Water requirements for ‘Hass’ avocado flowering and fruit development in New Zealand

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Abstract

The aim of this work was to determine water requirements for *Persea americana* 'Hass' avocado trees (mature, ca. 9 years and young, ca. 3 years) from November 2014 to October 2015 in Bay of Plenty, New Zealand. In addition, the effect of water deficit on flowering and early fruit development was investigated in young avocado (ca. 3 years) trees.

Reference evapotranspiration (ET_o) was calculated by the FAO Penman-Monteith equation based on weather information at the orchard. Crop evapotranspiration (ET_c) of avocado was measured by sap flow measurements using the compensation heat pulse method (CHPM), and daily crop evapotranspiration was also estimated from a soil water balance (E_{wb}). Avocado tree water requirements were calculated as crop coefficients (K_c - the ET_o/ET_c ratio).

- Monthly cumulative ET_o showed a gradual reduction from 145.9 mm in January to 27.7 mm in June. Monthly cumulative ET_c for both the mature and young plants was highest at 75 and 41.8 mm in January, and the lowest at 22.4 and 13.53 mm in July, respectively. The results showed a close correlation between ET_c and ET_o.
- E_{wb} was higher than ET_c by 22 % for the mature and 55 % for the young plants, probably caused by drainage which was not measured.

- In summer, monthly average K_c varied between 0.45-0.60 for the mature and 0.25-0.30 for the young plants. In winter, K_c increased to 0.9-1.0 for the mature and 0.45-0.55 for the young plants.

To investigate the effect of water deficit on flowering and fruit development, rainout shelters were set up under the young trees from mid-October 2014. During this treatment, the control plants were well irrigated and fertilized, while the drought plants received no irrigation, precipitation, or fertilizers. The rainout shelters were removed in early-May 2015, and monitoring was continued until late-October.

- During the rainout treatment, at a depth of 0-30 cm, soil water contents of the drought treated plants dropped to 0.13 m^3/m^3, while that of the control remained above 0.2 m^3/m^3. However, from March, soil water content of the drought plants was stable at 0.20 m^3/m^3 at a depth of 31-60 cm and 0.25 m^3/m^3 at a depth of 61-90 cm. Spatial soil moisture demonstrated the soil was drier close to the drought treated trees, but wetter near the edge of the rainout shelters. These measurements suggest the drought plants were able to obtain some water from the deeper soil or outside the shelters.
- Predawn leaf water potential (PLWP) of the drought plants was lower by 0.06-0.22 MPa than that of the control plants during the flowering season. However, in December, there was no significant difference in stomatal conductance (g_s) between the two treatments. The drought leaf water potential (LWP) and stem water potential (SWP) were more negative than the control LWP and SWP. In January and February, the drought LWP and SWP dropped to -0.43 MPa and -0.30 MPa, whereas the control LWP and

SWP were around -0.25 MPa and -0.23 MPa, respectively, assuming the rainout shelters caused moderate water stress in the drought-treated plants.

- Throughout the flowering season, in total, 1382 open female flowers on the control plants and 1515 flowers on the drought plants were marked and their fates monitored. About 21 % of control flowers and 23 % of the drought flowers remained on the tree 16 days after anthesis. 100 days after anthesis, only 4 control and 1 drought fruit from the monitored flowers were retained on the plants.
- The two treatments had significant fruit drop in summer, about 70 % of marked fruit dropped in January and February, and a second peak of fruit abscission occurred in winter, caused by frost. At harvest, the retention rate of fruit for the control and drought treatments were 15 % and 5 %, respectively. Moreover, the drought plants had smaller fruit size than fruit of the control plants by 21 % at harvest. The differences in fruit abscission and fruit size were probably caused by the combined effects of water deficit, nutrient deficiency, and crop load.
- The control had higher average yield at 36.4 ± 1.1 kg per plant than the drought plants at 27.8 ± 1.0 kg per plant. The dry matter content of the control fruit was 30.4 ± 0.3 %, 7% higher than that of the drought fruit.

The results can be used to develop irrigation recommendations, and show that under the conditions described here the trees were difficult to drought stress in spring and flowering did not appear to be very sensitive to drought stress. However, early fruit growth was very sensitive to water deficits, resulting in a large reduction in fruit size.

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Chapter 1: Introduction

1.1 Introduction

Avocado (*Persea americana* Mill.) is a perennial evergreen plant indigenous to the humid subtropical/tropical areas in central and northern South America. Valued as a fruit crop, today it is distributed throughout many countries from the tropics to Mediterranean climates (Bower & Cutting 1988; Wolstenholme & Whiley 1999; Carr 2013; Lahav et al. 2013). Its value as a fruit crop is demonstrated by the fact that world avocado production has continued to increase from 2.7 million tons in 2000 to 3.6 million tons in 2009 (Wolstenholme 2013b).

Avocado evolved under wet-summer conditions, so it is highly vulnerable to water deficit (Carr 2013; Lahav et al. 2013). It is believed that inadequate water supply in spring and summer can reduce fruit set, fruit retention and development (Lahav et al. 2013). Thus irrigation is widely used on avocado orchards in some regions, especially areas which have a dry-summer climate, such as California, Chile, Israel and Australia (Carr 2013).

Since water availability is one of the key factors controlling fruit quality and productivity of avocado, understanding plant-water relations is an important task which will allow the development of an irrigation strategy to optimise productivity. Water stress may negatively impact on fruit size due to water movement back to leaves from fruit (Schroeder & Wieland 1956). Fruit size is an important parameter for avocado production and fruit size reduction has a negative economic impact on the avocado industry (Kohne & Schutte 1991; Whiley et al. 1996a,b). Furthermore, carbohydrate produced from photosynthesis may be depleted because of stomatal closure in response to water deficit. Reduction of carbohydrates in a plant can result in a poor nutrient status in fruit (Lovatt 1987). In addition, water stress may increase flower (Lahav & Kalmar 1983) and fruit (Silber et al. 2012; 2013a.b) abscission rates, resulting in poor fruit yield (Bower & Cutting 1988; Lovatt 1990; Whiley & Wolstenholme 1990).

Excess water can also produce adverse effects in avocado plants. First, over-irrigation increases the risk of root rot caused by the pathogen, *Phytophthora cinnamomi* (Sterne et al 1978; Bower 1979; Whiley et al. 1986; Coffey 1987). Avocado is vulnerable to *P. cinnamomi* infection because the pathogen is not native to America, so avocado and the pathogen did not evolve together during their evolutionary history (Wolstenholme 1987). Second, excessive irrigation reduces soil aeration, and the condition of low soil oxygen in the upper soil layers significantly reduces nutrient uptake, resulting in lower concentrations of nitrogen (N), phosphorus (P), calcium (Ca), potassium (K), magnesium (Mg) and boron (B) in leaves, stems and roots of avocado plants (Labanauskas et al. 1978; Levinson & Adato 1991).

Efficient water management requires appropriate irrigation scheduling. There has been some research investigating the water requirements of avocado. For example, Lahav & Kalmar (1977a,b) studied irrigation management on avocado for six years in Israel that demonstrated water deficit trees had lower fruit yields while heavily irrigated trees had higher vegetative growth rates. They concluded that moderate irrigation is optimal as these plants had less trunk and height growth than heavy irrigated trees without any reductions in fruit yield or quality. Michelakis et al. (1993) also carried out irrigation research on avocado over six years in Greece, and their results were consistent with the finding of Lahav & Kalmar (1977a,b). More recently, Kiggundu et al. (2012) managed the amount of irrigation relative to reference evapotranspiration (ET_o) and soil water measurements over a four year period in Florida US. They exhibited an improvement of water-use efficiency by 93-87 % without any adverse effects on fruit yield, and demonstrated a significant reduction in nutrient leaching.

However the irrigation requirements for avocado orchards in New Zealand are poorly understood and the use of irrigation across the industry is inconsistent as a consequence. Inadequate irrigation may lead to irregular production linked to variable fruit set. Also, water is a limiting resource and excess irrigation can cause nutrient leaching. Thus, there is need for research investigating how much water avocado requires, and how water stress affects the process of flowering, fruit set and fruit development under New Zealand conditions.

This thesis focused on water use of avocado and the possible impact of water stress on flowering, fruit set and fruit growth.

1.2 Avocado reproduction

Flower and immature fruitlet abscission is a common occurrence on avocado. Despite avocado producing thousands of flowers, the final proportion of fruit set is very low (about 0.001 to 0.23 %) due to high rates of flower and immature fruit abscission (Inoue & Takahashi 1990; Garner & Lovatt 2008; Salazar-Garcia et al. 2013).

Avocado is a botanically unique type of plant. The avocado flower has an unusual behaviour called synchronous protogynous dichogamy – first opening as functionally female with a receptive stigma, then closing and opening again on the following day as a male with a non-receptive stigma and dehisced anthers (Bender 2012; Carr 2013; Salazar-Garcia et al. 2013) (Figure 1.1). This character is thought to be a mechanism that promotes outcrossing between two flowering genotypes; Type A (e.g. the cultivar 'Hass') and Type B (e.g. the cultivar 'Fuerte'). The two flowering types have different timings of flower opening in male and female phases. In the morning, when Type A cultivar's flowers open in a female phase, Type B cultivar's flowers open in a male phase. In the afternoon of the following day, when Type A cultivar's flowers reopen in a male phase, Type B cultivar's flowers open in a female phase (Salazar-Garcia et al. 2013).

Understanding avocado floral behaviour is important, because molecular studies of seeds have revealed that most final mature fruit are developed from outcrossing flowers (Degani et al. 1989; Garner et al. 2008). It is also known that low temperatures can disturb the normal flowering cycle, and temporal overlapping between female and male flowers of the same type could increase self-pollination rates and/or reduce cross-pollination rates (Sedgley & Grant 1983).

Figure 1. 1 Hass avocado flowers in a female phase (left) and a male phase (right). Yellow bars = 2 mm.

Another characteristic of the reproductive phenology of Hass avocado is alternate bearing – a seasonal cycle of ‘on (heavy)’ and ‘off (light)’ crops (Garner & Lovatt 2008; Salazar-Garcia et al. 2013: Escobedo-Solorzano & Lovatt 2014). A typical cycle of on and off crop is biennial, as a heavy crop year is followed by a light crop year, and vice versa. An alternate cycle can be triggered by an irregular bearing event driven by extreme climatic events such as low or high temperatures or drought condition which can accelerate flower/fruit abscission, causing an ‘off’ crop in the current year, and ‘on’ crop in the next year (Jonkers 1979). Also, a harvest delay can generate an off cycle in the following year, as a result of carbohydrate reduction in woody tissues caused by continuous growth of late maturing fruit (Whiley et al. 1996a,b). Alternate and irregular bearing are significant because they create fluctuations in annual production, with negative impacts on the avocado industry (Mickelbart et al. 2012a).

1.3 Avocado water use

Avocado tree water use (ET_c) largely depends on where they grow. ET_c of mature avocado in summer was reported to be 6 mm day^{-1} in the tropics, 4 mm day $^{-1}$ in California, (Lahav et al. 2013), and 3-5 mm day $^{-1}$ in other Mediterranean climates (Carr 2013). The crop coefficient (K_c) is needed to determine ET_c from ET_o, with K_c defined as the ratio of ET_c to ET_o. K_c depends on the crop, and each crop has a different K_c value depending on place, crop phenology, and season (Kuo et al. 2006). K_c of avocado in summer is generally between 0.4 and 0.6, but it is also location specific (Carr 2013). For example, in summer, standard K_c in California is 0.50-0.55, whereas K_c in Israel is 0.60-0.65 (Lahav et al. 2013). Once K_c is obtained, ET_c for specific crops can be accurately estimated with climatic information, and the water balance in a crop orchard can be evaluated daily, monthly or seasonally (Abdelhadi et al. 2000; Tyagi et al. 2000). However, despite the increasing importance of good irrigation management in many regions around the world, there have been few attempts to measure actual water use of avocado.

In New Zealand, avocado is mainly grown in warmer climate regions, such as Northland, the Bay of Plenty and Gisborne (Thorp et al. 1997). Irrigation of avocado is thought to be needed in these regions in spring and summer. However, there is lack of information of avocado water use, because climates and general growing conditions in New Zealand are different from other avocado growing regions around the world.

1.4 Site information

The research described in this thesis was conducted at an avocado orchard at 694 Katikati North Road, Katikati, Bay of Plenty, New Zealand (37°30’2.8”S, 175°55’33.1”E) (Figure 1.2). Katikati is located in a sub-tropical climate zone that typically has a warm humid summer and mild winter. The average daily maximum temperatures are about 22–26 °C in summer, and 12-17 °C in winter. Mean annual rainfall for the past 30 year period was 1198 mm with the highest in June at 120-140 mm and the lowest in January at 70-80 mm (Niwa. 2015).

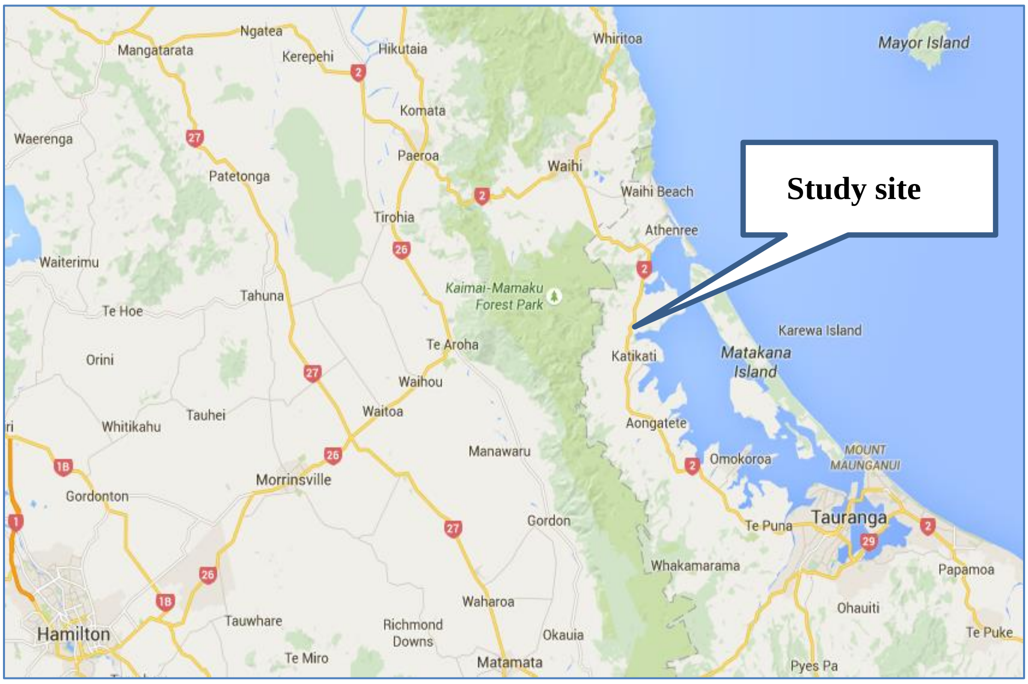

Figure 1. 2 Map showing the location of the study site in Katikati, Bay of Plenty, New Zealand (google maps).

1.5 Plant material – ‘Hass’ cultivar

Persea americana Hass Mill. is a late maturing variety and is the most common cultivar grown in New Zealand and worldwide. In New Zealand, ‘Hass’ avocado flowering season is spring from late-September to mid-November, and fruit becomes mature in August/September, although harvest may occur as late as March the following year.

Compared with other avocado-growning countries in the warm subtropics, New Zealand has a cooler climate and avocado requires a longer period for fruit maturity. Thus, during a specific period avocado commonly have two crops on the tree - immature fruit in the current season and mature fruit from the previous season (Whiley et al. 1996b).

1.6 Research objectives

The overall goal of this research was to investigate the water requirements of avocado growing in the major growing region of the Bay of Plenty, New Zealand. There were two objectives:

Objective One	Quantify the relationship between weather conditions and avocado tree water use.
Objective Two	Assess the effects of water deficits on flowering, fruit set and fruit development.

1.6.1 Objective one - Avocado tree water use

The aim of objective one is to estimate reference evapotranspiration (ET_o) using the FAO Penman-Monteith equation, and to compare ET_o with actual avocado tree water use (ET_c) estimated from sap flow measurements under well-irrigated conditions. The sap flow gauges were installed in three mature (ca. 9 years) and three (ca. 3 years) young trees - the two different age trees were chosen to reflect typical planting densities, tree size and leaf area in commercial avocado orchards. The measurements occurred continuously for a year from November 2014 to October 2015. Volumetric soil water content was also measured and a soil water balance (E_{wb}) calculated for comparison with climate based estimates of tree water use. At the end of the experiment, monthly crop factors (K_c) – the ET_o/ET_c ratio – were determined.

1.6.2 Objective two - The effects of water deficit on avocado flowering, fruit set and fruit development

The aim of objective two was to investigate the effects of soil water deficits on flowering, fruit set and fruit development of young (ca. 3 years) avocado trees. A rainfall-exclusion (‘rainout’) treatment was applied to 5 drought plants (rainout + non-irrigation) and the results were compared with 5 control plants (rainfed + irrigated). By drying of upper soil layers and imposing water stress on drought plants, the effects on the process of flowering, fruit set and fruit growth were observed.

1.7 Thesis layout

This thesis comprises four chapters.

1.7.1 Chapter one: Introduction

Chapter one provides an introduction that addresses general understanding of avocado, the importance of irrigation management, and previous research on avocado water use and irrigation. It then provides information of the study site and ‘Hass’ cultivar, and focuses on the research objectives and an outline of the thesis content.

1.7.2 Chapter two: Climate and avocado water use (Objective one)

Chapter two explores weather information obtained at the study site in the Bay of Plenty, New Zealand, and water use of the mature and young avocado throughout the year from November 2014 to October 2015. The FAO Penman-Monteith reference evapotranspiration (ET_o), measurements of crop evapotranspiration (ET_c), and crop coefficient (K_c) are briefly explained, and the theory and a basic methodology of the compensation heat pulse (CHPM) sap flow method is described. The result summarizes the weather conditions and actual plant water use under those conditions. Avocado tree water use is then evaluated relative to the local climatic conditions.

1.7.3 Chapter three: The effects of water deficit on flowering and fruit set and fruit development (Objective two)

Chapter three demonstrates the effects of water deficit during the period of flowering, fruit set and early fruit development, by applying rainout shelters to the drought treated plants from October 2014 to April 2015. This chapter briefly explains the importance of irrigation, avocado flowering and fruiting characteristics, and the possible effects of water stress, and describes the results of the experiment. The findings demonstrate the importance of tree water status and good irrigation management, particularly for early fruit growth and final yield.

1.7.4 Chapter four

Chapter four provides the overall discussion and conclusions of the two projects, and recommendation for further research on avocado water relations and irrigation management in New Zealand.

Chapter 2: Climate and avocado tree water use

2.1 Introduction

The term 'evapotranspiration (*ET*)' is used to define water loss from the land surface to the atmosphere by two processes; soil evaporation (*E*) and plant transpiration (*T*) (Allen 1986; Ham et al. 1990; Qiu et al. 1999). The most accepted way of achieving good agricultural water management requires good knowledge of crop evapotranspiration (ET_c) compared to reference evapotranspiration (ET_o) (Stockle et al. 2004).

Reference ET_o is defined as the evapotranspiration of grass of a uniform height under well-watered conditions (Allen 2000; Allen et al. 1998; 2006). ET_o can be calculated by the Penman-Monteith equation (Allen et al. 1998; 2006), recommended by the Food and Agricultural Organization of the United Nations (FAO). It is assumed to be independent of crop type or plant physiological factors, so it is influenced only by climatic conditions (Moran et al. 1996; Gong et al. 2006; Cai et al. 2007). The Penman-Monteith reference ET_o requires basic meteorological parameters; air temperature, solar radiation, relative humidity, rainfall and wind speed (Allen 2000: Allen et al. 1998; 2006).

ET_c is actual crop water use, and ET_c is expected to be correlated with ET_o, although it will vary with many factors (crop type, soil type, and stage of development etc.). Sap flow measurement is a plant-based water use indicator that measures plant water uptake by applying heat to the xylem tissues (Huber & Schmidt, 1937; Marshall, 1958; Swanson & Whitfield 1981). The advantages of using sap flow techniques are easy automation, and they can be used to estimate the continuous water use of individual trees (Granier 1987; Smith & Allen 1996; Burgess et al. 2001: Green et al. 2009). The compensation heat pulse method (CHPM) is applicable for non-destructive measurements and relatively inexpensive, and it requires only low electric power and simple equipment (Edwards et al. 1996: Green et al. 2003). Instead of continuous heating, in the CHPM short pulses of heat are applied periodically (Swanson & Whitfield 1981; Green et al. 1998; 2003). The CHPM has been commonly used in a variety of studies of crop water use, such as apple (Green & Clothier 1988), kiwifruit (Edwards & Warwick 1984), pear (Caspari et al. 1993; Goodwin et al. 2012), corn (Bethenod et al. 2000), apricot (Alarcon et al. 2000), olive (Tognetti et al. 2004; 2005), lemon (Alarcon et al. 2005: Ortuno et al. 2005; 2006), orange (Fernandez et al. 2006), walnut (Pereira et al. 2007), and grape (Fernandez et al. 2008; McClymont et al. 2009).

A soil water balance (E_{wb}) is another technique for estimating plant water use, because transpiration occurs as a soil-plant-atmosphere continuum. In a crop, the amount of water in soil depends on input and output of water and holding capacity in the soil. Input of water is basically rainfall and irrigation, and output is evaporation, crop transpiration, surface runoff and drainage (Palomo et al. 2002; Teixeira et al. 2008). The soil water balance approach requires quantification of all additional water input and losses, and water hold capacity in the soil. When drainage and surface runoff are not clear, the E_{wb} calculation might be suitable only for a few days at best (Smith & Allen 1996; Fernandez & Moreno 1999: Nicolas et al. 2005).

The purpose of this research was to define water requirements of avocado so that irrigation recommendations can be developed for New Zealand conditions. Meteorological information was obtained at the study site in Katikati, Bay of Plenty, and the daily, monthly and seasonally FAO Penman-Monteith $\mathrm{ET_O}$ was estimated. Water use of mature (ca. 9 years) and young (ca. 3 years) avocado plants were monitored over a year from November 2014 to October 2015. ET_c and E_{wb} were obtained from continuous measurements of sap flow and soil water content. With known monthly ET_o and ET_c, monthly K_c was calculated.

The hypothesis was that avocado tree water use is high when ET_o is high, and water requirements varies seasonally. During the flowering period, avocado requires more water due to an increase in the surface area contributed by inflorescences. Also, avocado tree water use in New Zealand was hypothesized to be different to estimates from overseas, because of different climatic conditions.

2.2 Methods

2.2.1 Meteorological station

A meteorological station was set up at a height of 2 m at the avocado orchard. Rainfall (TR-525I Rain gauge tipping bucket, Texas Electronics Inc. Dallas, TX), solar radiation (LI200 Pyranometer, LI-COR Inc, Lincoln, NE), air temperature and humidity (HMP50 Temperature and Relatively Humidity probe, Campbell

Scientific, Logan, UT), and wind speed (Vector A101M Anemometer, Vector Instruments, Denbighshire, UK) were recorded every minute and averages saved every 30 minutes with a data logger (CR1000, Campbell Scientific Inc., Logan, UT). Hourly and daily mean air temperature and rainfall were also calculated, and a modem was connected to the data logger. The measurements were made from late October 2014 to November 2015.

2.2.2 The Penman Monteith ET_o calculation

Based on the meteorological measurements, hourly potential evapotranspiration for a short grass canopy - 12 cm uniform grass surface - (ET_o) was calculated continuously by the datalogger using the Penman-Monteith equation (Allen et al. 1998; 2006) as:

$$ET_o = \frac{0.408\Delta(R_n - G) + \gamma \frac{C_n}{T_a + 273} u_2(e_s - e_a)}{\Delta + \gamma(1 + C_d u_2)}$$

Where ET_o is the standard reference evapotranspiration for grass (mm), R_n is net radiation, G is soil heat flux, T_a is average air temperature (˚C), C_n is the numerator constant for the reference crop type and time step (900 mm d^{-1} for daily or 37 mm h^{-1} for hourly), C_d is the denominator constant (daily = 0.34 MJ m^{-2} d^{-1}, hourly day-time = 0.24 MJ m^{-2} h^{-1}, and hourly – 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 and e_a is actual vapour pressure, Δ is slope of the saturated curve (kPa $^{\circ}C^{-1}$), and Υ is psychometric content (kPa $^{\circ}C^{-1}$). The monthly average temperatures and ET_o at the study site were compared with historical data for the previous 10 years for Te Puke, Bay of Plenty (CliFlo Database, NIWA, Auckland).

2.2.3 Plant materials

Three mature (ca. 9 years) and three young (ca. 3 years) plants of *Persea americana* 'Hass' avocado were used for this research. The mature plants were labelled B1, B2 and B3, and three young plants were labelled C1, C2, and C3 (Table 2.1). Plant spacing of the mature and young trees was 10 × 8 m and 5 × 8 m, respectively. These plants were well watered throughout the observation period, with the assumption that they experienced no water stress during this experiment.

Table 2. 1 Basic information (trunk diameter at 50 cm above the soil) of the experimental plants; three mature plants (B1, B2 and B3) and three young plants (C1, C2 and C3).

	plant no.	trunk diameter (cm)
mature plant	B1	42.0
	B2	34.2
	B3	26.4
	average	34.2
young plant	C1	12.6
	C2	13.4
	C3	12.3
	average	12.8

2.2.4 Sap flow measurements

Sap flow probe sets were installed on 7 November 2014. Firstly, the three vertically aligned holes for one set of heat-pulse probes were made radially into the main stem of the experimental trees with a 2.0 mm drill bit. Two sets of the sap flow probe sets were installed per tree (12 sets in total).

Each heat pulse set (model HP4TC-S, Tranzflo NZ Ltd., Palmerston North, New Zealand) consisted of two temperature probes and one heater. Temperature probes had four copper-constantan thermocouples positioned at depths of 5, 15, 25, and 40 mm, thus, a radial profile of sap flow velocity was measured. The heater probe was 1.8 mm in diameter and 30 mm in length. The sap flow probe sets were installed between 45 and 65 cm above the soil. The temperature probes were installed 10 mm downstream and 5 mm upstream from the heater probe in the experimental trees.

The sap flow probes were connected to a data logger and multiplexor (CR1000 and AM25T, Campbell Scientific Inc., Logan, UT). The duration of heat pulse was 3 seconds for the mature trees, and 2.5 seconds for the young trees, and heat was applied at 30 min intervals. After each heat pulse, temperatures were measured every 1.5 seconds over 8 min. The sap flows were recorded automatically every 30 min. The dataloggers were connected to wireless modems, and the data were automatically updated online. Heat pulse velocity was monitored over the year from November 2014.

2.2.5 Sap flow area and total leaf area

The sapwood area of the experimental trees was determined on 29 June 2015. For each tree, on the main trunk at about the same height as the sap flow probe sets, a hole was made using a drill and water-soluble food dye was injected. After two hours, the experimental trees were cored radially to the pith using an increment corer, approximately 4.5 cm above the dye injection site. One core was sampled per tree. Conductive sapwood areas were calculated for each tree based on the proportion of the core radius stained with dye. The bark thickness was measured and subtracted from diameter measured over bark.

For confirmation of sapwood areas relative to total basal area, additional medium to large branches were dye injected and sliced during pruning operations. Dye injection indicated that the entire radial depth of sapwood was conducting in these trees.

Leaf area to sapwood area ratio was determined from pruned branches (Figure 2.1), with leaf area measured using a leaf area meter (LI-3100, LI-COR, Lincoln, Nebraska). Total leaf area for all trees was estimated from a measurement of all branch diameters and the sapwood area to leaf area relationship.

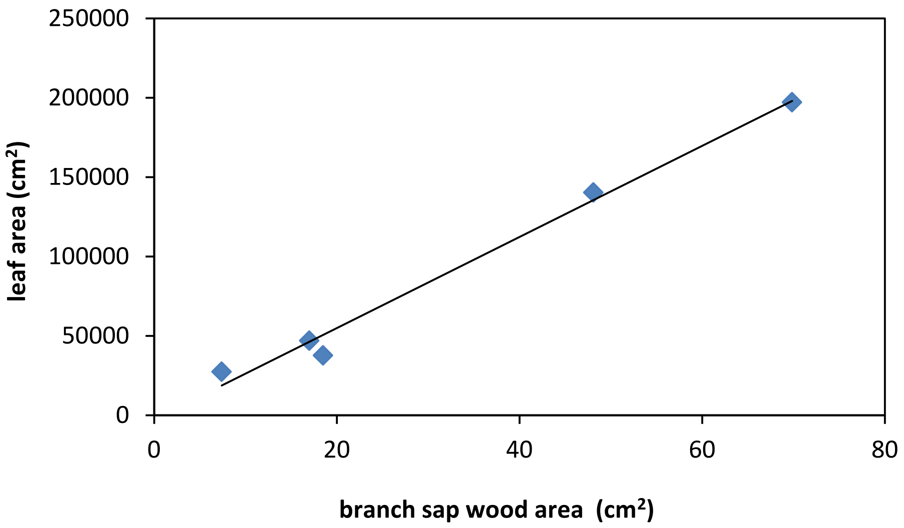

Figure 2. 1 Relationship between branch sap wood area and leaf area. The total leaf area of all tree branches, relative to sap wood area, was calculated by the equation of 'leaf area (cm^2) = 2871.4 × sap flow area (cm^2) – 2566.6' ($R^2=0.99$, $P<0.01$).

Heat pulse velocities were measured using the CHPM of Green et al. (2003). Firstly, the heat pulse velocity (V_h) was calculated from the cross-over time (T_z), when the temperatures of paired thermocouples (upstream and downstream) become equal in value after applying the heat pulse. The heat pulse velocity was derived from the calculation of Huber & Schmidt (1937) as:

$$V_h = \frac{X_d + X_u}{2T_z} 3600$$

Where V_h (cm h^{-1}) is the heat pulse velocity, X_d is the distance from the heater to the downstream temperature probe (X_d = 1.0 cm), and X_u is the distance from the heater to the upstream temperature probe (X_u = -0.5 cm). The distances of the two temperature (upstream and downstream) probes from the heater were not the same. This implies a time delay of the heat transport to the upstream probe, because at the cross-over time both probes are at the same temperature (Swanson & Whitfield 1981; Smith & Allen 1996).

Xylem sap flow is disrupted by the presence of probes and wounding from the sensor implantation. Hence, the heat pulse velocity should be corrected by an equation (Swanson & Whitfield 1983) as;

$$V = a_0 + a_1 V_h + a_2 {V_h}^2$$

Where V (cm h^{-1}) is corrected sap flow velocity, V_h (cm h^{-1}) is the raw heat pulse velocity (uncorrected), and a_0, a_1 and a_2 are correction factors. The wound width was estimated to be 2.8 mm, and the corrected V_h was calculated based on Green et al. (2003).

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

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

Where V_wood refers to volume fraction of the woody matrix in the xylem, and V_water is mean volume fraction of water. J_s was calculated based on $k = 0.505$, as the k factor is related to the thermal capacity of the woody matrix and the value is expected to be constant within and between species (Edwards & Warwick 1984; Alarcon et al. 2000). Sap flow velocities within a tree stem are generally not uniform, so J_s was calculated at four 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 by;

$$F = 2\pi \int_{H}^{R} r\,J_{\mathrm{s}}(r)\mathrm{d}r$$

Where R cambial radius (m) identified from the measurements of bark depths, and H is a heartwood radius (m) determined by dye injection. Because each plant had two probe sets, the sap flow per plant was taken as the average of two measurements.

For the calculation of daily transpiration on a ground area basis (mm), the area occupied by the tree was estimated to be 8 × 8 m for the mature trees and 5 × 5 m for the young trees. The proportion of the occupied area to the total orchard area was 100 % for the mature trees and 63 % for the young trees.

2.2.6 Soil moisture

For the mature plants, in early November 2014, 12 soil moisture probes (7 CS616 and 5 CS615; Campbell Scientific Inc., Logan, UT) were installed to measure volumetric soil moisture at depths of 0-30, 31-60, and 61-90 cm on the north, east, south, and west sides of tree B1. To account for potential spatial variation, soil moisture probes were positioned at a distance between 1.7 m and 2.5 m from the tree trunk. An 80 mm auger was used to install these probes, and the soil was replaced after sensor installation. These probes were connected to a data logger (CR1000, Campbell Scientific Inc., Logan, UT), and soil water content was measured every hour.

For the young plants C1 and C2, in mid-October 2014, soil moisture sensors CS616 (Campbell Scientific Inc., Logan, UT) were installed vertically at the three depths (0-30, 31-60, and 61-90 cm) midway between the trunk and dripline (60 cm from the trunk) on the northeast side of the tree. For installation of CS616 at depths of 31-60 and 61-90 cm, an 80 mm auger was used to make a vertical hole, and after installation of these sensors an 80 mm pipe with a cap on top was inserted into each hole instead of replacing the soil, making these sensors accessible during the experiment. The probes were connected to a multiplexor and data logger (CR1000 and AM16/32B, Campbell Scientific Inc., Logan, UT). Volumetric soil moisture content at each depth was recorded at one minute intervals and the average saved every hour. Daily averages of both soil moisture and soil temperature were calculated.

2.2.7 Water balance

Based on data obtained from volumetric soil moisture measurements, crop water use was calculated using a water balance equation as:

$$E_{\mathrm{wb}} = P + I - \Delta C - D$$

Where E_{wb} is the water consumption of the crop (mm), P is precipitation (mm), I is irrigation application (mm), D is drainage (mm) below the root zone, and ΔC is a change in soil water content (mm) obtained from weighted averages of the vertical soil moisture profiles.

The root depth was not measured, so the depth of the mature and young plant root zone was assumed to be 100 and 60 cm, respectively. The loss of water to drainage was unknown in this experiment, thus, D was excluded from the calculation. To minimise the effect of drainage, E_{wb} was estimated for periods when no rainfall or irrigation was occurring. These days were on 28-30 December

2014, 24-26 January, 19-21 February, 22-24 March, 2-4 April, 5-7 May, and 18-20 June 2015. E_{Wb} was not calculated from July 2015 due to the occurrence of frequent rainfalls.

2.2.8 Crop coefficient (K_c)

Over a year from October 2014 to September 2015, the crop coefficient (K_c) of the mature and young avocado was calculated as:

$$\text{Monthly } K_c = \text{Monthly average } ET_c / \text{ Monthly average } ET_o$$

ET_c and ET_o were calculated on a daily basis, and averaged for the month.

2.2.9 Statistical analysis

Statistical analyses were carried out using the statistical software STATISTICA (StatSoft Inc., Tulsa, OK, USA). Regression analysis was used for comparison between ET_c of the mature/young plants and reference ET_o, sap flow area and leaf area index, sap flow measurements and sap flow area, and sap flow measurements and soil moisture measurements. Statistical comparisons were considered to be significant when $P<0.05$.

2.3 Results

2.3.1 Weather summary

From November 2014 to October 2015, January was the hottest month with a mean daily maximum temperature of 26.1 ˚C and a mean minimum temperature of 14.7 ˚C (Figure 2.2a). These values were slightly higher than the January average maximum temperature of 25 ˚C and minimum temperature of 13.8 ˚C of the past 10 years in Te Puke (CliFlo Database, NIWA, Auckland). In 2015 July was the coldest month with a mean maximum temperature of 15.3 ˚C, higher than the July average maximum of 14.4 ˚C, even though the minimum temperatures were lower at 4.6 ˚C than the July average minimum of 5.7 ˚C.

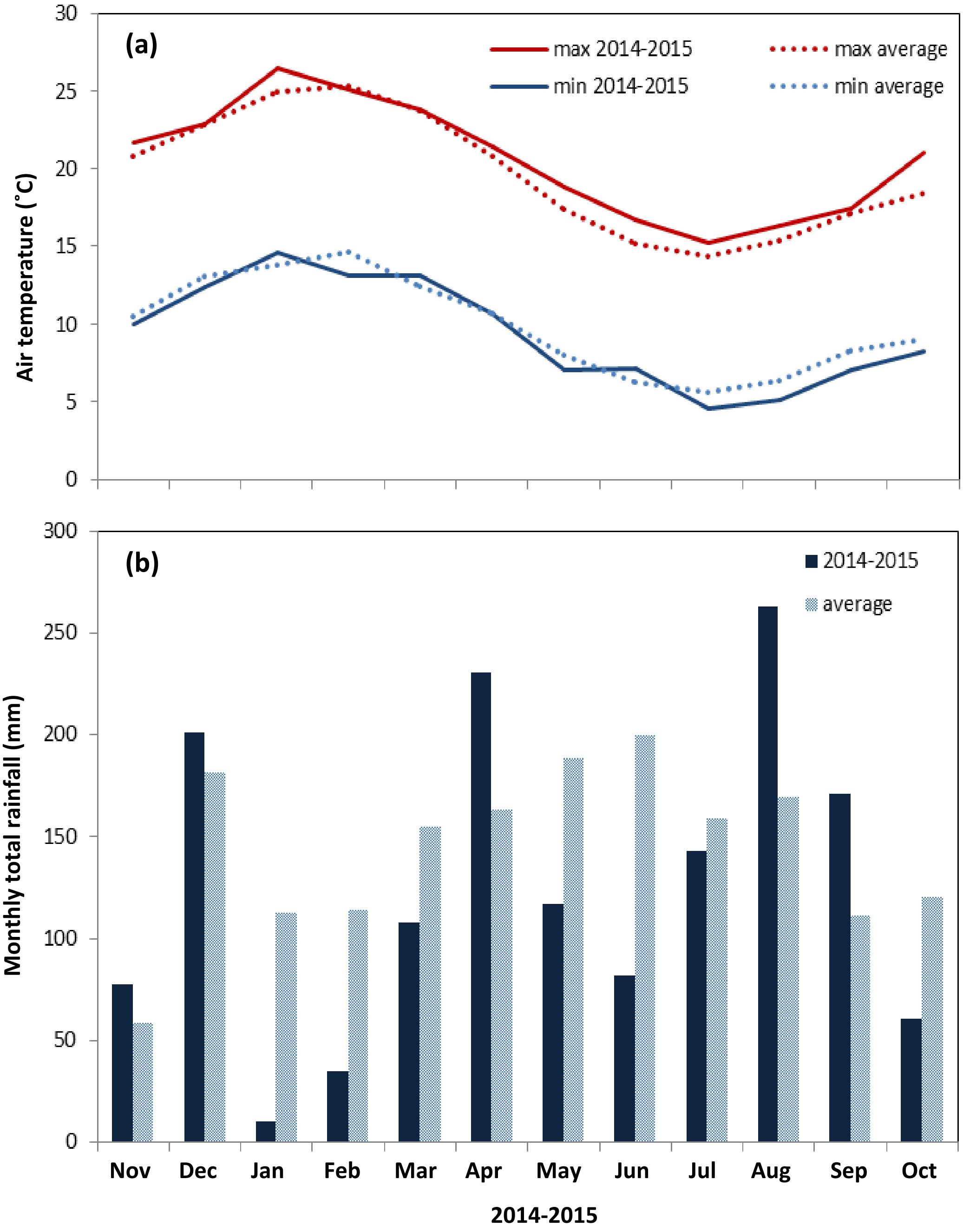

Figure 2. 2 Monthly average maximum and minimum temperatures (a) and monthly total rainfall (b) at the study site in Katikati from November 2014 to October 2015. The graphs show comparison with the historical averages for the past 10 years in Te Puke, Bay of Plenty.

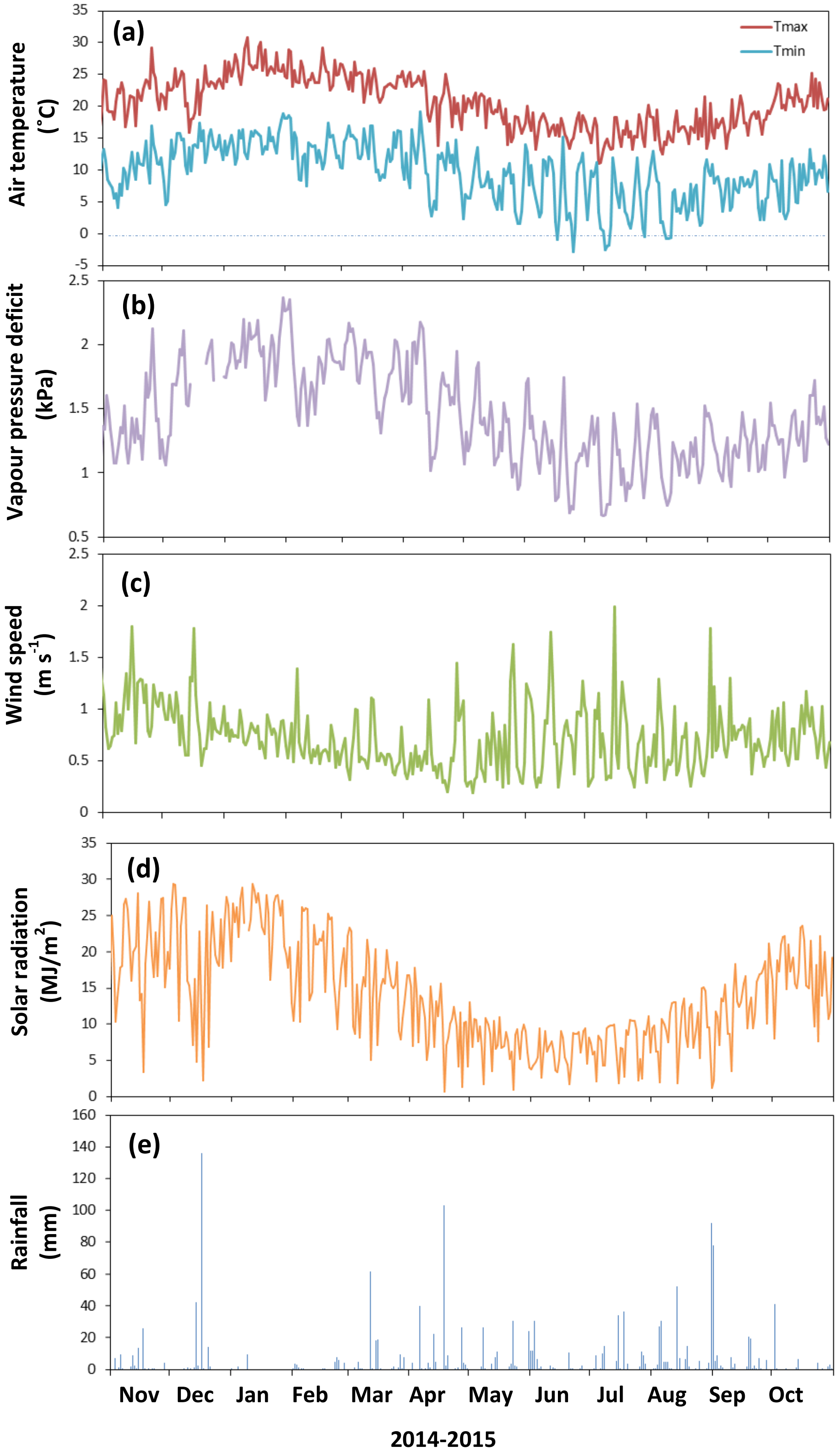

Figure 2. 3 Daily meteorological conditions at the Katikati study site; maximum and minimum air temperatures (a), and mean vapour pressure deficit (b), wind speed (c), solar radiation (d) and rainfall (e), measured from November 2014 to October 2015.

The annual total rainfall from November 2014 to October 2015 was 1495 mm at the study site, similar to the long term average, despite less precipitation in summer (Figure 2.2b). In January and February 2015, monthly rainfall was only 10 mm and 34.6 mm, respectively.

Daily weather observation provided more detail. In summer some days had maximum daily temperatures rising over 30 ˚C, whereas in winter there were frosts with minimum temperatures falling below 0 ˚C (Figure 2.3a). The vapour pressure deficit was generally above 1.0 kPa (Figure 2.3b). The wind speed was mostly light to moderate between 0.2 and 1.5 m s^{-1} throughout the year, with increased variation in winter (Figure 2.3c). The mean daily solar radiation was highest in January at 24 MJ/m^{-2}, and lowest in June at 6 MJ/m^{-2} (Figure 2.3d). Except for January and February, rainfall was frequent over the year (Figure 2.3e).

2.3.2 Sap flow measurements

Daily sap flow differed noticeably between seasons (Figure 2.4). As expected, the transpiration rate of mature plants was three or four times that of the young plants, and there was a strong relationship between reference ET_o and sap flow.

The mature and young plants had a maximum ET_c value in January at 3.0 and 1.7 mm day^{-1}, respectively. ET_o was also high in January with a maximum value of 6 mm day^{-1}. With a progressive reduction of ET_o from summer to winter, ET_c was slightly decreased. In July, when ET_o was just above 1.0 mm day^{-1}, a maximum ET_c was approximately 1.0 mm day^{-1} for the mature plants, and 0.9 mm day^{-1} for the young plants.

Typical hourly sap flow patterns for both mature and young plants, along with the reference Penman-Monteith ET_o for a 10 days period in mid-summer (11-20 January) (a), autumn (11-20 April) (b), mid-winter (11-20 July) (c), and spring (11-20 October) (d) are presented in Figure 2.5. Sap flow increased sharply in the morning, reached a peak around midday, then declined in the evening. Sap flow did occur at night however this was very small in comparison.

Sap flow rates were highest in summer (15.0 L h^- mature plants and 3.3 L h^{-1} young plants), when ET_o was high (0.6-0.7 mm h^{-1}). In autumn, ET_o decreased to 0.3-0.5 mm h^{-1}, and daily maximum sap flow of the mature and young plants fluctuated with the maximum value of 12.0 and 2.5 L h^{-1} respectively. In winter, when ET_o was low at around 0.2 mm h^{-1}, plant sap flow was below 7.6 L h^{-1} for the mature and 1.5 L h^{-1} for the young plants. ET_o went up to 0.5 mm h^{-1} on the sunny days in spring, and the sap flow of the mature plants increased to 10 L h^{-1}, but the sap flow of the young plants did not increase and was about 1.5 L h^{-1}.

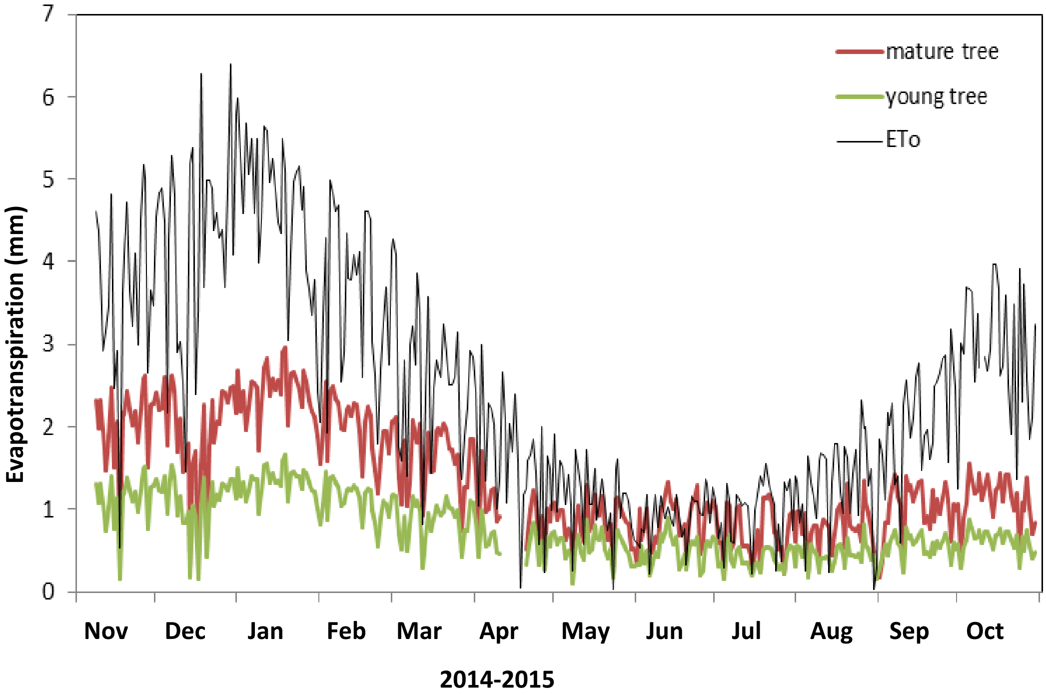

Figure 2. 4 Daily mean sap flow measurements (ET_c) of three mature and three young plants with daily Penman-Monteith ET_o at the orchard over the experimental period between November 2014 to October 2015.

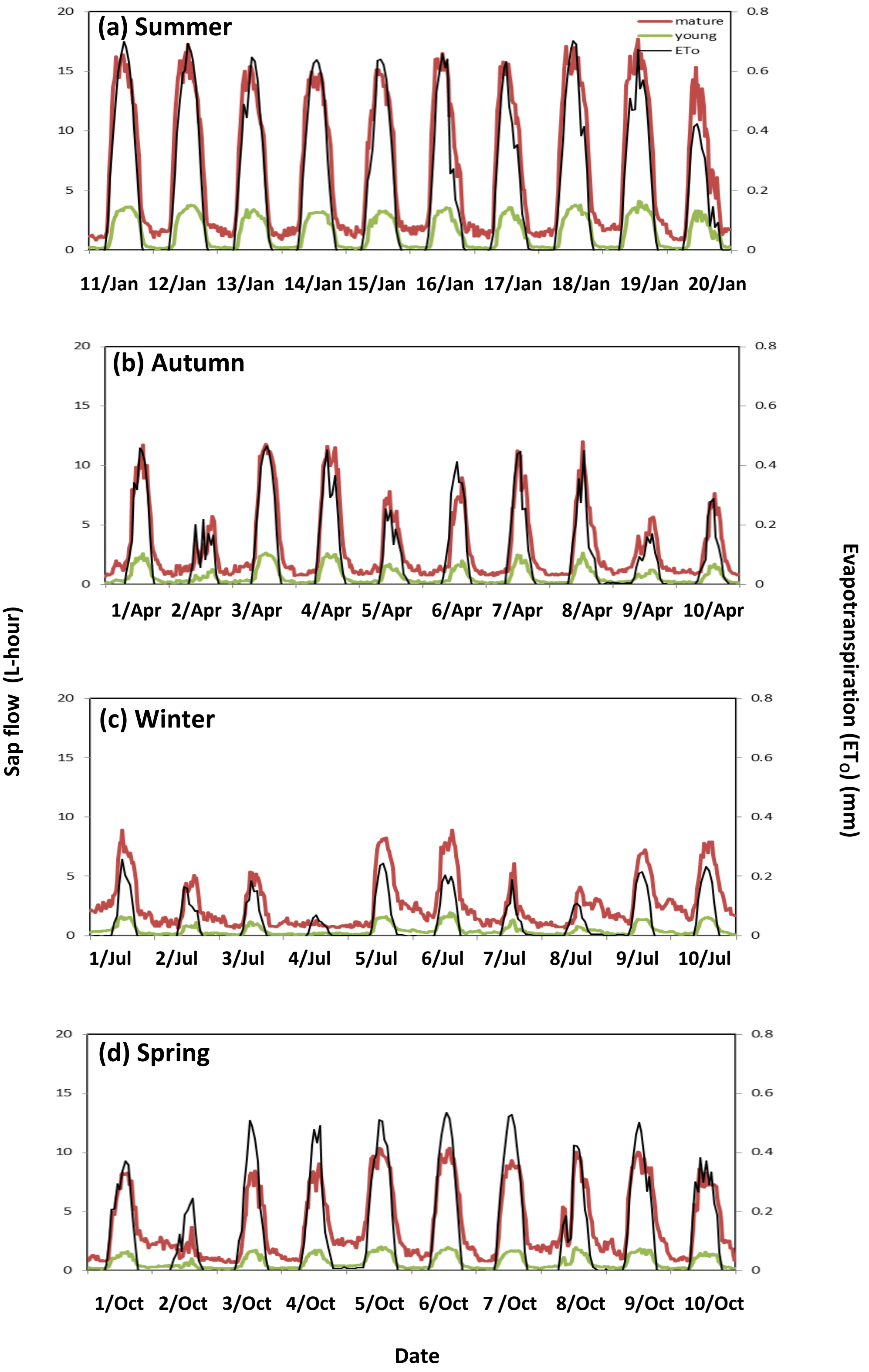

Figure 2. 5 Hourly mean sap flow measurements of three mature and three young trees on typical days in mid-summer (a), autumn (b), winter (c), and spring (d) with estimated FAO Penman-Monteith evapotranspiration ET_o (mm hour^{-1}), obtained from the met station at the study site.

2.3.3 Leaf area and sap flow area

There was a close correlation between leaf area and sap flow of the mature and young plants (Figure 2.6). The relationship between these two variables was highly significant ($P<0.01$).

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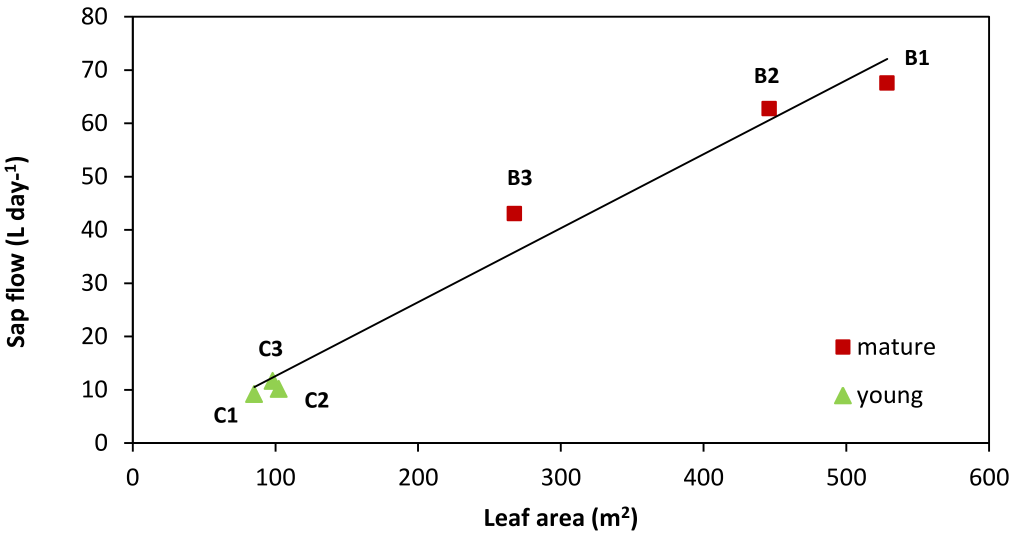


Figure 2. 6 Relationship between leaf area and sap flow of the mature (B1, B2, B3) and young (C1, C2, C3) plants. The values of sap flow were the daily average in July 2015. Sap flow (L day^{-1}) = 0.1388 × leaf area (m^{2}) – 1.324 ($R^2 = 0.98$, $P<0.01$).

3.3.4 Soil moisture

Throughout the year from November 2014 to October 2015, mean volumetric soil water content of the mature and young plants showed soil moisture fluctuations in response to rainfall/irrigation, and plant uptake/drainage. Due to the greater root zone of the mature plants, soil moisture was measured on the four different sides of the trees, the results showing soil moisture was homogenous. The soil moisture profile of two young plants was also similar to each other (Figure 2.7a,b).

Soil water content increased with depth, and fluctuated less with increasing depth. The mature and young plants presented a similar trend. Both had lower soil moisture during summer from early-January to mid-March due to significantly less rainfall. There was an occurrence of a heavy rainfall event in December 2014, and it increased soil water content dramatically before the drought condition started. In autumn and winter, soil moisture increased in a response to frequent rainfall. Soil moisture fluctuated more at all depths in the young trees, relative to the mature trees. There was a period in summer when little drainage to the bottom layer occurred.

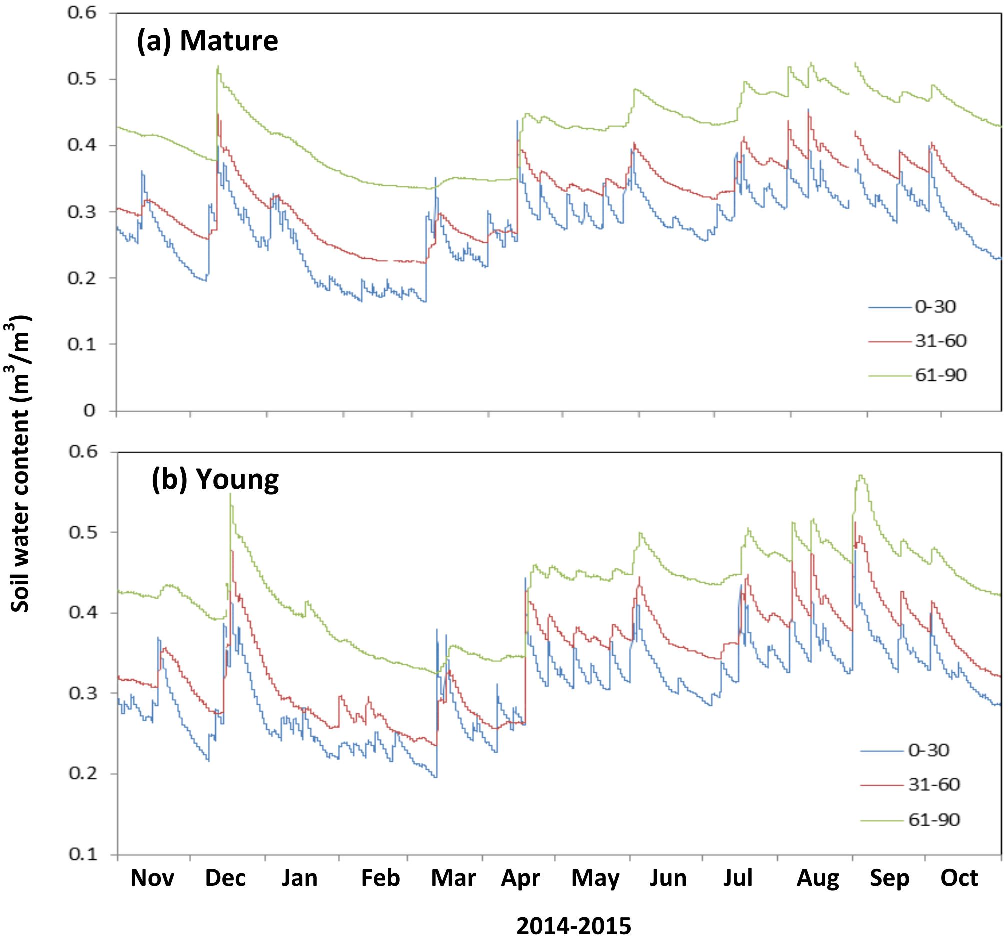


Figure 2. 7 Average of volumetric soil water content beneath the mature (a) and young (b) trees at a depth of 0-30, 31-60, and 61-90 cm, from November 2014 to October 2015.

2.3.5 Sap flow measurements (ET_c) and water balance (E_{wb})

Daily crop evapotranspiration was also estimated from the volumetric soil water balance (E_{wb}) and E_{wb} values were compared with ET_c (Figure 2.8a,b). The mature and young plants had higher E_{wb} than ET_c, by 22 % and 55 % respectively. Regression analysis showed that a correlation between ET_c and E_{wb} of the mature plants was statistically significant (R^2=0.57, P=0.02), but that of the young plants was weak (R^2=0.42, P=0.08).

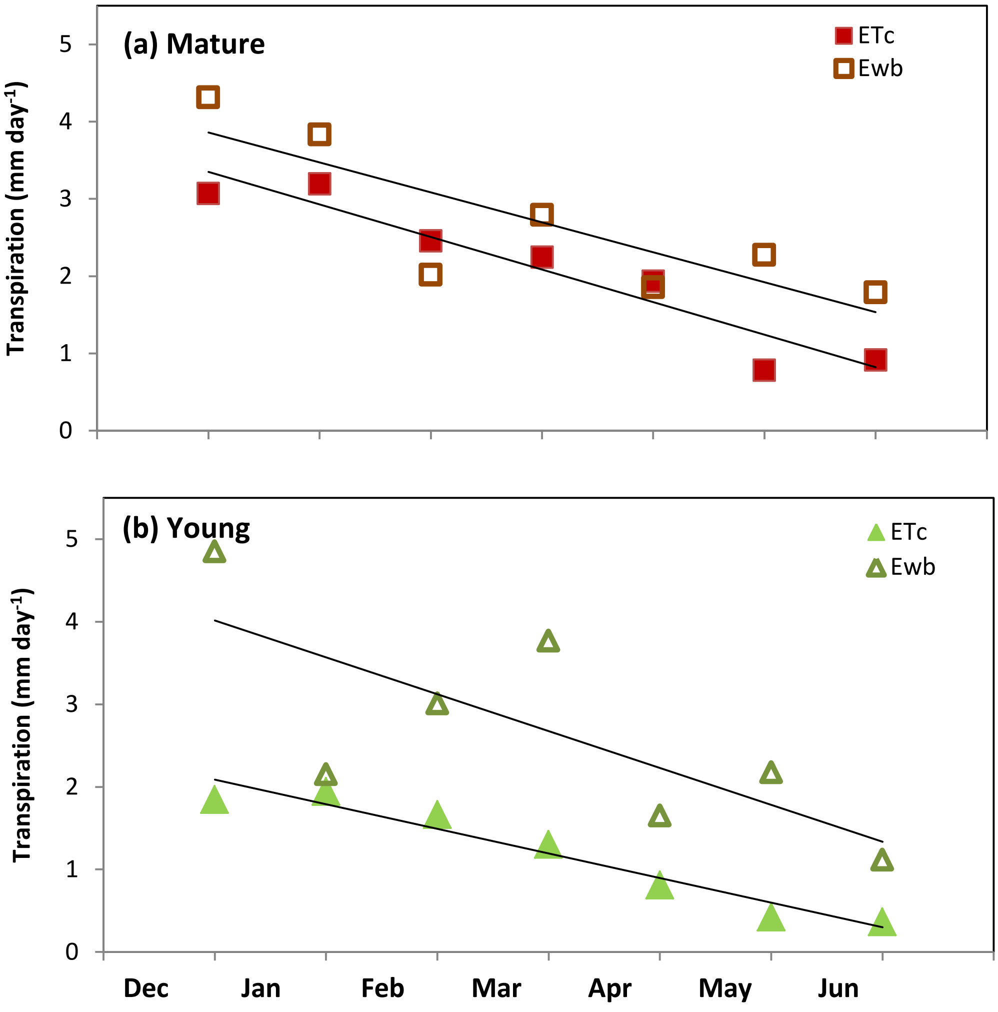


Figure 2. 8Comparison between ET_c (sap flow measurements) and E_{wb} (soil water balance) of the mature (a) and young (b) plants. The points were calculated as an average of two to three days each month from December 2014 to June 2015.(mature P<0.05, young P>0.05).

2.3.6 Monthly water use and crop coefficient

From December 2014 to October 2015, monthly cumulative ET_o, ET_c and K_c of the mature and young plants were calculated (Table 2.2).

The monthly cumulative reference ET_o was highest at 145.9 mm month^{-1} in January and lowest at 27.7 mm month^{-1} in June. ET_c of the mature and young plants were correlated to ET_o. The maximum ET_c values of the mature and young plants were 75.5 and 41.8 mm month^{-1} in January, respectively. In July, the mature plants had a minimum value of 22.4 mm month^{-1}, while the young plants had the lowest ET_c value of 12.8 mm month^{-1}.

The monthly average K_c of the mature and young plants differed seasonally. In summer, the K_c values ranged between 0.45-0.60 for the mature plants and 0.25-0.30 for the young plants. On the other hand, in winter, the K_c values increased up to 1.00 for the mature plants and 0.55 for the young plants. These increases were due to a significant decline in ET_o values in winter at the study site.

Table 2. 2 Monthly cumulative reference ET_o at the study site, and ET_c and K_c of the mature and young plants from November 2014 to October 2015.

Year	Month	ET_o (mm)	mature plant		young plant	
			ET_c (mm)	K_c	ET_c (mm)	K_c
2014	Nov	107.0	60.5	0.60	34.1	0.30
	Dec	133.3	60.4	0.45	34.0	0.25
2015	Jan	145.9	75.5	0.50	41.8	0.30
	Feb	100.8	56.1	0.60	31.4	0.30
	Mar	81.0	50.7	0.70	27.7	0.35
	Apr	49.1	30.4	0.70	18.7	0.40
	May	35.5	26.3	0.90	17.1	0.50
	Jun	27.7	26.3	1.00	15.3	0.55
	Jul	29.2	22.4	0.85	12.8	0.45
	Aug	41.1	24.5	0.60	14.2	0.35
	Sep	62.5	30.1	0.50	17.1	0.30
	Oct	94.9	35.4	0.40	19.6	0.20
average		70.4	39.9	0.60	22.7	0.35

2.4 Discussion

The results provide the first estimates of monthly crop factor for avocado in New Zealand. The data demonstrated that avocado water use is correlated with ET_o, but K_c varies more seasonally than that of other countries. The results also showed that water use is strongly correlated with tree leaf area, which can potentially be used to predict how avocado water requirements may vary between orchards (e.g.

with tree age and planting density). It was also possible to estimate tree water use from soil moisture measurements, but this approach is less reliable than ET_c based estimates, probably because drainage and spatial variation in soil moisture content were not accounted for.

2.4.1 Weather summary

In the Bay of Plenty, summer 2015 had higher temperatures with less rainfall than the normal years, and these conditions contributed to a higher ET_o value. However, winter 2015 was slightly colder with more frequent rainfall, but ET_o was about the average. When comparing with the ET_o values at Te Puke (CliFlo Database, NIWA, Auckland), cumulative monthly ET_o at the study site was the same in summer but was higher by 10-20 % in winter, possibly the result of local difference in daily temperatures, solar radiation, humidity and wind speed.

Rana & Katerji (2000) and Villalobos et al. (2000) highlight the difficulties in obtaining accurate ET_o estimation in some cases, due to variation in weather conditions, soil and plant type, and topography. However, in this research, the reference Penman-Monteith ET_o calculated from the weather data at the study site was reasonable. On a daily basis, solar radiation, air temperature, relative humidity and rainfall obtained at the study site were similar to the reference meteorological data from Te Puke. Wind speed at the study site was 30-40 % lower throughout the year, probably because of wind shelters surrounding the orchard. Except for wind speed, the meteorological measurements were representative for the region. Thus, the measurements of meteorological data and the Penman-Monteith method were appropriate in this experiment.

2.4.2 Plant water use

In recent years, sap flow measurements have been widely used to investigate whole plant water use by installing sap flow probe sets into the trunk of woody species. Practically, no uniform pattern has been identified in the stem xylem, thus Swanson (1994) and Fernandez et al. (2001) highly recommended using two to four sap flow probe sets per plant. Some studies (e.g. Dye et al. 1996; Bethenod et al. 2000) reported that the CHPM sometimes provides estimates of sap flow that are inconsistent to the actual tree transpiration in the field conditions, even when multiple sap flow probe sets were installed. These errors may be due to variability in sap flow velocity and wound widths between probes (Cohen & Li 1996; Dye et al. 1996; Fernandez et al. 2001).

In this work, the results from sap flow probes appear to be robust. The experiment had two sap flow probe sets per plant, and the results of sap flow measurements of two different positions on each plant were statistically similar, indicating the sap flux density within avocado stems is relatively consistent. Also, for the analysis of sap flow measurements, wound correction factors from Green et al. (2003) were used, and it was appropriate to measure actual plant water use on both the mature and young avocados.

According to the measurements, a small amount of sap flow occurred at night time, however, the CHPM sap flow technique has a limitation in measuring low sap flow (Becker 1998), and it cannot measure zero sap flow. Even though plants usually stop transpiration at night, low sap flow may be observed. Nocturnal sap flow might occur for replenishing of tissue water storage, after water in the plant tissues are withdrawn during day time transpiration (Goldstein et al. 1998; Nicolas et al. 2005). Plant water potential and stomatal conductance were not measured for this aspect of the project, and the experimental plants were well watered, so these trees did not have water stress. However, from this observation, it cannot be concluded that the nocturnal sap flow did not occur. Further measurements would be necessary to determine this.

The results showed a good agreement between hourly/daily ET_c and reference Penman-Monteith ET_o. The relationship between sap flow measurements and weather conditions were investigated in other crops, for example, apricot (Alacon et al. 2000), lemon (Ortuno et al. 2005; 2006), and olive (Tognetti et al. 2005). These studies reported that plant transpiration was largely influenced by climatic conditions, hence it is possible to estimate plant water use with known ET_o. The results of this experiment were consistent with their findings, indicating sap flow measurements using the CHPM are appropriate for estimating transpiration by avocado trees, and the results can be used to improve irrigation management. There were also strong positive relationships between sap flow measurements and leaf area. The leaf area and the plant transpiration rates are correlated in other species, such as walnuts, apples and olives (Pereira et al. 2007). With further research it should therefore be possible to use estimates of leaf area per tree, leaf area index or canopy gap fraction to predict avocado water use for orchards with different tree ages, tree spacing, or canopy condition.

The correlation between ET_c and E_{wb} calculated from volumetric soil water content of the mature plants was significant ($P<0.05$), but that of the young plants was weak ($P>0.05$). This could be because surface runoff and drainage were uncertain (Castel et al. 1987; Smith & Allen 1996; Nicolas et al. 2005). The days which did not have rainfall nor irrigation were selected for the E_{wb} calculation. However, the E_{Wb} values were higher than the ET_c values. The plant water use based on the soil water budget was probably overestimated, because drainage was occurring, resulting in higher estimated E_{wb} than actual plant transpiration. Compared with the results of the mature plants, the young plants had far higher E_{wb} than ET_{c} with large fluctuations between months, indicating the soil drainage was greater in the young plants due to smaller root zones.

2.4.3 Seasonal changes

How much irrigation is required for a crop largely depends on plant size and local weather conditions (Petillo & Castel 2007). As expected, the results of this experiment showed that the mature plants had higher K_{c} values than the young plants.

There were also seasonal variations in ET_{c} and K_{c}. In November 2014, both the mature and young plants had higher K_{c} values, relative to the following month of December. This can be explained by an increase in water requirement due to flowering. Comparing between the ET_{o} and ET_{c} values in other months, the estimated increase in plant water use during flowering was 8-10 %. Whiley et al. (1988) reported that water loss from floral organs was about 13 %, and the results of this experiment were consistent with the finding of Whiley et al. (1988).

In this experiment, the estimated K_{c} values were lower in summer and higher in winter, due to an increase or decrease in the monthly ET_{o} values. In summer from December 2014 to February 2015, the monthly average K_{c} values were lower at 0.45-0.60 for the mature and 0.25-0.30 for the young plants. In contrast, in June, the mature and young plants had the highest K_{c} value of 1.00 and 0.55 respectively. These K_{C} values were more variable, compared to other countries.

For example, K_C of mature avocado ranges from 0.55 and 0.40 in California (Lahav et al. 2013), and from 0.42 to 0.68 in Israel (Lahav & Kalmer 1983).

Castel et al. (1987) stated that K_c is strongly associated with ET_o and relative humidity. In winter, when ET_o is low and relative humidity is high, higher K_c was observed in other evergreen fruit crops, for example, citrus (Castel et al. 1987; Petillo & Castel 2007). This might be because of partial stomatal closure in dry air (Kaufmann 1977). The results of this research were similar to their findings.

Penman-Monteith reference ET_o was strongly correlated to plant water use. However, in spring 2015, ET_c of the mature and young plants was lower than expected, by 10-20 % in September and 30-40 % in October. The ET_c values for the final months of the measurement period may have been underestimated due to gradual increases in wounding and lignification around the sap flow probe sets.

2.4.4 Conclusions

Continuous measurements of the local weather conditions and sap flow revealed actual water use of avocado plants in Katikati, Bay of Plenty, New Zealand. Sap flow measurements using the CHPM were in reasonable agreement with reference ET_o over the year of observation, thus, this technique was adequate for the avocados. K_c obtained in this research presented more seasonal variations, compared with K_c in other countries (e.g. California and Israel). Further research will require re-installation of sap flow probes in the same orchard to identify year to year variation, or in a range of orchards and regions to observe any differences related to variation in local conditions.

Chapter 3: Water stress during flowering, fruit set, and fruit development

3.1 Introduction

Many fruit crop plants require additional water throughout the time of anthesis and fruit development. This is because the transpiration rate of flowers is generally higher than that of leaves (Blanke & Lovatt 1993; Chapotin et al. 2003; Boyer & Westgate 2004), and fruit growth is largely associated with water accumulation (Li et al. 1989; Genard & Huguet 1996).

In isohydric species, water deficit can cause stomatal closure and reduce photosynthetic activity (McDowell et al. 2008). If drought conditions occur during flowering and fruit development, it might negatively influence the reproductive process and fruit growth of isohydric plants, by a shortage of carbohydrate. For example, in maize, lowering leaf water potential during flowering causes inhibition of photosynthesis, which leads to depletion of carbohydrate reserves, and failure of ovary development (Westgate & Boyer 1986; McLaughlin & Boyer 2004a,b; Makela et al. 2005). In pear, water stress over early fruit development caused reduction of sugar and organic acid accumulation in fruit (Hudina & Stampar 1999).

Avocado responds to water stress by stomatal closure. In the first stage of water stress stomatal conductance tends to decrease (Blanke & Whiley 1995;

Chartzoulakis et al. 2002; Celedon et al. 2012), and increases rapidly after re-watering (Gil et al. 2008). Therefore, avocado can be considered an isohydric species. Flowering and fruit growth are a major event during plant ontogeny, but are energy expensive. Avocado is thought to be highly sensitive to water deficit for the flowering and early fruiting period, when water demand increases with a rise in canopy surface area caused by flowering (Whiley et al. 1988; Corbet 1990; Blanke & Lovatt 1993), and when fruit grow rapidly during early fruit development (Blanke & Lovatt 1993; Blanke & Whiley 1995; Lahav et al. 2013).

Water deficit may influence flower and immature fruit abscission, and fruit growth. Silber et al. (2012) reported that the water-stressed avocado had lower CO_2 assimilation, and as a result, fruit abscission increased although there were no obvious effects on vegetative growth. Early fruit growth also requires a large amount of carbohydrate (Scholefield et al. 1985). Interestingly, Alcaraz et al. (2010; 2013) found that flowers with higher starch accumulation in the pistil had higher rates of retention and fruit set. If this is the case, photosynthesis inhibition caused by water deficit could affect flower retention, fruit set, and fruit growth of avocado.

Imposing water deficit and measuring the level of water stress is a common technique to assess the role of water-plant status during fruit development (Neuhaus et al. 2009; Lahav et al. 2013). By eliminating water supply to the drought treated plants, it is possible that mild drying of upper soil layers, combined with dynamic water stress within distal leaves and inflorescences, enhanced by the added water loss from inflorescences, could affect the process of flowering, fruit set and early fruit development.

The aim of this project was to determine the effects of water stress on flowering, fruit set and fruit development, by applying a rainout treatment. Ten young avocado trees (ca. three years from planting) – five control (rainfed + irrigated) and five drought (rainout + non-irrigated) plants were chosen, and the rainout shelters were applied on the drought plants over 6 months from October 2014 to April 2015. Over the observation period, soil moisture was monitored continuously, and plant stress levels were determined by regular measurements of predawn, midday leaf and stem water potential, and stomatal conductance. In addition, during the flowering season, timing of female flower opening and flower abscission were observed. After fruit set, the occurrences of fruit abscission and fruit growth were monitored until harvest.

The hypothesis was that insufficient water supply would increase the occurrence of flower and immature fruit abscission, change the timing of flower opening and closing, and reduce early fruit growth, and consequently reduce fruit yield at harvest. The drought plants would exhibit water stress, either the direct impacts of water deficits on growth and flowering, or indirect mechanisms such as starch depletion in flowers as a result of photosynthesis reduction, or inability of carbohydrate transport to flowers or fruit.

3.2 Methods

3.2.1 Plant materials

Young trees (ca. three years) of *Persea americana* 'Hass' avocado were chosen for the rainout experiment. These plants were spaced approximately 5 m apart within rows and 8 m apart between rows. In early-October 2014, at the orchard, 10 similar size adjacent plants on the flat area were selected as five control plants (C1 to C5) and five drought treated plants (D1 to D5).

Throughout the experimental period, weeds and insects were controlled by mowing and herbicides, and insecticide sprays, respectively. Each tree had two sprinklers, and the irrigation interval was 4-6 days depending on precipitation. Irrigation levels were determined by the orchard owner, based on previous experience and observation of precipitation and tensiometer measurements at the site, and irrigation was not applied when there was enough rainfall. Over the observation period, the experimental plants were irrigated from early-December 2014 to mid-March 2015.

When the rainout shelters were put on the drought plants, only control plants were fertilized according to standard industry recommendations, with approximately monthly applications of solid complete fertilizer (YaraMila COMPLEX) supplemented by foliar applications of urea and boron (Yaravita Bortrac), and fertigated applications of soluble boron and NPK (Hakaphos K Max).

The soil type at the site is Katikati sandy loam (Typic Orthic Allophanic Soil). In 2014, in this orchard, avocado flowered between mid-October and late-November, and the harvest was in November the following year.

3.2.2 The rainout treatments

The rainout shelter was installed on 20 October 2014. For the drought plants, sprinklers were blocked and light grey painted corrugated iron sheets (4.2 × 4.9 m) were placed over the root zones. To create air movement under the rainout shelters, the iron sheets were placed 10-20 cm above the ground with a slight gradient for drainage. During the rainout treatment, the drought plants did not receive either irrigation or precipitation from the top soil, whereas the control plants were well irrigated. The rainout shelters were in place for over 6 months. On 1 May 2015, the shelters were removed and the drought trees supplied with irrigation and precipitation at the same rates as the control trees.

3.2.3 Vertical soil moisture and soil temperature measurements

Soil moisture profiles of two control trees (C1, C2) and two drought trees (D1, D2) were monitored from mid-October 2014. At each tree, three soil moisture sensors CS616 (Campbell Scientific Inc., Logan, UT) were installed vertically at three depths (0-30, 31-60, and 61-90 cm) midway between the trunk and the dripline (60 cm from the tree) on the northeast side of the tree. For installation of sensors at depths of 31-60 and 61-90 cm, an 80 mm auger was used to make a vertical hole, and after installation of these sensors an 80 mm pipe with a cap on top was inserted into each hole instead of replacing the soil. Therefore, these sensors were accessible during the experiment.

Additional soil moisture sensors of a different type (ECH_2O, Decagon Devices, Inc. WA) were also used to monitor soil moisture of all experimental trees. One sensor per tree was installed vertically at the depth of 0-20 cm midway between the trunk and the dripline on the northeast side of each tree.

Soil temperature probes (Model 107 Temperature probe, Campbell Scientific Inc, Logan, UT) were installed under three control trees (C1, C2, C3) and two drought trees (D1, D2) at the depth of 10 cm midway between the trunk and the dripline on the northeast side of each tree.

The soil moisture and soil temperature probes were connected to a multiplexor (AM16/32B, Campbell Scientific Inc., Logan, UT) and a data logger (CR1000, Campbell Scientific Inc., Logan, UT). Volumetric soil moisture contents at each depth and soil temperature were recorded at one minute intervals and the average saved every hour. Daily averages of both soil moisture and soil temperature were calculated. The measurements were carried out until September 2015.

3.2.4 Spatial soil moisture measurements

To investigate the effectiveness of the rainout shelters, on 19 December 2014, spatial variation in soil moisture contents beneath one control (C2) and two drought (D2, D3) plants in the top 10 cm were measured manually, using a portable soil moisture probe (Field Scout TDR 100, Spectrum, Technologies, Inc. Plainfield, IL). On 19 March 2015, spatial soil moisture content of one control (C2) and two drought (D1, D2) plants were measured again. The soil moisture probe used for the previous measurement was not available after the first measurement, thus, for the second measurement, Decagon sensors (ECH$_2$O 10-HS, Decagon Devices, Inc. WA) were used instead. A systematic grid point sampling method was used for the measurement with a 60 × 60 cm grid within a sampling area of 4.2 × 4.9 m. As necessary, the tin sheets for the rainout treatment were lifted up during the measurements.

3.2.5 Predawn and midday leaf and stem water potential

Predawn (0500-0700 hours) leaf water potential (PLWP) was measured once or twice a week from late-October 2014 to mid-December 2014 using a pressure chamber (PMS Instrument Co. Ltd., Corvallis, OR). For each tree, two healthy mature leaves were chosen randomly, enclosed in a plastic bag, removed from the plant with a razor blade, and immediately measured.

Midday (1200-1600 hours) leaf water potential (LWP) and stem water potential (SWP) were measured from October 2014 to August 2015. Three leaves per plant were measured at approximately one weekly intervals during a period of flowering and fruit set from October to December 2014, at two to three weekly intervals from January to April 2015, and at four weekly intervals from May to August 2015. At each time, for LWP measurements sun-exposed fully developed leaves were chosen and put in a plastic bag before removal from the plants, while for SWP measurements leaves were put in a plastic bag and covered by aluminium foil at least two or three hours before the measurements.

3.2.6 Stomatal conductance

On 12 December 2014, at the end of the flowering period, stomatal conductance (g_s) was measured with a portable photosynthesis system (LI-6400, LI-COR Inc. Lincoln, NE) at the same time as LWP measurements. These measurements were made on sunny day at 1200-1600 hours. At each time, after g_s measurements, the leaves were put in a plastic bag and taken off from the tree by a razor blade and immediately used for the LWP measurements. These measurements were replicated five times per plant.

3.2.7 Floral intensity

Based on visual observation during the peak of the flowering season in October 2014, floral intensity of each experimental tree was categorized into five classes.

1. Very light flowering
2. Light flowering
3. Intermediate flowering
4. Heavy flowering
5. Very heavy flowering

3.2.8 Flower abscission

Open female flowers were marked on 6 dates (31 October, and 9, 15, 16, 22, 27 November, 2014) to determine what proportion were retained to form fruitlets and fruit. In preparation, 4-6 north-facing floral branches were selected and flagged. Then, on each day, 50 female flowers per tree on these branches were marked using colour acrylic paint. Approximately two and half weeks after marking, paper jewellery tags were used to more permanently mark the remaining marked flowers, then these tags were recounted each week. The marked flowers were monitored for up to 100 days after anthesis. One control tree, C5, had no flowers later in the flowering season, therefore, on 22 and 27 November, C5 was excluded from flower abscission monitoring.

3.2.9 Female flower opening behaviour

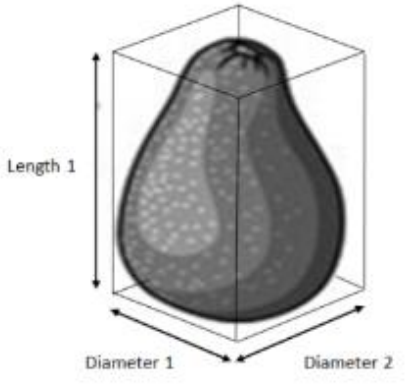
The timing of female flower opening was observed to identify if the timing of opening during the day was affected by water stress. Monitoring occurred on three different days late in the flowering season; 19, 23 and 28 November, 2014. Observation was made on the north side of the tree at a height of 100-130 cm on four control trees and five drought trees (C5 was excluded for this monitoring because it had no flowers on these dates). On each day, monitoring started when female flowers started opening, and finished when most female flowers were closed. During the observation, the number of flowers in the female phase was counted every half hour, and recorded.

3.2.10 Fruit abscission

The monitoring of fruit abscission started from 26 December 2014. Initially, 8-16 fruiting branches per tree were selected and flagged, and over 200 fruit per tree were counted (Two control plants, C3 and C5, had significantly fewer fruit than the other trees, so the sample size was reduced to 54 fruit on C3 and 84 fruit on C5). After the first count, the remaining fruit on the flagged branches were recounted every one to two weeks, and the percentage of fruit retention calculated. Fruit abscission monitoring continued until late-October 2015.

3.2.11 Fruit growth

Fruit growth was measured non-destructively from January 2015. Thirty fruit per plant were randomly selected and tagged, and one length and two diameters of these tagged fruit were measured using digital callipers every two weeks until harvest on 1 November 2015 (Figure 3.1). Prior to harvest, any fruit lost were replaced by selecting and marking another fruit.



Length	Maximum fruit length
Diameter1	Maximum diameter perpendicular to length 1
Diameter2	Maximum diameter perpendicular to diameter 1

Figure 3. 1 Non-destructive fruit size measurement. One length and two diameters were measured periodically from December 2014 to October 2015.

Fruit weight was predicted from the multiple of L x D_1 x D_2 (LDD), after obtaining the relationship between LDD and actual fruit weight from fruit destructively harvested from nearby trees. Throughout fruit development, 20 fruit of a range of sizes were sampled periodically, and fruit weight of the monitored fruit was estimated from LDD, based on the actual LDD to weight relationship of the harvested fruit (Figure 3.2).

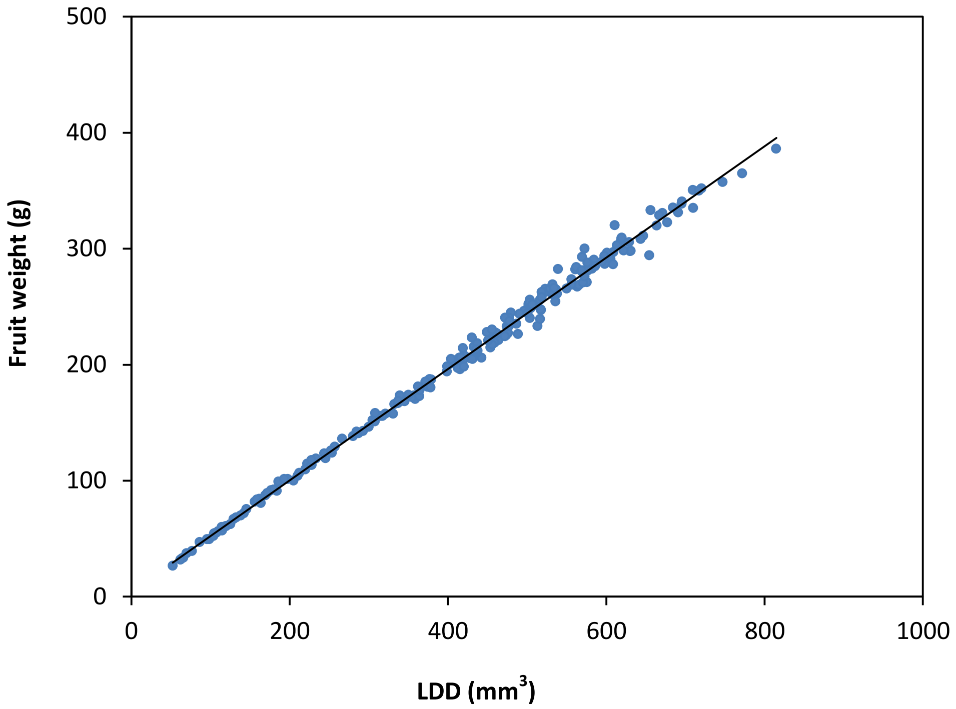


Figure 3. 2 Relationship between fruit size (LDD) and fruit weight (g). Fruit weight was calibrated by the equation of 'fruit weight (g) = 0.48 × LDD (mm^3) + 4.2762' (R^2=0.9955, $P<0.01$).

3.2.12 Leaf nutrients

The nutritional status of the experimental trees was assessed by leaf analysis according to standard commercial practise. On 19 April 2015, 10 fully expanded healthy leaves per tree were selected from non-fruiting shoots on nodes 3-5 from the north, east and west sides of the tree, bagged immediately and stored at 4 °C until analysis.

Leaf nutrient analysis was conducted by Hill Laboratories. These leaves were washed then dried in an oven at 62 ˚C overnight, and ground with a 1.0 mm screen before measurement of concentrations of 12 elements on a dry weight basis: Nitrogen (N), Phosphorus (P), Potassium (K), sulphur (S), Calcium (Ca), Magnesium (Mg), Sodium (Na), iron (Fe), Manganese (Mg), Zinc (Zn), Copper (Cu), Boron (B), and Chloride (Cl), using nitric acid/hydrogen peroxide digestion followed by inductively coupled plasma – optical emission spectroscopy (ICP-OES; Integra XL, GBC, Hampshire IL, USA).

3.2.13 Harvest

All fruit were harvested from the experimental trees on 1st November 2015, approximately 12 months after fruit set. One week before harvest, the total fruit number per plant was counted, and based on the fruit growth measurements, the fruit yield per plant was calculated. At harvest, for analysis of dry matter content, 10 fruit per tree were placed in a plastic bag and carried to the laboratory.

In the laboratory, LDD and fresh weight were measured, and a longitudinal core of flesh (10 mm diameter) was taken from the stem end and the rounded base. The skin and seed were removed from the core, and the remaining flesh weighed and dried at 60 ˚C for 3 days before reweighing. Percent dry matter content was calculated as the ratio of dry weight to fresh weight. Based on percent dry matter content, oil content of each fruit was estimated using the formula provided by Hoffman et al. (2013).

3.2.14 Statistical analysis

Statistical analyses were carried out using the statistical software STATISTICA (StatSoft Inc., Tulsa, OK, USA). For the effects of treatments, differences in WP and g_s were tested with a T-test, and the relationships among variables were tested with regression analysis. Statistical comparisons were considered to be significant when $P<0.05$.

3.3 Results

3.3.1 Soil moisture and soil temperatures

The vertical soil moisture profiles of a mean of two control and two drought plants showed increasing volumetric soil water content with increasing depth. The control had daily fluctuations in soil water content at a depth of 0-30 and 31-60 cm in response to rainfall and irrigation (Figure 3.3a). However, at a depth of 61-90 cm, soil water content fluctuated only in a response to the heavy rainfall events. Throughout the year of observation from October 2014 to September 2015, there was seasonal variation in soil moisture. The soil water content was lower in summer at 0.3-0.20 m^3/m^3 from the surface to 60 cm deep, and 0.35-0.40 m^3/m^3 below 61 cm. In autumn and winter, the soil moisture increased by approximately 20 % to above 0.3 m^3/m^3 at 0-60 cm deep, and to above 0.4 m^3/m^3 at 61-90 cm deep.

The drought treated plants received neither precipitation nor irrigation from October 2014 to April 2015, resulting in a near continuous decline in soil moisture at a depth of 0-30 cm from 0.25 m^3/m^3 to 0.13 m^3/m^3 (Figure 3.3b). However, a slight increase in soil moisture content at a depth of 31-60 and 61-90 cm occurred in late-December after the heavy rainfall, Also, from March, the soil water content became stable at 0.20 m^3/m^3 at a depth of 31-60 cm and 0.25 m^3/m^3 at a depth of

61-90 cm. From May, soil moisture content of the drought treatment increased dramatically after removal of the rainout shelters.

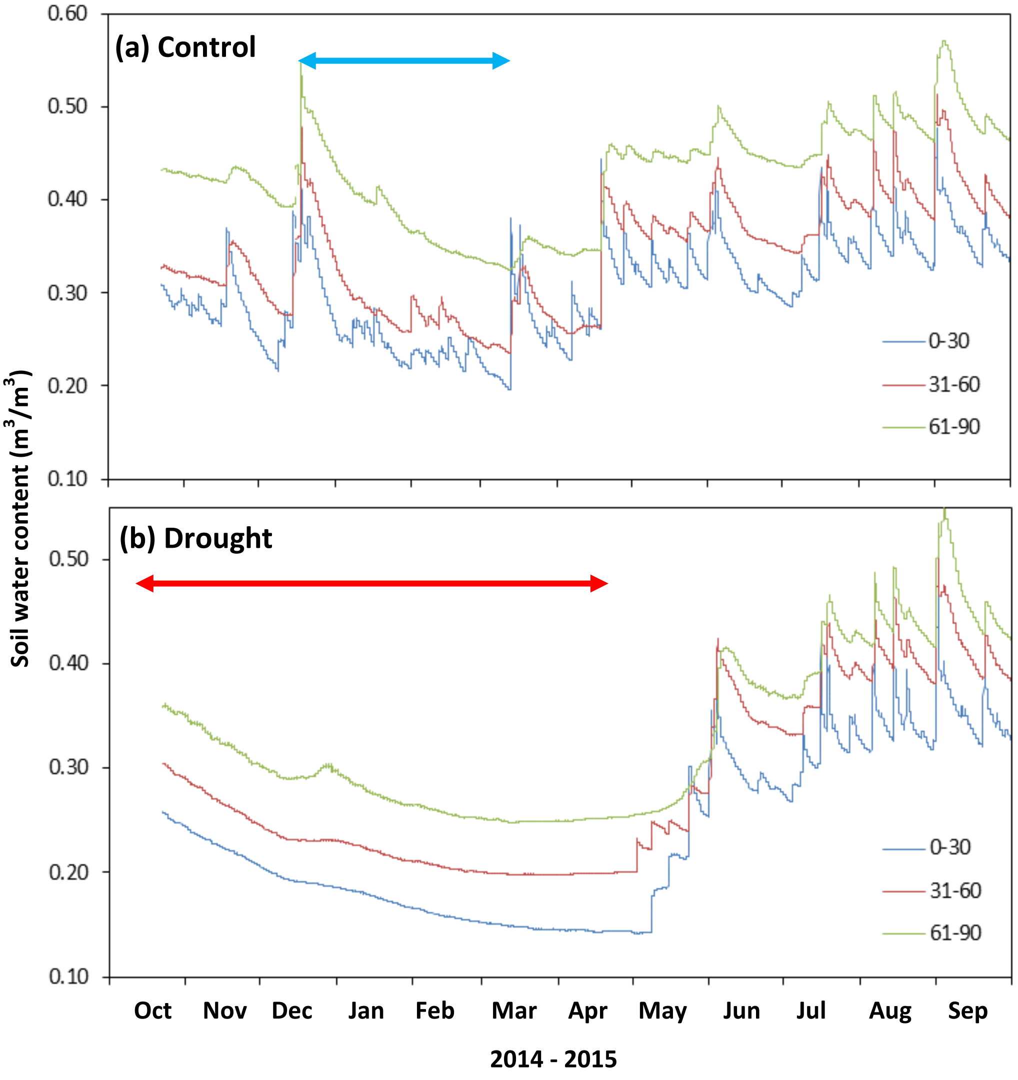


Figure 3. 3 Vertical soil moisture profiles of the means of the two control and two drought treatment trees, observed from October 2014 to September 2015, for three depths of 0-30, 31-60 and 61-90 cm. The blue arrow represents the period of irrigation, and the red arrow represents the period of the rainout treatment.

Additional soil moisture probes, installed under every tree in the top 20 cm from the surface also showed that soil water contents of the control trees were constant at 0.2-0.3 m^3/m^3 throughout the year, with a fluctuation caused by the occurrence of rainfall and irrigation (Figure. 3.4). In contrast, soil moisture of the drought trees declined continuously to between 0.2 m^3/m^3 to 0.15 m^3/m^3 over the rainout experimental period. After removal of the rainout shelters in early May, soil water content of the drought treatment increased significantly. In winter, there was no difference in soil moisture between the two treatments.

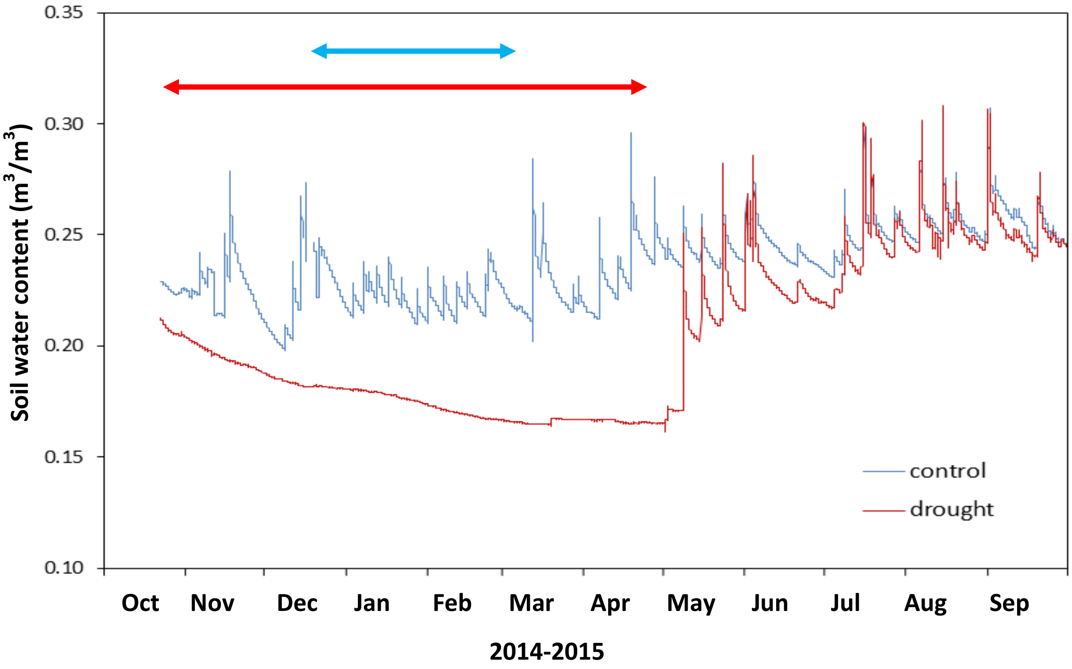


Figure 3. 4 Mean soil water content of the five control and five drought treated plants at a depth of 0-20 cm, measured from October 2014 to September 2015. The blue arrow presents the period of irrigation, and the red arrow presents the period of the rainout treatment.

Soil temperatures at the depth of 10 cm fluctuated from a high of 21 ˚C in January, to a low of 7 ˚C in July (Figure 3.5). There was no difference in soil temperatures between the control and drought treatments throughout the year.

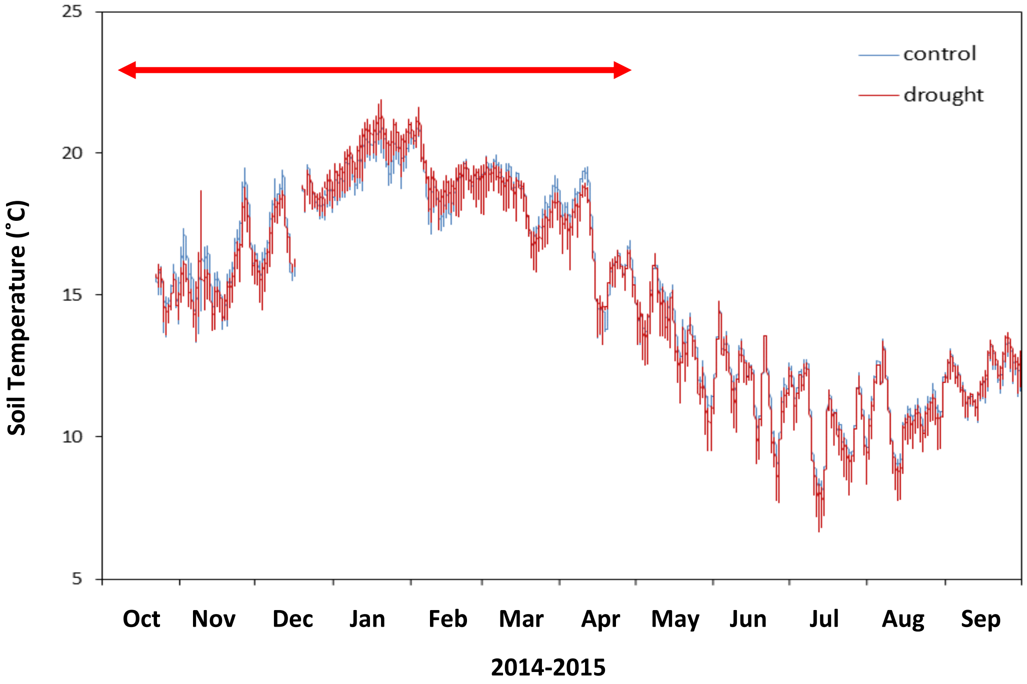


Figure 3. 5 Mean daily soil temperatures of the three control and two drought plants at the depth of 10 cm, measured from October 2014 to September 2015. A red arrow presents a period of the rainout treatment.

Soil water content was evenly distributed around the control tree (Figure 3.6 and 3.7). Under the rainout shelters, soil moisture of two drought plants was lower only surrounding the trees and increased toward the edge of the shelters. The soil moisture content at the edges of the rainout shelters were higher compared to the same locations around the control plants, indicating water moved under the rainout shelters from outside. There were also areas of higher moisture content under the western ends of the rainout shelters.

Spatial Soil moisture (19 Dec 2014)

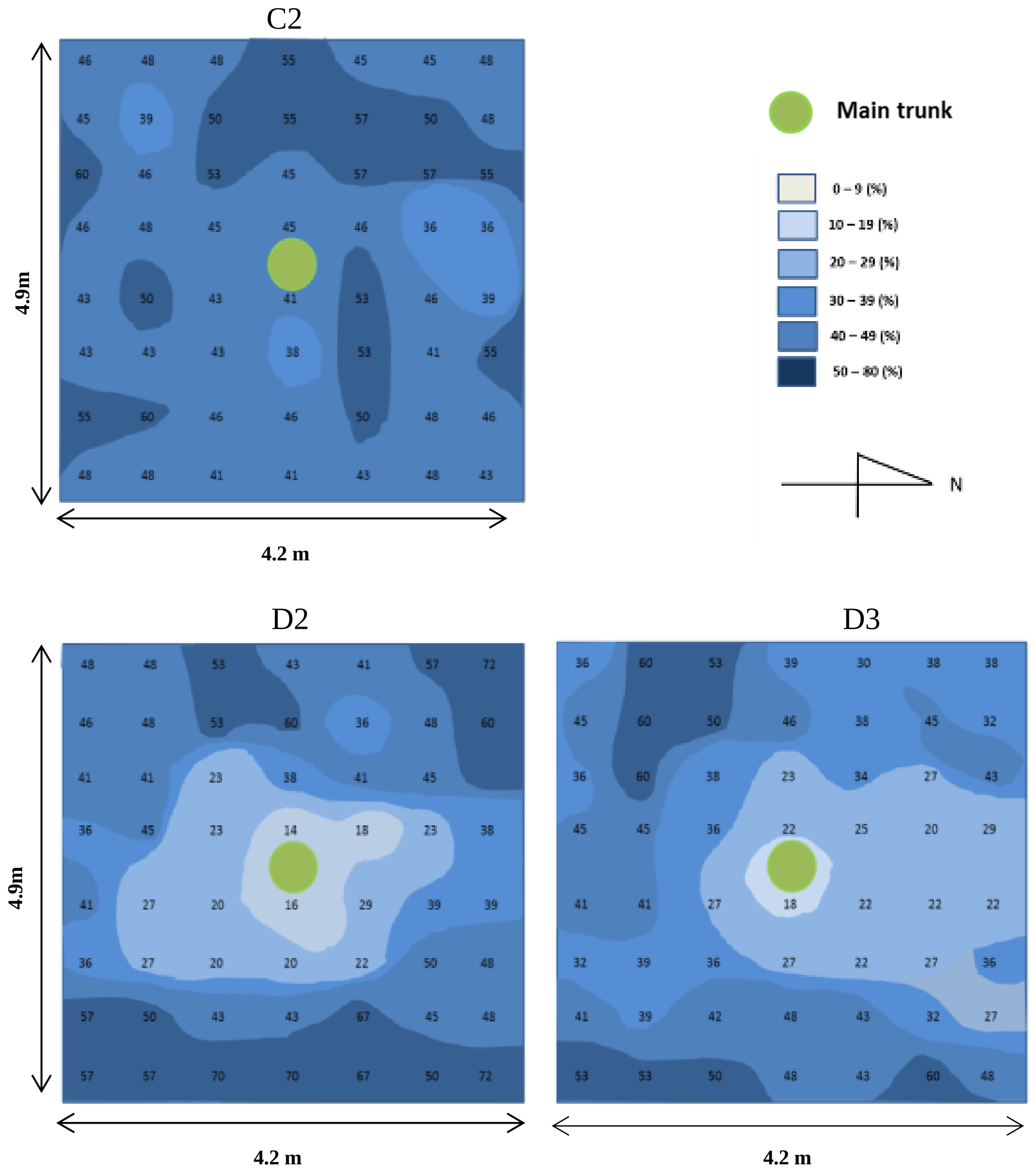


Figure 3. 6 Spatial soil moisture distribution in the top 10 cm of soil of one control (C2) and two drought trees (D2, D3), measured on 19 December 2014 (after heavy rain). The values shown are the actual soil moisture measurements, and the colours have been drawn to approximate zones of similar water content.

Spatial Soil moisture (19 Mar 2015)

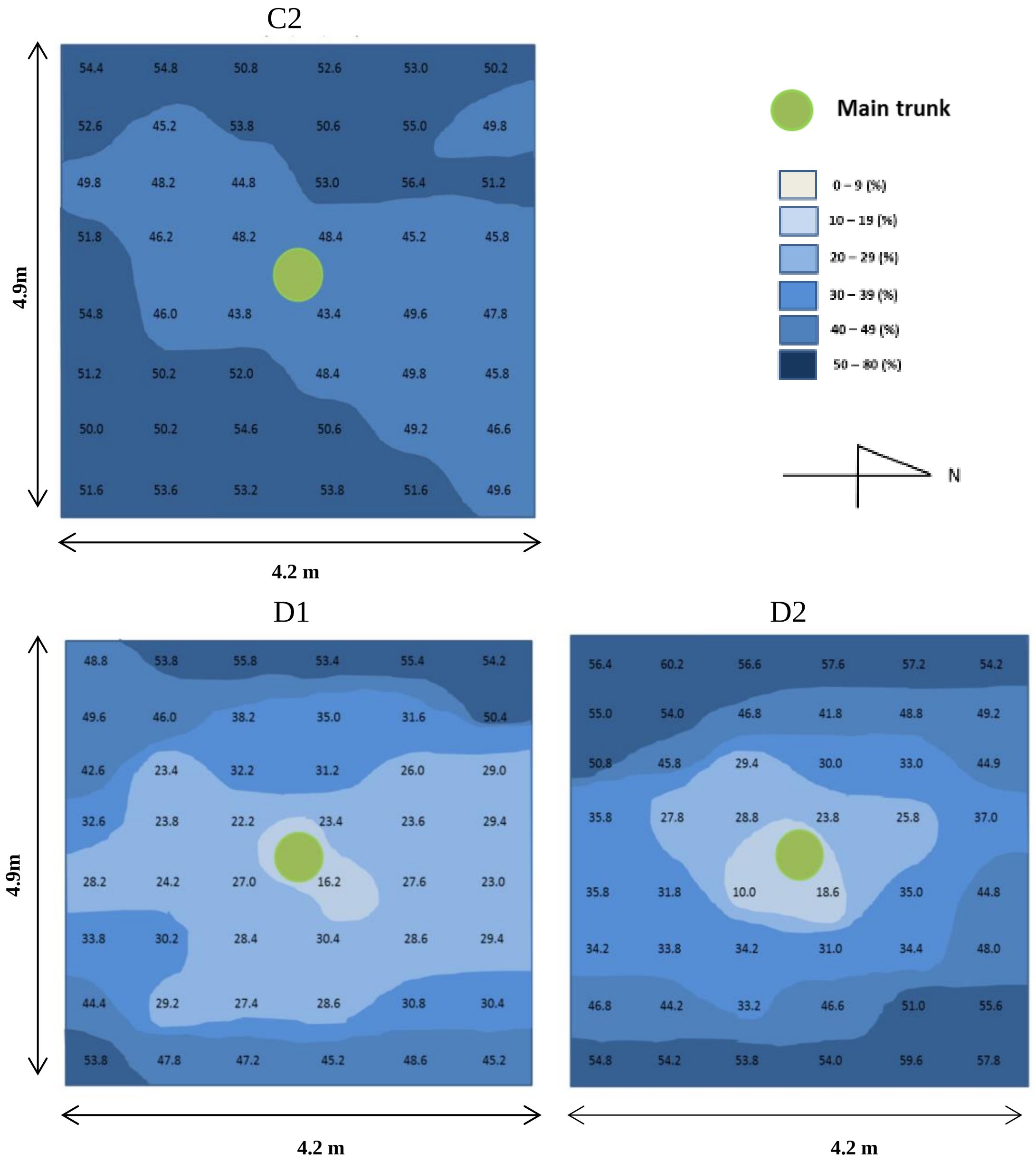


Figure 3. 7 Spatial soil moisture distribution in the top 10 cm of soil of one control (C2) and two drought trees (D1, D2), measured on 19 March 2015 (after heavy rain). The values shown are the actual soil moisture measurements, and the colours have been drawn to approximate zones of similar water content.

3.3.2 Predawn, stem and leaf water potential and stomatal conductance

PLWP was measured from late-October to mid-December 2014, during the main flowering season (Figure 3.8a). Overall, the control PLWP was around -0.05 MPa, and the drought PLWP was consistently lower than the control PLWP by between 0.06 and 0.22 MPa ($P<0.05$).

LWP and SWP were measured from October 2014 to August 2015 (Figure 3.8b,c). As expected, LWP and SWP were more negative in spring and summer, and increased in autumn and winter, and SWP was more consistent than LWP, because SWP was less affected by leaf to leaf variation with the weather.

During the rainout treatment, the drought plants had slightly lower LWP and SWP values, relative to the control plants ($P<0.05$). The difference between the control and the drought LWP was greater in summer, when the drought LWP was mostly below -0.3 MPa while the control LWP fluctuated around -0.25 MPa. On one date in mid-January the drought LWP decreased significantly to -0.43 MPa, while the control was relatively unchanged at -0.26 MPa. Similarly, from November 2014 to February 2015, the drought SWP was slightly more negative than the control SWP, at approximately -0.25 and -0.20 MPa respectively. Regardless of the rainout shelter application, from late-March 2015 the LWP and SWP values of the two treatments increased to above -0.20 MPa ($P>0.05$). From May, after the removal of the rainout shelters, there were no significant differences in LWP and SWP between the two treatments.

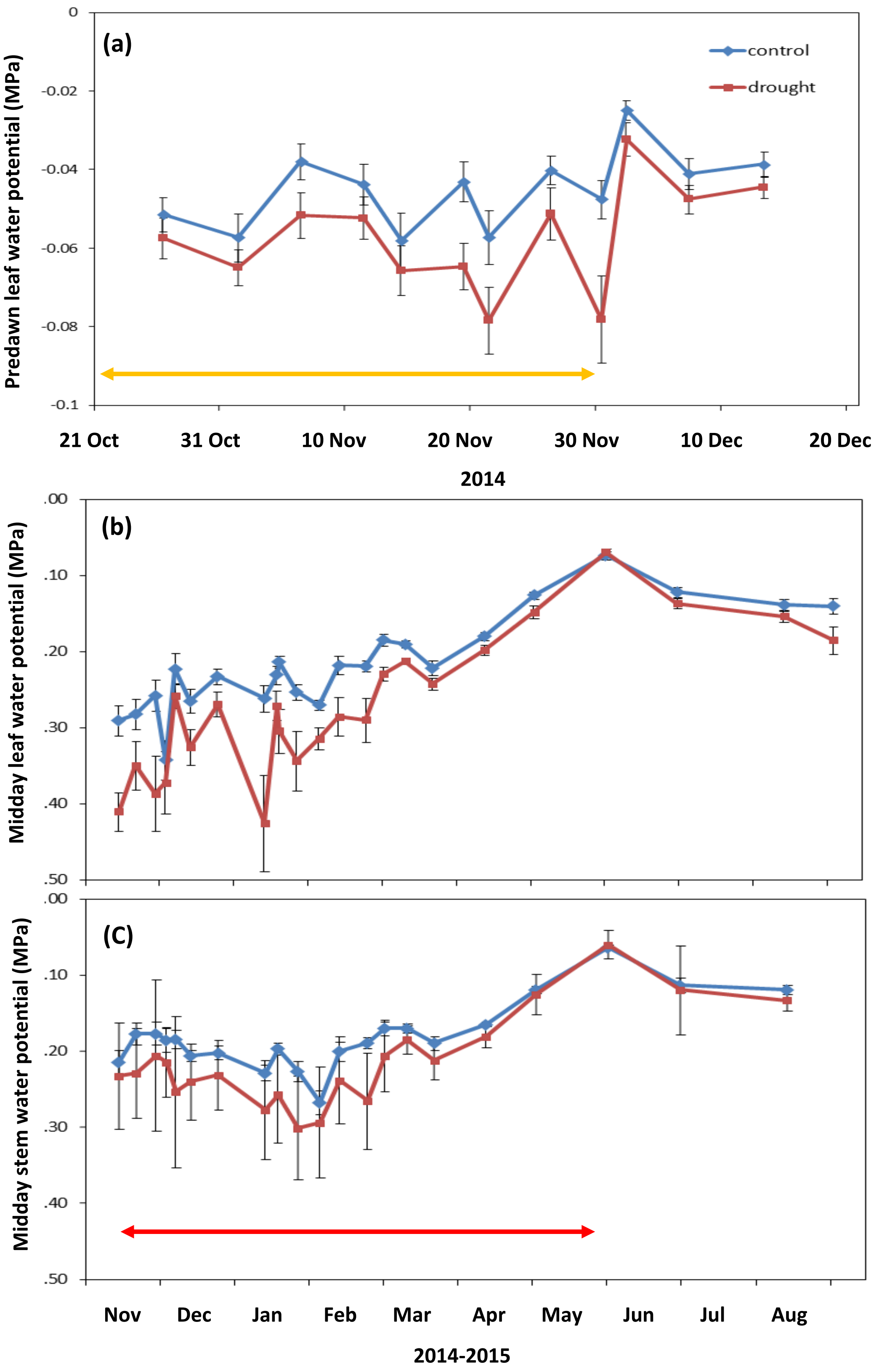


Figure 3. 8 Predawn leaf water potential (PLWP) of the control and drought treatments (n=10), measured over the flowering period from October to December 2014 (a), and midday leaf water potential (LWP) (n=15) (b) and stem water potential (SWP) (n=15) (c), measured from October 2014 to August 2015. The values are averages with standard error bars. An orange arrow presents the flowering season, and a red arrow presents the period of the rainout treatment.

In December 2014, the control treatments had a mean g_s value of 0.223 mol m^{-2} s^{-1} with a mean LWP value of -0.337 MPa, whereas the drought had a g_s of 0.208 mol m^{-2} s^{-1} with LWP of -0.359 MPa. The control had slightly higher g_s and LWP relative to the drought treatment, however, there was no significant difference between the two treatments (P>0.05).

3.3.3 Flower monitoring

Floral intensity was set before treatments were applied and varied from tree to tree (Table 3.1). Flowering was less intense in C3 and C5 compared to the others. Tree C5 produced the least flowers and had a shorter flowering period, however, vigorous vegetative growth was observed. In contrast, the floral intensities of C2, C4, D1, D3 and D4 were high, with D3 producing the most floral shoots.

Table 3. 1 The level of floral intensity that categorized 5 classes; 1(low) to 5 (high).

Treatment	**Control**					**Drought**				
plant no.	**C1**	**C2**	**C3**	**C4**	**C5**	**D1**	**D2**	**D3**	**D4**	**D5**
floral intensity	**3**	**4**	**1**	**4**	**1**	**4**	**2**	**5**	**4**	**3**

The retention rates of flowers marked on 6 different days were observed over 100 days (Figure 3.9). In brief, there was no significant difference in the rate of flower abscission between the control and drought treatments, although the control flowers marked on 9th and 16th November 2014 dropped slightly more than the drought flowers. Overall, only 21 % of marked flowers on control trees, and 23 %

of flowers on drought trees, survived for 16 days after opening as female, and only approximately 2 % of both treatments survived up to 40 days after anthesis. After 100 days of observations, four marked flowers remained on the control trees and one flower on the drought trees.

The timing of daily female flower opening was monitored on three different days in the late flowering season (Figure 3.10). On these days, the duration of female flower opening was approximately 6-7 hours. Under the conditions at the study site on 19, 23, and 28 November 2014, the female flowers started opening in the late-morning, and reached the highest numbers open 2-3 hours after the first open female flower was observed, and then gradually closed in the afternoon. There were not differences in the timing of flower opening between the control and drought treatments.

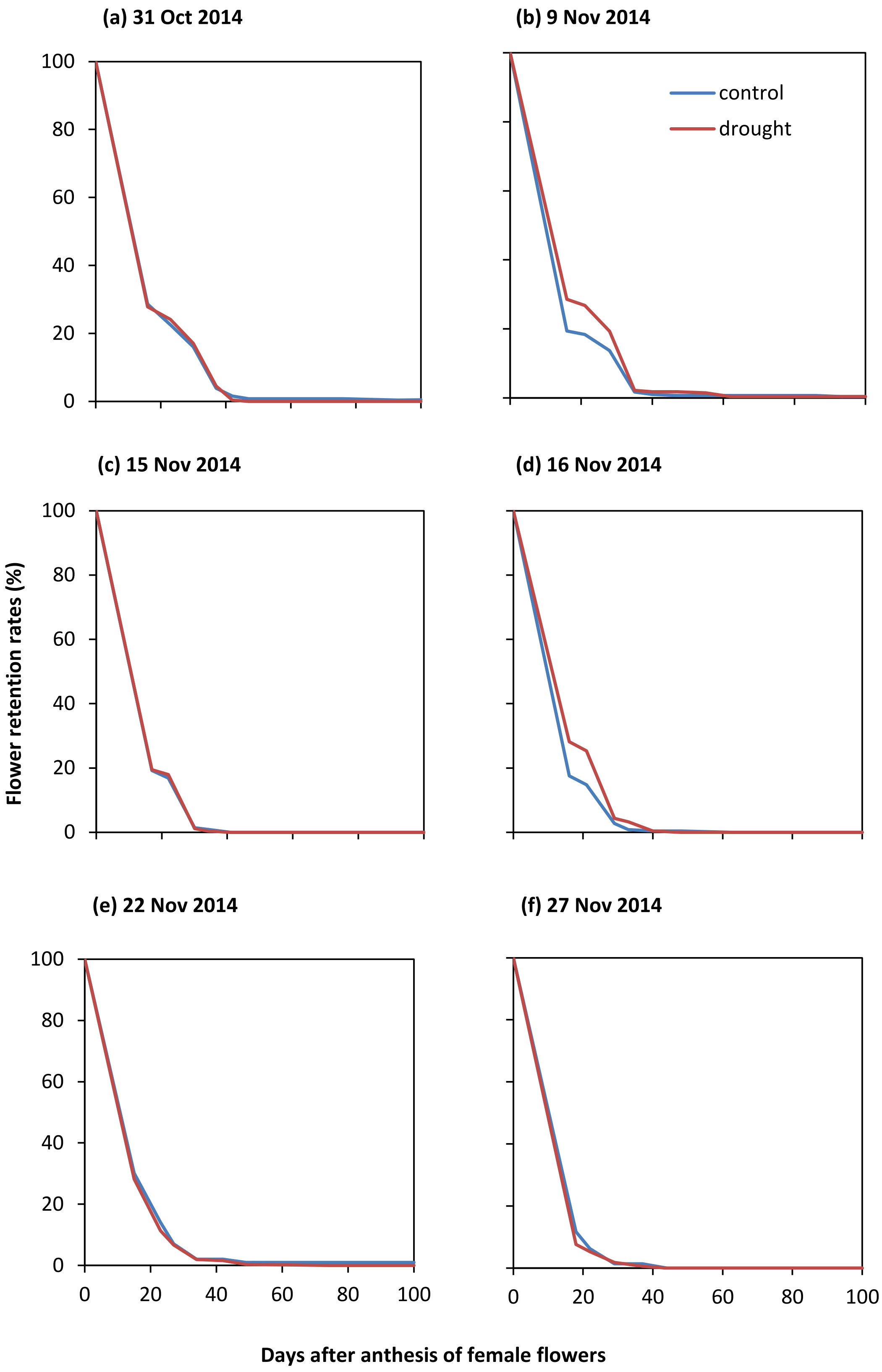


Figure 3. 9 Flower abscission for flowers marked in November 2014. On each starting date, 50 open female flowers per tree were marked (n=250). Marked flowers were recounted each week for 100 days. There was no significant difference in the final proportion of flowers abscised between the control and drought treatments.

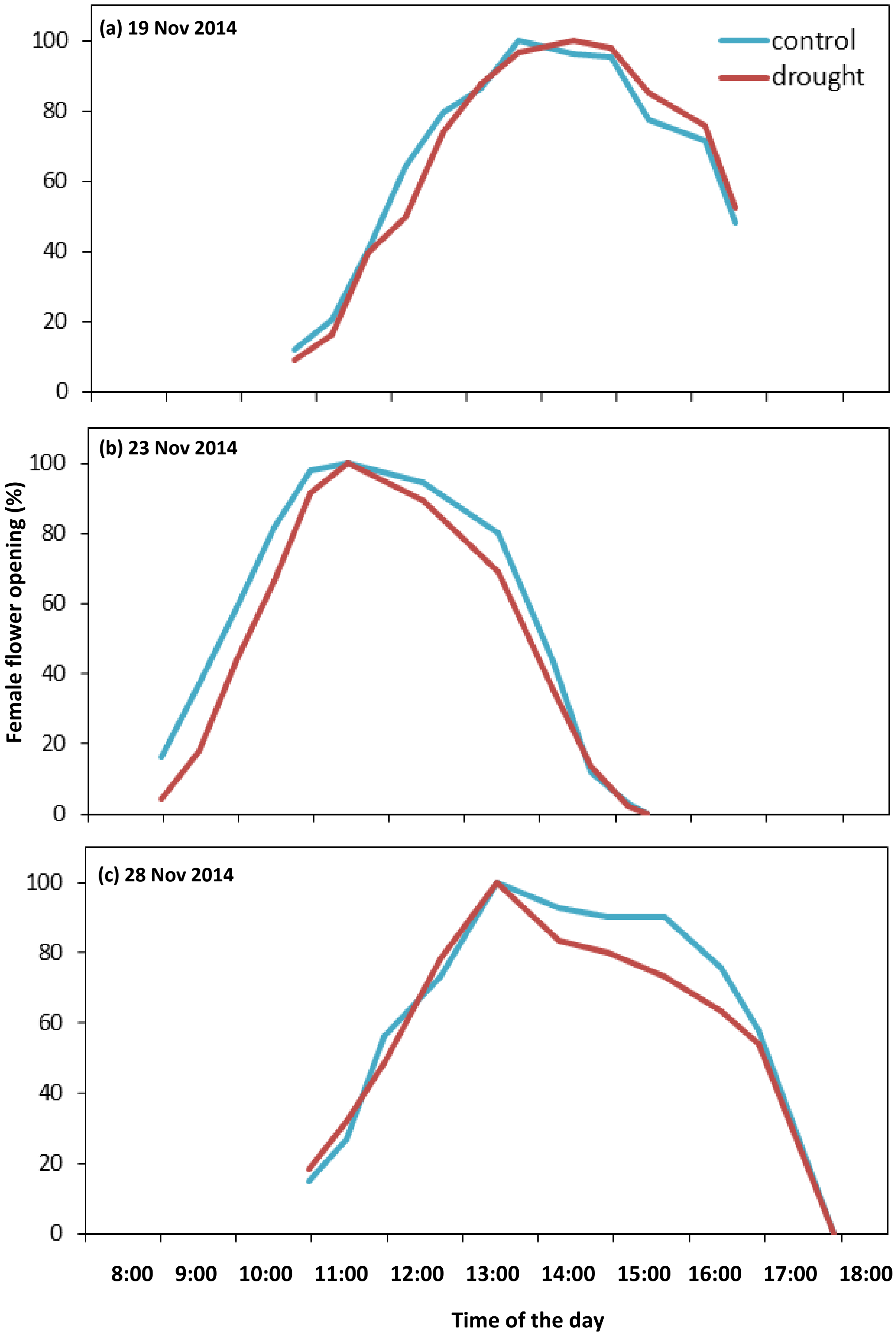


Figure 3. 10 The timing of female flower opening of control and drought treatments, measured on 19 Nov (a) (control n=278, drought n=375), 23 Nov (b) (control n=141, drought n=223) and 28 Nov (c) (control n=41, drought n=60). The numbers were expressed relative to the maximum count of open females for the day.

3.3.4 Fruit monitoring

The fruit grew continuously from January to October 2015 (Figure 3.11a). There were two developmental stages; stage 1: rapid fruit growth, stage 2: slow growth. Although the initial fruit weight of the control and drought treatments were similar at about 30 g in January ($P>0.05$), control fruit increased in size more rapidly, relative to the drought fruit. From late-March to late-October, the drought fruit were 21 % smaller than control fruit ($P<0.05$). There was also a strong relationship between the total number of fruit and individual fruit weight per plant (Figure 3.11b) ($P<0.01$).

Monitoring of fruit abscission was initiated in late-December 2014, approximately one month after the end of the flowering period (Figure 3.12a). Both treatments had high abscission rates in January and February 2015. On average, about 60 % of the fruit marked in December had abscised by late-January, and another 10 % abscised in February. From March fruit abscission became negligible regardless of the treatments, and until July the fruit retention rates of the control and drought treatments were stable at 19 % and 22 %, respectively ($P>0.05$). Relative to the other experimental plants, two of the control plants had significantly less flowers, and consequently had set less fruit, but these plants had higher fruit retention rates (50 %), contributing to the higher fruit retention rates of the control treatment.

Significant fruit abscission occurred in July in response to a severe frost, with larger fruit loss from the drought treated trees. Plants with a higher crop load were more severely impacted by frost, and the three trees with the highest crop loads and greatest loss of fruit in response to frost belonged to the drought treatment (Figure 3.12b). At harvest in early-November, the final retention rates of

December marked fruit in the control and drought treatments were 15 % and 5 %, respectively.

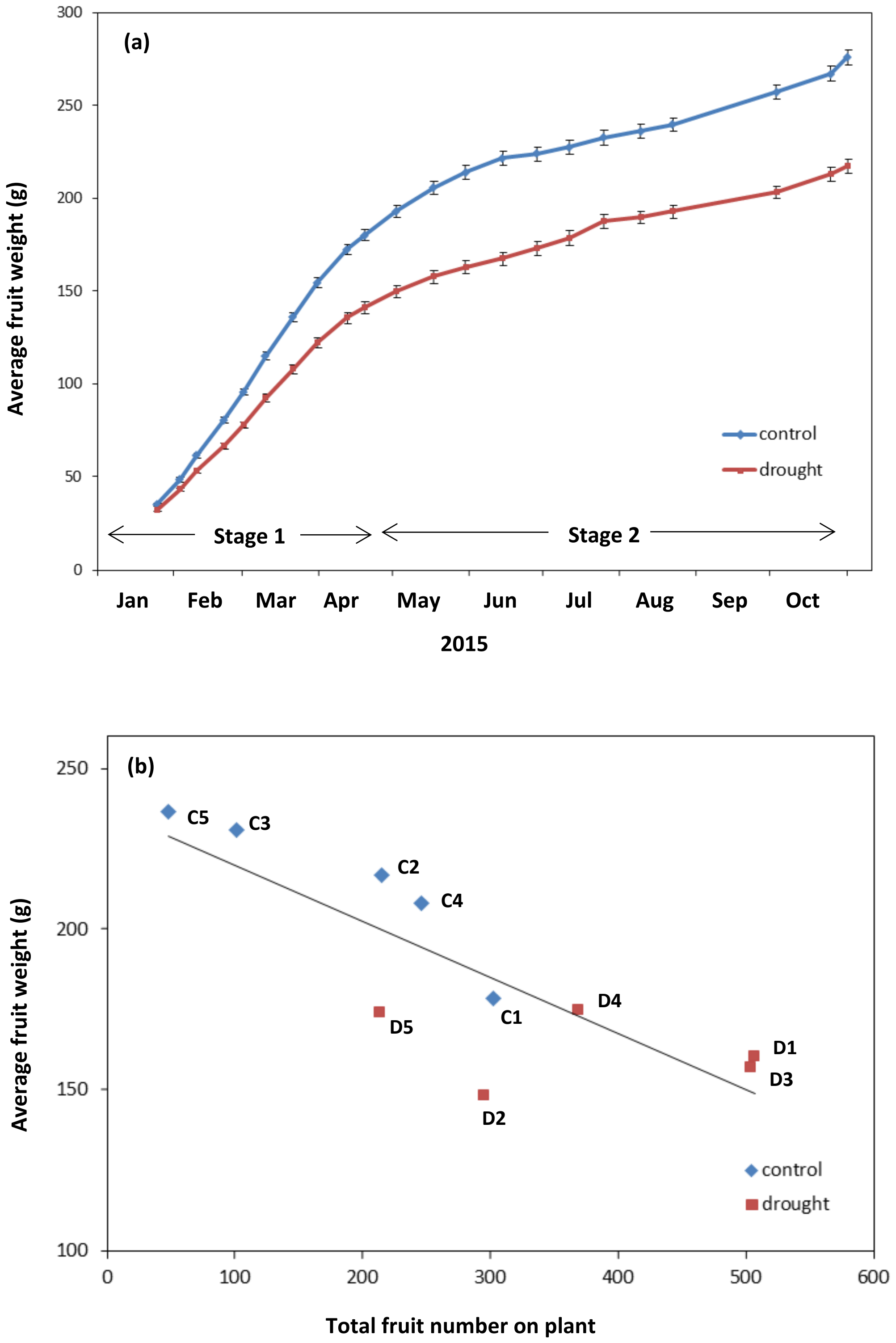


Figure 3. 11 Average fruit weight (g) from non-destructive measurements, with standard error bars (n=150) (a) and the relationship between total fruit number on the plants and fruit weight (g), measured on 24 May 2015, after fruit abscission became negligible and before the frost event (Average fruit weight = -0.1736 × total fruit number + 237.25, R^2=0.68, P<0.01).

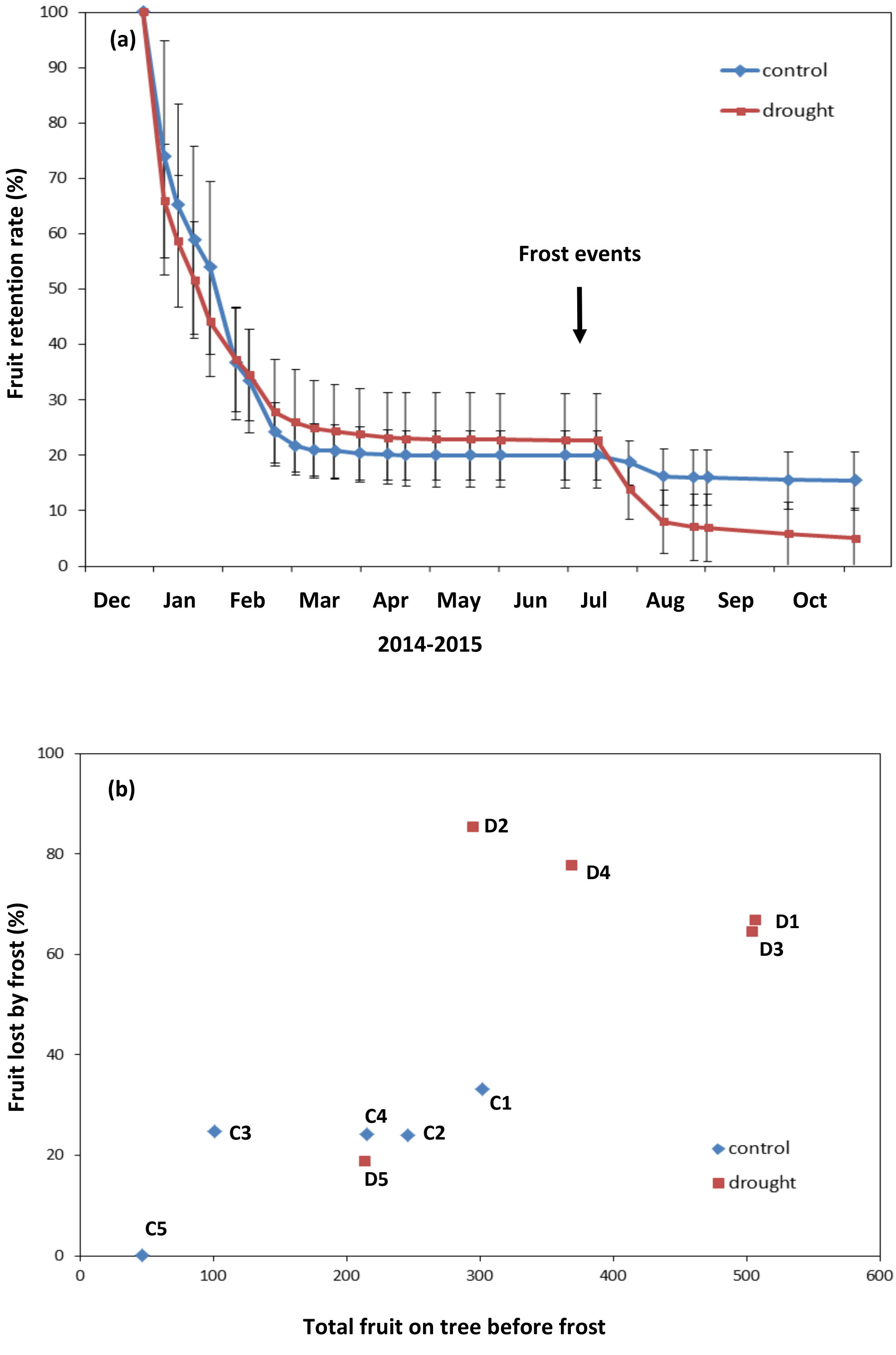


Figure 3. 12 The fruit retention rates of five control and five drought trees (control n=761, drought n=1024) with standard error bars, measured from late-December 2014 to late-October 2015 (a), and the relationship between total fruit on the tree before the frost and the percentage of fruit lost to frost (b).

3.3.5 Leaf analysis

According to the analysis of leaf samples, the drought trees had lower nutrient status than the control trees (Table 3.2). Except for Ca and Mg and Cl, the nutrient status of leaves from drought trees was lower than that of the control trees. In particular, the differences in concentrations of N, P, K and B between the control and drought treatments were statistically significant (P<0.05), even though N and P values of the drought plants were within an appropriate range. The most significant effect was on the concentration of K in the drought plants, which exhibited below a medium range of 0.9-1.2 %. No significant effect on the Cl concentrations was observed, as the Cl concentrations of all experimental plants were within the normal range reported for avocado (0 and 0.25 %, P>0.05).

Table 3. 2 Nutrient analysis of leaf samples that were taken on 22 April 2015. The values are an average of five control and five drought trees (n=50) ± S.E. of means. A percentage of Nitrogen(N), Phosphorus(P), Potassium(K), Sulphur(S), Calcium(Ca), Magnesium(Mg), Sodium(Na), Chloride(Cl), and the average weight (mg/kg) of Iron(Fe), Manganese(Mg), Zinc(Zn), Copper(Cu), Boron(B) are shown. * P<0.05, ** P<0.01

Nutrient	Medium range levels for NZ avocado	control	drought	P value (T-test)
N (%)	2.4-2.9	**2.78 ± 0.102**	**2.46 ± 0.103**	*
P (%)	0.12-0.18	**0.164 ± 0.007**	**0.142 ± 0.007**	*
K (%)	0.9-1.2	**1.02 ± 0.049**	**0.86 ± 0.024**	**
S (%)	0.2-0.3	0.246 ± 0.010	0.232 ± 0.012	
ca (%)	1.2-2.0	1.228 ± 0.057	1.41 ± 0.156	
Mg (%)	0.3-0.55	0.286 ± 0.014	0.316 ± 0.019	
Na (%)	0-0.25	0.0028 ± 0.0002	0.0024 ± 0.0002	
Fe (mg/kg)	40-100	53.4 ± 7.65	41 ± 3.56	
Mn (mg/kg)	80-300	151.4 ± 17.28	127.6 ± 16.5	
Zn (mg/kg)	25-50	25.6 ± 0.75	23.6 ± 2.20	
Cu (mg/kg)	5-15	81.8 ± 8.06	72 ± 4.30	
B (mg/kg)	30-50	**26.6 ± 2.56**	**16.2 ± 0.37**	**
Cl (%)	0-0.25	0.058 ±0.004	0.064 ± 0.012	

3.3.6 Fruit yield and dry matter contents

Fruit were harvested on 01 November 2015 (Table 3.3). The total number of fruit was the same in the two treatments, as the control had 135 fruit/plant whereas the drought had 129 fruit/plant. However, the control had larger fruit on average, relative to the drought treatment, thus, the average yield of the control was approximately 24 % higher than the yield of the drought treatment, at 36.4 (S.E.±1.1) and 27.8 (S.E.±1.0) kg/plant, respectively ($P<0.05$). The dry matter and predicted oil content of the fruit from control trees were 7 % higher than that of the drought treatment ($P<0.05$).

Table 3. 3 The total fruit number, average fruit weight and estimated yield per treatment plant at harvest on 1 November, 2015 (the mean with standard error). Fruit weight n=30, dry matter content n=10.

Treatment	Plant no.	Fruit count	Fruit weight (g)	Yield (kg)	Dry matter (%)	Oil content (%)
Control	C1	202	253.9 ± 6.8	51.3 ± 1.4	29.2 ± 0.8	18.4 ± 0.8
	C2	187	264.9 ± 7.6	49.5 ± 1.4	30.4 ± 0.5	19.5 ± 0.4
	C3	76	289.3 ± 9.5	22.0 ± 0.7	31.7 ± 0.3	20.7 ± 0.3
	C4	163	277.9 ± 8.9	45.3 ± 1.5	29.6 ± 0.8	18.7 ± 0.7
	C5	47	294.1 ± 11.8	13.8 ± 0.6	31.0 ± 0.7	20.0 ± 0.6
	average	**135**	**276.0 ± 7.5**	**36.4 ± 1.1**	**30.4 ± 0.3**	**19.5 ± 0.3**
Drought	D1	168	191.5 ± 6.2	32.2 ± 1.0	26.3 ± 0.5	15.6 ± 0.5
	D2	43	214.0 ± 7.0	9.2 ± 0.3	28.3 ± 0.6	17.6 ± 0.6
	D3	179	211.3 ± 8.7	37.8 ± 1.6	25.2 ± 0.8	14.7 ± 0.8
	D4	82	242.7 ± 7.2	19.9 ± 0.6	28.9 ± 0.7	18.1 ± 0.7
	D5	174	229.6 ± 9.2	39.9 ± 1.6	32.6 ± 0.7	21.5 ± 0.6
	average	**129**	**217.8 ± 8.7**	**27.8 ± 1.0**	**28.3 ± 0.5**	**17.5 ± 0.4**

3.4 Discussion

The rain out shelters did not completely prevent wetting of the soil, but did have some impact upon tree yield. Overall, the evidence suggests that flowering of avocado is not particularly sensitive to water stress, or the timing of flowering makes water stress during flowering unlikely under New Zealand growing conditions. However, fruit growth was very sensitive to the imposed stress. Rainfall and irrigation exclusion resulted in slower fruit growth and reduced leaf nutrient content. Lower fruit yield in the drought plants was caused by a combination of fruit size reduction and a higher fruit abscission rate caused by frost.

3.4.1 Soil moisture and water stress on plants

Despite over 6 months of rain/irrigation exclusion by the rainout treatment (mid-October 2014 to late-April 2015), vertical soil moisture measurements showed soil moisture only declined dramatically up to a depth of 31 cm. Also, spatial soil moisture measurements in the top 10 cm showed high soil water content where leaks were present and at the edge of the rainout shelters. Presumably, the drought trees were able to obtain some water from deeper or surrounding soil.

Hass avocado is known to have a relatively shallow root system with few or no root hairs (Michelakis et al. 1993; Chanderbali et al. 2013). Most roots of mature avocado are concentrated in the soil from the surface to 120-140 cm deep with a highest root density at a depth of 20-60 cm, and the horizontal root extension from a tree trunk is generally 250-300 cm, depending on soil types (Salgado & Cautin 2008; Carr 2013). Young (ca. 3 years) trees were examined in this research, therefore, the root system of these trees was expected to be shallower and narrower than that of mature trees. However, at the end of the rainout treatment, the presence of some roots was identified beyond the rainout shelters. In general, a flush of root growth occurs in early summer and autumn (Whiley 2013). There is a possibility that the drought plants developed new roots and were withdrawing water from the soils at deeper layers or nearer to the edges of the rainout shelters.

During the rainout treatment, the drought plants had slightly lower water potentials (PLWP, LWP and SWP) than the control plants had, indicating that the drought plants experienced moderate water stress. The PLWP measurements showed some fluctuations, associated with the weather conditions at the site rather than changes in soil water stress levels. For example, on 02 December 2014, control and drought leaves had a higher mean PLWP value, compared with the values on other days. On this day, overnight minimum air temperature was lower, decreasing below 4.5 ˚C, causing leaves to be wet with dew in the early morning.

LWP also corresponded with temperature. Over the observation period, the drought plants had the lowest LWP value of -0.425 MPa on 12 January 2015, the hottest day of the summer, with the maximum air temperature reaching over 30 ˚C during measurement. A decline in WP of the drought plants may have been observed if the weather had stayed hot for few more days.

Some previous studies have observed a decrease in g_s in avocado as a response to water deficit (Carr 2013). For example, Bower (1978) and Scholefield et al. (1980) reported a decline in g_s when SWP became -0.4 MPa, and stomata closed when SWP was -1.0 to -1.2 MPa. In this experiment, the g_s measurement was carried out on a sunny day in mid-December 2014, approximately two months after the rainout shelters were applied. g_s was not significantly different between the two treatments because the rainout treatment had not yet caused considerable water stress.

3.4.2 Floral observation

Temperature is known to influence avocado flower opening behaviour (Salazar-Garcia et al. 2013), but the effects of water stress on the daily flowering process have not been described. No difference in the floral opening process between the control and drought treatments was detected in this study, and it is concluded that the soil had not yet become dry enough to have much impact.

There was also no significant difference between control and drought flower abscission rates. In total, 1382 control flowers and 1515 drought flowers were monitored over the flowering season, and only one control flower developed into a mature fruit at harvest, while none of the drought flowers survived as fruit until harvest. The high flower abscission rate and tree to tree variation of avocado makes detection of treatment effects difficult without monitoring an even larger number of flowers.

In this experiment, starch content in pistils was not analysed, because the drought trees had experienced only moderate water stress, and there were no effects of the treatment on any other aspects of flowering. There might be a positive correlation between flower survival rates and starch content in pistils, as large amounts of carbohydrates are required for flowering and fruit set (Alcaraz et al. 2010; 2013). If the drought plants had been experiencing severe water stress, decreasing starch accumulation may have caused an increase in the occurrence of flower abscission, caused by reduction of photosynthetic activities and/or an impact on phloem transport.

3.4.3 Fruit monitoring

The difference in fruit size between the control and drought treatments appeared during early fruit development. By the time the rainout shelters were removed in early May the fruit growth rate was similar in the two treatments. However, the drought plants had significantly smaller fruit at harvest. The results suggested that water status during early fruit development influences final fruit size and yield, and that alleviation of water stress later in fruit growth does not result in compensatory increases in fruit growth in the stressed plants.

Many fruit, such as grape, apple, and pears, exhibit a double sigmoid curve with three distinct phases during fruit development (e.g. Matthews et al. 1987). Unlike these fruit, avocado showed a single sigmoid growth pattern with two different phases (Zilkah & Klein 1987; Silber et al. 2012). In the first phase, fruit shows rapid growth after fruit set with high sugar accumulation. In the second phase, fruit growth becomes slower and oil accumulation increases instead of sugar accumulation (Liu et al. 1999). Throughout the two fruit developmental stages, avocado fruit increase in size as a result of the continuous occurrence of cell division (Schroeder 1958; Cowan et al. 2001).

There are some studies that have defined the relationship between fruit size and irrigation levels. For instance, Adato & Levinson (1988) found dry treatment plants had smaller fruit by 13 %, compared with wet treatment plants. In the experiment described in this thesis, fruit grew rapidly until March (25-30 weeks after fruit set), then grew more slowly until harvest in November, and this means the drought plants had water stress in the first phase of fruit development. Water status in the first phase may be most important (Hoffman & Plessis 1999). Slowing fruit growth during the first phase was reported by Bower (1985), explaining it was a result of Ca uptake reduction caused by water stress. Even though avocado exhibits cell division throughout fruit development, its cell division occurs most rapidly during the first phase, and cell division required Ca uptake (Barmore 1976). It is possible that the rainout treatment restricted the Ca uptake during a critical period of fruit growth.

There is a relationship between fruit weight and crop load, such that fruit size decreases with an increase in crop load (Michelakis et al. 1993; Salazar-Garcia et al. 2013). In this experiment, the drought plants had a heavier crop than the control plants, and a significant negative correlation between fruit weight and crop load was observed. However, there was some overlap between treatments, with trees carrying the same crop load (e.g. C1 and D2), and the drought plants with a light crop still had smaller fruit size relative to the control plants.

The results of this study showed higher fruit abscission rates in the drought plants, relative to the control plants. In general, the occurrence of fruit abscission in avocado is most intense within the first three months after the end of the flowering season (Wolstenholme et al. 1990; Lahav & Zamet 1999; Garner & Lavatt, 2008). In this experiment, high fruit abscission was observed until mid-February 2015, so these results were consistent with previous findings.

There was a second peak of fruit abscission in July and August due to extreme low temperatures below 0 ˚C on some days (see Chapter 2), and significant fruit abscission started two-three weeks after the first frost event. Fruit loss was much more significant in the drought treated trees. It could be because of a combination of pre-existing differences in flower and crop load, and the treatment effects on nutrient status, shoot vigour and carbohydrate levels. Although the plants with a heavy crop load had higher fruit abscission, treatment effects cannot be eliminated because fruit abscission in response to frost was much higher in drought treated trees, even when trees with similar crop loads were compared (Figure. 3.13b).

In this experiment, there were negative correlations between fruit loss by the frost events and the N, P, and B concentrations ($P<0.05$). Thus, lower nutrient status on trees possibly resulted in severe frost damage. The low nutrient status may have resulted from the rainout treatment effects, drying the topsoil root zone and reducing nutrient uptake through early fruit development. In addition, the drought plants probably had lower carbohydrate levels due to the reduced vegetative growth and photosynthesis caused by the rainout treatment. Lower temperature below 0 ˚C is a rare case at the study site, therefore, the second peak of fruit abscission was unexpected. However, avocado is known to be sensitive to low temperatures, and winter temperatures may accelerate fruit abscission (Lahav et al. 2013).

There was no significant difference in the total number of fruit per tree between the two treatments at harvest. If the severe frost event had not occurred the drought treatment would have had a higher yield of smaller fruit than the control treatment. Tree to tree variation, typical of avocado and caused by variation in floral intensity and total fruit number per tree, makes it difficult to determine the actual loss of yield caused by the drought treatment.

Maturity can be examined by the percentage of dry matter content, because dry matter is highly correlated with oil content (Morris & O’Brien 1980; Lee et al. 1983; Ozdemir & Topuz 2004; Hoffman et al. 2013). Dry matter content of mature avocado fruit should be above 24 % for the New Zealand market (Burdon et al. 2013). The results of this research demonstrated that the mean dry matter contents of both treatments was higher than 24 %. Therefore, both treatments reached maturity, although the control had higher dry matter content than the drought plants.

3.4.4 Vegetative growth

This study focused on flower and fruit development, and vegetative growth was not measured. However, there was a clear visual difference in vegetative growth between the two treatments. At the end of the rainout experiment in late-April 2015, the control plants had more new shoot growth, longer shoots and larger leaves, relative to the drought plants. Certainly, the drought treatment restricted vegetative growth on the drought plants.

If moderate water stress caused shoot growth to be slower, there might be two possible reasons. One possibility is that resource was allocated toward fruit growth, resulting in less resource availability for vegetative growth. Hsiao et al. (1976) reported that greater resources would be moved to the fruit when plants have water stress and experience resource limitation. Avocado fruit in particular contain high oil content, so high energy is required for fruit development (Liu et al. 1999).

Another possible reason is that the drought plants invested more in root development rather than shoot development. On avocado, shoot flushes alternate with root flushes, and vegetative shoot flush occurs after the flowering period when root growth is inactive (Scholefield et al. 1985; Mickelbart et al. 2012b). Also, plants develop more roots when they have water stress (Carr 2013). This rainout experiment showed the upper soil of the drought plants was dried out, which could have caused an increase in root biomass, resulting in lower growth of other parts.

There is evidence that water stress has resulted in reduction of plant growth, and consequently crop yields, examined in Chile (Celedon et al 2012), Israel (Lahav and Kalmar 1977b) and Greece (Michelakis et al 1993; Chartzoulakis et al 2002). In this study, the control trees were receiving irrigation and fertilizer while the drought trees were not. Nutrient uptake will have been affected by the exclusion of fertilizer, but the drought treatment would also have affected nutrient uptake because it makes nutrients less mobile. The shoot growth reduction in droughted plants observed in this study was likely caused by drought stress effects on photosynthetic resource allocation and nutrient availability in the soil. However, due to the application of fertilizer to control plants only, it cannot be dismissed that nutrient levels in the soil also affected vegetative growth.

3.4.5 Conclusions

In conclusion, this research demonstrated that water deficit in summer significantly affected fruit yield and quality, even though no obvious effects were observed during the flowering season. Increasing fruit yield was due to the larger size of fruit. In this experiment, the rainout shelters did not completely eliminate water supply. To impose water stress on drought plants more dramatically, the rainout treatment could be modified by ensuring gaps are closed and by installing plastic skirts in trenches on the edges of the shelters. Fruit monitoring may be best in the field, but the influence of water stress on flowering should be studied with plants in pots with more control over conditions as a short term water stress.

Furthermore, due to tree to tree variation, there was difficulty in determining the effects of water stress on fruit yield. To overcome this problem, the number of trees included in the trial should be increased.

Chapter 4: Discussion and Conclusions

4.1 Final discussion

This thesis has demonstrated water requirements of avocado under the New Zealand weather conditions. It quantified actual water use of mature and young avocado plants in Katikati, Bay of Plenty, and presented the effects of water stress during the period from flowering to early fruit development. The findings of the two objectives were to quantify seasonal variation in the crop factor (K_c) for avocado in New Zealand, and that water stress during early fruit development influenced final fruit yield at harvest.

Based on local weather monitoring and sap flow measurements over the year, monthly ET_c and K_c were provided. According to the calculation, ET_c increased by 8-10 % in spring, probably as a result of increasing canopy surface area caused by blooming (Chapter 2). However, the rainout experiment showed that the avocado plants did not show any signs of water stress in spring because of high soil water content caused by frequent rainfalls in winter (Chapter 3). In contrast, in summer, the water use of avocado was the highest (Chapter 2), soil water decreased in response to rainfall exclusion, and the drought plants had significantly lower LWP and SWP values relative to the control plants (Chapter 3).

Under New Zealand conditions, irrigation is thought to be important from October to March. From the results of this study, I conclude that water supply is not a limiting factor in spring, thus, additional water may not be necessary during the flowering season in Katikati, Bay of Plenty. However, irrigation is probably required in summer to increase soil water content in the orchard, especially in January when air temperature and plant transpiration are high. Without irrigation avocado plants may experience water stress on hot days.

4.1.1 Avocado water use under the New Zealand weather conditions

Among avocado growing areas within the world, New Zealand is the coolest country. For example, California and Israel have a mean annual temperature of 16-18 ˚C, whereas in northern New Zealand the mean annual temperature is about 14 ˚C (Wolstenholme 2013a). The New Zealand summer is moderate and maximum daily temperatures are rarely over 30 ˚C. Although lower temperature causes a slower fruit growth rate over a year, it also reduces the potential for water deficits under the New Zealand climatic conditions, if summer daily maximum temperatures are about normal.

Annual rainfall in the Bay of Plenty is moderate but often unevenly distributed with a drier summer and wetter winter, and this seasonal variation was observed in this experiment. This study measured actual water use of the mature and young avocado plants and provided data that can be used as the basis for irrigation recommendations for avocado in the Bay of Plenty. K_c can be used to estimate irrigation requirements in other orchards, using web based applications and standard meteorological data. However, it should be noted that the irrigation schedule depends on some factors such as soil type, topography and plant age and physiological characteristics. Careful management should be paid to the amount of water applied to avocado trees.

K_c also depends on local weather conditions, while other avocado growing regions in New Zealand may have different weather conditions from Bay of Plenty. Relative to the weather in Bay of Plenty, Gisborne has a drier climate with an annual average rainfall of less than 1000 mm. Northland has slightly higher air temperature throughout the year (Niwa 2015). The ET_c and K_c presented here would be applicable only to orchards in Bay of Plenty.

4.1.2 Water stress in avocado

In the rainout experiment, it was difficult to completely eliminate water supply to the drought plants, because of water movement from gaps in the rainout shelters, and from outside the shelters via the soil. Even though it may be possible to improve the rainout treatment by ensuring gaps are closed and by installing plastic skirts in trenches on the edges of the shelters, water could still move sideways within the deeper soil layers. Unless using lysimeters or large, moveable overhead shelters, complete water exclusion in the field is probably unrealistic.

From the observations, there is frequent rainfall in winter. According to weather data, ET_o is approximately 1.3 mm per day in August, and 2.0 mm in September. K_c of a young avocado plant was 0.2-0.3. This suggests that water requirements will be 1 to 1.5 mm per day in August and September. Based on the on-going soil moisture measurements, in winter, the soil water content of 0-100 cm depth is high at 0.4 m^3/m^3. If approximately half of this water is available, this means there is a reservoir of around 200 mm available to the trees prior to flowering.

Therefore, water stress is not likely to be a problem during the flowering season at the study site, and rainfall exclusion would have to occur for a long period prior to flowering if an effective drought treatment were to be imposed.

However, according to the results of plant water potential and fruit monitoring, the negative effects of the rainout treatment did appear in summer, when air temperatures increased and there was significantly less rainfall. The impact of water deficit during early fruit development was significant, as it affected the final fruit yield in November 2015.

This study was one year of observation, but the short term water deficit may cause longer term effects in avocado. Firstly, the rainout treatment eliminated water supply from the top soil of the drought plants over 6 months, and it may have reduced root growth or damaged the root system. Because the most active avocado feeder roots are found in the upper layer of soil (Lahav et al. 2013), changing root density in the top soil may affect nutrient uptake. Secondly, a visual reduction in shoot growth of the drought plants was observed in this experiment. This restricted whole plant growth, resulting in a significant reduction in new leaf development. Reduction of leaf area may decrease photosynthetic activity, and it may also have contributed to the increased vulnerability of the drought treated trees to frost. For these reasons the drought plants may show further treatment effects on yield in the following year.

4.2 Conclusions and recommendations

In conclusion, this study has investigated actual avocado water use with the local weather conditions in New Zealand, and the effects of water deficit during a period from flowering to early fruit development. Avocado plant water use was maximum in January and adequate rainfall and irrigation clearly provided positive benefits in terms of fruit size and quality.

In this research, the rainout experiment imposed water stress on the drought plants by eliminating irrigation and precipitation. In reality, plants receive precipitation but irrigation depends on a management scheme. Investigating the levels of irrigation may be more important than rain water exclusion. The effect of excess water on avocado was not considered, but there is a possibility of excess soil water from irrigation application during flowering, on top of already high water content in winter and spring, Over-irrigation will affect the reproductive process because inadequate aeration affects nutrient uptake from roots. Avocado plants may show a different response to different water levels, therefore, comparing the levels of irrigation would be essential to determine optimal irrigation in avocado.

There was difficulty in determining the effects of water stress, because the floral intensity, fruit set, and crop load were variable from tree to tree. Therefore, increasing the number of plants included in a trial is necessary. Also, year to year variation is a common problem with avocado. Continuous monitoring over several years may provide better results that confirm whether irrigation would be needed in spring.

Monitoring drainage may be also necessary to improve irrigation management, because fertilizers are commonly applied in the irrigation water, and there is a potential risk of nutrient leaching, especially N and P. Over irrigation can cause dissolved nutrients to move from agricultural fields to ground water, change soil characteristics, and decrease water quality. Declining water quality has become a serious worldwide environmental problem (Jiao et al. 2004: Kiggundu et al. 2012), including in New Zealand (Hart et al. 2004).

In the rainout experiment, fruit quality was assessed by measuring dry matter content because dry matter content is highly correlated with oil content. However, nutrient content in fruit is also important to evaluate fruit quality. In particular, Ca concentration in the fruit mesocarp can be a useful indicator for the potential for physiological damage or disease after harvest (Hoffman et al. 2002; Neuhaus et al. 2009). Thorp et al. (1997) found that fruit with lower Ca concentration had a higher occurrence of vascular browning at harvest. Therefore, analysis of fruit nutrient content should be included in future research.

This study has highlighted water requirements of avocado in Katikati, Bay of Plenty, New Zealand, but avocado water use will vary between locations. To improve the irrigation design and to obtain more reliable estimates of K_C, further research could consider crop water use in other growing regions of New Zealand.

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Appendix

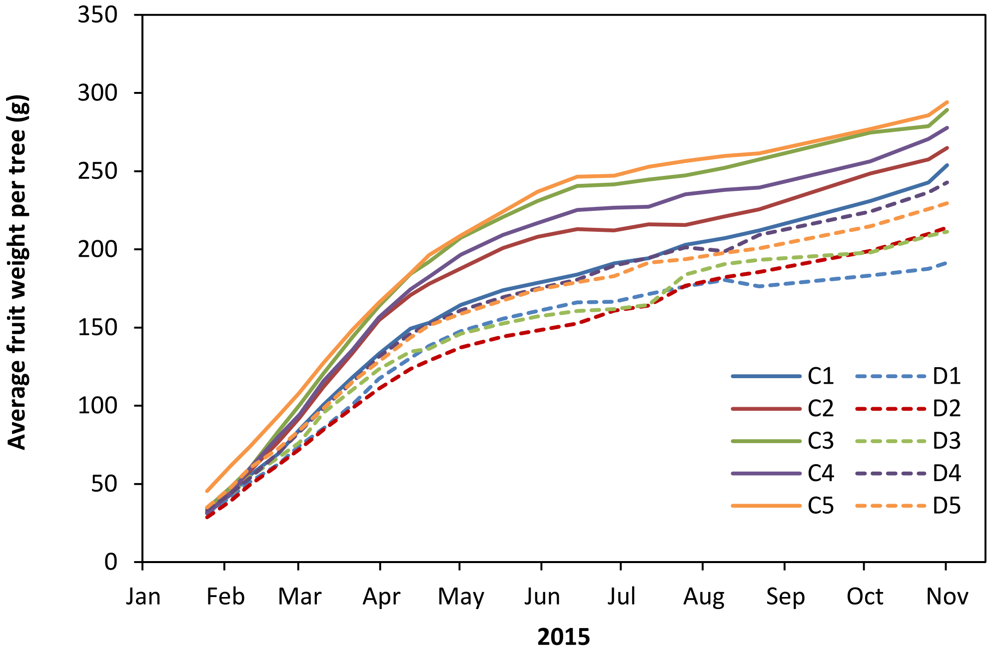

Figure 5.1 Average fruit weight (g) per tree from non-destructive measurements.

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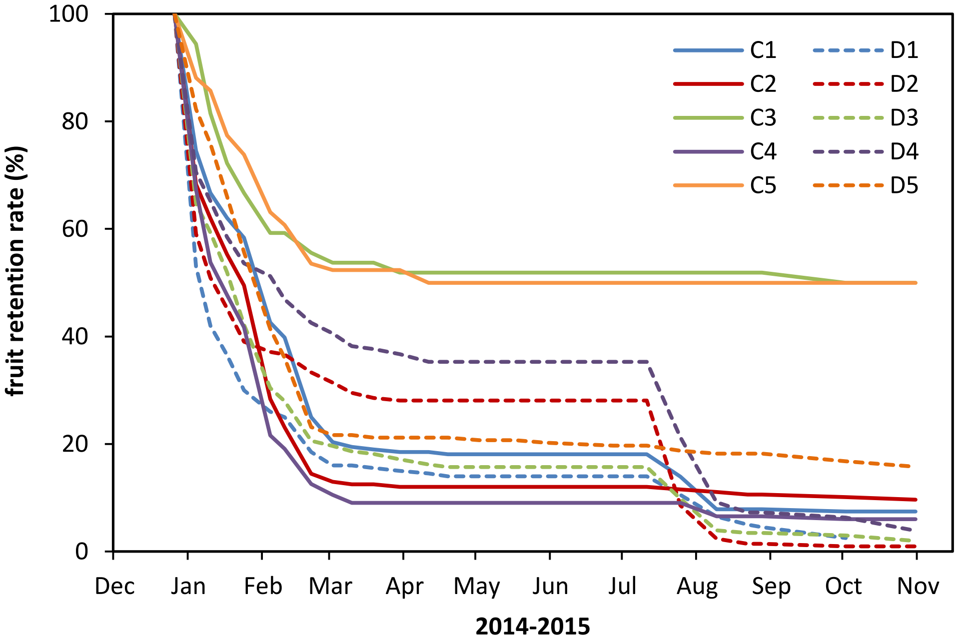

Figure 5.2 13 Fruit retention rate per tree.