 2018-19, non-irrigated avocado trees exhibited isohydric stomatal behaviour, reducing stomatal conductance (g_s) by 20%, maintaining a constant stem water potential (Ψ_{stem}) above -0.25 MPa, but decreasing photosynthesis and potential carbohydrate production. Carbohydrate supply is important for fruit growth. Higher photosynthetic rate has been observed during fruit growth in some fruiting species, such as apple (Wibbe & Blanke, 1995) and citrus (Iglesias *et al.*, 2002), and avocado (Silber *et al.*, 2013a). Isohydric stomatal behaviour limits carbohydrate accumulation in fruit, and this effect may be critical for heavy crop trees.

The findings of Objective 2 implied that avocado fruit weight will be reduced if avocado trees are not irrigated in the Bay of Plenty, particularly if there is a reduction in rainfall over two to three weeks during early avocado fruit development. However, the question remains as to why avocado fruit size is reduced by water deficits. Thus, Objective 3 investigated water flow through the vascular tissues and observed changes in water inflow and outflow through the shoot stems and fruit pedicels.

5.2.3 The water balance of the ‘Hass’ avocado plant

There has been very limited research investigating actual water flow through the vascular tissues of fruiting shoots, such as for example, in mango (Higuchi & Sakuratani, 2005, 2006), and kiwifruit (Clearwater *et al.*, 2013). These studies quantified water flow through the fruit pedicels using external sap flow gauge, although actual vascular flows are unknown in most fruit, including avocado. Thus, Objective 3 of this thesis examined the water balance of ‘Hass’ avocado potted plants and fruit during early fruit development by comparing between well-watered plants (control) and water-stressed plants (drought). (Chapter 4). This was the first study that quantified water flow through the small shoot stems and fruit pedicels of ‘Hass’ avocado plants, and demonstrated changes in vascular flows caused by water stress and its impacts on avocado fruit growth.

Water flow in the small shoot stems (approximately 7 mm in diameter) and fruit pedicels (approximately 6 mm in diameter) was measuring using external sap flow gauges, and fruit growth was monitored by linear transducer measurements. The results showed that water flows in one direction through shoot stems from the parent stem to leaves, while flow occurred in both directions through the fruit pedicels. Not only did water move into the fruit during periods of fruit growth, but outflow was observed in the morning when leaf stomata started to open. The results also demonstrated a clear difference in water flow through the fruit pedicels between well-watered and water-stressed conditions.

Objective 3 provided meaningful information that helps in understanding how water flows changes in response to soil water deficits. Under well-watered conditions, avocado fruit increased in size by 1.4 (± 0.09 [S.E.]) cm^3 d^{-1}. However, the fruit growth rate was 0.4 (± 0.06) cm^3 d^{-1} during the severe water-stressed conditions. This reduction in growth rate was because water inflow to fruit decreased by 31% and the outflow from fruit increased by 65%. However, there was a substantial recovery in fruit size when water stress ended because xylem inflow increased to higher than normal levels. This suggests that Ψ_{fruit} declines over time, but changes in sap flow to and from the fruit during simulated drought are minimized by the isohydric stomatal behaviour. At the end of this experiment, there was no significant difference in fruit size between the two treatments, because the drought treatment was relatively short. In reality, dry periods can be prolonged in the field, and fruit size reduction is more likely to appear in response to long-term water-stressed conditions (Chapter 3). The fact that fruit size can recover to some degree after water stress may also have contributed to the difficulty of detecting a stress effect on fruit growth in the orchard against a background of tree to tree variation in fruit load and fruit size (Chapter 3). There are some studies that investigate how fruit grow, for example, in peach (Constantinescu *et al.*, 2020) and grape (Zhu *et al.*, 2019), but the growth of freshly fruit is complex and is still poorly understood. The avocado fruit water balance provided in this thesis will contribute a better understanding of the physiological mechanism of avocado plants.

5.3 Overall conclusion

This thesis provided valuable insights about water use of ‘Hass’ avocado plants and the effects of water stress on avocado fruit development. The findings are directly relevant to the need to properly manage water resources in the horticultural industry. Therefore, the findings of the thesis are important economically and ecologically.

Due to climate change that may lead to limitation of water resources and increasing air temperatures, irrigation management has become a significant challenge for growers and their crop production. In particular, avocado has unique characteristics that create year-to-year and tree-to-tree variation. Therefore, maintaining consistent avocado crop production is a difficult task (Carmen *et al.*, 2018). Even though many features (e.g. weather conditions, tree health conditions, and nutrient status) influence avocado productivity, soil water availability is one of the key factors for successful crop management. However, it is difficult to manage water application without good knowledge.

In this thesis, estimates of avocado tree water use have been provided along with the reasons why irrigation is important for avocado under New Zealand conditions. To the author’s knowledge, this thesis is the first work that provides this information from a broad perspective, from quantifying water use of mature avocado trees in the orchard to measuring water flow through the vascular tissues of small stems and fruit pedicels on potted plants. It is an important first step towards improving irrigation management within avocado orchards in this country. Local weather conditions and soil type were important, but fruit load was also a big driver of avocado tree water use. In particular, avocado tree water use in the early stages of developmental was most crucial. If avocado trees experience water stress during such an important period, avocado fruit growth slows down because of loss of water from the fruit skin and outflow through the xylem of the pedicel. Therefore, I would recommend that avocado growers monitor soil water content in their orchards, and apply irrigation from November to March, at rates based on the crop coefficients provided in this thesis, if there is not enough rainfall.

5.4 Recommendations for further work

There are further questions in terms of ‘Hass’ avocado tree water use and effects of water deficit on fruit growth.

First, Objective 1 provided the ET_c and K_c values for 'Hass' avocado under New Zealand conditions. However, further assessment may be required if irrigation water demand for avocado increases in response to climate change. Also, plant density is a factor that strongly influences irrigation management. In recent years, high-density avocado orchards have been developed in the Far North, with a plant spacing of 3 m × 5 m. High-density avocado orchards are known to improve irrigation efficiency due to root growth restriction (Winer, 2007). Plant physiological conditions in a high-density orchard may be different from low-density orchards, therefore, these types of orchards may require a different irrigation strategy. Further research is also required to strengthen our estimates of how orchard water requirements vary with tree size and spacing.

Second, Objective 2 provided evidence about how water deficits impact avocado fruit development in New Zealand if avocado trees are not irrigated. However, the effect of varying the timing and method of water application was not assessed. Water, nutrients and light are the three major resources for plant growth and crop production, and of these, water is the most limited in many areas of the world. While water scarcity is becoming a serious problem in the drier regions in New Zealand, detailed research will be required to conserve water resources and allow sustainable water use at avocado orchards. Deficit irrigation (application of water below full crop water requirement) has been investigated on avocado in some countries, for example, Chile (Gardiazabal *et al.*, 2003) and Western Australia (Neuhaus *et al.*, 2007), but the study of deficit irrigation has been limited. Further work, therefore, should be conducted to examine whether deficit irrigation (e.g., 75% or 50% to ET_c) is acceptable for avocado in New Zealand, including under what conditions and when during the cropping cycle. Deficit irrigation could be used during the off cropping year.

Third, Objective 3 was conducted on potted avocado plants to identify the effect of soil water restriction on water flows in the vascular tissues of the shoot stems and fruit pedicels. The results showed clear diurnal patterns of water flow under well-watered and water-stressed conditions, and it is essential to understand why water stress affects avocado fruit size. However, water flow identified in this experiment was the total flow in the xylem and the phloem. For better understanding, xylem and phloem transport should be quantified separately. Phloem flow might be quite a large contributor to total water supply to the avocado fruit, because of the high carbohydrate demand of such an energy dense fruit. It may be identified by applying girdling and eliminating the phloem transport (Guichard *et al.*, 2005; Morandi *et al.*, 2014; Savage *et al.*, 2016), or other methods such as isotope based tracer methods (Cernusak *et al.*, 2002). In addition, like other fruit such as tomato (Liu *et al.*, 2007), kiwifruit (Hall *et al.*, 2013), and grape (Zhu *et al.*, 2019), a mechanistic fruit growth model could be developed for avocado based on the water balance calculated from water flow through the xylem and phloem. Development of such a model will contribute to understand how water and carbohydrates accumulate in the avocado fruit.

Finally, further work recommended here will fine tune how the main findings presented in this thesis can be used by avocado growers. This thesis revealed that water deficit influences fruit size and weight, hence, knowledge of avocado tree water use is directly linked to economic benefit. However, there is generally a gap between the scientific and grower perspectives. The author hopes that the insights presented in this thesis will help improve the links that pass knowledge between avocado growers and researchers, and will contribute to more sustainable water management in horticulture.

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Appendix A

Site information

Study site	Orchard owner	Address
Bay of Plenty	Maria and Andrew Watchorn	62 Prole Road, Omokoroa, Bay of Plenty
Whangarei	John Wiessing	326 Whatitiri Rd, Whangarei
Far North	Jason and Jackie McLarnon	97B Burnage Rd, Pukenui

(a) Bay of Plenty

Figure 6.1: The avocado orchards in the Bay of Plenty. The yellow circles present the experimental trees for Objective 1, and the red square presents the experiment for Objective 2 (Adapted from Google Maps, 2020).

(b) Whangarei

Figure 6.2: The avocado orchards in Whangarei. The yellow circles present the experimental trees for Objective 1 (Adapted from Google Maps, 2020).

(c) Far North

Figure 6.3: The avocado orchards in the Far North. The yellow circles present the experimental trees for Objective 1 (Adapted from Google Maps, 2020).

Reference: Google Maps. (2020). [Website]. https://www.google.co.nz/maps/.

Appendix B

Soil moisture probe calibration

Soil moisture probe (CS615, CS616, CS650, were calibrated Campbell Scientific Inc., Logan, UT) were calibrated for Chapter 2 (Objective 1: the Bay of Plenty, Whangarei, the Far North) and Chapter 3 (Objective 2: the Bay of Plenty).

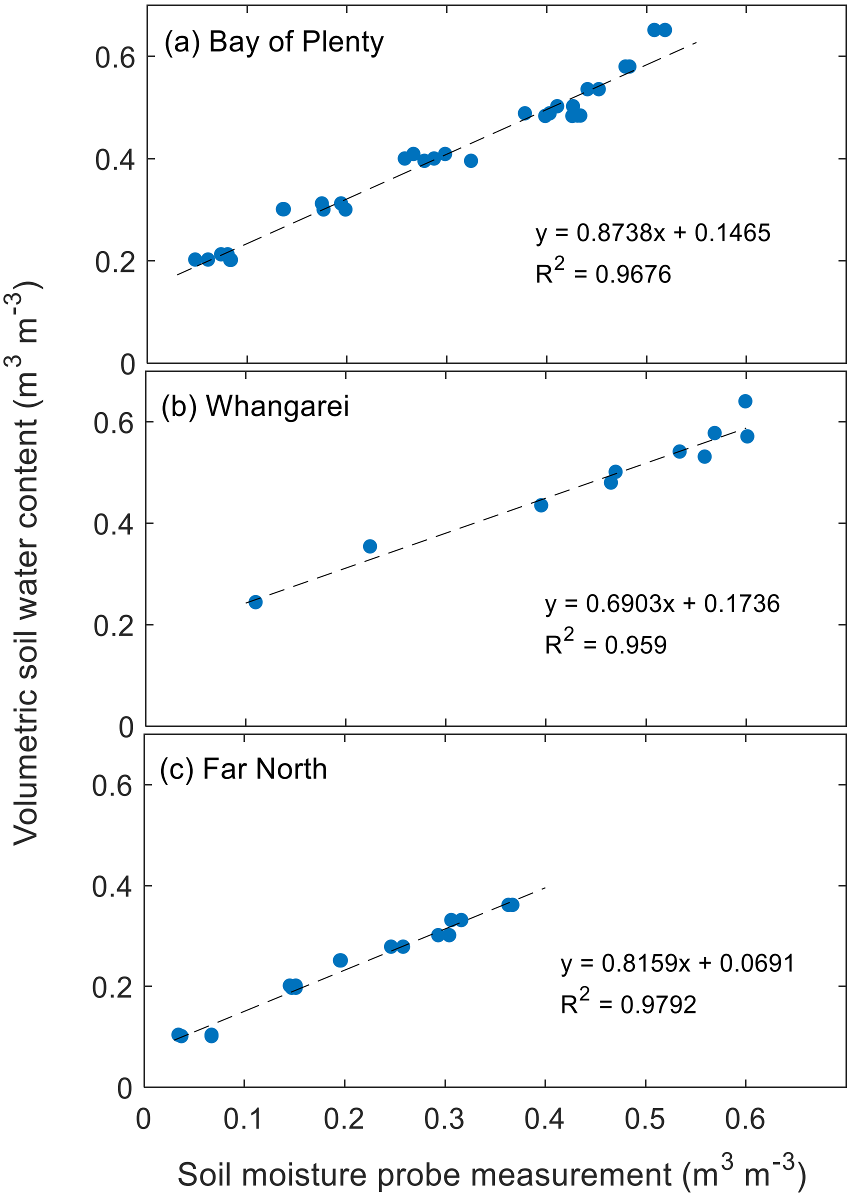

Figure 6.4: Relationship between soil moisture probe probes (CS615, CS616, and CS650, Campbell Scientific Inc., Logan, UT) measurement and actual volumetric soil water content (m3 m-3) for allophanic soil in the Bay of Plenty (a), clay soil in Whangarei (b), and sandy soil in the Far North (c).

Appendix C

Non-destructive perdition of fruit size, volume and surface area

From the $L_1D_1D_2$ measurements, fruit weight was calculated for Chapter 3 (Objective 2), and fruit volume and fruit surface area was estimated for Chapter 4 (Objective 3).

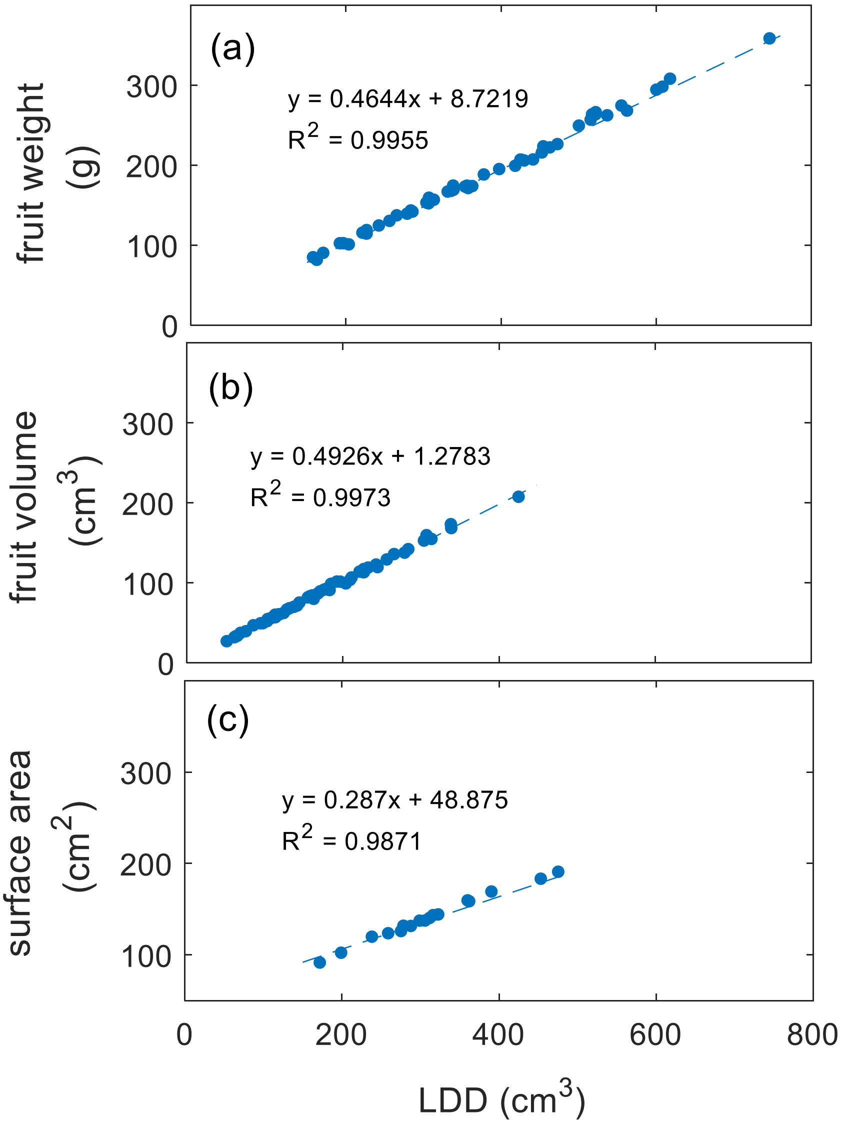

Figure 6.5: Fruit calibration from $L_1D_1D_2$ measurements to fruit weight (g) (a), to fruit volume (cm^3) (b), and fruit surface area (cm^2) (c).