Seasonal water use dynamics of *Macadamia* (F. Muell) orchards

by

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DECLARATION

I, Theunis Gerhardus Smit, declare that the thesis/dissertation, which I hereby submit for the degree PhD Horticulture at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signed: Theunis Gerhardus Smit

Date: 30 April 2020

PREFACE

The research contained in this thesis was completed by the candidate while based in Department of Plant and Soil Sciences of the Natural and Agricultural Science Faculty, University of Pretoria, Pretoria, South Africa. The research was financially supported by South Africa's Water Research Commission (Project K5/2552//4, Water use of avocado and macadamia orchards), with co-funding from South African Macadamias.

ABSTRACT

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Increased demand for sustainably produced, healthy, and nutritious food has seen certain segments of the world agricultural sector flourish in the past few decades. The macadamia nut industry in particular has expanded at a tremendous rate, with more than 10 000 hectares of trees being planted annually across a range of environments. The greatest portion of these expansions occur in semi-arid areas, which are characterized by highly variable rainfall patterns, and are as a result irrigated to minimize the risk of yield, quality and income losses, brought about by water stress. The recently commercialized nature of the crop, in combination with lack of water use research specific to macadamia, has created great uncertainty amongst producers. This study has therefore firstly aimed at gaining a fundamental understanding of leaf gas exchange and macadamia transpiration (E_c) in response to a range of environmental and physiological variables, in an attempt to identify the driving variables of transpiration. Secondly, the study aimed to identify crop water use models that best incorporate the driving variables of E_c, in order to transfer results obtained from this study, to a range of growing environments. Measurements of leaf gas exchange, hydraulic conductance, canopy dimensions, weather, and E_c were made over an approximate three year period, in a fully irrigated commercial mature bearing (MB) and immature bearing (IB) macadamia orchard in the Mpumalanga province of

South Africa. Leaf gas exchange measurements, included, but were not limited to net CO_2 assimilation rate (A) and stomatal conductance (g_s). Transpiration measurements were obtained using sap flow measurements using the heat ratio method of the heat pulse velocity technique. Macadamia A was found to be slightly lower than that of other evergreen subtropical crops, which is largely attributed to substantial stomatal and non-stomatal limitations to A. Non-stomatal limitations to A were linked to an internal light limitation resulting from the sclerophyllous nature of leaves. Stomatal limitations stem from the predominantly isohydric nature of macadamias, where g_s is carefully controlled in order to maintain midday leaf water potential within certain safety margins. Isohydric behaviour suggested an underlying hydraulic limitation, which was found to exist within the stem to leaf interface of macadamias. Responses of g_s to leaf vapour pressure deficit ($\mathrm{VPD_{leaf}}$) showed that g_s declined as $\mathrm{VPD_{leaf}}$ exceeded 2.5 kPa. The response of g_s to $\mathrm{VPD_{leaf}}$, however, varied substantially throughout the season, being significantly higher during fruiting periods compared to non-fruiting periods, implying isohydrodynamic behaviour and emphasizing the influence of phenology on leaf gas exchange. Similar results were found on both fruiting and non-fruiting branches implying that an upregulation of g_s at leaf level would most likely lead to an upregulation at the canopy level, which would lead to increased E_c. During fruiting periods, macadamia E_c was ~20% higher compared to non-fruiting periods, with no significant difference in weather variables or canopy size, which could act as confounding factors. Increased E_c during fruiting periods was associated with a greater response of E_c to air vapour pressure deficit ($\mathrm{VPD_{air}}$) in the 0.0 – 3.0 kPa range, which was similar to the observed increases of g_s in response to $\mathrm{VPD_{leaf}} > 1.5$ kPa. An examination of transpiration crop coefficients ($\mathrm{K_t}$), confirmed that increased E_c during fruiting periods stem from a physiological upregulation of g_s and subsequently canopy conductance (g_a). Besides physiological and phenological variables influencing macadamia E_c, physical attributes (i.e. canopy size) and weather variables remained the key driving variables of E_c. Macadamia E_c increased in a linear fashion when $\mathrm{VPD_{air}} < 0.8$ kPa, solar radiation ($\mathrm{R_s}$) <0.3 MJ $\mathrm{m^{-2}\ h^{-1}}$ and reference evapotranspiration ($\mathrm{ET_o}$) <0.13 mm $\mathrm{day^{-1}}$, but failed to increase at the same rate when these limits were exceeded. The reduction in the rate of E_c in response to increases in environmental evaporative demand under non-limiting soil water conditions, indicates that E_c in macadamias is a supply-controlled system. Supply controlled E_c was confirmed upon examination of maximum daily recorded E_c ($E_\mathrm{c\ max}$) in response to increases in the

aforementioned weather variables, with daily $E_{c\ max}$ failing to increase at $VPD_{air} > 1.5$ kPa, $R_s > 15$ MJ m^{-2} day^{-1} and $ET_o > 3.5$ mm day^{-1}. The response of E_c and $E_{c\ max}$ to these weather variables did not vary between the two orchards, the magnitude of both E_c and $E_{c\ max}$, however, differed between orchards, being highest in the MB orchard. Higher E_c in the MB orchard was largely attributed to a ~60% larger canopy, with E_c in the MB orchard being ~60% more than E_c in the IB orchard. Transpiration measured in this study, however, remains site specific, and identification and validation of crop water use models were therefore needed to extrapolate data to a broader range of growing environments. The study therefore evaluated three models including the widely used FAO-56 dual crop coefficient, a canopy conductance (g_c) model in conjunction with the Penman-Monteith equation, and a canopy transpiration model. The study showed, that a poor estimation of daily K_t and subsequently E_c was obtained using the FAO-56 dual crop coefficient model, which was largely attributed to overestimation of K_t and therefore E_c when daily reference evapotranspiration (ET_o) rates exceeded 4.0 mm day^{-1}, and an underestimation of K_t and E_c when $ET_o < 2.0$ mm day^{-1}. The model, however, provided reasonable estimates of K_t and E_c on a monthly or seasonal basis, with only slight discrepancies observed between measured and simulated K_t and E_c from January to April in each season, which was attributed to physiological upregulation of E_c in the presence of fruit. The g_c estimations in conjunction with the Penman-Monteith equation, provided more accurate estimates of daily E_c in both the MB and IB orchards, compared to the empirical FAO-56 dual crop coefficient model, but was particularly sensitive to seasonal changes in leaf area index (LAI), with adjustments of maximum canopy conductance ($g_{c\ max}$) being required to achieve accurate estimates of $E_{c.}$ An adjustment for variations in LAI, however, failed to provide increased estimates of E_c during the January to April period reaffirming the phenological and physiological influence of fruit on g_c and E_c during this period. Measurements of macadamia g_c in this study was rather low (0.3 – 0.7 mm s^{-1}) in relation to g_a (37 - 75 mm s^{-1}), confirming that macadamias are well coupled to the atmosphere. The high degree of coupling in macadamias implies that changes in g_c would lead to direct changes in E_c, which contributed to the success of the use of a simplified E_c model. This model provided reasonable estimates of daily E_c without multiple adjustments for canopy size being needed within each of the orchards. The E_c model, similar to the other models tested, however, failed to provide reasonable estimates of E_c during the January to April period. The results from this study have shown that macadamias are predominantly isohydric in nature, a trait which ultimately dictates leaf gas exchange and E_c in this recently domesticated subtropical crop. Strict stomatal control in response to increased atmospheric evaporative demand, is also evident in the supply controlled nature of macadamia E_c, which has added to the success of mechanistic models in accurately estimating macadamia E_c. Although the study has reaffirmed that E_c is largely driven by environmental demand and canopy size, it demonstrated that physiological and phenological factors can have a significant effect on leaf level gas exchange and subsequently E_c of macadamias.

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Mumford and Sons song perfectly embodies what I have learned from you, “what's behind, I can clearly see, but that beyond, that's beyond me”.

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LIST OF ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS

ABA	Abscisic Acid
ANOVA	Analysis Of Variance
AQE	Apparent Quantum Efficiency
AWS	Automatic Weather Station
DIS	Dry In Shell
EC	Eddy Covariance
ET	Evapotranspiration
FAO	Food And Agriculture Organization Of The United Nations
GPS	Global Positioning System
IB	Immature Bearing
IRGA	Infrared Gas Analyser
LAI	Leaf Area Index
LCP	Light Compensation Point
LSD	Least Significant Difference
MB	Mature Bearing
NF	Non-Fruiting
NIH	Nut In Husk
OPEC	Open Path Eddy Covariance
PAR	Photosynthetically Active Radiation
PFTE	Polytetrafluoroethylene
PGA	Phosphoglyceric Acid
PPFD	Photosynthetic Photon Flux Density
R^2	Coefficient Of Determination
REML	Restricted Maximum Likelihood Estimation
RH	Relative Humidity
RWC	Relative Water Content
SAMAC	South African Macadamia Association
SPAC	Soil-Plant-Atmosphere-Continuum
TDR	Time-Domain Reflectometer

TPU	Triose Phosphate Use
VEPAC	Variance Estimation, Precision & Comparison
VPD	Vapour Pressure Deficit
WIS	Wet In Shell
WRC	Water Research Commission
WUE	Water Use Efficiency
WUP	Water Use Productivity

SYMBOLS

Symbol	**Description**	**Units**
A	net carbon dioxide (CO_2) assimilation rate	µmol m^{-2} s^{-1}
A_{max}	maximum net carbon dioxide (CO_2) assimilation rate	µmol m^{-2} s^{-1}
c_w	specific heat capacity of the wood matrix	J kg^{-1} °C^{-1}
c_s	specific heat capacity of the sap	J kg^{-1} °C^{-1}
C_i	internal CO_2 concentration	
C_p	specific heat capacity of air	J kg^{-1} K^{-1}
E_c	canopy transpiration	mm
$E_{c\ max}$	maximum canopy transpiration	mm
E_{leaf}	leaf transpiration rate	mm
ET_c	crop evapotranspiration	mm
ET_o	reference evapotranspiration	mm
f_{ceff}	effective fraction of ground covered or shaded by vegetation near solar noon	Unitless
f_{IPAR}	fraction of intercepted photosynthetically active radiation	unitless
F_r	adjustment factor relative to stomatal control	unitless
G	Ground heat flux	W m^{-2}
g_a	aerodynamic conductance	mm s^{-1}
g_s	stomatal conductance	mmol m^{2} s^{-1}
g_c	canopy conductance	mm s^{-1}
$g_{c\ max}$	maximum canopy conductance	mm s^{-1}

g_{m}	mesophyll conductance	mmol m^{2} s^{-1}
h	mean maximum plant height during the midseason period or full cover period	m
J_{max}	electron transport rate	µmol m^{-2} s^{-1}
J	sap flux	cm h^{-1}
k	thermal diffusivity of green (fresh) wood	cm^{2} s^{-1}
k	hydraulic conductance	mmol m^{-2} s^{-1} MPa^{-1}
k_{L}	leaf specific hydraulic conductance	mmol m^{-2} s^{-1} MPa^{-1}
$k_{soil\text{-}stem}$	hydraulic conductance within the soil to the stem pathway	mmol m^{-2} s^{-1} MPa^{-1}
$k_{stem\text{-}leaf}$	hydraulic conductance within stem to the leaf pathway	mmol m^{-2} s^{-1} MPa^{-1}
K_{c}	crop coefficient	unitless
K_{cb}	basal crop coefficient	unitless
$K_{cb\ full}$	basal crop coefficients for fully grown orchard	unitless
K_{d}	crop density coefficient	unitless
K_{e}	soil water evaporation coefficient	unitless
K_{t}	transpiration crop coefficient	unitless
l	stomatal limitation	%
m_{c}	moisture content of sapwood sample	%
M_{L}	parameter that simulates the physical limits imposed on water flux through the plant root, stem and leaf systems	unitless
N	number of samples	unitless
P_{a}	atmospheric pressure	kPa
RH_{min}	minimum relative humidity	%
r_{leaf}	mean leaf resistance	s mm^{-1}
r_{s}	mean surface resistance	s mm^{-1}
R_{d}	mitochondrial respiration rate	µmol m^{-2} s^{-1}
R_{n}	total net radiation flux density	W m^{-2}
R_{s}	solar radiation	MJ m^{-2}
R_{so}	solar radiation under clear sky conditions	MJ m^{-2}

T_{air}	mean daily air temperature at 2 m height	°C
T_{leaf}	leaf surface temperature	°C
T_L	lower temperature limit to transpiration	°C
T_U	upper temperature limit to transpiration	°C
ψ_{leaf}	leaf water potential	MPa
ψ_{pd}	pre-dawn leaf water potential	MPa
ψ_{stem}	stem water potential	MPa
$\psi_{sun\ leaf}$	sun exposed leaf water potential	MPa
ψ_{soil}	soil water potential	MPa
u_2	wind speed at 2 m height	$m\ s^{-1}$
v_1	increase in temperature of upper thermocouple after the heat pulse is released	°C
v_2	increase in temperature of the lower thermocouple after the heat pulse is released	°C
V_c	corrected heat pulse velocity	$cm\ h^{-1}$
V_h	heat pulse velocity	$cm\ h^{-1}$
VPD_{air}	air vapour pressure deficit	kPa
VPD_{leaf}	leaf vapour pressure deficit	kPa
V_s	sap velocity	$cm\ h^{-1}$
V_w	volume of wood sample	cm^3
w	wounding width	cm
Δ	slope of the vapour pressure curve	$kPa\ K^{-1}$
ρ_a	density of dry air	$kg\ m^{-3}$
ρ_b	basic density of wood	$g\ cm^{-3}$
ρ_s	density of water	$kg\ m^{-3}$
γ	psychrometric constant	$kPa\ K^{-1}$
λ	the latent heat of vaporization	$J\ kg^{-1}$
Δ	slope of the vapour pressure curve	$kPa\ K^{-1}$
$\propto$	fraction of sunlit canopy leaf area	unitless

CHAPTER 1: GENERAL INTRODUCTION

The modern day consumer is progressing towards healthier, fresher, and more sustainable produce, with increased emphasis being placed on products with a so-called “clean label” (Asioli et al. 2017). Although, no clear definition exists for the “clean label” product, it generally refers to production practices involved in producing a said product, with products produced in a system which has little to no impact on the natural environment being favoured above products that exploit scarce natural resources (Garnett et al. 2013). The conscious decision to support more sustainably produced products is especially evident in more affluent consumers (Kearney 2010), which is of particular interest to the continuously growing macadamia nut market (Scheepers 2018).

The macadamia nut, which is known to be one of the most expensive tree nuts, is a fruit produced by two *Macadamia* (F. Muell) species consisting of *M. integrifolia* (Maiden & Betche) and *M. tetraphylla* (L.A.S. Johnson) and hybrids thereof. These species originate from coastal Australia, where they have been commercially cultivated for the past fifty years (Nock et al. 2019). Commercial orchards have also extended to other parts of the world, with South Africa, Kenya, and China currently producing a large portion of the world macadamia crop. The rapid expansion of the macadamia industry has, however, only commenced in the past ten years, with expansion in South Africa being approximately 6000 ha in 2018 alone according to the South African macadamia industry body (SAMAC, pers. comm.). Expansion is mostly driven by the global demand and accompanying high prices, but further expansion in many producing countries is limited to some extent by the availability of suitable land, and more specifically by the availability of fresh water suitable for irrigation.

The largest macadamia producing countries, Australia and South Africa, have faced extreme water shortages in the past few decades and more dry weather extremes are expected in years to come (Malherbe et al. 2016; Baudoin et al. 2017; Deo et al. 2017). Shifts in rainfall patterns have seen supplementary irrigated macadamia orchards becoming fully irrigated, if water is available, and rain-fed macadamia orchards becoming rather unproductive. The value of macadamia nuts has, however, seen growers acquiring increased volumes of water, by either purchasing fresh water at exorbitant prices or applying for increased water licencing allocations. Both of these options, although feasible, create a range of social problems, especially when considering that in times of drought, communities are also faced with water restrictions and human consumption of water takes precedence over commercial agriculture. Furthermore, in the era of food labelling (Asioli et al. 2017), macadamias would not be seen as a sustainably produced product given the aforementioned scenario and may in fact result in decreased demand by its affluent consumers.

Further complications to the aforementioned scenario are linked to the limited amount of water use and irrigation research in macadamias, creating a large amount of uncertainty regarding the water needs of the crop. Most knowledge used by producers has, as a result, been gathered from either trial and error or from studies conducted in Australia (Stephenson et al. 1989; Lloyd 1991; Lloyd et al. 1991; Trochoulias and Johns 1992; Stephenson et al. 2003). Even though this information has guided producers in the management of irrigation in macadamia orchards, these studies never attempted to provide more insight into the dynamic relationship between crop physiology and the environmental variables driving macadamia water use. An in-depth understanding of this dynamic relationship could prove to be fundamental to the sustainable production of this high value crop.

Our current understanding of the water requirements of this recently commercialized crop is limited to a few published studies, which have reported water use (evapotranspiration) figures ranging from 750 – 1200 mm per season (Stephenson et al. 2003; Gush and Taylor 2014). Anecdotal evidence has also shown that high yields have been reported in areas such as Hawaii, where annual rainfall exceeded 2000 mm (Stephenson and Trochoulias 1994). Although these ranges don’t provide clear cut answers to either scientists or producers, it is surprising that such a large range of water use estimates exist. This raises the question of which factors drive macadamia water use, specifically transpiration, and how these factors subsequently influence dry matter accumulation and yield. For example, in areas where annual rainfall exceeds 2000 mm (Stephenson and Trochoulias 1994), is the volume of water alone driving production or is the increase in humidity accompanying the well distributed rainfall leading to increased production? Currently available research, although limited, would suggest that humidity and more specifically vapour pressure deficit is one of the key driving variables of stomatal conductance (Lloyd 1991; Lloyd et al. 1991) and would therefore also be a key driving variable of macadamia water use. There is, however, no research on macadamias that have investigated the manner in which other driving variables impact water use, such as canopy size, crop phenology, and crop physiology, which are fundamentally important to constructing practical and comprehensive irrigation and water requirement guidelines.

Given the lack of research into water use and water relations of macadamia, in combination with the rapid expansion of the industry in areas outside that of the crop's natural environment, which are often faced with limited fresh water resources for irrigation, it is absolutely critical that crop water use models, which are not only accurate but are easily parameterized and user friendly, be studied and evaluated. To date, no such models have been used in the macadamia industry, which is most likely due to the lack of information needed to successfully parameterize these models (Carr 2013). Successful parameterization of a crop water use model specific to macadamias, would not only aid growers in selecting sites which are more suitable for production, but will aid them in managing water in existing orchards with greater care, which would subsequently increase efficiencies and sustainability of the crop (Liu et al. 1998; Nguyen et al. 2017; Thorp et al. 2018).

Although various publications have suggested that macadamias are well adapted to a range of environments and have attributes that make them tolerant to extended periods of water shortages (Searle and Lu 2002; Stephenson et al. 2003; Carr 2013; Stephenson and Searle 2014), the current value of the crop, in combination with increased variability in rainfall, dictates a risk aversion strategy amongst macadamia growers, which increases their reliance on fresh water. It is also highly unlikely that this strategy will change in the foreseeable future, due to the continuous growth in demand, and the lag between research and expansion. The increased reliance on fresh water, and lack of water specific research in the macadamia industry therefore motivates the need for this study, which not only addresses some of the current knowledge gaps in the field of macadamia water research, but will attempt to provide more insight into the driving variables of macadamia water use. This would serve as a foundation for future research in the field, whilst being fundamental to water licencing bodies, and most importantly farmers, in understanding water requirements of macadamias.

1.1 Hypotheses

The main hypotheses that were formulated and tested in this study were as follows:

- Given that macadamias are an evergreen, sclerophyllous species which originated in an area with a distinct dry period, they would have a conservative or isohydric water use strategy. This trade-off for safety over efficiency contributes to significant non-stomatal limitations to photosynthesis and strong stomatal control over gas exchange, and is attributed to a hydraulic limitation within the root to leaf pathway. However, as this is an oil storing nut with a high assimilate demand, it was further hypothesised that non-stomatal limitations would vary between fruiting and non-fruiting phenological stages.
- Net CO_2 assimilation rate (A) and stomatal conductance (g_s) would be higher in both fruiting and non-fruiting branches in the presence of developing fruit, and a downregulation of A and g_s can be expected upon fruit removal.
- Leaf level increases in g_s, brought about by the presence of fruit, would translate into subsequent increases in canopy transpiration (E_c), given that a degree of branch dependence exists in macadamia, g_s is expected to increase throughout the canopy on bearing and non-bearing branches, resulting in a significant increase in canopy transpiration in the presence of fruit.
- Given the isohydric nature and subsequent strict stomatal control of macadamia, E_c is a supply controlled system, which would result in an upper limit of E_c under conditions of high evaporative demand, with its magnitude dictated by physical orchard attributes (i.e. canopy size). Given the hypothesized upper limit of E_c, the study further hypothesized that variations in seasonal values of K_t can be expected in macadamias, given that the rate of E_c is unlikely to increase at the same rate as the increase in ET_o and given the significant effect of physiology and phenology on leaf gas exchange and E_c.
- Mechanistic modelling approaches would provide more accurate estimates of E_c in macadamias, especially on a daily basis, where high degrees of environmental variation exist, compared to an empirical model based on the premise of demand limited E_c.

- Macadamias are highly coupled to the atmosphere, and the high degree coupling would allow for the direct estimation of E_c using a E_c model.

1.2 Aim of the study

The aim of this study was to determine the key environmental and physiological driving variables of transpiration for macadamia, which is a relatively new, and understudied economically important crop. Through an increased understanding of macadamia physiology the study further aimed to select, parameterise, and evaluate crop water use models, which would provide irrigators and researchers with increased insight into the water relations of macadamia across a range of different environments.

1.3 Study objectives

1. To study the response of leaf gas exchange, specifically A and g_s, of field-grown macadamias in response to a range of environmental conditions and variations in crop phenology;
2. To study diurnal and seasonal evolution of macadamia A, g_s and leaf water potential of macadamias and to determine its water relations under non-limiting soil water conditions;
3. Determining whole-tree and segmented (i.e. soil-root, root-stem and stem-leaf interfaces) hydraulic conductivity of macadamias;
4. Measuring unstressed E_c of macadamia orchards varying in canopy size, whilst monitoring environmental variables, crop phenology, and variations in canopy size throughout the growing seasons;
5. Determining the effect of developing macadamia fruits on leaf gas exchange and E_c;
6. To select and successfully parameterize a suitable E_c model for macadamias, based on insights gained from ecophysiological and E_c measurements;
7. Parameterizing and validating, the FAO-56 dual crop coefficient model for the estimation of E_c in macadamia;
8. Parameterizing and validating, a canopy conductance and E_c model for the estimation of E_c in macadamia;

9. Evaluating and comparing the accuracy of canopy conductance and E_c modelling approaches to the empirical crop coefficient modelling approach, in estimating E_c of macadamias;
10. Determining the degree of coupling in macadamia orchards.

1.4 Thesis outline

The thesis consists of a total of eight chapters, with Chapter 1 consisting of a general introduction, as well as the objectives and hypotheses of the study. A literature review of currently available macadamia specific research and related ecophysiological and water use studies is captured in Chapter 2. This is followed by a general materials and methods section (Chapter 3), containing details of the study sites and methods used for the determination of weather parameters, leaf gas exchange, canopy dimensions, transpiration, and evapotranspiration that is applicable to all results chapters. The first of four working chapters (Chapter 4) investigates the seasonal regulation of macadamia leaf gas exchange, whilst providing an in-depth look at the water relations of field grown macadamias. Building on the results from Chapter 4, the second working chapter (Chapter 5) attempted to establish if water use of macadamia trees is dynamically regulated by the presence or absence of fruit. Chapter 6 provides a detailed analysis of macadamia transpiration and the driving variables of macadamia transpiration. These three chapters provided a solid foundation on which transpiration model selection could be based and subsequently Chapter 7 has focussed on parameterizing and assessing some of the most widely implemented tree water use models. The eighth and final chapter of the thesis contains a concise summary and integrated discussion of results presented in each of the working chapters, whilst providing suggestions for future research in the field of water use and ecophysiology of macadamias.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Water use of recently domesticated subtropical fruit tree crops have been largely understudied, making irrigation water management difficult, especially in semi-arid regions where water is a major limiting factor to crop production (Wullschleger et al. 1998; Lu 2000; Williamson et al. 2015; Durán Zuazo et al. 2019; Moreno-Ortega et al. 2019; Nhamo et al. 2019). The shortage of information could in part be attributed to the relatively young nature of such species (Jackson et al. 2011; Fuller 2018), but is also due to the long-term nature and complexity of research required in tree crops (Wullschleger et al. 1998; Klein et al. 2015; Gasque et al. 2016; Nicolás et al. 2016). For *Macadamia* F. Muell and more specifically *M. integrifolia* (Maiden & Betche) and *M. tetraphylla* (L.A.S. Johnson) (Proteaceae*)*, the problem is not limited to that of water use, but extends to other fields of agricultural research, including crop morphology and physiology. It compels researchers to prioritise research according to the needs of the industry as a whole, whilst also trying to obtain a better understanding of the dynamic and interrelated nature of crop morphology, physiology, and the environment.

When examining the overall state of macadamia research, most studies have been conducted in either Australia or Hawaii. Hawaii, being the first country to produce macadamias commercially (Stephenson 2005a), has focused its research efforts on the development of a sound understanding of the botany of the crop (Shigeura and Ooka 1984). This differs substantially from research conducted in Australia, which has prioritized research linked to canopy management (McConchie et al. 1999; Huett 2004; Wilkie et al. 2010), and improvements in macadamia yields through cultivar selection (Trochoulias 1990) and increased pollination and fertilization efficiency (Stephenson and Gallagher 1986; Trueman and Turnbull 1994; Wilkie et al. 2009b).

Both countries have not prioritised water-use and irrigation-related research of macadamia, which is most likely due to the limited number of irrigated orchards (Trochoulias and Johns 1992). Studies by Trochoulias and Johns (1992), which concluded that macadamias responded poorly to irrigation in high rainfall areas of Australia, might also have contributed to the low priority given to this research. Nevertheless, with the high growth rate of macadamia production in South Africa, where most orchards are irrigated, and Australia which is progressing towards an industry more dependent on irrigation due to irregular rainfall (Hajani and Rahman 2018), water-related research has become a priority. However, before allocating resources to such research, it is important to critically examine existing research and to identify the potential shortfalls and gaps in research. This will help guide new research projects to deliver applicable and practical results pertaining to water use in this recently commercialized crop.

Obtaining a clear understanding of each component in the soil-plant-atmosphere-continuum (SPAC) is therefore fundamental to quantifying and improving crop water use (Katul et al. 2012; Reichardt and Timm 2020). Within the SPAC, the soil acts as the reservoir for water and nutrients, and as a result dictates the supply of water to both the plant and the atmosphere (Cowan 1965). The physical and chemical characteristics of soil, which dictates water availability to the plant and the atmosphere can be determined fairly easily using a range of well-defined experimental methods (Doran and Parkin 1994). In a similar fashion, measurements and determination of atmospheric variables, which often quantifies the demand component of the SPAC, can be obtained with relative ease, albeit at a cost, and typically conform to well-defined and universally accepted methods (Allen et al. 1998; Katerji and Rana 2014).

The plant component of the SPAC varies considerably and consists of multiple components, each with the capability to significantly impact the supply of water from the soil in accordance to that demanded by the atmosphere. The difficulty in quantifying variables of the plant component arises from the inherent genetic variability between and within species, which is further exacerbated in recently commercialized crops. Nevertheless, technological advances have aided scientists in measuring a range of ecophysiological parameters, such as stomatal conductance, hydraulic conductance, and water potential, which allow researchers to examine crop specific components which dictate crop water use within the SPAC (Fernández et al. 1997; Hernandez-Santana et al. 2016; Landsberg et al. 2017). By determining hydraulic conductance in plants, we are able to better understand water movement through plants whilst attempting to establish possible constraints within the plant itself, which will in turn have significant impacts on the entire SPAC (Hatfield and Prueger 2016; Knauer et al. 2018; Dubbert and Werner 2019).

Even under conditions where each component of the SPAC is quantified and understood, data is often site specific and the extrapolation of this data to other orchard environments can be rather challenging due to the large variability in soil type, varietal differences and environmental variation. Fortunately, a range of crop water use models, with varying degrees of complexity, can be utilized to simulate complex soil (Radcliffe and Simunek 2010), plant (Zweifel et al. 2007; DeJong et al. 2011), and atmospheric (Allen et al. 1998) variability. Most models require crop and site specific parameters, but model selection, especially crop water use models, is often dictated by the crop behaviour which would imply that an in-depth understanding of the crop is required before any component can be modelled with confidence (Boote et al. 1996; Annandale et al. 1999; Bonan et al. 2014).

This literature review therefore aims to examine currently available macadamia research, in combination with relevant research for other fruit tree crops, to establish a baseline of knowledge on tree water-use in complex orchard systems. It further aims to examine existing ecophysiology studies for macadamia and other subtropical fruit tree species in order to identify the driving variables and regulation of water-use, which should contribute to informed decisions regarding the choice of water use models.

2.2 Stomatal behaviour and water use strategies

Native to sub-tropical regions of Australia, the evergreen macadamia tree has gained substantial popularity in the global agricultural sector, due to the substantial economic value of its fruit. This popularity has seen macadamia trees planted in environmental conditions which are distinctly different from its native coastal rainforest environment. In environments that are often drier and more marginal than those of its natural habitat, macadamia has shown to be resilient as a commercial crop, sustainably producing economically viable yields (Rogan 2000; Hardner et al. 2009; Neal et al. 2010). It would therefore seem as if macadamias have the capability to grow and produce good quality fruits in environments outside of its natural habitat which is rather significant considering the fact that commercially cultivated macadamias have only recently been domesticated (Hardner et al. 2009; Nock et al. 2019).

The adaptability of macadamias to drier and more marginal environments could potentially stem from a large degree of whole tree and more specifically leaf morphological plasticity and/or as a result of strict control of leaf gas exchange and carbon/water relations in response to environmental variables (Lavorel and Garnier 2002; Chaves and Oliveira 2004). The long-lived hypostomatous, sclerophyllous leaves and specialised proteoid roots of macadamia (Dinkelaker et al. 1995; Syvertsen et al. 1995; Stephenson 2005b) could also aid the trees in not only surviving but flourishing in a range of environments. These leaf morphological characteristics are believed to have evolved in response to conditions, where either climate, water or nutrient supply imposed significant constraints on species survival (Turner 1994; Wright and Cannon 2001; Neumann and Martinoia 2002; Miller 2005; Gerke 2015). Such suboptimal conditions are, however, rarely present in agricultural systems, and these evolutionary adaptations could potentially place an upper-limit on both crop yield and quality.

In an attempt to better understand leaf gas exchange of macadamia, Lloyd (1991) developed and tested a range of models to study responses of stomatal conductance (g_s) to climatic variables. It was established that g_s is largely controlled by leaf to air vapour pressure deficit (VPD_{leaf}) at high irradiances. At low irradiances g_s tended to be more sensitive to leaf temperature (Lloyd 1991), most likely due to the temperature dependence of metabolic pathways. This distinct response of g_s to VPD_{leaf} is well documented for a range of subtropical tree crops (Flore and Lakso 1989; Lloyd and Howie 1989; Jifon and Syvertsen 2003). There is, however, still considerable uncertainty regarding the mechanisms involved in these responses (Losch and Tenhunen 1981; Assmann and Shimazaki 1999; Davies et al. 2002; Brodribb and McAdam 2011; Buckley 2019), as well as the distinct differences observed between and within species (Jarvis 1976b; Brodribb et al. 2003; Hetherington and Woodward 2003; Schultz 2003).

Research suggests that the mechanisms involved in stomatal responses, especially in response to VPD, are mediated by both mechanical and chemical changes within plants. This concept is the premise by which plant water management strategies are classified (Jones 1980; Smart and Coombe 1983; Mrad et al. 2018), and is encapsulated by the ecophysiological classification of Tardieu and Simonneau (1998) into isohydric or anisohydric plant behaviour. Isohydric plants attempt to maintain favourable plant water status and therefore attempt to prevent leaf water potential (ψ_{leaf}) from dropping below a minimum value, irrespective of soil water content and atmospheric demand, and is achieved by means of strict stomatal control. This strategy is in contrast with anisohydric behaviour where a greater diurnal decline in ψ_{leaf} is observed in response to increased atmospheric demand, compared to isohydric plants, and in general midday ψ_{leaf} for anisohydric plants will be substantially lower than that of its isohydric counterparts under the same set of environmental conditions. It should also be noted that the decline in ψ_{leaf} in anisohydric crops is significantly higher in water stressed plants compared to well-watered plants (Schultz 2003).

These two distinct water management strategies are based on the role of stomata in maintaining the balance between the plant’s ability to supply water to its leaves and the prevailing atmospheric evaporative demand. It is, therefore, intuitive that in the case of isohydric crops, strict stomatal control under rising evaporative demand, especially during increases in VPD_{leaf}, is necessitated by the inability of the crop to maintain the balance between water supply to the leaves and water lost from the leaves, whilst also attempting to maintain a favourable water status within the tree in an attempt to avoid xylem embolism (Sperry et al. 2008). Results from Schultz (2003) suggest that the inability to supply sufficient water to the leaves, stems from inherent hydraulic limitations of the xylem. These limitations can in-part be attributed to the complexity of hydraulic architecture of dicotyledonous species, but is mostly as a result of significant resistance within xylem vessels (Tyree and Zimmermann 2002). Due to the serial nature of interconnected xylem vessels the term hydraulic conductance is frequently used. If evaporative demand for water exceeds the upper limit of whole tree hydraulic conductance, water potentials decrease significantly (i.e. increased negative pressures) causing an increase in tension between water molecules within xylem vessels, thereby increasing the risk of xylem embolism, which if occurring frequently, could lead to ultimate dysfunction of the hydraulic continuum (Tyree and Sperry 1988; Tyree and Sperry 1989; Urli et al. 2013).

In subtropical regions, such as those where macadamias originated from and are currently cultivated, the microclimate is often characterized by high rates of atmospheric evaporative demand which often tends to exceed precipitation, especially during distinctly dry winter periods in summer rainfall regions. The evolutionary fitness of especially evergreen subtropical species is believed to be linked to the avoidance of runaway embolism mediated by strict stomatal control (Jones and Sutherland 1991). A risk-avoiding strategy is also evident in the anatomical structure of xylem vessels of these species. Tyree and Zimmermann (2002) reported that these species generally have xylem vessels which are significantly narrower than those of temperate species. The significance of these narrow vessels is that more vessels are present per unit area compared to wider vessels. If, and when embolism does occur, the percentage loss of conducting tissue per unit area will therefore be less in species with narrower vessels, thereby avoiding the risk of complete hydraulic failure (Sperry et al. 2008). It should, however, be emphasized that a decrease in vessel diameter leads to a significant decrease in hydraulic conductance (Tyree and Sperry 1989; Tyree and Zimmermann 2002). Combining the risk-avoiding nature of evergreen subtropical species with hydraulic inefficiency, it could be hypothesized that a large portion of these species may be isohydric.

The function of stomata as regulatory valves for the hydraulic continuum is balanced against their other significant function as gateways for carbon uptake and assimilation (Cowan and Farquhar 1977). Although Buckley (2005) proposed that cavitation avoidance and maximal carbon gain per unit water loss are not competing goals of stomata, it could be said that the upper maximum of carbon gain is a function of cavitation avoidance. This is substantiated by the fact that stomata tend to operate close to the upper limit of plant specific hydraulic capabilities (Tyree and Sperry 1988; Brodribb and Holbrook 2003). Reduced cavitation risk mediated by stomatal closure will therefore also lead to reduced rates of carbon assimilation (Koch et al. 2004; McDowell 2011). McDowell (2011) further proposed that in evergreen *Pinus edulis*, known to follow a predominantly isohydric water management strategy, carbon limitation and hydraulic failure are coupled. Studies have also demonstrated that isohydric plants are more prone to cavitation than their anisohydric counterparts (Sperry et al. 2008; Meinzer et al. 2009). There could therefore be an inherent limitation to carbon gain in isohydric evergreen subtropical tree species under hot and dry conditions.

Low net carbon dioxide (CO_2) assimilation rates (A) are not uncommon for evergreen trees (Warren and Adams 2004) and can also be low for a range of subtropical tree crops including macadamias, especially when compared to deciduous fruit tree species (Flore and Lakso 1989; Reynoso et al. 2000; Medina et al. 2002; Jifon and Syvertsen 2003; Huett 2004). Low A values could be due to various factors, as outlined by Warren and Adams (2004). Care should, however, be taken when comparing measurements of A, seeing that the method of determination could vary significantly between studies. Most studies, however, use infra-red gas analysis of heteroatomic molecules such as CO_2 and H_2O, to determine vapour fluxes in a controlled chamber (Hunt 2003). Conditions inside the chamber, including humidity, temperature, CO_2 concentration and photosynthetic active radiation can be controlled to suit the specific needs of both the plant and experiment, but the correct setup and maintenance of the chamber and its parameters is essential for making accurate measurement of leaf gas exchange, with Hunt (2003) and Field et al. (2000) outlining a range of potential errors which can occur if setup is incorrect. Most infra-red gas analysers have the ability to determine on of the most fundamental components of photosynthesis, being the relationship between A and internal CO_2 concentration (C_i) (Farquhar and Sharkey 1982), which can be used to study a range of fundamental leaf gas exchange dynamics. Of particular interest to this discussion are stomatal limitations, but it should be noted that significant non-stomatal limitations could also result in low net CO_2 assimilation rates (Warren 2007). Macadamias have rather low mesophyll conductance (g_m) (Lloyd et al. 1992), a trait believed to be linked to the sclerophyllous nature of the leaf.

Sclerophyllous leaves are characterized by thick cuticles and highly lignified cell walls. This combination results in a high leaf density per unit leaf area and a high leaf specific dry mass (Niinemets et al. 2009; Tosens et al. 2012). These anatomical adaptations give rise to an increased path length between the sub-stomatal cavity and site of carboxylation, thereby resulting in low g_m. A low g_m in combination with strict control of g_s in response to a narrow range of VPD_{leaf} poses significant constraints to the assimilation of CO_2 at the site of carboxylation. This has been shown by Lloyd et al. (1992) for a range of citrus species and for *M. integrifolia*, all of them being sclerophyllous species. What was evident from the study by Lloyd et al. (1992) was that macadamia had the lowest g_s, g_m and A of all species measured due to its high leaf tissue density.

The literature discussed so far has highlighted the importance of gaining a clear understanding of ecophysiological traits with evolutionary underpinnings, especially in the context of better understanding tree water relations. The most significant of these ecophysiological traits in macadamia is likely to be its proposed isohydric nature, possibly indicative of a water supply limitation within the stem to leaf interface. Such a water supply limitation from the soil to the atmosphere often disturbs the well-defined linear relationship between water supply and demand on which some of the most widely used crop water use models are based (Allen et al. 1998; Kumar et al. 2012; Mencuccini et al. 2019). The degree of linearity between water supply and demand therefore has a significant influence on the selection and accuracy of crop water use models (Meinzer 1993; Jones and Tardieu 1998; Lai and Katul 2000). This will be further discussed in section 2.4.

Given the dual function of stomata in facilitating CO_2 assimilation and transpirational water loss, an understanding of the factors influencing the former is also important. Strict stomatal control presents challenges to leaf level carbon assimilation, which although difficult to scale to canopy level, could lead to the realization of an upper limit of biomass accumulation and more importantly yield. Attempts to quantify and model macadamia water use and water use efficiency therefore also necessitates a review of carbon balance considerations in macadamia and other subtropical tree crops.

2.3 Macadamia water relations

Plant water relations involve the absorption of water, ascent of sap, loss of water by transpiration, and the internal water balance of the tree. Plant water relations are also inherently linked to carbon gain at both leaf and canopy level via the function of the stomata. One of the fundamental stomatal functions is to maintain a favourable trade-off between carbon gain and water loss, described as instantaneous water use efficiency or transpiration efficiency (WUE_t) at leaf level, and as water use efficiency (WUE) at either a canopy or orchard level. At the canopy level, the WUE is often referred to in terms of the harvestable yield per unit of water lost through either transpiration or evapotranspiration, and although yield may increase with increased water use, commercial agriculture tends to find more value in expressing water use in terms of income generated per unit of water used, a term often referred to as water use productivity (WUP)(García et al. 2012; Kool et al. 2014). Although these terms have been criticized in regards to their transferability between seasons and locations (Binkley et al. 2004; Blum 2009), they are widely used to estimate how effectively water is used in the production of harvestable crop and income, and provide a benchmark which can be improved upon (Wallace 2000; Howell 2001; Condon et al. 2004). Given the mathematical relationship between biomass gain and water loss, increases in WUE can be made by either increasing biomass gain at constant water loss, reducing water loss for a given biomass gain (by means of reduced evaporation and deep percolation of irrigation water), or by a combination of both. However, WUE is generally a conserved trait in plant species, as the above discussion on stomatal regulation would suggest.

The inherently conservative nature of water use amongst isohydric plants could imply that carbon gain is equally conservative. Macadamia nuts have a high oil content (>75%) (Macfarlane and Harris 1981) and therefore a high carbon and energy demand during the fruiting stages. South African orchards can produce nut in shell yields in excess of 20 kg tree^{-1} (Stephenson et al. 1986b), which is considered to be high in the global macadamia industry. It therefore seems unlikely that a conservative carbon gain strategy can support this high demand at certain phenological stages. It is possible that canopy level carbon gain from a high leaf area is more than sufficient to support the substantial demand for carbon, as has been demonstrated in avocado and citrus (Wolstenholme and Whiley 1999; Testi et al. 2008; Iglesias et al. 2013). Alternatively, macadamia could deviate from its assumed conservative isohydric nature during periods of high carbon demand. Deviation from a pure isohydric strategy to a more dynamic isohydric/anisohydric strategy is not uncommon amongst fruit tree crops (Palmer 1992; Naor et al. 2013; Silber et al. 2013; Sade and Moshelion 2014), especially in the presence of a high fruit load.

Relating whole tree carbon demand to tree water use is complicated by the fact that non-environmental effects, such as sink and source relations and carbon metabolism and portioning, are masked by dominating environmental variables (Lakso 1989; Nebauer et al. 2013). At a leaf level it is, however, possible to study responses of leaf gas exchange to environmental variables and how these responses vary with phenology. Very few studies have focussed on this aspect of subtropical fruit tree crops, which is rather surprising since phenology plays a significant role in tree water use and irrigation requirements, especially when considering that water stress during certain phenological stages, such as flowering and fruit growth has a greater effect on yield and quality compared to some less sensitive periods, including floral initiation and late fruit maturity stages (Li et al. 1989; Stephenson et al. 2003; Hutton et al. 2007; Savé et al. 2012; Silber et al. 2013; Martínez-Lüscher et al. 2016). Studies on deciduous tree crops, such as peach (Marsal and Girona 1997), and apple (Pretorius and Wand 2003), and subtropical crops such as avocado (Silber et al. 2013), for example, have provided valuable insight into differences in leaf gas exchange and water relations during different phenological stages.

These studies have also highlighted the numerous opportunities to apply an ecophysiological understanding to irrigation and water management and thereby make significant contributions towards increased yield and quality. Such opportunities include the use of regulated deficit irrigation, which, could be used to manipulate vegetative growth especially during periods of high assimilate demand (i.e. fruit growth) which could in turn reduce competition between fruit and vegetative flushes for a limited pool of assimilates thereby possibly reducing premature nut drop and subsequently increasing yield in macadamias (Stephenson et al. 1986a; Lloyd et al. 1991; Searle and Lu 2002; Wilkie et al. 2009a; Stephenson and Searle 2014). Studies by Stephenson et al. (2003) and Lloyd et al. (1991) suggest that macadamia may be highly suited to the practice of regulated deficit irrigation given the limited effects of mild water stress during certain phenological stages on leaf gas exchange, yield and nut quality. Linking with previous discussions in this chapter, the proposed isohydric nature of macadamia could further motivate the implementation of such strategies. The studies of Stephenson et al. (2003) and Lloyd et al. (1991), provides us with the best insight with regards to carbon and water relations in macadamia, and will therefore be used in conjunction with other literature to explore possible research shortcomings and opportunities with regards to water relations and the factors affecting these relations.

2.3.1 Water relations under conditions of soil moisture stress

Studies of container-grown macadamias have revealed that complete stomatal closure (g_s =0.00 mol m^{-2} s^{-1}) occurs at ψ_{leaf} of < -2.0 MPa (Stephenson et al. 1989a). Although these results cannot be extrapolated to field grown macadamias, given that ψ_{leaf} of < -2.0 MPa has rarely ever been measured in field grown macadamias (Stephenson and Searle 2014), Lloyd et al. (1991) reported that a significant decrease in g_s was observed at ψ_{leaf} of < -1.2 MPa. This decrease in g_s resulted in a significant decrease in *A*, with Stephenson et al. (2003) reporting values of <2.2 µmol CO_2 m^{-2} s^{-1} (average *A* of macadamias at non-stress conditions being 8 – 10 µmol CO_2 m^{-2} s^{-1}) at ψ_{leaf} of between -1.8 to -2.0 MPa. It was surprising that in the study of Stephenson et al. (2003), only slight differences in the diurnal course of g_s was found between water stressed and unstressed trees, and that g_s responded in a similar fashion to VPD_{air}, increasing to a maximum when VPD_{air} was favourable, regardless of ψ_{leaf}. These results confirm findings by Lloyd et al. (1991), who stated that stomata respond mainly to VPD_{air} and to a lesser extent to other environmental variables. The same study by Lloyd et al. (1991), however, showed that in field-grown macadamia trees, g_s of water stressed trees was lower than that of well-watered trees exposed to the same set of environmental conditions, but there was little to no difference in minimum ψ_{leaf} for water stressed and well-watered trees. In this sense, it emphasizes the point by Sperry (2000), which states that stomata act as pressure regulators in plants.

Net CO_2 assimilation rates reported by Stephenson et al. (2003) were, however, lower in water stressed compared to unstressed trees between 08:00 – 14:00h. This was surprising since these reductions were not accompanied by disproportionately lower g_s. This decrease in *A* could be attributed to the more negative ψ_{leaf}, resulting in reductions in relative water content (RWC) and subsequent reductions in *A* mediated by reduced enzymatic activity, as has been shown in other plants (González and González-Vilar 2001; Lawlor and Cornic 2002; Bacelar et al. 2004; Reddy et al. 2004). The reduction of *A* as a result of reduced RWC, mediated by enzymatic impairment is a rather complex phenomenon. Reddy et al. (2004), however, suggested that the most common reduction in *A* is mediated by the reduced regeneration of Ribulose 1,5-bisphosphate (RuBP) to 3-Phosphoglyceric acid (3-PGA) under water stress conditions. In circumstances where RuBP is limited, *A* would also be limited seeing that a linear relationship exists between RuBP content and *A* (Tezara et al. 1999; Vu et al. 1999; Reddy et al. 2004). Reductions in RWC are also known to acidify the chloroplast stroma, resulting in inhibited rubisco activity and subsequent reduction in *A* (Meyer and Genty 1999).

The reduction in g_s as a result of limiting soil water is more often than not as a result of either chemical or physical changes within the leaf. The most common chemical response resulting in stomatal closure is mediated by abscisic acid (ABA) (Lind et al. 2015; Cai et al. 2017; Tee 2018; Abrams and Loewen 2019). ABA is typically synthesized in the roots in response to drying soil (Sharp and LeNoble 2002) and subsequently transported via the xylem towards the leaf, where receptor sites receive the transported ABA and stomatal closure proceeds (Wang and Zhang 2007; McCourt and Creelman 2008; Seo and Koshiba 2011). Although these responses are well documented in subtropical crops such as citrus (Gomes et al. 2004; Rodríguez-Gamir et al. 2011) and olive (Guerfel et al. 2009; Torres-Ruiz et al. 2013; Torres-Ruiz et al. 2015), very little is known about these responses in macadamias. Further complicating this matter is the lack of information regarding macadamia roots, with reports from Firth et al. (2003) suggesting that although macadamias have taproots, which can exceed 1.0 meters in length, the majority of the roots are shallow (<0.4 meters depth) and fibrous in nature. Given the shallow nature of macadamia roots, it would not be unreasonable to assume that the shallow root zone would quickly be depleted of water during periods of increased atmospheric demand, which could lead to an increased probability of ABA being synthesized in macadamia roots under suboptimal water management conditions.

2.3.2 Water relations under non-limiting soil moisture

Stomatal responses under water stressed conditions can vary significantly between and within species. These variations are, however, not limited to water stressed conditions and under non-limiting soil water conditions stomata and subsequently ψ_{leaf} tend to differ in their response to environmental variables on a daily basis. The most commonly observed variation in both g_s and ψ_{leaf} under non-stress conditions is found in the hysteresis loop response (Körner and Cochrane 1985; Bai et al. 2015; Buckley 2019). The hysteresis loop in g_s and ψ_{leaf} could be explained by means of a three phase process (Körner and Cochrane 1985, Von Willert et al. 1989) during which g_s increases in in the morning in response to increasing solar radiation, which causes a significant drop in ψ_{leaf} (Phase 1), where after ψ_{leaf} stays fairly constant whilst g_s decreases in response to increasing VPD_{leaf} (Phase 2). The final phase is characterized by a stable recovery of ψ_{leaf} with g_s remaining low, whilst ψ_{leaf} increases.

Based on the aforementioned process, the key components driving g_s and ψ_{leaf} in most plants, including macadamias, are solar radiation and VPD_{leaf}. Both Stephenson et al. (2003) and Lloyd et al. (1991) have reported briefly on the response of g_s to VPD_{leaf}. The most significant of these responses was observed in the study by Lloyd et al. (1991), which showed that the highest g_s in macadamias does not correspond to the highest VPD_{leaf} and that under circumstances of non-limiting soil water and saturating levels of solar radiation, g_s can decline rapidly when atmospheric evaporative demand exceeds the maximal rate of water supply out of the leaf. Depending on the growing environment and the VPD_{leaf} cut-off point where g_s ceases to increase as VPD_{leaf} increases, this phenomenon is often referred to as midday depression of g_s and subsequently A and transpiration in plants, and although this has not been extensively reported in macadamias, this has been shown in subtropical fruit trees such as citrus (Vu and Yelenosky 1988; Veste et al. 1999; Hu et al. 2009; Rana and Ferrara 2019), olive (Angelopoulos et al. 1996; Bacelar et al. 2007; Bacelar et al. 2009) and mango (Elsheery et al. 2007; Jutamanee et al. 2008; Jutamanee and Onnom 2016). Midday depression was noted in the study by Lloyd et al. (1991), with diurnal macadamia g_s, on a day with high VPD_{leaf} (30 October 1989), revealing the lowest values of g_s under high levels of irradiance occurring between 1200h and 1300h. This reduction in g_s could possibly lead to decreased A as reported in both citrus and olive (Loreto and Sharkey 1990; Jifon and Syvertsen 2003; Raveh et al. 2003) and offers an opportunity to increase crop growth and yield either through selecting sites which have less diurnal fluctuations in VPD or by means of intermittent overhead sprinklers which will reduce leaf temperature and $VPD_{leaf.}$ The latter approach has been shown to increase macadamia kernel yield (Allan et al. 1994), whilst increased *A* has been reported for citrus (Hu et al. 2009).

Although both proper site selection and evaporative cooling of the canopy can be used to alleviate conditions where $\mathrm{VPD_{leaf}}$ leads to significant reductions in g_s, an in-depth analysis of the response of g_s to $\mathrm{VPD_{leaf}}$ is required to establish the optimal $\mathrm{VPD_{leaf}}$ range for macadamias. To date, no studies have investigated this aspect of macadamias, but the potential to increase g_s and subsequently *A* is a promising aspect to potentially increase growth, yield, and quality of macadamias. It should, however, be noted that stomatal behaviour is not solely under the control of $\mathrm{VPD_{leaf}}$, and environmental and physiological variables such as solar radiation, temperature, wind speed and internal CO_2 concentration (C_i) can also have a significant effect on stomatal behaviour (Jarvis 1976a; Brodribb et al. 2003). To further complicate matters leaf phenological aspects such as leaf age and history, could also have a profound impact on response of stomata to environmental variables (Huett 2004; Choat et al. 2006; Marias et al. 2017).

One of the most studied aspects of macadamias, being the effect of increased solar radiation interception on yield (Huett 2004; McFadyen et al. 2004; McFadyen et al. 2011), has to date not investigated the response of g_s to increased radiation interception and the possible effects this has on whole tree water use. Results from Lloyd et al. (1991), although limited, have shown that g_s reached a maximum fairly early in the morning, which in the study corresponded with a photosynthetic photon flux density (PPFD) of 800 µmol m^{-2} s^{-1}, and g_s failed to increase with increases in PPFD above this point, suggesting that PPFD becomes non-limiting to g_s above the reported value. Although Stephenson and Trochoulias (1994), never reported the response of g_s to increases in solar radiation, they reported that *A* is saturated at rates greater than 800 µmol m^{-2} s^{-1}, which considering the well-defined relationship between *A* and g_s could provide some anecdotal evidence suggesting that maximal g_s is achieved at this point and further limitations to g_s is most likely attributed to other environmental or physiological factors. Nevertheless, the reported value is not dissimilar from that previously reported in citrus (~750 µmol m^{-2} s^{-1}) (Jifon and Syvertsen 2003). It is therefore not surprising that increases in yield and quality of macadamias have been reported with increased solar radiation interception by the canopy (Huett 2004; McFadyen et al. 2004; McFadyen et al. 2011), given that canopy conductance (g_c) and A would increase.

Regardless of how both g_s and ψ_{leaf} can vary within or between species, or with phenology, microclimate, and orchard specific conditions, it is established that, in fruit tree crops stomata respond primarily to VPD_{leaf} to maintain a favourable plant water status, with decreases in g_s occurring without substantial changes in ψ_{leaf} (Flore et al. 1984; Klein 2014; Miner et al. 2017). Transpiration volumes are therefore likely to be controlled by g_s at the leaf level, and g_c when scaled to the canopy level. Although atmospheric evaporative demand would determine the rate of water loss from the leaf or canopy, the rate of water loss would not increase linearly with increased evaporative demand (i.e. supply controlled/limited transpiration) due to the strong stomatal influence present in these crops. This is supported by the results of Stephenson et al. (2003) showing no differences in mean daily water use between non-stressed and mildly stressed macadamia trees even though ψ_{leaf} differed between the two treatments, indicating that g_s and factors driving g_s, which according to the current knowledge available is largely VPD_{leaf}, dictates water use in macadamias. It will therefore be critical to select crop water use models that account for physiological control over water use and is not solely dictated by atmospheric demand.

2.4 Macadamia water-use and water use modelling

Water use, defined as total evapotranspiration (ET) (Allen et al. 1998), serves as the baseline for a crop’s irrigation water requirements. For macadamias, it was initially believed that in order to achieve high yields, a well-distributed rainfall exceeding 2000 mm per annum was needed (Liang et al. 1983). Trochoulias and Johns (1992) later reported that additional irrigation reduced yields of macadamias in areas receiving rainfall ranging between 1200-2000 mm per annum. It was therefore believed that a mean minimum annual rainfall of 1200 mm would be sufficient for macadamia production. This estimate of macadamia water requirement was, however, obtained by assessing WUE and not necessarily WUP and/or actual measurements of evapotranspiration of macadamias.

Nevertheless, with the exception of ET measurements using large through-draining lysimeters conducted by Stephenson et al. (2003), very few studies have been published regarding macadamia ET. The study by Stephenson et al. (2003) found that well-watered mature macadamia trees had an average daily ET of ranging between 50 - 80 L tree^{-1} day^{-1}. Given these values of ET, and assuming a South African industry standard spacing of 8 x 4 m (32 m^2 per tree) the total seasonal ET of macadamias according to Stephenson et al. (2003) should be approximately 570 - 900 mm. This estimated total ET is considerably lower than the initial estimates of the rainfall requirements (~1200 mm) for macadamia and highlights the lack of water use research in the macadamia industry whilst also emphasizing the need for seasonal measurements of water use in macadamias.

The only other report of macadamia water use was published by Gush and Taylor (2014), who used a combination of sap flow and short term measurements of total evapotranspiration to determine ET of field grown macadamias in South Africa. Total season ET calculated in this study was approximately 720 mm, which was highly comparable with that reported by Stephenson et al. (2003), given the fact that variations in canopy size and prevailing weather conditions between the two studies were likely. Gush and Taylor (2014) further reported that the 1200 mm of rain and irrigation applied/received over the 2011/2012 season exceeded ET by approximately 500 mm and exceeded ET_o at the site (1162 mm). It was concluded that the orchard was over-irrigated. The volume of water (1200 mm) applied/received in this study, however, agreed with the minimum rainfall volume required by macadamias as published by Trochoulias and Johns (1992), which considering the fact that the volume applied/received in the study by Gush and Taylor (2014), exceeded both total ET and ET_o it could be said that if guidelines by Trochoulias and Johns (1992) are to be followed indiscriminately as an irrigation guideline, a large portion of orchards could be over-irrigated. Regardless of these discrepancies between published studies, there have been no published studies which have measured seasonal water use and/or transpiration of macadamias, which has in return led to large uncertainty regarding the water requirements of the crop.

The lack of, but high demand for, irrigation related information specific to macadamia creates a need for research which can be extrapolated to a wide range of growing regions. The successful extrapolation of site specific data can be achieved by means of crop water use modelling, but requires proper parameterization if accurate results are to be obtained (Boote et al. 1996; Allen et al. 1998; Allen et al. 2011). The most common modelling approach used, not only by researchers but also by farmers, is the relatively simple FAO 56 crop coefficient (K_c) (Allen et al. 1998). With this model, crop ET can be determined by calculating reference ET of an unstressed and uniform short grass reference surface (ET_o) (Allen et al. 1998), from site specific weather data, and multiplying it with a suitable K_c (Equation 2.1). The K_c encompasses crop specific characteristics and relates these characteristics to that of a reference short grass surface.

One of the major limitations of this model in macadamia is the lack of suitable K_c values, given the lack of macadamia ET data which is required for the calculation of K_c. Reported K_c values for macadamia were approximately 0.65 (Carr 2013), which was in the range (0.50 – 0.78) reported by Gush and Taylor (2014). Although crop coefficients are meant to be transferable across a range of conditions, they can be highly variable and are especially influenced by canopy cover, accompanying vegetation characteristics and varying managing practices, including irrigation and pruning (Allen et al. 1998).

$$ET = K_c \times ET_o \qquad \textbf{Equation 2.1}$$

The FAO 56 crop coefficient model, in its simplest form, assumes a large degree of linearity between ET_o and ET. The degree of linearity, however, becomes less significant when comparing two distinctly different cropping surfaces, i.e. uniform short and smooth reference grass surface and tall, rough orchard canopies (Annandale and Stockle 1994). The transferability of K_c values obtained from one site/orchard to that of multiple sites/orchards is therefore limited to similar climatic zones and orchard characteristics. Possible solutions to the limitations of extrapolation of K_c values have been published (Allen and Pereira 2009; Rosa et al. 2012), and therefore still makes the K_c model a promising model to use, especially in strategic water planning where estimates of seasonal or long term water use is more sought after than daily or hourly estimates. Given the lack of water use studies on macadamias, the successful parameterization of this model could be labelled as a research priority, as the simplistic nature of the model and ease of use by both farmers and irrigation consultants could significantly improve current water management and also aid in better irrigation system design.

One of the technical advantages of the K_c model is the fact that a dual crop coefficient approach could be used to distinguish between the two main components of ET, namely evaporation and transpiration. The dual crop coefficient approach is an extension of Equation 2.1 and separates K_c into the basal crop coefficient (K_{cb}) or transpiration component and the soil evaporation component (K_e) as outlined in Equation 2.2. Partitioning ET between these components allows for more accurate estimations of crop ET, on a daily basis and throughout the growing season, as the fraction of canopy cover, which changes over the season (

Figure 2.1), and irrigation wetting patterns which significantly influences both K_{cb} and K_e, can both be accounted for. Allen et al. (1998), suggested that crop coefficients should be divided into the initial-stage, mid-stage and end-stage of crop development. In mature evergreen crops, such as macadamia and citrus, the canopy size changes significantly less over a season compared to both deciduous annual crops, and therefore the difference in crop coefficients between the crop developmental stages is rather small (Allen et al. 1998). In citrus for example, both K_c and K_{cb} changed by 0.05 between the initial-stage, mid-stage and end-stage of crop development for the same percentage of canopy cover (Allen et al. 1998). A range of other citrus studies have shown that K_c changes on average by 0.07 between autumn, summer, winter and spring (Castel et al. 1987; Castel 1996; Petillo and Castel 2007; Snyder and O'Connell 2007). Gush and Taylor (2014), showed that K_c of macadamia showed greater changes with values in the first season of 0.50 – 0.78 and decreasing in the second season to 0.60 – 0.78.

$$ET=(K_{cb}+K_e) \text{ x } ET_o \qquad \textbf{Equation 2.2}$$

These changes in the crop coefficient can largely be attributed to environmental conditions, as well as the changes in canopy size and the accompanying aerodynamic changes. However, it may be interesting to consider whether the changes in the crop coefficients could be driven by physiological factors such as stomatal regulation of water use. Allen and Pereira (2009), have included a term (F_r) in the estimation of K_{cb}, to account for the degree of stomatal control on transpiration, but currently no studies on macadamias have reported any variation in F_r over a season or across phenological stages. By considering the discussion in Section 2.2 on the variable responses of g_s to increases in VPD_{leaf} in the presence or absence of a considerable sink (i.e. fruit load), and assuming that g_c responds in a similar fashion to that of g_s (Lhomme et al. 1998; Irmak et al. 2008), it is not unreasonable to attribute some of this variation in K_c to physiological factors and it would be advisable to investigate changes in g_c and subsequently F_r over a season.

Determining the contribution of physiological factors to the K_c is rather difficult when considering the timeframe of physiological changes (days to weeks) relative to that of reported K_c values (months). In crops that exert significant stomatal control over transpiration, such as found in crops following a predominantly isohydric strategy, which likely includes macadamia, the K_c model might provide reasonable estimates of seasonal ET, given the reduction in variation of model input parameters brought about by averaging, but may fail to give reasonable and reliable estimates of daily or weekly ET. The K_c model is therefore sometimes replaced by models which incorporate crop physiological parameters, such as the Penman-Monteith model (Monteith and Unsworth 1990) often referred to as “big leaf” models. These models have one major assumption being that entire crop fields or orchards are treated as a single surface with uniform characteristics.

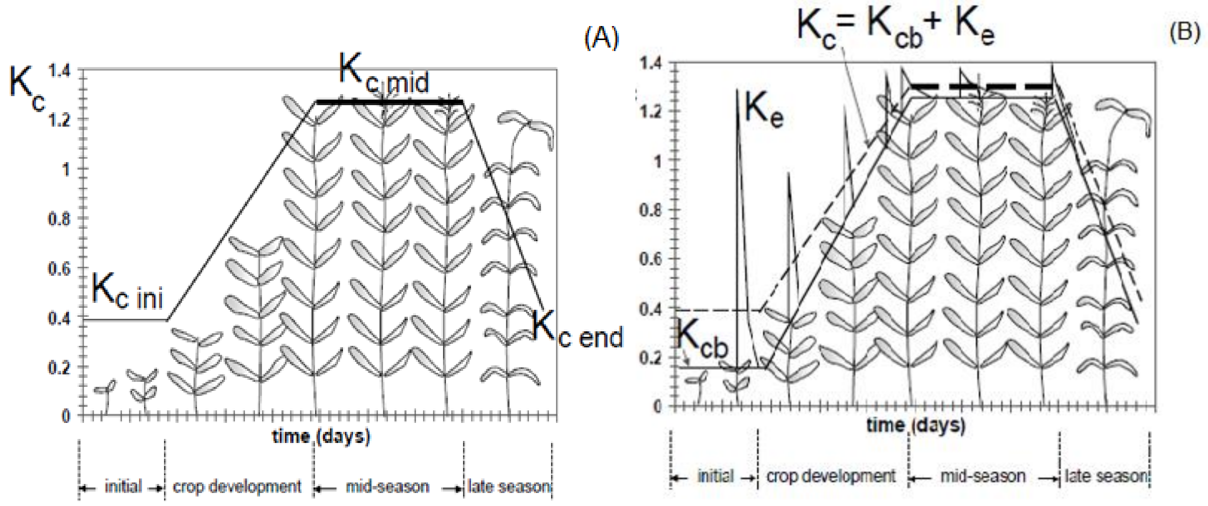

Figure 2.1: (A) General single crop coefficient (K_c) curve and (B) variation in dual crop coefficients including basal crop coefficient (K_{cb}) and soil evaporation coefficient (K_e) throughout the various crop stages as adapted from (Allen et al. 1998)

Nevertheless, the Penman-Monteith equation (Monteith and Unsworth 1990) is shown in Equation 2.3, where λ is the latent heat of vaporization of water (J kg^{-1}), E_c is canopy transpiration (kg m^{-2} s^{-1}), Δ is slope of the vapour pressure curve (kPa K^{-1}), R_n is net radiation at the crop surface (W m^{-2}), G is soil heat flux (W m^{-2}) taken as 10 % of R_n, ρ_a is the density of dry air (kg m^{-3}), C_p is the specific heat capacity of the air (J kg^{-1} K^{-1}), VPD is saturation vapour pressure deficit (kPa), γ is the psychrometric constant (kPa K^{-1}), g_a is the aerodynamic conductance (m s^{-1}) and g_c is the canopy conductance (m s^{-1}).

$$\lambda E_c = \frac{\Delta(R_n - G) + \rho_a C_p g_a VPD}{\Delta + \gamma\left(1 + \frac{g_a}{g_c}\right)}$$

Equation 2.3

Even though a large portion of the parameters required to solve Equation 2.3 can be obtained from an automated weather station, g_a, g_c and R_n are often estimated or modelled. The most widely used models for g_c is that proposed by Jarvis (1976). This model, and various extensions of the model, are often used in conjunction with the Penman-Monteith equation to generate reasonable values of E_c. It should also be noted that E_c is often measured by means of sap flow or eddy covariance techniques and g_c is then calculated by means of the inversion of Equation 2.3 (Granier and Bréda

1996; Lu et al. 2003; Oguntunde et al. 2007). In most applications of Equation 2.3, the Jarvis (1976) type model (Equation 2.4) and varitions of this model is also required to quantify a set of seasonal response terms describing the functional relationships among g_c, R_s, VPD, air temperature (T_{air}) and soil water potential (θ), to give modelled predictions of g_c, which are needed in Equation 2.3. The functional relationships describing the response of g_c to R_s, VPD, T_{air} and θ can be assessed mathematically as has been described by Whitley et al. (2008), Stewart (1988), Wright et al. (1995) and Harris et al. (2004). In most studies irrigated studies of tree water use, θ is often neglected from the Jarvis (1976) type models (Equation 2.4) seeing that θ would have a limited impact on g_c and the functional terms of the Jarvis type model can be described as outlined in Equations 2.5 – 2.8. These mathematical relationship of g_c as encapsulated by Equation 2.4, weights maximum g_c ($g_{c\ max}$) with each response function (Equations 2.5 – 2.8) which have values between 0 and 1, and the maximum value of 1.0 is attained only at certain optimum conditions, which is rarely the case (e.g. Jarvis,1976; Wright et al., 1995) and in such a way $g_{c\ max}$ is rarely achieved.

$$g_{c,j}=g_{c\ max}f(S_R)f(VPD_{air})f(T_{air}) \quad \textbf{Equation 2.4}$$

$$f(R_s)=\frac{R_s}{R_m}\left(\frac{R_m+k_R}{R_s+k_R}\right) \quad \textbf{Equation 2.5}$$

$$f(T_{air})=\frac{(T_a-T_L)(T_H-T_a)^t}{(k_T-T_L)(T_H-k_T)} \quad \textbf{Equation 2.6}$$

$$t=\frac{T_H-k_T}{k_T-T_L} \quad \textbf{Equation 2.7}$$

$$f(VPD)=k_{e1}VPD_{air}\exp(-k_{e2}VPD_{air}) \quad \textbf{Equation 2.8}$$

Equation 2.4 describes the radiation response, showing an asymptotic saturating function that plateaus at R_m, which is approximately 1000 W m^{-2}, with k_R (W m^{-2}) describing the curvature of the relationship. Hyperbolic saturating functions describing R_s have been applied extensively at leaf, tree and canopy scales for conductance (Kelliher et al., 1993; Granier et al., 2000) and for tree water use (Komatsu et al., 2006). The temperature response function in Equation 2.6 typically describes the physiological response of g_c to temperature with parameters T_L and T_H in Equations

2.6 and 2.7 being the lower and upper temperature limit to g_c, and is often fixed at 0°C and 45 ^oC, respectively as this is the physiological temperature limits to most crops. The modelling parameters k_{e1} and k_{e2} of Equation 2.8, describe the rate of change in g_c at low and high atmospheric demand and has been used successfully in native Australian forests by Whitely et al. 2009. There are, however, multiple variations to Equations 2.5 – 2.8 and assessing the response of g_c to each of the environmental variables is critical to ensure optimal model performance.

Nevertheless, in other crops exhibiting strict stomatal control over transpiration, including citrus (Kriedemann and Barrs 1981; Sinclair and Allen 1982) and olive (Fernández et al. 1997; Giorio et al. 1999), a Jarvis-type model has provided accurate estimates of g_c (Cohen and Cohen 1983; Villalobos et al. 2000; Testi et al. 2006; Oguntunde et al. 2007). It would, therefore, be logical to test such models on macadamias, as reasonable estimates of g_c could then be utilized in solving E_c using Equation 2.3.

One of the major limitations to using g_c to obtain reliable estimate of E_c, especially in so called "big leaf" models, is engrained in the fact that most g_c estimates scale leaf level g_s to an entire canopy by using average measurements of leaf area index (LAI). This poses an array of problems, considering that unequal distribution of solar radiation within the canopy and variations in leaf age and angle, in combination with microclimatic variations within the canopy, could lead to some erroneous estimates of g_c when simply scaled by means of LAI. In an attempt to overcome these limitations, Leuning et al. (1995) has developed a multilayer approach in which the canopy is divided into various layers, g_s is estimated for each layer, and weighted with the LAI for the layer. This approach still uses averages of LAI in scaling g_s from a leaf level to a canopy level, which would subsequently lead to erroneous estimate in g_c.

Acknowledging the limitations linked to the scaling of g_s to g_c through the use of LAI, another approach for modelling g_c has been developed by Villalobos et al. (2013). In this approach, g_c is modelled directly using measurements of E_c, and is based on the concept that E_c is directly proportional to radiation interception. In well coupled sclerophyllous tree crops such as olive (Villalobos et al. 2000; Orgaz et al. 2007), this modelling approach has been shown to be rather effective and could prove to be equally effective for sclerophyllous macadamias, although no such studies have been published to date. Nevertheless, this direct approach for estimating g_c is used to determine crop specific modelling parameters a and b (Equation 2.4) by means of linear regression of (f_{IPAR}*R_s)/g_c against VPD_{air}. After mathematical determination of parameters, a and b, direct estimates of daily E_c (mm day^{-1}) can be obtained using the Equation 2.4:

$$E_c=0.3708\frac{f_{IPAR}R_s}{a+b\,VPD_{air}}\frac{VPD_{air}}{P_a} \qquad \textbf{Equation 2.4}$$

where f_{IPAR} is the fraction of photosynthetically active radiation intercepted by the canopy (dimensionless), R_s is the total daily solar radiation (J m^{-2} d^{-1}), P_a is the atmospheric pressure (kPa), and the coefficient 37.08×10^{-3} incorporates the conversion of units for Joules of R_s to mol quanta and from mol to kg of H_2O.

As far as we are aware, only Gush and Taylor (2014) attempted to model macadamia water use. They reported that under conditions of high atmospheric evaporative demand, the FAO 56 crop coefficient model tended to overestimate macadamia water use. It was proposed that a model including the driving variables of canopy conductance and transpiration would be better suited to macadamias. There are, however, no published studies for macadamia that have successfully parameterized canopy conductance models feeding into equations calculating canopy transpiration. This presents researchers within the field of tree crop water use with an opportunity to contribute to improved science-based water management theory and practice.

From the current assessment of available literature on water use modelling of macadamia, it is concluded that models incorporating g_c should be investigated if reasonable estimates of macadamia ET are to be achieved. Although g_c is rather difficult to measure, models combining the work of Lloyd (1991) with the derivatives of Jarvis-type models (Cohen and Cohen 1983; Villalobos et al. 2000; Testi et al. 2006; Oguntunde et al. 2007) and Villalobos et al. (2013) could potentially be used to obtain reliable measures of g_c.

2.5 Conclusion

The evaluation of available literature confirms the substantial lack of macadamia specific physiological information, especially in the fields of water use and ecophysiology. Consequently, very little is known about the irrigation and water requirements of this evergreen subtropical crop, which is surprising considering the tremendous expansion of macadamia plantings in semi-arid countries, including South Africa and Australia. The lack of information regarding the physiological and morphological components of macadamias has led to a large amount of speculation regarding the carbon assimilation, water use and drought tolerance of the crop (Stephenson et al. 2003; O’Hare et al. 2004; Stephenson and Searle 2014).

The literature examined suggested that physiological and morphological adaptations, including leaf sclerophylly and the hydraulic architecture typical of subtropical trees, contribute to a predominantly isohydric water management strategy in macadamia. Crops following isohydric water management strategies tend to exercise strict stomatal control to maintain the hydraulic continuum within safe limits and minimize the risk of hydraulic embolism. Strict stomatal control could also be associated with limited leaf level carbon gain, which could place limitations on the supply of energy-rich assimilates to the oil storing crop. It is possible that macadamias can deviate from a pure isohydric strategy to a more dynamic isohydric/anisohydric strategy in the presence or absence of a significant sink (i.e. developing fruit).

Such a deviation could be facilitated by changes in stomatal responses to environmental variables, which in turn, could be linked to variable responses of stomata to either physical or chemical signals. If stomatal conductance can be extrapolated to canopy conductance, a variable response of stomata to environmental changes could significantly influence the accuracy and reliability of crop water use models. Models, such as the commonly used FAO-56 crop coefficient model, may provide relatively accurate seasonal estimates of macadamia water use, but would possibly fail to provide a reasonable estimate of weekly or even monthly water use. Models incorporating canopy conductance, although difficult to parameterize, could potentially provide more accurate estimates of macadamia water use compared to the FAO-56 crop coefficient model.

CHAPTER 3: GENERAL MATERIALS AND METHODS

The aim of this chapter is to describe the materials and methods shared by each of the working chapters within this thesis. Specific details pertaining to the materials and methods used in each of the working chapters, including but not limited to, experimental layout, data and statistical analysis, will be described in detail in each of the respective chapters. The general materials and methods chapter will therefore consist of sections describing the study site, weather data collection, measurements of canopy dimensions and yield as well as details pertaining to the measurement and determination of transpiration, evapotranspiration, and leaf gas exchange.

3.1 Measurement strategy in macadamia orchards

3.1.1 Site and Orchard description

The trial was conducted on a commercial macadamia farm located approximately 35 km west of Nelspruit in the Schagen Valley, Mpumalanga, South Africa (25°21'50.36" S, 30°46'46.47" E, approximately 900 m.a.s.l.). The area has a seasonally dry sub-tropical climate ideal for macadamia production, although environmental conditions can vary considerably. On average the area has an annual precipitation of approximately 750 – 850 mm and has an annual average temperature of 23°C, with January typically being the hottest month (Schulze 1997).

The trial consisted of two orchards, one consisted of fully irrigated mature bearing (MB) macadamia trees, whilst the other orchard consisted of fully irrigated, immature bearing (IB) macadamia trees (Table 3.1). The MB orchard was planted in 2005 and was approximately 3.8 ha in size, planted at a population of 312 trees ha^{-1} (spacing 8 x 4 m i.e. 32 m^2 per tree). Trees were planted in an approximate north-south orientation and were allowed to form a complete hedgerow, with height being controlled below 6.0 m by means of annual hand pruning. Pruning practices included selective limb removal of the tallest vertical branch and a single major side branch. These trees had an average leaf area index (LAI) of 5.25 m^2 m^{-2} and an estimated canopy cover of 0.72 at the start of the measurement period (10 August 2016). Furthermore, the orchard was irrigated using one 50 L h^{-1} (5.5 mm h^{-1}) microsprinklers per tree, with a wetted diameter of 1.7 m and irrigation was typically scheduled once a week with soil water content monitored using DFM capacitance probes. This was historically a high yielding orchard, with up to 6 t ha^{-1} dry in-shell (DIS) previously recorded in this orchard. Inter-rows consisted of grass cover, interspersed with a variety of weeds. Tree rows were free of weeds as a result of shading under the trees (Figure 3.1).

The IB orchard was located approximately 4 km North-West of the MB orchard and consisted of 5-year old (Planted 2012) macadamia trees, planted at a population of 312 trees ha^{-1} (spacing 8 x 4 m, i.e. 32 m^2 per tree). The trees were planted in an approximate east-west orientation and were characterized by canopies which had not yet formed a hedgerow (i.e. canopies not touching within the row). Trees within this orchard were lightly pruned by means of selective limb removal to maintain an approximate cone shape and had an average leaf area index (LAI) of 1.44 m^2 m^{-2} and an estimated canopy cover of 0.28 at the start of the measurement period (16 August 2017). Furthermore, this orchard was irrigated by means of one 50 $L.h^{-1}$ microsprinkler per tree (wetted diameter of 1.4 m), placed in close proximity to tree stems. No irrigation scheduling equipment (i.e. capacitance probes) was present at the intermediate orchard site and a fixed irrigation regime of approximately 150 L $tree^{-1}$ $week^{-1}$ was maintained. Given that these orchards had just started producing, historic yields were low (between 1 and 2 t ha^{-1}) but were within industry norms. Tree rows were kept clean by means of regular herbicide applications, with shortly mown inter-rows consisting of a mixture of weeds and grasses (Figure 3.2)

3.1.2 Weather variables

A WS-GP1 Delta-T (Delta-T Devices Ltd, Cambridge, United Kingdom) weather station was installed close to the MB orchard (Figure 3.1). The station collected weather data including solar radiation (R_s), windspeed (u_2) and direction, air temperature (T_{air}), air relative humidity (RH) and rainfall at 20-minute intervals for approximately 3 years (10 August 2016 – 5 August 2019). Air vapour pressure deficit (VPD_{air}) was calculated from T_{air} and RH. Reference evapotranspiration (ET_o) was calculated using the FAO Penman-Monteith equation for a short grass reference surface, as described by Allen et al. (1998) and Pereira et al. (2015).The weather station was installed over a dry short grass surface and was within 50 m of irrigated

MB orchard. There was natural vegetation to the north and east of the weather station, which consisted mostly of short grass. Thus, depending on wind direction, the ET_o was either well estimated or slightly overestimated. When wind blows over the natural vegetation towards the AWS the air is likely to be fairly dry air, resulting in an overestimation of ET_o as opposed to the well-watered reference surface. Quality assessment and quality control of the data was performed according to the procedures described by Allen (2008). Throughout the entire data set, only corrections to R_s were required, with a multiplication factor of 1.27 applied to measured values. This value was used based on assessments of R_s on clear days, which fell significantly below the computed R_s under clear sky conditions (R_{so}).

Figure 3.1: The location of the mature bearing (MB) 'Beaumont' macadamia orchard and the automatic weather station on Mayo Estate in the Schagen Valley close to Nelspruit.

Figure 3.2: The intermediate bearing (IB) macadamia orchard with short, mown inter-rows consisting of grasses and a variety of weeds.

Table 3.1: Details of the mature and immature bearing orchards used in the trial.

Orchard Reference	Mature Bearing (MB)	Intermediate Bearing (IB)
Cultivar	'Beaumont' Macadamia	'Beaumont' Macadamia
Rootstock	'Beaumont'	'Beaumont'
Planting date	2005	2012
Orchard block area	3.8 ha	3.0 ha
GPS co-ordinates	25°21'50.36" S, 30°46'46.47" E	25°21'2.01"S, 30°43'44.30"E
Tree spacing	4 m x 8 m (32 m^2), planted on ridges	4 m x 8 m (32 m^2)
Row orientation	North-South	East-West
Irrigation - Type - Delivery rate - Wetted diameter	 Microsprinkler 50 L h^{-1} 1.7 m	 Microsprinkler 50 L h^{-1} 1.4 m
Canopy dimensions*	Height – 5.7 m Width – 5.8 m Breadth – 4 m - Hedgerow	Height – 4.2 m Width – 3.0 m Breadth – 3.0 m
Canopy cover*	0.72	0.28
Leaf area index* – orchard ($\bar{x}$ = 4 measurements) – individual trees	 5.25 m^2 m^{-2} 1 – 5.49 m^2 m^{-2} 2 – 5.25 m^2 m^{-2} 3 – 4.95 m^2 m^{-2} 4 – 5.33 m^2 m^{-2}	 1.44 m^2 m^{-2} 1 – 0.88 m^2 m^{-2} 2 – 1.64 m^2 m^{-2} 3 – 1.75 m^2 m^{-2} 4 – 1.49 m^2 m^{-2}
No of experimental trees	4	4
Stem circumferences of trees instrumented with sap flow equipment*	1 – 46.2 cm 2 – 45.6 cm 3 – 44.6 cm 4 – 50.9 cm	1 – 35.2 cm 2 – 37.8 cm 3 – 37.2 cm 4 – 36.5 cm

* Variables measured at the start of the trial

3.1.3 Canopy dimensions and fractional cover

Canopy dimensions (height, width, and breadth) were measured throughout the trial in both orchards. Additional measurements of canopy dimension were made using drone imagery and processing software developed and maintained by Aerobotics (Cape Town, Western Cape, South Africa). Drone technology has been shown to provide reasonable estimates of tree height and canopy dimension measurements (Dempewolf et al. 2017; Panagiotidis et al. 2017), and was as a result deemed as acceptable for this study. Canopy volume was calculated by assuming that macadamia canopies have an ellipsoid shape. Measurements of leaf area index (LAI) and fractional interception of photosynthetically active radiation (PAR) were performed randomly throughout the duration of the trial using a Decagon AccuPAR LP-80 ceptometer (Decagon Devices, Pullman, WA, USA). Sampling of PAR below the canopy was conducted across and within the row (covering the total area allocated to one tree) at pre-determined 1 m intervals (Figure 3.3), whilst full sun measurements were taken in an open area next to each orchard. All measurements were taken between 12:00 and 14:00, under clear sky conditions.

Figure 3.3: Grid system for fractional interception of PAR measurements in the mature bearing (MB) macadamia orchard.

3.1.4 Yield and quality determination

Yield was measured on an individual tree basis by means of hand harvesting trial trees in both the MB and IB orchard. Yields including nut in husk (NIH), wet in shell (WIS) and dry in shell (DIS) weights were recorded separately. Harvested nuts were de-husked using a single de-husking plant to ensure that similar WIS recovery rates were obtained from each tree. Following the de-husking process, WIS nuts were dried at a temperature of 34°C for approximately 14 days, where after the DIS weight was measured. Quality assessments were conducted by the processing facility staff by means of hand sorting kernel according to industry developed standards.

3.1.5 Sap flow

Sap flow measurements were performed using the heat ratio method of the heat pulse velocity technique as developed by Burgess et al. (2001) and described in citrus by Taylor et al. (2015) using the locally manufactured equipment. This technique was used on four sample trees in each orchard based on a stem circumference survey conducted at each of the respective orchards described in Section 3.1. Four custom made heat pulse probe sets were inserted at four different depths which were 1, 2, 3 and 4.5 cm beyond the cambium in the MB trees and 1, 2, 3 and 4 cm beyond the cambium in the IB orchard. Depths selected in each tree trunk were used to account for the radial variation in sap flux within the conducting sapwood. Each probe set consisted of two Type T (copper/constantan) thermocouples (embedded in 2.0 mm outside diameter polytetrafluoroethylene (PFTE) tubing) placed equidistantly (0.5 cm) upstream and downstream of the heater probe inserted into a brass collar (2.5 mm). These probe sets were inserted above the rootstock in the scion and below the lowest branch, with probes being equally spaced around the trunk and randomly arranged, taking care to avoid any abnormalities in the trunk. The heat pulse velocity (V_h) in cm h^{-1} for each probe set was calculated following Marshall (1958) as:

$$V_h=\frac{k}{x}\ln\left(\frac{v_1}{v_2}\right)*3600 \qquad \textbf{Equation 3.1}$$

where k is the thermal diffusivity of green (fresh) wood (assigned a value of 2.5 x 10^{-3} $cm^2\ s^{-1}$ (Marshall 1958)), x is distance in cm between the heater and either the upper or lower thermocouple, v_1 and v_2 are the maximum increases in temperature after the heat pulse is released (from initial temperatures) as measured by the upstream and downstream thermocouples and 3600 converts seconds to hours. Heat pulse velocities were measured and logged on an hourly basis using a CR1000 data logger and an AM16/32B multiplexer (Campbell Scientific Ltd, Logan, Utah, USA). Conversion of heat pulse velocities to sap flux densities, taking into account wounding, were performed according to Burgess et al. (2001). Wounding corrections were performed by using wounding coefficients *b, c*, and *d* obtained from a numerical model developed by Burgess et al. (2001) using the following equation:

$$V_c=bV_h+cV_h^2+dV_h^3$$ **Equation 3.2**

where V_c is the corrected heat pulse velocity. The functions describing the correction coefficients in relation to wound width (w) were as follows:

$$b = 6.6155w^2+3.332w+0.9236$$ **Equation 3.3**

$$c = -0.149w^2+0.0381w-0.0036$$ **Equation 3.4**

$$d = 0.0335w^2-0.0095w+0.0008$$ **Equation 3.5**

The wound width was assessed through visual inspection and subsequent measurement of the outer diameter of the wound. These measurements were made at the end of the measurement campaign in both the MB and IB orchards, with measurements of wounding in both orchards being minimal and were on average 0.30 cm total width of probes and wound (Table 3.2). In order to assess the accuracy of the determined wounding factor measurements of evapotranspiration made alongside measurements of transpiration during periods with no rainfall and/or irrigation (20 - 31 July 2018, i.e. little to no soil evaporation) were used. This assessment revealed that estimates of transpiration obtained from sap flow data and evapotranspiration measurements made using eddy covariance method were fairly similar when using the measured wounding width (0.30 cm). If a wounding width greater than 0.30 cm was used in the calculation, measurements of transpiration during this period, would exceed measurements of total evapotranspiration (Figure 3.4). Although, measurements of transpiration, using a wounding width of 0.30 cm, was on average 0.15 mm day^{-1} lower than that of total evapotranspiration, it is unreasonable to assume that no evaporation or transpiration from the plant cover between the rows (both weeds and grass) would have occurred.

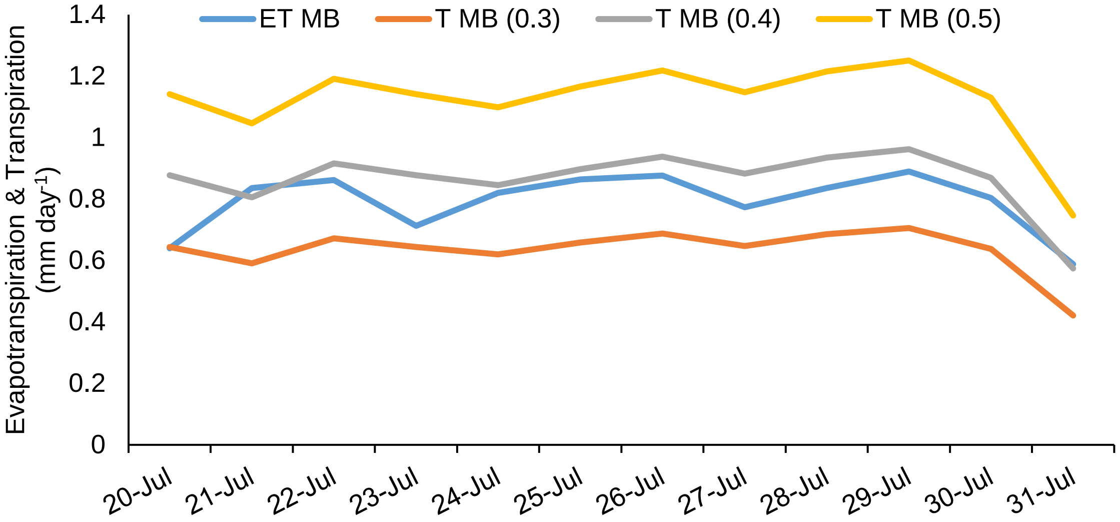

Figure 3.4: Measurements of evapotranspiration (ET) and transpiration (T) using three different wounding widths (0.30, 0.40, and 0.50 cm) in the mature bearing (MB) orchard from 20 July 2018 – 31 July 2018.

The presence of heartwood was determined by taking wood cores with an incremental borer. These core samples were stained using safranin, with unstained areas being marked as non-conducting wood. As there was no change in colour of the wood from the outside (youngest wood) to the centre of the core (oldest wood) in any of the samples taken in each orchard, it was assumed that no heartwood (non-conducting xylem) was present. This was confirmed by the uniformity of the safranin stain across the wood sampled. Other wood characteristics, including sapwood moisture content (m_c) and density (ρ_b) were determined from additional core samples taken during the measurement period (Table 3.2).

Table 3.2: Parameters used for transpiration estimates obtained from destructive measurements in the mature bearing (MB) orchard

Parameter	Value used
Wood density (ρ_b)	0.69 g cm^{-3}
Wood moisture content (m_c)	62.0 %
Wound width*	0.30 cm

*Wound width was taken as an average of measurements made from the four experimental trees, in both the MB and IB orchard, and was assumed that wound width was radially constant. Wound width was measured as the total width of the probe and additional wounding that occurred as part of the installation process.

Following the determination of m_c and ρ_b, sap velocity (V_s) was calculated from the corrected heat pulse velocity using the equation suggested by Marshall (1958) that was later modified by Barrett et al. (1995):

$$V_s=\frac{V_s\rho_b(c_w+m_c c_s)}{\rho_s c_s} \qquad \textbf{Equation 3.6}$$

where c_w and c_s are specific heat capacity of the wood matrix (1200 J kg^{-1}°C^{-1} at 20 °C (Becker and Edwards, 1999) and sap (water, 4182 J kg^{-1}°C^{-1}) at 20 °C (Lide, 1992), respectively, and ρ_s is the density of water (1000 kg m^{-3}). Volumetric flow for individual probes was calculated as the product of V_s and its cross-sectional area of conducting sapwood. Whole stem flux (Q) was calculated, by means of a weighted average of heat pulse velocity with depth (Equation 3.7), as applied by Hatton et al. (1990).

$$Q=\pi\left[r_1^2*v_1+\left(r_2^2-r_1^2\right)*v_2+\left(r_3^2-r_2^2\right)*v_3+\left(r_4^2-r_3^2\right)*v_4\right] \qquad \textbf{Equation 3.7}$$

where v_x is the heat pulse velocity measured by sensor x, placed between radii r_{x-1} and r_x. Integrated volumetric sap flow of the individual trees (L day^{-1}) was converted to transpiration (mm day^{-1}) using the ground area allocated to each tree in the orchard i.e. 32 m^2. Orchard transpiration was calculated as a weighted average of sampled trees as suggested by Hultine et al. (2010), based on a stem circumference survey at the start of the study.

3.2 Ecophysiology measurements

Ecophysiological measurements were restricted to only the MB orchard, where clear phenological changes and the large canopy of trees within in the MB orchard were ideal for the measurements. Measurements of leaf gas exchange including but not limited to, net assimilation CO_2 rate (A), stomatal conductance (g_s), leaf transpiration rate (E_{leaf}) and intercellular CO_2 concentration (C_i) were measured using an infra-red gas analyser (IRGA) (Model: LI-6400 XT, LI-COR, Lincoln, Nebraska, USA). Sensors inside the cuvette monitored leaf surface temperature (T_{leaf}) and leaf-to-air vapour pressure deficit (VPD_{leaf}). All measurements of leaf gas exchanges were made on healthy, mature, sun exposed and hardened-off macadamia leaves. Supplementary measurements of stomatal conductance (g_s) were also measured using an AP4 porometer (Delta-T Devices Ltd, Cambridge, United Kingdom) and/or an SC-1 leaf porometer (Decagon Device Inc, Pullman, WA, USA). Stomatal conductance was sampled at 30-minute intervals throughout the day. These measurements were assumed to be a representative sample of leaves on the tree and should approximate canopy conductance.

Measurements of pre-dawn leaf water potential (ψ_{pd}) were made in both the MB and IB orchard using a Scholander pressure chamber (Model 600, PMS Instrument Company, Albany, OR, USA). These measurements were made the same four trees used for sapflow measurements in order to assess the water status of trees in an attempt to eliminate water stress as a confounding factor in any of the results presented. A total of 180 ψ_{pd} measurements were made in the MB orchard and 63 ψ_{pd} measurements in the IB orchard throughout the duration of the trial. Water stress is believed to occur at ψ_{pd} exceeding -0.5 MPa based on research from Stephenson 2003.

3.3 Evapotranspiration measurements

Two sets of evapotranspiration (ET) measurements were obtained in the MB orchard, with the first set commencing in September 2017 and continued until October 2017, whilst the second set of measurements commenced May 2018 and concluded in September 2018. Fluxes of latent (LE) and sensible heat (H) were measured with an extended open path eddy covariance (OPEC) system, comprising an IRGASON open-path analyser and sonic anemometer (Campbell Scientific Inc., Logan, Utah, USA), which was mounted on a lattice mast 7.5 m above the soil surface (1.5 m above the canopy). Upwind and downward fetch of the prevailing north westerly and south easterly winds was 150 m. Air temperature and humidity were measured using a HygroClip2 HC2-S(3) thermohygrometer probe (Rotronic Instruments, Bassersdorf, Switzerland). Net radiation (R_n) was measured using an NR-Lite net radiometer (Model 240-110 NR-Lite, Kipp & Zonen, Delft, Netherlands) 7.5 m above ground. Four soil heat flux plates (model HFT-S, REBS, Seattle, Washington, USA) were used to measure soil heat flux (G) at a depth of 80 mm under the trees and between the rows, and four TCAV-L soil temperature averaging probes (Campbell Scientific Inc., Logan, Utah, USA) at depths of 20 and 60 mm were used to calculate the heat stored above the plates. Volumetric soil water content in the first 60 mm of the soil surface was measured using two-time domain reflectometer (CS616, Campbell Scientific Inc., Logan, Utah, USA) placed near the heat flux [pates. Measurements were sampled at a frequency of 10 Hz and logged on a CR3000 data logger (Campbell Scientific Inc., Logan, Utah, USA) using the Easyflux-DL software from Campbell Scientific. The program applies the most common open-path EC corrections to fluxes.

CHAPTER 4: THE SEASONAL REGULATION OF GAS EXCHANGE AND WATER RELATIONS OF FIELD GROWN MACADAMIA

4.1 Abstract

Macadamia F. Muell is a recently domesticated nut crop characterized by a certain amount of drought tolerance. Whilst stomatal regulation of gas exchange and water relations has been documented in macadamia, there are no reports on non-stomatal limitations to photosynthesis and if changes occur over a season in relation to different phenological stages. It was hypothesised that in order for macadamias to be adapted to a seasonally dry native environment the trees would be characterised by strong stomatal control over gas exchange, indicating an isohydric water use strategy that is related to a hydraulic limitation within the tree. However, due to high assimilate demand during nut filling, the level of stomatal control would vary between fruiting and non-fruiting phenological stages. Gas exchange and water relations measurements were made for 18 months on irrigated mature macadamia trees (cv. HAES 695, 'Beaumont') in a subtropical region of South Africa. Results confirmed that macadamias had relatively low light-saturated net CO_2 assimilation rates (A_{max}) (8.34 ± 1.21 µmol CO_2 m^{-2} s^{-1}). The low A_{max} values resulted from relatively high stomatal and non-stomatal limitations and decreasing stomatal conductance (g_s) in response to increasing leaf-to-air vapour pressure deficit (VPD_{leaf}) above ca. 2 kPa. Strict stomatal control and nearly constant midday leaf water potential (ψ_{leaf} = -1.16 ± 0.43 MPa) confirmed the predominantly isohydric nature of the crop, which seemed to be a result of low hydraulic conductance in the stem to leaf pathway. Significant differences in leaf gas exchange capacity were, however, observed between the fruiting and non-fruiting phenological stages. The presence of fruit resulted in significantly higher A_{max} (10.27 ± 2.23 µmol CO_2 m^{-2} s^{-1}) compared to non-fruiting periods (A_{max} = 6.58 ± 2.00 µmol CO_2 m^{-2} s^{-1}). Increases in A_{max} were mediated by increased rates of electron transport (J_{max}) and triose phosphate use (TPU). Fruiting stages were also characterized by variable responses of g_s to increases in VPD_{leaf}, which indicated varying degrees of isohydricity. This study reaffirms that macadamias are inherently adapted to seasonally dry environments, characterized by strict stomatal control, yet under humid conditions or in the presence of developing fruit, macadamias deviate from a purely isohydric water management strategy.

Keywords: Hydraulic conductance, Isohydric, Non-stomatal limitation, Light saturation

4.2 Introduction

Macadamia F. Muell and more specifically *M. integrifolia* (Maiden & Betche) and *M. tetraphylla* (L.A.S. Johnson) (Proteaceae), have gained popularity in the global agricultural sector due to their highly valued and nutritious kernel. Despite the significant growth of this recently domesticated horticultural crop (estimated 1930's (Stephenson 2005)) both within and outside the species' natural distribution in seasonally arid, subtropical south-eastern Australia, very little is known about the gas exchange and water relations of field grown *Macadamia* species. Whilst detailed gas exchange modelling studies have been performed in potted trees and mature trees (Lloyd, 1991; Lloyd et al. 1991; Syvertsen et al. 1995), these studies did not assess the dynamic and interrelated nature of leaf gas exchange and water relations. In order to optimally cultivate macadamias, a holistic understanding of leaf gas exchange and water relations, which account for potential shifts in photosynthetic capacity, stomatal regulation of carbon gain, plant water status, and crop phenology is essential.

Despite the lack of long term macadamia specific leaf gas exchange studies, there is sufficient evidence to suggest that as a result of leaves being long lived, hypostomatous and sclerophyllous (Hardner et al. 2009) there may be both stomatal and non-stomatal limitations to photosynthesis (Lloyd 1991, Lloyd et al. 1991, Lloyd et al. 1992). Sclerophyllous leaves typically have low mesophyll carbon dioxide (CO_2) conductance (Lloyd et al. 1992; Syvertsen et al. 1995), and a high level of internal self-shading (Read et al. 2006; Marchi et al. 2008), which is often accompanied by lower photosynthetic rates. Although adaptations such as leaf sclerophylly are advantageous under seasonally dry and drought conditions, these conditions rarely exist in well managed, irrigated agriculture, and may impose a constraint on yield and quality of an oil storing crop such as macadamia, which has a high assimilate demand.

In addition to significant non-stomatal limitations to gas exchange, such species may also show dynamic stomatal responses to achieve a favourable ratio of carbon gain to transpirational water loss (instantaneous water use efficiency, WUE_i) (Farquhar and Sharkey 1982) and to control leaf water potential within certain limits to prevent loss of hydraulic conductivity through xylem cavitation (Sperry 2000; McDowell et al. 2008). The stomatal regulation strategies employed are generally closely attuned to the hydraulic capabilities of the tree from the roots to the leaves (Sperry et al. 1993; Nardini and Salleo 2000). Hydraulic limitations to leaf gas exchange may differ significantly between and within plant species (Sperry 2000; Schultz 2003) depending on the need to avoid hydraulic failure in stressful environments. This trade-off between WUE_i and hydraulic safety, as controlled by stomatal conductance (g_s) and leaf water potential (ψ_{leaf}), is broadly classified into two strategies, with plants typically exhibiting isohydric or anisohydric behaviour (Tardieu and Simonneau 1998). Anisohydric plants show a distinct diurnal decline in ψ_{leaf} with increased atmospheric evaporative demand under well-watered conditions (Schultz 2003). Isohydric plants, on the other hand, are characterized by near constant midday ψ_{leaf} under various soil water regimes and atmospheric evaporative demands, as a result of strict stomatal control, which is indicative of a more conservative water management strategy. It should, however, be noted that by analysing a number of plant species Klein (2014) found a continuum between isohydric and anisohydric behaviours rather than a dichotomy and within species, these behaviours may differ between contrasting seasons or in response to fruiting (Sade and Moshelion 2014).

Macadamia water stress trials in Australia (Lloyd et al. 1991), demonstrated an approximate 30% reduction in g_s in non-irrigated trees, whilst daytime ψ_{leaf} remained fairly constant and highly comparable to that of irrigated trees for approximately eight weeks following the cessation of rains. In addition, irrigation was reported to have no effect on daytime ψ_{leaf} throughout the trial. This seems to imply that macadamias follow a predominantly isohydric water management strategy. However, the finding by Lloyd et al. (1991) of high hydraulic conductance of macadamia trees relative to a range of other crops, including citrus, olive, apple, and peach, seems to be in contradiction to other isohydric species which are generally characterized by low whole tree hydraulic conductances (Sperry et al. 1993; Nardini and Salleo 2000). As the methodology used by Lloyd et al. (1991) to determine hydraulic conductance is not comparable to many other studies and seems to be in contrast to other studies, a more detailed examination of components of whole tree hydraulic conductance is needed to identify if any hydraulic limitations are present, which could aid in the explanation of strict water potential control in macadamias.

This study therefore aimed to examine leaf gas exchange and water relations of field-grown macadamia in response to varying environmental conditions, and between fruiting and non-fruiting periods, in an attempt to identify water use strategies and possible limitations to net carbon assimilation. It was hypothesised that as this evergreen, sclerophyllous species originated in an area with a distinct dry period, it would have a conservative or isohydric water use strategy. This trade-off for safety over efficiency contributes to significant non-stomatal limitations to photosynthesis and strong stomatal control over gas exchange, and is attributed to a hydraulic limitation within the root to leaf pathway. However, as this is an oil storing nut with a high assimilate demand, it was further hypothesised that non-stomatal limitations would vary between fruiting and non-fruiting phenological stages.

4.3 Materials and Methods

4.3.1 Site description, weather variables and tree phenology

The trial was conducted on a commercial macadamia farm located approximately 35 km west of Nelspruit in the Schagen Valley, Mpumalanga, South Africa (25°21'50.36" S, 30°46'46.47" E, approximately 900 m.a.s.l.). The area has a seasonally dry sub-tropical climate ideal for macadamia production, although environmental conditions can vary considerably. The orchard consisted of mature bearing (MB) macadamia trees under irrigation (cv. HAES 695, 'Beaumont', a *M. tetraphylla* x *M. integrifolia* hybrid). Four trees in the centre of the 3.80 ha block were chosen as representative samples based on general tree health and a stem circumference survey conducted at the start of the trial. These trees had a mean leaf area index (LAI) of 5.25 $m^2\ m^{-2}$ and an estimated canopy cover of 0.70 at the start of the trial. A WS-GP1 Delta-T (Delta-T Devices Ltd, Cambridge, United Kingdom) automatic weather station was installed over a dry short grass surface within 100 m of the orchard. Data was collected at 20-minute intervals for solar radiation, wind speed, and direction, air temperature (T_{air}), air relative humidity (RH) and rainfall. Air vapour pressure deficit (VPD_{air}) was calculated from T_{air} and RH.

Fruiting and non-fruiting phenological stages were visually determined. Fruiting (F) stages (November to April) were regarded as periods after premature nut drop and before harvest, whilst non-fruiting stages (NF) (May to October) were regarded as periods after harvest until premature nut drop. Although small nuts were present in October, macadamias tend to abort some fruit directly after initial nut set and final fruit load is only established by November. A high assimilate demand during the fruiting stages was reflected in the annual yield of 21.5 kg tree^{-1} in 2016/2017 and 19.4 kg tree^{-1} in 2017/2018 dry in shell (1.5% moisture content).

4.3.2 Leaf gas exchange

Leaf gas exchange spot measurements were made on the four sample trees during eleven data collection campaigns from August 2016 to May 2018. Measurements were made on randomly selected mature, hardened-off leaves, which were fully exposed to the sun prior to measurement and typically situated on the outside of the canopy, within 2 m of the ground surface. Measurements were made between 09:00 h and 16:00 h, during which either the western or eastern face of the canopy was exposed to direct sunlight. Environmental conditions during each of the measurement campaigns are provided in Table 4.1.

The gas exchange parameters measured included net light-saturated CO_2 assimilation rate (A_{max}), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i), obtained using a photosynthesis system (Model: LI-6400 XT, LI-COR, Lincoln, NE, USA). Sensors inside the cuvette monitored photosynthetically active radiation (PAR) and leaf temperature (T_{leaf}). Chamber CO_2 concentration was maintained at 400 µmol mol^{-1}, the flow rate was 400 µmol s^{-1}, PAR inside the chamber was maintained between 1500 – 2000 µmol m^{-2} s^{-1} (LI-6400 XT LED light source), and RH was maintained at more than 50% (to prevent stomatal oscillations). Leaf-to-air vapour pressure deficit (VPD_{leaf}) was calculated by the LI-COR software. Measurements were typically recorded as soon as *A* stabilized, usually within two minutes of leaf insertion.

Table 4.1: Mean weather variables, including air temperature (T_{air}), air vapour pressure deficit (VPD_{air}), solar radiation (R_s), and estimated fruit bearing status during each of the 11 leaf gas exchange measurement campaigns. Fruiting periods were regarded as periods after premature nut drop and before harvest, whilst non-fruiting periods were regarded as periods after harvest to premature nut drop (i.e. containing no nuts). *N* is the number of replicate gas exchange measurements.

Measurement Date	*N*	T_{air} (°C)	VPD_{air} (kPa)	R_s (MJ m^{-2} day^{-1})	Fruiting
2016/08/09	140	21.9	1.7	16.0	No
2016/09/01	31	21.1	1.8	16.5	No
2016/10/13	23	17.4	0.4	19.6	No
2017/03/23	26	27.9	2.2	18.3	Yes
2017/05/11	21	25.2	2.3	13.8	No
2017/07/13	48	23.5	2.1	11.3	No
2017/12/08	38	21.6	1.1	25.5	Yes
2018/02/03	44	23.3	0.5	10.0	Yes
2018/02/04	52	23.9	0.5	20.2	Yes
2018/02/05	35	22.3	0.4	9.0	Yes
2018/03/19	45	20.5	0.6	13.0	Yes

The auto program function of the LI-6400 XT was used to obtain photosynthetic light and CO_2 response (A/C_i) curves using mature sun-exposed leaves on the four sample trees in the orchard. A/C_i curves were generated on 2 September 2016 and 12-13 October 2016 (NF, nine replicate curves) and 23 March 2017 and 5/7 December 2017 (F, 13 replicate curves). Light response curves were generated on 8 August 2016, 1 September 2016, 13 October 2016, 11 May 2017 and 13 June 2017 (NF, 18 replicate curves) and 8 December 2016, 14 February 2017, 23 March 2017 and 5 April 2017 (F, 12 replicate curves). Light and A/C_i curves were performed by altering the PAR (2000, 1500, 1000, 600, 400, 200, 100, 50, 0 µmol m^{-2} s^{-1}) and CO_2 concentration (400, 300, 200, 150, 100, 50, 0, 400, 600, 700, 1000, 2000 µmol mol^{-1}) within the chamber. For light response curves the CO_2 concentration was controlled at 400 µmol mol^{-1}, whilst PAR was set at 1500 µmol m^{-2} s^{-1} for A/C_i curves. T_{leaf} was controlled within 5°C of ambient by Peltier coolers, and RH within the chamber was maintained at more than 50%. Data points were logged within one to two minutes, and all measurements were made based on a stability factor where A had a standard deviation of less than 0.5 µmol m^{-2} s^{-1} and a rate of change per minute less than 0.1 µmol m^{-2} s^{-1}. Curve fitting and analysis was done using the monomolecular function for light response curves as described by Causton and Dale (1990) and by fitting the model described by Sharkey et al. (2007) for CO_2 response curves. CO_2 response curves were also used to calculate stomatal limitation (*l*) as described by Long and Bernacchi (2003). Light saturation point for macadamia was calculated from light response curves as the light level where A was approximately 90% of $A_{\max}$ as determined using the model of Causton and Dale (1990).

4.3.3 Water potential, transpiration and hydraulic conductance

Leaf (ψ_{leaf}) and stem (ψ_{stem}) water potential were measured on the four sample trees using a Scholander pressure chamber (Model 600, PMS Instrument Company, Albany, OR, USA) from before sunrise to sunset on selected days. For each tree, measurements were taken on the hour for three randomly selected mature sun-exposed leaves (ψ_{leaf}), three shade leaves on the inside of the canopy (ψ_{leaf}) and three shade leaves (enclosed) on the inside of the canopy (ψ_{stem}) (36 measurements per cycle). The latter were enclosed in aluminium covered bags (PMS Instrument Company, Albany, OR, USA) for at least 30 min prior to measurement. Pre-dawn leaf water potential (ψ_{pd}) was measured on a weekly basis from October 2016.

Sap flow measurements were performed using the heat ratio method of the heat pulse velocity technique as described by Burgess et al. (2001) and Taylor et al. (2015) on the same four sample trees used for leaf gas exchange and water potential measurements. Four custom made heat pulse probe sets were inserted at four different depths (0.5, 1.5, 2.5 and 3.5 cm below the cambium) in each tree trunk to account for the radial variation in sap flux within the conducting sapwood. Each probe set consisted of two Type T (copper/constantan) thermocouples (embedded in 2.0 mm outside diameter PFTE tubing) placed equidistantly (0.5 cm) upstream and downstream of the heater probe inserted into a brass collar (2.5 mm). These probe sets were inserted above the rootstock in the scion and below the first branch, with the probes being equally spaced around the trunk and randomly arranged, taking care to avoid any abnormalities in the trunk. Whole stem sap flux (assumed to be equal to transpiration based on the lack of a time lag between measured transpiration and evapotranspiration) was calculated as a product of sap flux density and weighted sapwood cross-sectional area represented by each probe set.

Wound width was calculated as 3.0 mm, and was determined by means of destructive sampling at the end of the trial. Volumetric sap flow of the individual trees (L h^{-1}) was converted to transpiration per unit leaf area (L m^{-2} h^{-1}) using the leaf area allocated to each tree, where after transpiration was converted to molar units of mmol H_2O m^{-2} s^{-1}. Seasonal adjustments in canopy leaf area were made based on canopy dimensions measured during five measurements campaigns throughout the duration of the trial. It should, however, be noted that difference in canopy size were negligible throughout the growing seasons.

Hydraulic conductance (k) was estimated according to (Moreshet et al. 1990) where k was separated into the pathway from the soil to the stem ($k_{\text{soil-stem}}$) and from the stem to the leaves ($k_{\text{stem-leaf}}$). Daily values of k were calculated as a mean of daytime hourly values. The root-stem interface was calculated using Equation 4.1, where J is sap flux and ψ_{soil} was assumed to be equal to pre-dawn leaf water potential under well-watered conditions, as were present in this study. The hydraulic conductance between the stem and leaf interface was calculated based on Equation 4.2, with the fraction of sunlit canopy leaf area ($\propto$) estimated using visual inspections of the tree canopy in a similar fashion to that described by Moreshet et al. (1990). Whole tree hydraulic conductance ($k_{\text{soil-leaf}}$) was calculated using Equation 4.3, with whole plant leaf specific hydraulic conductance (k_L) calculated using Equation 4.4, as outlined by Hubbard et al. (2001). It should be noted that estimates of k_L were obtained by using measurements of g_s (measured using the LI-6400 XT) and ψ_{leaf} obtained from the same leaf and VPD_{air} obtained from the weather station, with the assumption that macadamias are well-coupled to the atmosphere being a tall, rough surface.

$$k_{\text{soil-stem}} = J/(\psi_{\text{soil}} - \psi_{\text{stem}}) \qquad \textbf{Equation 4.1}$$

$$k_{\text{stem-leaf}} = J/(\psi_{\text{stem}} - (\propto\psi_{\text{sun leaf}} + (1-\propto)\psi_{\text{shade leaf}})) \qquad \textbf{Equation 4.2}$$

$$k_{\text{soil-leaf}} = J/(\psi_{\text{soil}} - (\propto\psi_{\text{sun leaf}} + (1-\propto)\psi_{\text{shade leaf}})) \qquad \textbf{Equation 4.3}$$

$$k_L = g_s/((\psi_{\text{soil}} - \psi_{\text{sun leaf}})/VPD_{\text{air}}) \qquad \textbf{Equation 4.4}$$

4.3.4 Statistical analysis

To analyse the influence of T_{leaf} and VPD_{leaf} on A_{max} and g_s, data from all measurement dates were grouped into five T_{leaf} categories spanning 5°C, and eight categories of VPD_{leaf} spanning 0.5 kPa. Using repeated measures ANOVA with Restricted Maximum Likelihood estimation (REML) in the Variance Estimation, Precision & Comparison methodology (VEPAC) of Statistica (TIBCO Software Inc. Version 13.3), a test for A_{max} and g_s was conducted. The individual tree replicate was a random variable so that N=4. Using LSD multiple comparisons, the treatment means were regarded as different if $p \leq 0.05$.

Statistical assessment of the seasonal changes in A_{max}, g_s and VPD_{leaf} (from leaf gas exchange measurements) and corresponding VPD_{air} and T_{air} (from the automatic weather station) were also analysed using repeated measures ANOVA with REML in VEPAC (Statistica, TIBCO Software Inc. Version 13.3). Five of the measurement dates had data for all four trees (i.e. containing full data sets) and could be used for this analysis. Using LSD multiple comparisons, the treatment means were regarded as different if $p \leq 0.05$.

To assess the influence of the presence of absence of fruit on leaf gas exchange and particularly g_s and A_{max}**,** data from all measurement dates were grouped, as described above, into eight VPD_{leaf} categories. These were sub-categorised into two groups representing fruiting and non-fruiting phenological stages. Repeated measures analysis using REML in VEPAC was then conducted using VPD_{leaf} (category) and fruiting/non-fruiting as factors, as well as their interaction. Individual tree was used as a grouping variable so that N=4. Using LSD multiple comparisons, the means were regarded as different if $p \leq 0.05$.

4.4 Results

4.4.1 Seasonal Weather and Tree Phenology

Mean air temperature throughout the study period was 20.1°C, with a mean daily maximum of 26.4°C and an mean daily minimum of 12.2°C (Figure 4.1). Predictably, daily solar radiation reached a low point during the autumn/winter period (April – August), but this period was also associated with lower variations in daily solar radiation, due to mostly clear skies. In the spring/summer period (August – March), large variations in daily solar radiation were observed as a result of the summer rainfall pattern of the region, leading to more frequent overcast conditions. Air vapour pressure deficit, although highly variable, had a seasonal mean of 1.01 kPa. Maximum daily VPD_{air} was highest (reaching 2.83 kPa on 16 September 2016 and 3.01 kPa on 2017/09/13) in the period September to November in 2016 and 2017. Total rainfall throughout the trial was 1877 mm, with rainfall during the first year (August 2016 – July 2017) amounting to 1182 mm, which was higher than the long term mean of ~750 mm per annum.

Tree phenology, including vegetative flushing patterns and fruit development, followed a similar pattern to that outlined by Stephenson et al. (1986) and Wilkie et al. (2009). Important crop physiological stages (from visual observations) and harvest times are outlined in Figure 4.2. With the exception of the early summer vegetative flushes, most phenological events occurred at similar periods in each year of the trial. The onset of flowering typically occurred in early August and was followed by nut set in September to October. Following nut set, premature nut drop occurred during late October and was followed by a vegetative flush in middle November. Increases in relative assimilate demand and oil accumulation were included in Figure 4.2 according to phenological stages, using the results of Stephenson et al. (2003). Relative assimilate demand was assumed to increase as vegetative growth and oil accumulation, as outlined by Stephenson et al. (2003), increased. Oil accumulation was assumed to commence after shell hardening (end December) and continue until harvest (mid-April). Peak relative assimilate demand (February to April) also coincided with a major vegetative flush at the end of January/beginning February.

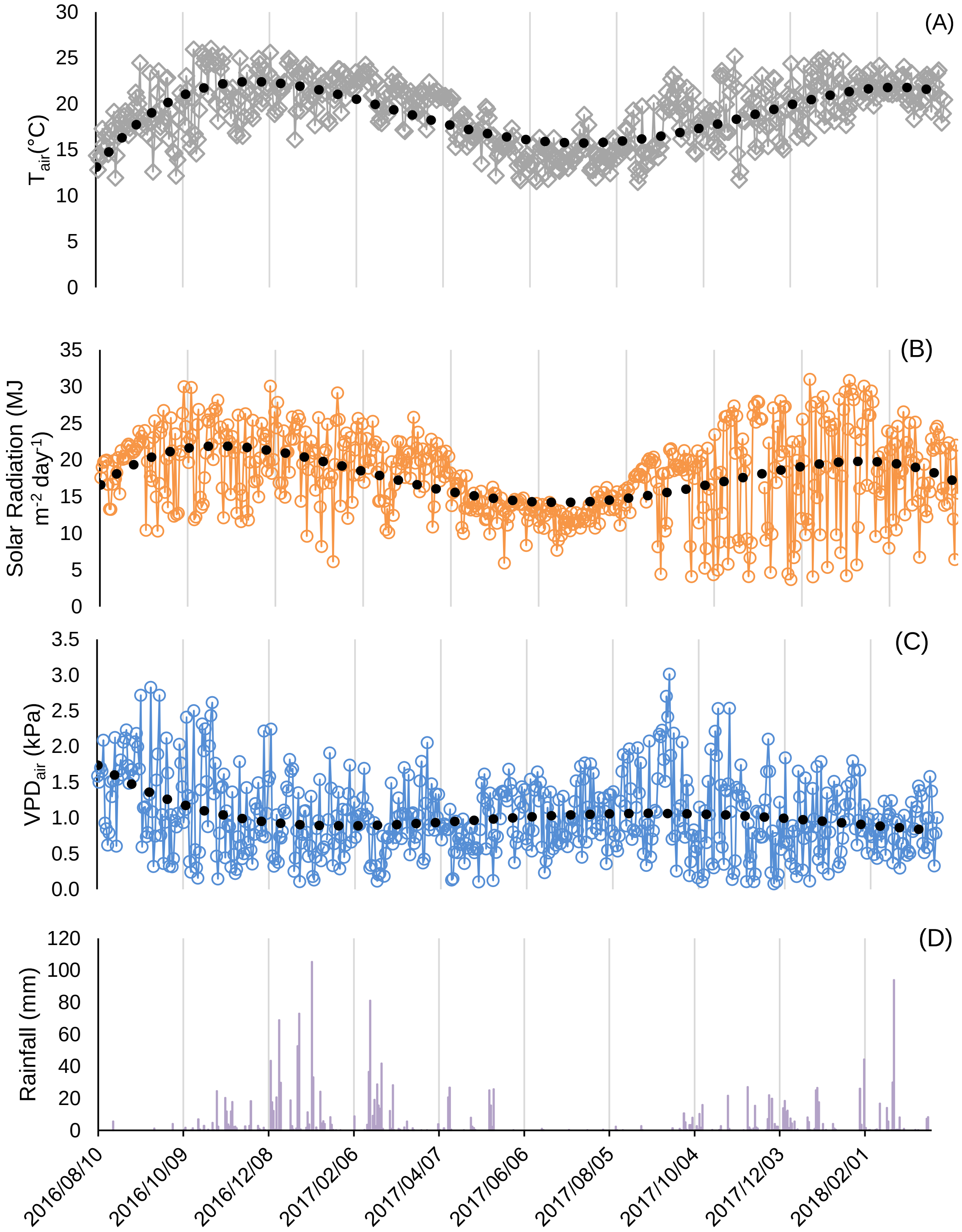

Figure 4.1: Daily weather variables recorded at the study site including (A) T_{air}, (B) Solar radiation, (C) VPD_{air} and (D) Rainfall. Dotted lines are polynomial trend lines fitted to daily data.

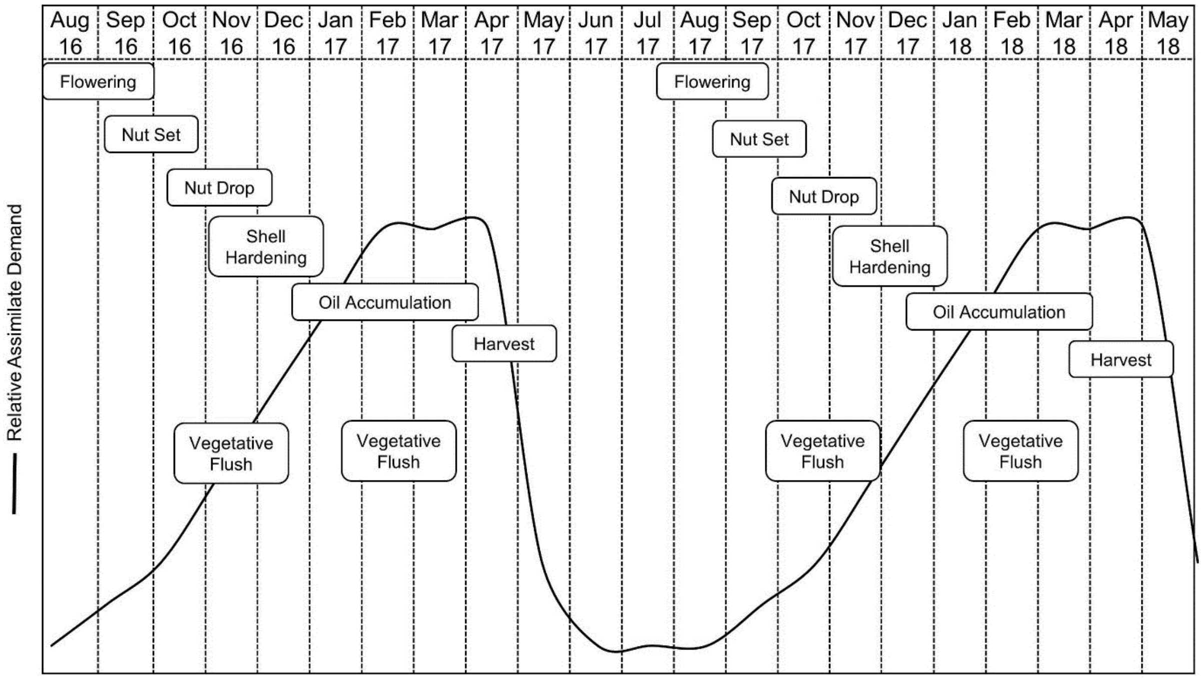

Figure 4.2: Major phenological events and harvest time of macadamia trees from August 2016 to May 2018 based on visual observations. Increases and decreases in relative assimilate demand throughout this period were estimated based on results by Stephenson et al. (1989) and approximately coincide with the presence or absence of fruit and especially the oil accumulation period.

4.4.2 Leaf gas exchange

4.4.2.1 Responses to environmental variables

Light-saturated rate of net CO_2 assimilation (A_{max}) reached optimum levels when leaf temperature ranged from 20-30°C, declining thereafter, with significantly lower A_{max} measured at leaf temperatures above 35°C compared to the optimum range between 20 and 30 °C (Figure 4.3 A). In response to increasing VPD_{leaf}, A_{max} reached a maximum between 1.0 – 2.5 kPa (Figure 4.3 B), declining at higher VPD_{leaf}. A similar response was observed between g_s and VPD_{leaf}, with g_s reaching a maximum between 1.0-2.5 kPa, where after it declined in a similar manner to that of A_{max} in response to increasing VPD_{leaf} (Figure 4.3 C). The relationship between A_{max} and g_s (Figure 4.3 D) was non-saturating under the measured values of g_s.

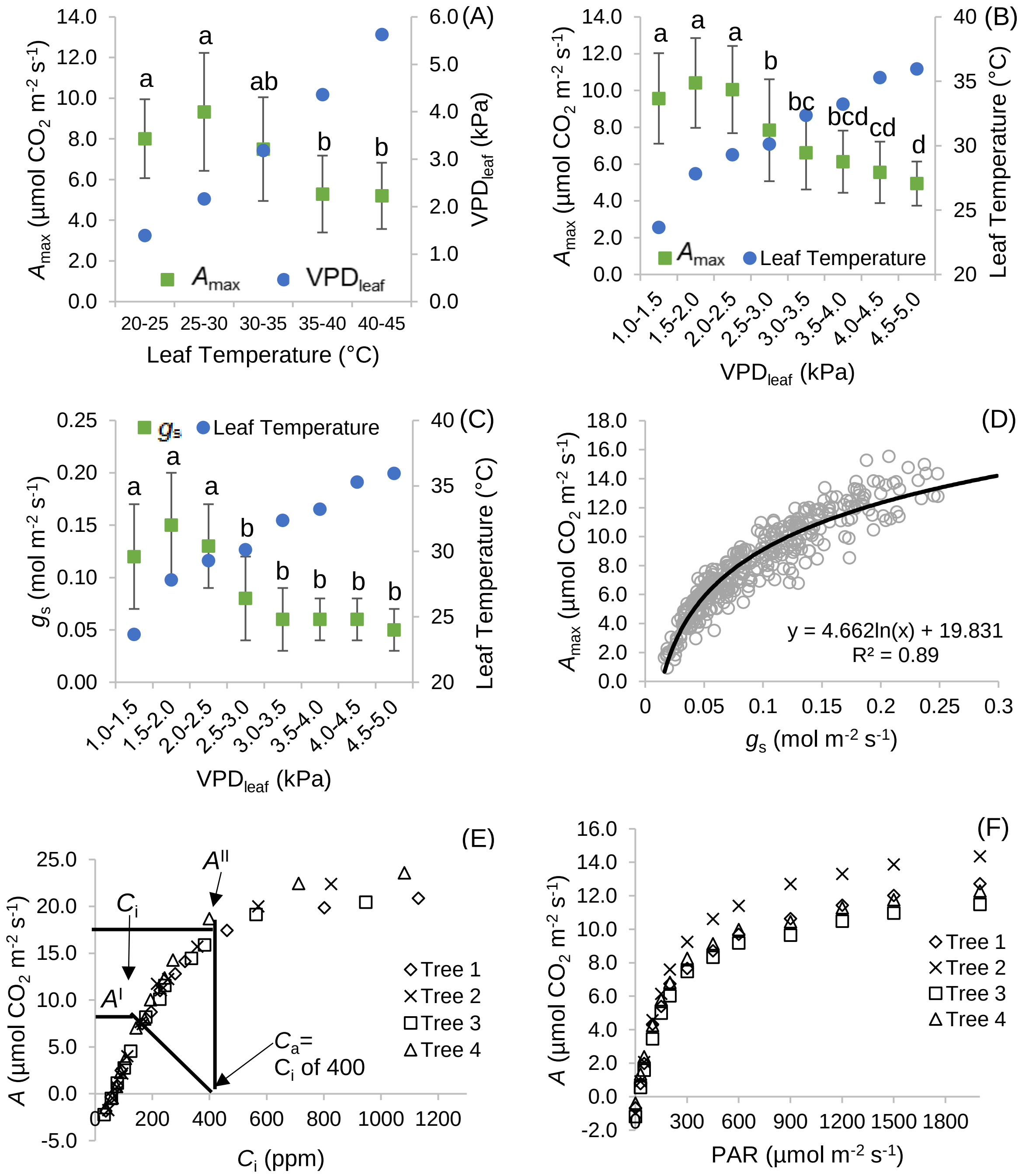

Figure 4.3: Response of A_{max} to increasing (A) T$_{leaf}$ (N= 500) and (B) VPD$_{leaf}$ (N= 500), (C) the response of g_s to VPD$_{leaf}$ (*N*= 500), and (D) the response of A_{max} to g_s (*N*= 500). (E) Representative responses of *A* to intercellular CO$_2$ concentration (C_i) showing the method used to calculate stomatal limitations ($l = (A^{II} – A^{I})/A^{II}$) as outlined by Long and Bernacchi (2003) of four experimental trees (*N*= 51) measured on 2017/12/07. (F) Response of *A* to PAR in fully sun-exposed leaves of four trees (*N*= 52) measured on 23 March 2017. Means followed by the same letter are not significantly different (p = *0.05*) as analysed using repeated measures ANOVA. Data from figures A-D were pooled data obtained from several measurement campaigns.

In response to increasing C_i, A increased linearly up to approximately 180 µmol mol^{-1}, after which the response flattened out during the shift from the CO_2-limited region to the RuBP-limited region (Figure 4.3 E). At C_i concentrations above 400 µmol mol^{-1} the mean A was approximately three times higher (17.16 µmol m^{-2} s^{-1}) than the mean value of A^l (A at C_i when C_a= 400 µmol mol^{-1}), which was approximately 6.64 µmol m^{-2} s^{-1} (Figure 4.3 E). Stomatal limitation as calculated from these response curves amounted to approximately 33% of all possible limitations to photosynthesis. Macadamias reached A_{max} at PAR levels of >900 µmol m^{-2} s^{-1} (examples of photosynthetic light response curves are given in Figure 4.3 F). Mean A_{max} obtained from light response curves (8.77 ± 2.49 µmol m^{-2} s^{-1}) was comparable to mean seasonal spot measurements of A_{max} (8.34 ± 1.21 µmol m^{-2} s^{-1}) made at PAR levels of 1500 µmol m^{-2} s^{-1}.

4.4.2.2 Influence of fruit on leaf gas exchange

Mean A_{max} varied throughout the data collection period between a minimum of 6.03 µmol m^{-2} s^{-1} (2016/08/09) and a maximum of 11.21 µmol m^{-2} s^{-1} (2017/12/08) (Table 4.2). Intermediate A_{max} values were recorded on the other measurement dates. Differences in A_{max} and g_s throughout the season typically agreed with significant differences in mean T_{leaf} and VPD_{leaf}, However, these differences were not consistent throughout the season. Despite similar VPD_{leaf} on 2016/08/09 and 2017/03/23, A_{max} and g_s were significantly higher in March than August. This variation in A_{max} and g_s corresponded to the presence of oil accumulating fruit in March, as opposed to August when the trees were in flower (Figure 4.2).

Table 4.2: Mean light saturated net CO_2 assimilation rate (A_{max}), stomatal conductance (g_s), leaf-to-air vapour pressure deficit (VPD_{leaf}), leaf temperature (T_{leaf}) and the corresponding daylight (6:00 – 18:00) air vapour pressure deficit (VPD_{air}) and air temperature (T_{air}) for spot measurements. Measurement dates falling within the fruiting period are denoted by a *. Means in columns were separated by LSD at 5% when P≤0.05 according to repeated measures ANOVA.

Date	*N*	A_{max} (µmol CO_2 m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	VPD_{leaf} (kPa)	T_{leaf} (°C)	VPD_{air} (kPa)	T_{air} (°C)
2016/08/09	140	6.03 c	0.05 c	3.2 ab	31.0 b	1.7 b	21.9 c
2016/10/13	23	7.88 bc	0.11 b	1.4 c	22.2 d	0.6 d	17.4 d
***2017/03/23**	**26**	**8.64 b**	**0.12 b**	**3.5 a**	**34.6 a**	**2.3 a**	**27.9 a**
2017/05/11	21	8.43 b	0.10 b	2.6 b	32.3 b	2.3 a	25.2 b
***2017/12/08**	**38**	**11.21 a**	**0.15 a**	**1.7 c**	**26.8 c**	**1.2 c**	**21.6 c**
Average		7.48	0.08	2.77	30.02	1.6	22.4

Although A_{max} during fruiting (F) periods remained higher than A_{max} during non-fruiting (NF) periods at similar leaf temperatures, the difference was only significant within the 25-35°C range (Figure 4.4 A). A_{max} during F periods was significantly higher than A_{max} during NF periods within the 1.0 – 2.0 kPa and 2.5 – 3.5 kPa VPD_{leaf} ranges (Figure 4.4 B). During F periods, g_s was significantly higher compared to g_s during NF periods at VPD_{leaf} ranging from 1.5 – 3.5 kPa (Figure 4.4 C). The majority of higher A_{max} and corresponding g_s values were obtained during the F period (Figure 4.4 D). Higher *A* values were achieved for similar C_i levels during F periods compared to NF periods (Figure 4.4 E). However, responses of *A* to increases in PAR (Figure 4.4 E) were similar between F and NF periods.

Mean values of A_{max} and g_s, and associated T_{leaf}, T_{air}, VPD_{leaf} and VPD_{air} from spot gas exchange measurements are shown in Table 4.3. Mean air temperature was 3.6°C higher during F periods compared to NF periods (with no difference in mean VPD_{air}), yet leaf temperature was 1.6°C lower during F periods (Table 4.3). A greater g_s during F periods was therefore associated with a lower T_{leaf} and VPD_{leaf}. Parameters derived from A/C_i curves ($V_{c\ max}$, J_{max}, TPU, R_d and *l*) measured at similar T_{leaf} and VPD_{leaf}, for NF and F periods, are also shown in Table 4.3. Estimates of $J_{max,}$ and TPU were higher during F periods compared to NF periods (Table 4.3). Mitochondrial respiration rate (R_d) was lower for periods of F compared to periods of NF. No differences were found between the two periods for $V_{c\ max}$ and *l.* No significant differences were found between F and NF periods for the parameters derived from the photosynthetic light response curves (Table 4.3).

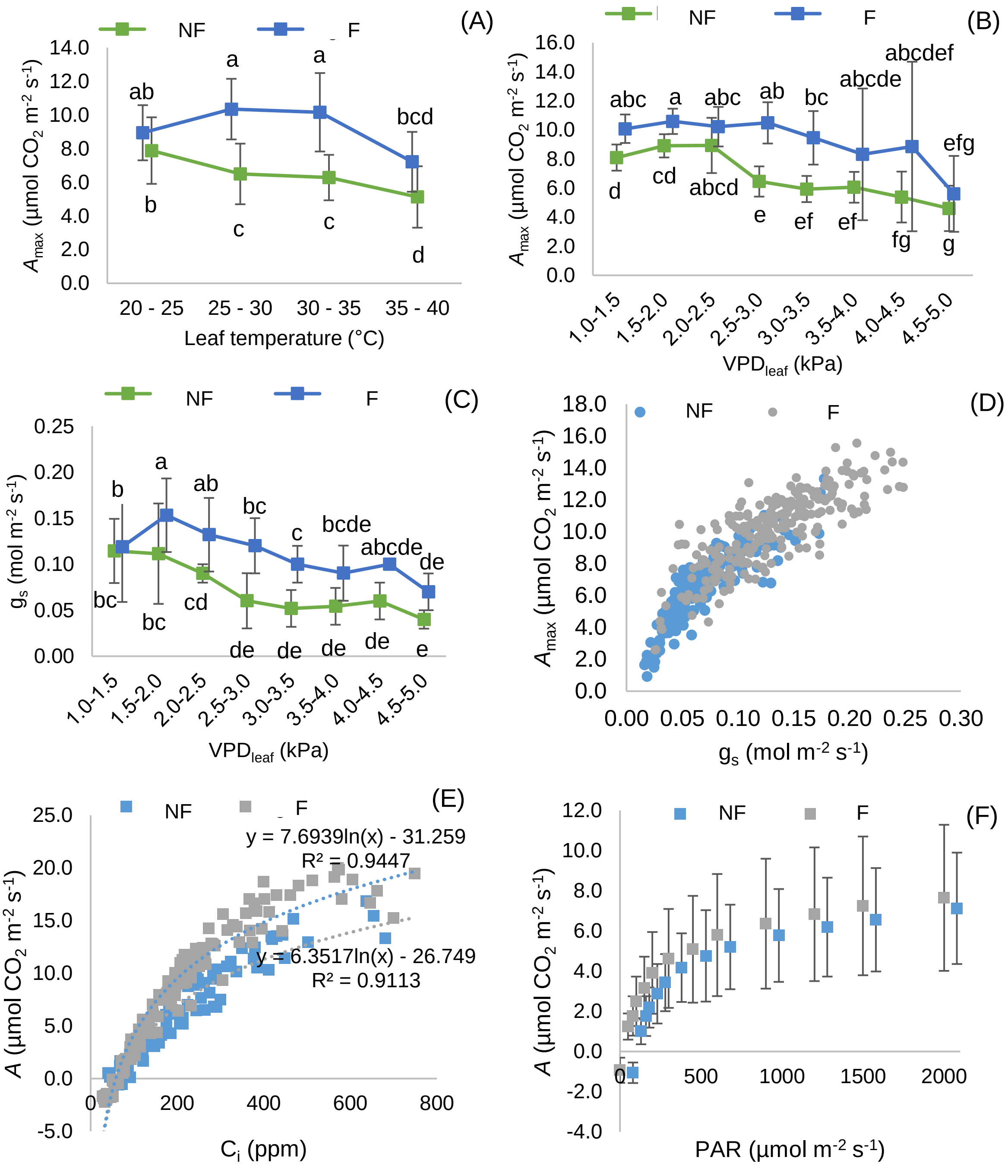

Figure 4.4: Response of A_{max} to increasing (A) T_{leaf} and (B) VPD_{leaf}, and (C) the response of g_s to VPD_{leaf}, and (D) the response of A_{max} to g_s during non-fruiting (NF) (N= 261) and fruiting (F) (N= 239) stages. (E) Responses of A to intercellular CO_2 concentration (C_i) during non-fruiting stages (N= 78) and fruiting stages (N= 138) stages. (F) Response of A to PAR in fully sun-exposed leaves of all experimental trees during non-fruiting stages (N= 368) and fruiting stages (N= 272) stages. Means were separated by LSD at 5% when P<0.05 according to repeated measures ANOVA.

Table 4.3: Average values and standard deviations (SD) for light saturated net CO_2 assimilation rate (A_{max}), stomatal conductance (g_s), maximum velocity of Rubisco for carboxylation ($V_{c\ max}$), maximum rate of electron transport (J_{max}), rate of triose phosphate use (TPU), mitochondrial respiration rate (R_d) and stomatal limitation (l), leaf temperature (T_{leaf}), daylight (6:00 am – 6:00 pm) air temperature, leaf-to-air vapour pressure deficit (VPD_{Leaf}), air vapour pressure deficit (VPD_{air}), light compensation point (LCP), apparent quantum efficiency (AQE) and day respiration rate between periods of high and low sink strength. The parameters $V_{c\ max}$, J_{max}, TPU, R_d, l, LCP, AQE and day respiration rate were obtained from analysis of A/C_i and PAR response curves at T_{leaf} ranging from 25 – 30 °C. Averages were separated by LSD at 5% when $P \leq 0.05$. *N* is the number of measurements.

	N	High Sink (Average ± SD)	*N*	Low Sink (Average ± SD)
		Spot Measurements		
A_{max} (µmol CO_2 m^{-2} s^{-1})	228	10.27 ± 2.23^a	196	6.58 ± 2.00^b
g_s (mol m^{-2} s^{-1})	228	0.13 ± 0.05^a	196	0.07 ± 0.03^b
T_{leaf} (°C)	228	28.66 ± 2.27^b	196	30.28 ± 3.45^a
T_{air} (°C)		23.58 ± 1.36^a		19.93 ± 2.59^b
VPD_{leaf} (kPa)	228	1.91 ± 0.61^b	196	2.97 ± 0.80^a
VPD_{air} (kPa)		1.04 ± 0.40^a		1.37 ± 0.62^a
		A/C_i Response Curve Analysis		
$V_{c\ max}$ (µmol m^{-2} s^{-1})	9	72.80 ± 14.31^a	13	55.92 ± 25.51^a
J_{max} (µmol m^{-2} s^{-1})	9	90.24 ± 10.39^a	13	73.35 ± 12.86^b
TPU (µmol m^{-2} s^{-1})	9	6.53 ± 1.05^a	13	4.77 ± 0.78^b
R_d (µmol m^{-2} s^{-1})	9	0.35 ± 0.25^b	13	1.01 ± 0.45^a
l (%)	9	35.23 ± 8.96^a	13	29.24 ± 9.32^a
		PAR Response Curve Analysis		
LCP (µmol PAR m^{-2} s^{-1})	7	7.73 ± 5.83^a	13	11.01 ± 9.81^a
AQE (mol mol^{-1})	7	0.05 ± 0.01^a	13	0.03 ± 0.02^a
Day respiration rate (µmol m^{-2} s^{-1})	7	0.37 ± 0.24^a	13	0.33 ± 0.30^a

4.4.3 Water relations

Pre-dawn leaf water potentials (ψ_{pd}) throughout the duration of the trial revealed only minimal variation, with an mean value of -0.13 ± 0.04 MPa (data not shown). During the course of a day (selected days shown in Figure 4.5), VPD_{air} increased to a maximum around 13:00 in summer (2016/12/08), between 12:00 and 13:00 in autumn (2017/04/15, 2018/04/18) and around 12:00 in winter (2017/07/14) (Figure 4.5 A). On these days, mean minimum sun exposed leaf water potential ($\psi_{sun\ leaf}$) was reached between 10:00 – 11:00 daily, with a mean minimum value of -1.21 ± 0.22 MPa (Figure 4.5 B). Thereafter, $\psi_{sun\ leaf}$ recovered gradually to a mean value of -0.73 ± 0.21 MPa at 15h00. Consecutive days of measurements confirmed that ψ_{leaf} recovered completely during the night, with ψ_{pd} of consecutive days being similar (data not shown). Leaf water potentials lower than -2.0 MPa were rarely recorded throughout the duration of the trial. Midday $\psi_{sun\ leaf}$ remained relatively constant across all measurement days, despite distinctly different environmental conditions (Figure 4.5 A & B). Whole tree transpiration rate (*E*) showed an approximate linear increase from 8:00 – 11:00 daily, where after *E* remained relatively constant as $\psi_{sun\ leaf}$ started to decrease from 11:00 onwards (Figure 4.5 C). Diurnal trends in $\psi_{sun\ leaf}$ and g_s, measured on the same leaf confirmed that the lowest value for $\psi_{sun\ leaf}$ and highest value for g_s typically occurred between 10:00 and 11:00 (Figure 4.6 A). This corresponded to a VPD_{leaf} of approximately 2.1 – 2.9 kPa (Figure 4.6 B).

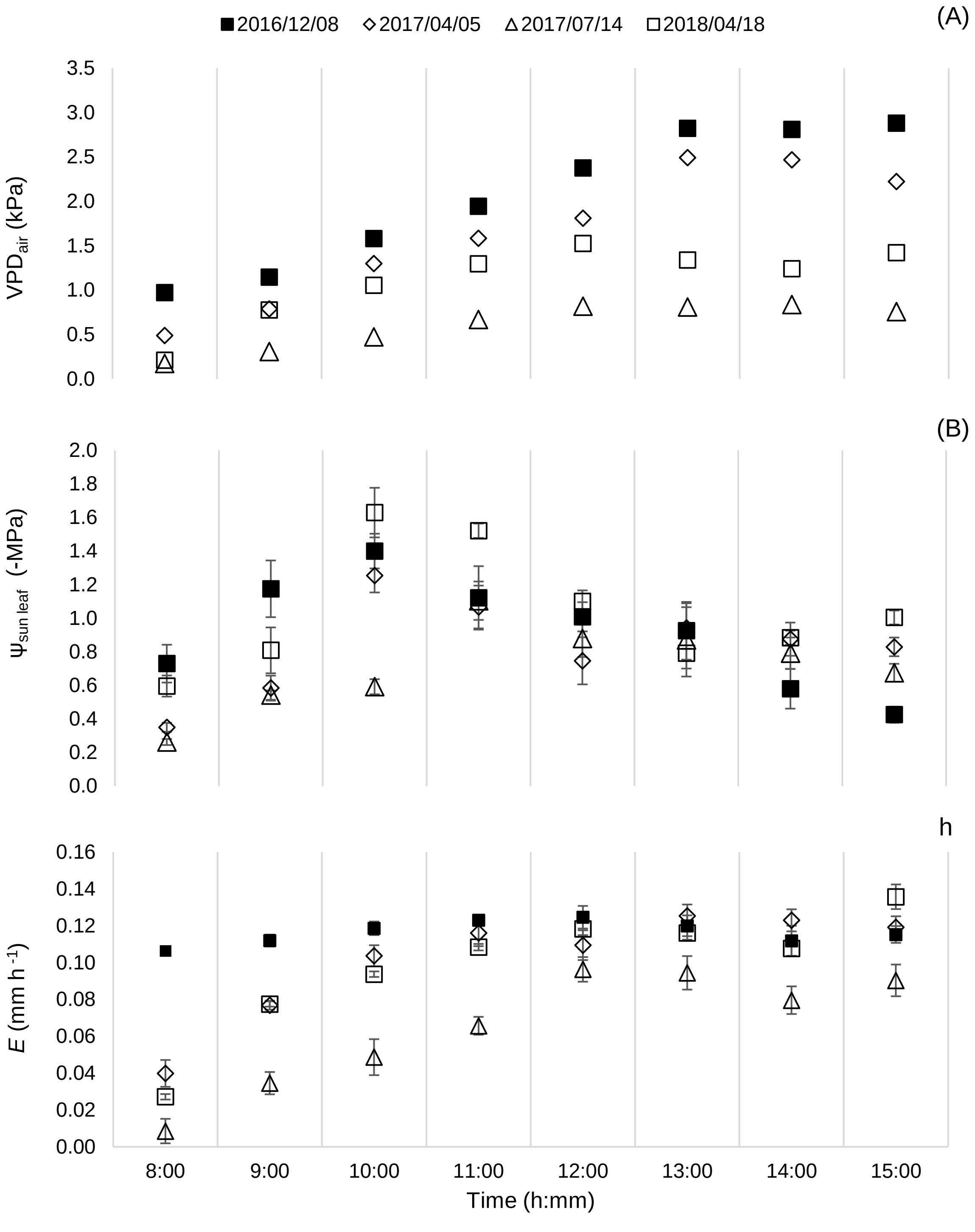

Figure 4.5: Diurnal progression of (A) air vapour pressure deficit ($\mathrm{VPD_{air}}$), (B) sun exposed leaf water potential ($\psi_{\mathrm{sun\ leaf}}$) obtained from random canopy sampling, and (C) whole tree transpiration rate (*E*) for four measurement days with contrasting weather conditions. Measurements of $\psi_{\mathrm{sun\ leaf}}$ (*N* = 384) and *E* (*N* = 128) are means of four trees (± standard deviation).

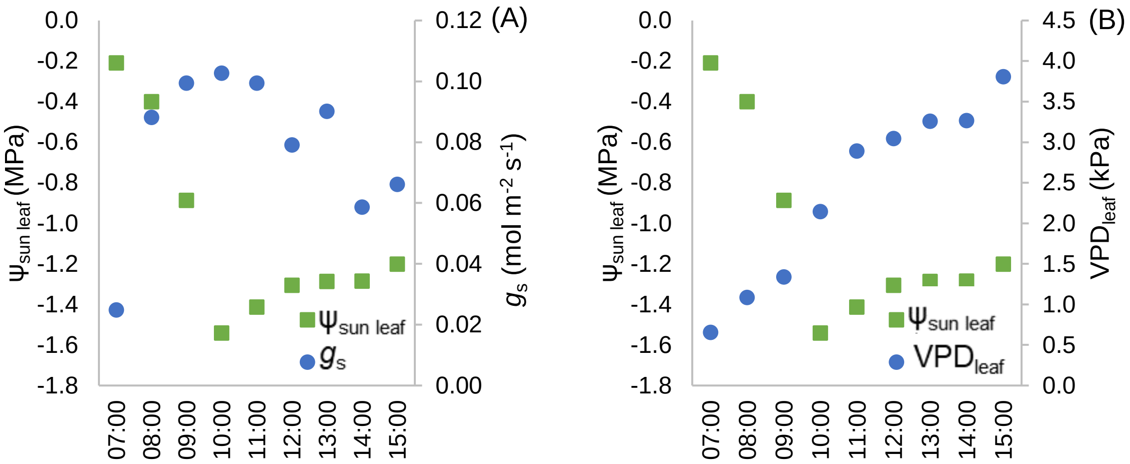

Figure 4.6: The diurnal trends in (A) $\psi_{sun\ leaf}$ and g_s and (B) $\psi_{sun\ leaf}$ and VPD$_{leaf}$, measured on the same leaf (2017/07/13).

The seasonal mean of whole tree hydraulic conductance ($k_{soil\text{-}leaf}$) was estimated at 3.44 ± 2.13 mmol m^{-2} s^{-1} MPa^{-1}, with an mean conductance at the stem-leaf interface ($k_{stem\text{-}leaf}$) of 7.43 ± 5.05 mmol m^{-2} s^{-1} MPa^{-1}, and an mean conductance at the soil-stem interface ($k_{soil\text{-}stem}$) of 7.85 ± 4.48 mmol m^{2} s^{-1} MPa^{-1}. However, large variation in hydraulic conductance was found, with estimates varying between individual trees and between consecutive days of measurements (Figure 4.7 A). An analysis of the diurnal variation of the various components of the hydraulic pathway revealed that both $k_{soil\text{-}leaf}$ and $k_{soil\text{-}stem}$ increased slowly until 10:00, where after the increase was more rapid (especially $k_{soil\text{-}stem}$), before slowly decreasing after 15:00 (Figure 4.7 B). In contrast, $k_{stem\text{-}leaf}$ increased rapidly until 08:00, then decreased rapidly from 8:00 – 10:00, before increasing from 11:00 onwards until 14:00 (Figure 4.7 B). Maximum g_s was reached between 8:00 – 11:00, after which g_s decreased rapidly. The mean $k_{soil\text{-}leaf}$, obtained from the inverse of the slope of the linear relationship (Figure 4.7 C) between whole tree transpiration rate (E) and $\psi_{sun\ leaf}$ was slightly higher (3.95 ± 2.24 mmol m^{-2} s^{-1} MPa^{-1}) than that determined from Equation 4. (3.44 ± 2.13 mmol m^{-2} s^{-1} MPa^{-1}). There was a positive linear relationship between leaf specific conductance (k_L) and g_s (Figure 4.7 D).

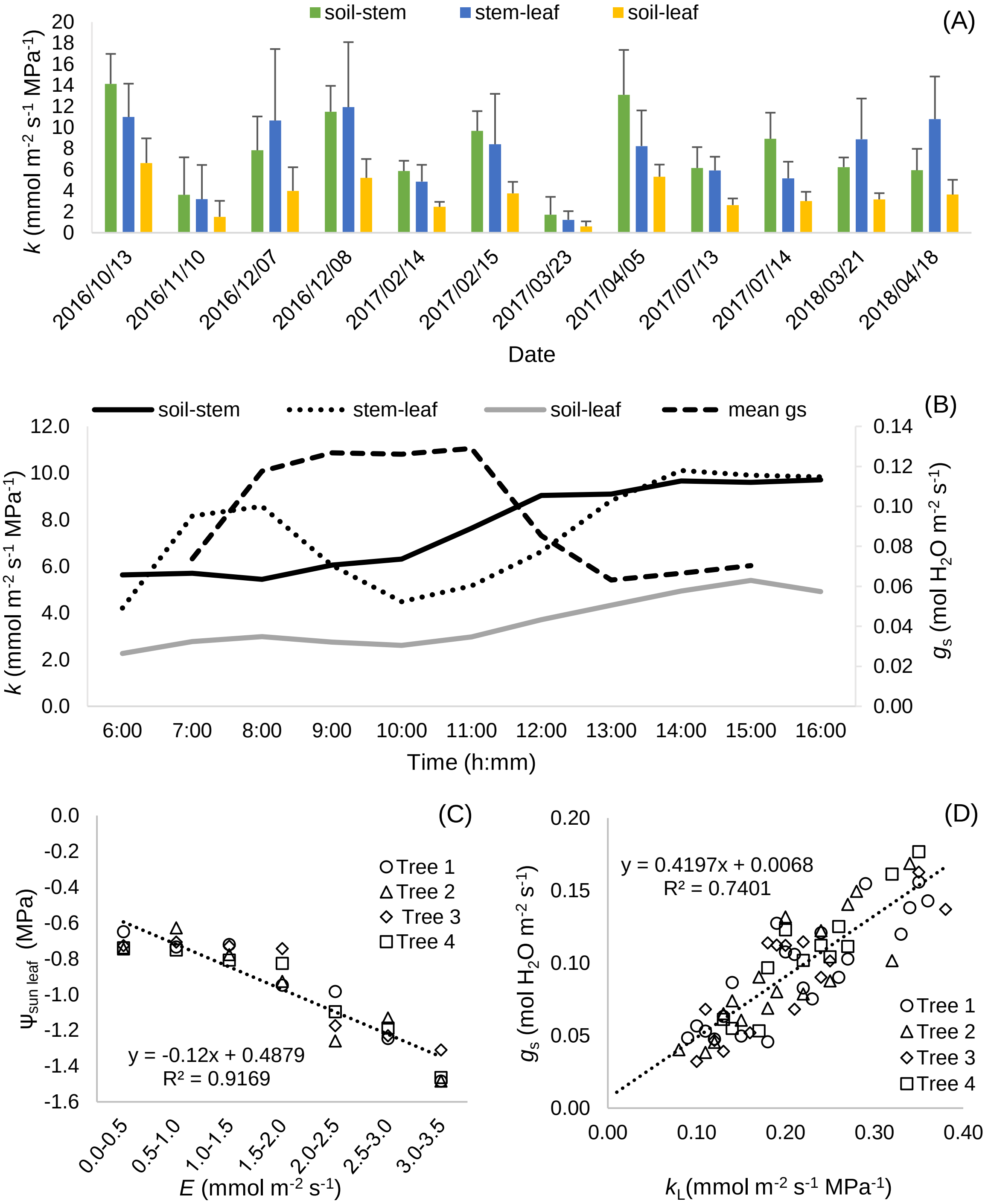

Figure 4.7: **Means (± standard deviation) of (A) daily seasonal and (B) diurnal progression of individual components of hydraulic conductance, including soil to stem ($k_{soil\text{-}stem}$), stem to leaves ($k_{stem\text{-}leaf}$), whole tree hydraulic conductance ($k_{soil\text{-}leaf}$) of four study trees (N = 102) and mean stomatal conductance (g_s) from random spot measurements made during the same measurement day (N = 52). (C) Linear relationship between mean sun leaf water potential ($\psi_{sun\ leaf}$) and mean whole tree transpiration rate (E) for four study trees on 18 April 2018. (D) Linear relationship between mean leaf specific conductance (k_L) and mean stomatal conductance g_s for four study trees measured on 2017/07/13(Niinemets et al. 2009).**

4.4 Discussion

This study has reaffirmed that macadamias have low rates of carbon assimilation, compared to other fruit trees crops, which is attributed to both stomatal and non-stomatal limitations, with stomatal limitations accounting for approximately one third of the total limitation to carbon assimilation. Although environmental conditions are an important driver for macadamia leaf gas exchange, net assimilation rates remained low despite the optimal growing conditions experienced throughout the duration of the trial, with mean annual temperature falling within the optimal 20 – 25°C range and annual precipitation and irrigation exceeding 1000 mm, as prescribed by Stephenson and Trochoulias (1994).

Seasonal mean A_{max} of macadamias, obtained from spot measurements of leaf gas exchange in this study, was 8.34 µmol CO_2 m^{-2} s^{-1}, which is similar to values reported by Huett (2004) (8-10 µmol CO_2 m^{-2} s^{-1}). Net assimilation rates for macadamia were low compared to temperate, deciduous fruit and nut crops, such as apple, pear, plum, pecan and almond, all of which have mean A_{max} values greater than 15 µmol CO_2 m^{-2} s^{-1} (Flore and Lakso 1989). Compared to other evergreen subtropical crops, such as citrus, macadamia seems to be slightly lower in terms of both A_{max} and g_s (Syvertsen et al. 2003). This could be partly attributed to slightly higher estimated stomatal limitations of macadamia (33%) than that of citrus (23.3%) (Jifon and Syvertsen 2003), but various non-stomatal limitations also seem to result in lower assimilation rates.

Non-stomatal factors seem to be related to low mesophyll conductance to CO_2 and internal light limitations attributable to the sclerophyllous nature of macadamia leaves. Even though mesophyll conductance was not determined in this study, Lloyd et al. (1992) estimated mesophyll conductance to CO_2 for *M. integrifolia* as 1.1 µmol m^{-2} s^{-1} Pa^{-1}, which was lower than that of sclerophyllous citrus leaves (1.7 – 2.2 µmol m^{-2} s^{-1} Pa^{-1}) and approximately half of that estimated for peach (3.1 – 4.0 µmol m^{-2} s^{-1} Pa^{-1}). Low mesophyll conductance could also explain the substantial increase of A in response to increasing C_i in this study, which has previously been observed by Flexas et al. (2008) and Niinemets et al. (2009). Increased C_i levels, up to 180 µmol mol^{-1}, led to a linear increase in A, with values greater than 17 µmol CO_2 m^{-2} s^{-1} being recorded at these levels (Figure 4.3 E). Internal CO_2 concentrations greater than 400 µmol mol^{-}

^{1} resulted in no substantial increases in A_{max}, which is most likely due to the realization of the upper limit of RuBP regeneration, often referred to as a limitation in triose phosphate utilization (TPU-limitation).

The lower J_{max} of macadamias relative to apple (Gindaba and Wand 2007b) and other sclerophyllous shrubs (Wullschleger 1993) suggests that low electron transport capacity may also be contributing to the low photosynthetic rates observed in macadamia, which could be related to light limitations within the internal leaf space, as the AQE for macadamia was also fairly low compared to a range of other tree crops (Higgins et al. 1992). These low values for AQE and J_{max}, were similar to that of shaded apple leaves (Gindaba and Wand 2007a; Gindaba and Wand 2007b). A high light saturation point of >900 µmol m^{-2} s^{-1} was also observed in this study, with similar results being reported by Huett (2004) and in other sclerophyllous species such as citrus (Medina et al. 2002) and olive (Sofo et al. 2009). The high light saturation point further supports the proposed internal light limitation in sclerophyllous macadamia (Niinemets and Sack 2006) and emphasises the need for intensive macadamia canopy management practices aimed at increasing solar radiation distribution throughout dense and shaded macadamia canopies. In fact, increases in A_{max}, together with increases in yield and quality, have been reported in sclerophyllous crops, including macadamia, as a result of increased light distribution within tree canopies (Medina et al. 2002; Huett 2004; Cherbiy-Hoffmann et al. 2012).

Stomatal limitations to macadamia leaf exchange are largely attributed to the predominantly isohydric behaviour of the crop, which was confirmed by diurnal $\psi_{\text{sun leaf}}$ which rarely reached levels lower than -2.0 MPa, despite rather contrasting environmental conditions, resulting in a range of atmospheric evaporative demands, during measurements and non-limiting soil water conditions (Figure 4.5). Strict stomatal control in macadamias resulted in the maintenance of favourable $\psi_{\text{sun leaf}}$, irrespective of atmospheric evaporative demand. Lloyd et al. (1991) found similar results, with $\psi_{\text{sun leaf}}$ of both irrigated and non-irrigated macadamias being very similar over a 2-month period and rarely reaching levels below -1.5 MPa. There were, however, differences in g_{s} between the two treatments, with g_{s} in the non-irrigated treatment dropping significantly to maintain ψ_{leaf}. The maintenance of high ψ_{leaf}

through stomatal closure is believed to be an underlying hydraulic safety factor used to avoid xylem cavitation under high evaporative demands (Sperry 2000; Schultz 2003) and suggests that there is a hydraulic limitation to water flow through certain segments within the tree (Gleason et al. 2016).

The report by Lloyd et al. (1991) of high hydraulic conductance of macadamia trees relative to most other fruit trees seems to be at odds with the isohydric nature of the tree. However, results from this study suggest a lower value for $k_{\text{soil-leaf}}$ than those reported by Lloyd et al. (1991), which possibly reflects different techniques for estimating $k_{\text{soil-leaf}}$. The values from this study ($k_{\text{soil-leaf}} = 3.44 \pm 2.13$ mmol m^{-2} s^{-1} MPa^{-1}), using whole tree transpiration, are more comparable with other fruit tree species, such as apple (4.4 mmol m^{-2} s^{-1} MPa^{-1}, Cohen and Naor 2002) and kiwifruit (2.44-3.83 mmol m^{-2} s^{-1} MPa^{-1}, Clearwater et al. 2004). Importantly, the separation of the hydraulic pathway from the soil to the stem and from the stem to the leaf, provided more insight into the hydraulic characteristics of macadamia trees and suggested slightly lower conductance in the stem to leaf pathway, than the soil to stem pathway throughout the season. This was confirmed through the analysis of the diurnal progression of $k_{\text{stem-leaf}}$ and $k_{\text{soil-stem}}$, which revealed that there was a reduction in $k_{\text{stem-leaf}}$ in the morning (Figure 4.7 B), which roughly corresponded with an increase in g_s. This suggests that as stomata open, water held within storage tissues in the leaf is lost through transpiration. As there was a negligible time lag between transpiration estimated via sap flow and evapotranspiration estimated using Eddy Covariance measurements (data not shown), it was assumed that the storage capacity in the trees was low and thus transpiration at the start of the day would have depended predominantly on stored water in the leaves. After the depletion of these stored water reserves, and increased transpiration rates, significant resistance to water flow occurs within the stem to leaf interface. From this point onwards results suggest that resistance within the stem to leaf interface places significant constraints on macadamia leaf gas exchange, by means of changes in leaf water status, as demonstrated in ponderosa pine by Hubbard et al. (2001). This is complemented by the linear relationship between k_L and g_s (Figure 4.7 D), which is not only indicative of near isohydric behaviour, but also suggests that a reduction in bulk leaf water status, or even a single portion of the leaf as a result of reduced k_L, could lead to reduced g_s, According to Hubbard et al. (2001) this slight reduction in g_s returns ψ_{leaf} to its original levels and the continuous nature of this response in both time and space results in a near constant ψ_{leaf} irrespective of atmospheric conditions.

Although results from this study suggest that macadamias are predominantly isohydric and exhibit strict stomatal control in response to increasing VPD_{leaf}, these responses were not consistent throughout the duration of the trial. The observed variation of both g_s and A_{max} in response to increasing VPD_{leaf} corresponded to the absence or presence of fruit on trees (Figure 4.4 B & C). Both A_{max} and g_s were significantly higher during fruit bearing periods compared to periods when the tree lacked fruit (Table 4.3). During fruit bearing periods g_s and A_{max} were significantly higher than during non-fruit bearing periods, especially within the higher VPD_{leaf} range of 2.5 – 3.5 kPa (Figure 4.4 B & C). These results suggest that macadamias have the capability to maintain g_s for longer under high evaporative demands during fruit bearing periods than during non fruit bearing periods.

Given the significant assimilate demand by the oil storing fruit of macadamia (Stephenson et al. 1989), an upregulation of photosynthesis during fruit bearing periods is not only highly likely but also necessary. Whilst the upregulation of A_{max} in response to the presence of fruit observed in this study can be linked to the concomitant increase in g_s, it is also most likely linked to reduced mitochondrial respiration rates and increased Calvin cycle kinetics, as J_{max} and TPU were higher during fruiting periods (Table 4.3). Similar increases in g_s and/or A_{max} in response to crop load have been noted in a number of fruit crops (Naor 2001; Pretorius and Wand 2003; Silber et al. 2013), with Sade and Moshelion (2014) suggesting a switch between isohydric and anisohydric behaviours over the course a season as a result of shifts in hydraulic and stomatal regulation in response to sink strength. It is therefore plausible that macadamias do not follow a purely isohydric water management strategy, but rather a more plastic isohydric strategy, which depends on the presence or absence of developing nuts.

Unfortunately, the mechanisms by which stomata respond to sink strength are not well understood. Whilst Hölttä et al. (2017) has proposed a steady state stomatal model balancing gas exchange with plant hydraulics and source-sink flux, Mitchell et al. (2016) linked an increase in ABA with the build-up of non-structural carbohydrates in source tissue, which lead to a reduction in g_s. A dual role for ABA in reduced g_s was suggested Pantin et al. (2013), with a direct impact on guard cells and an indirect action through reduced k_L by decreasing water permeability of the leaf vascular tissue. Finally, Sade and Moshelion (2014) suggest that aquaporins play an important role in controlling leaf water status and therefore whether a plant will behave in a more isohydric or anisohydric fashion.

This study has demonstrated that the net CO_2 assimilation rates of macadamias over a production season are fairly low, which seems to be linked to a quick response of stomata to increasing VPD_{leaf}, in order to maintain midday leaf water potential within certain safety margins to avoid cavitation. These safety margins seem to be dictated by a hydraulic limitation within the stem to leaf interface and suggest that macadamias predominantly follow an isohydric strategy. Whilst xylem safety remains a priority throughout the season, the response of g_s to VPD_{leaf} is dynamic and is influenced by the presence or absence of oil storing fruit on the tree. There are, however, also non-stomatal limitations to photosynthesis linked to a possible internal light limitation and the previously reported low mesophyll conductance. Planting macadamias in more humid regions and pruning to ensure adequate light interception throughout the canopy should be considered in order to maximise canopy photosynthesis and possibly boost production.

CHAPTER 5: WATER USE OF MACADAMIA TREES IS DYNAMICALLY REGULATED BY THE PRESENCE OR ABSENCE OF FRUIT

5.1 Abstract

Macadamia, an oil rich fruit producing tree, is characterized by an isohydric and therefore conservative water management strategy enforced by means of strict stomatal control. The presence of fruit, a significant sink, has been shown to increase both net assimilation rate (A) and stomatal conductance (g_s) in a range of crops. However, no reports relating sink strength to leaf gas exchange in macadamia have been published. It is also unclear if increases in A and g_s at a leaf level would lead to increased canopy transpiration (E_c) in the presence of fruit. This study therefore aimed to show that the presence of fruit would lead to an increase in A, g_s and E_c. In order to examine the effect of fruit on leaf gas exchange, the study used fruiting and non-fruiting branches, in combination with phloem girdling to extrapolate possible effects of fruit on leaf gas exchange to a canopy level. Measurements of leaf gas exchange were made on four different treatments including non-fruiting (NF), fruiting (F), girdled non-fruiting (GNF), and girdled fruiting (GF) branches, over a 7-month period in a mature commercial macadamia orchard. Independent measurements of E_c, using sap flow measurements, were made across two seasons from August 2016 - August 2018 in the same orchard. No significant differences in A_{max} and g_s were observed between GF branches and that of non-girdled treatments approximately 2-months after girdling, whilst A_{max} and g_s of GNF were significantly reduced during the same period. Fruit removal on GF branches, resulted in significant reduction in both A_{max} and g_s compared to F and NF treatments. In contrast, the presence of fruit lead to a ~25% increase in g_s at leaf vapour pressure deficits (VPD_{leaf}) > 1.50 kPa. There were also no significant differences in g_s between F and NF branches throughout the trial, suggesting that increases in g_s, in the presence of fruit can be scaled to a canopy level. Subsequent examination proved that E_c was ~20% higher during fruiting compared to non-fruiting periods. Increased E_c during fruiting compared to non-fruiting periods was unrelated to changes in canopy size and weather and was largely attributed to significantly higher E_c in response to air vapour pressure deficit within the 0.0 – 3.0 kPa range.

Keywords: Phloem-girdling, Sink strength, Leaf gas exchange, Transpiration

5.2 Introduction

Macadamia F. Muell is characterized by a conservative water management strategy, often referred to as isohydric plant behaviour (Schultz 2003; Sade et al. 2012), and is characterized by strict stomatal control in response to increased atmospheric evaporative demand (see Chapter 4) in an attempt to maintain a nearly constant minimum daily leaf water potential. This contrasts with anisohydric behaviour, where less strict stomatal control leads to increased stomatal conductance (g_s) in response to increases in atmospheric evaporative demand, followed by substantial decreases in leaf water potential. The strict stomatal control in macadamias results in lower net CO_2 assimilation rates (A) in comparison to a range of other fruit tree crops (Flore and Lakso 1989). It is, however, highly unlikely that predominantly isohydric oil storing tree crops, such as macadamia and olive, which are typically grown in high evaporative demand environments, can meet whole-tree photoassimilate demand without employing a more dynamic water management strategy at critical times in the growing season. Sade and Moshelion (2014) therefore suggested that fruit trees can shift between water management strategies, resulting in so-called isohydrodynamic behaviour, which is influenced by the fruit load. Of particular interest to the field of water use is how fruit load influences stomatal behaviour.

Stomatal conductance (g_s) of apple (Palmer 1992; Pretorius and Wand 2003) and coffee (Vaast et al. 2005) for example, differed in their response to increased vapour pressure deficit (VPD) depending on the presence or absence of fruit. In citrus, Syvertsen et al. (2003) reported that both A and g_s were approximately 40% higher for fruit bearing trees compared to trees where fruits were removed. The degree to which leaf gas exchange is influenced by source-sink relations has been a matter of debate for decades (Sweet and Wareing 1966; Körner 2015). A range of widely used assimilation and stomatal models (Ball et al. 1987; Leuning 1995), which are based on the premise that g_s optimizes carbon uptake relative to water loss via transpiration (E), have been shown to adequately describe stomatal behaviour (Hari and Mäkelä 2003). If the aforementioned premise holds true, increases in g_s observed in the presence of fruit, stem from increased carbon assimilation capacity, implying that source-sink relationships have a significant impact on not only g_s, but also on E. A range of recent studies have also highlighted that although hormonal and physical control of g_s have been studied extensively, very little is known about the biochemical (i.e. organic anions) control of g_s (Kottapalli et al. 2018; Lima et al. 2018; Lawson and Matthews 2020).

Further complicating the intricate relationship between A, g_s and E in relation to source-sink relationships, is the fact that E has a direct influence on assimilate transport from source leaves to sinks (Hölttä et al. 2006). Nevertheless, the failure to transport assimilates away from source leaves results in the accumulation of simple sugars and eventually starch in leaf tissues, ultimately leading to downregulation of A and g_s (Paul and Pellny 2003; Ryan and Asao 2014; Sharkey 2019; Tombesi et al. 2019). The continuous transport of assimilates from source leaves to sinks, is therefore essential for the upkeep of photosynthetic production. In trees, which consist of an intricate matrix of fruiting and non-fruiting branches (i.e. proximal and distal sinks), and where phloem loading is a predominantly passive process (Turgeon 2010a; Turgeon 2010b), long distance transport of assimilates from source to sink would have to occur if leaf gas exchange, of especially non-fruiting branches, is to be maintained at levels which would meet whole tree assimilate demand.

Studying the effect of source-sink relations on leaf gas exchange is, however, difficult when considering the complexity of source-sink dynamics within tree canopies. As a result, most studies have used branch isolation methods, which include cincturing or phloem-girdling of a single branch, to study these effects (Schaper and Chacko 1993; Krapp and Stitt 1995; Williams et al. 2000; Goren et al. 2004; Urban et al. 2004; Urban and Alphonsout 2007). The aim of phloem-girdling is often to understand the influence of assimilate accumulation and transport within a single stem or branch, and to extrapolate these findings to a larger canopy. Furthermore, branch isolation can be used successfully to generate hypothetical scenarios of sink and source relationships by means of fruit and leaf removal, which in the context of this study would aid us in understanding the effect of sink strength, brought about by developing macadamia fruit, on specifically A, g_s, and E. Caution should, however, be taken that girdling might induce hormonal changes which might significantly influence leaf gas exchanges.

Linking with results discussed in Chapter 4, it was found that both A and g_s of macadamias varied in response to VPD throughout the crop's phenological cycle, increasing as sink strength of developing fruit increased. These results were obtained from completely randomized spot measurements of leaf gas exchange on both fruit bearing and non-fruit bearing branches, which suggests that changes in stomatal behaviour in response to sink strength occur at a canopy level. Given the well-defined relationship between A and g_s (Ball et al. 1987; Leuning 1995), this study hypothesized that A and g_s would be higher in both fruiting and non-fruiting branches in the presence of developing fruit, and that downregulation of A and g_s can be expected upon fruit removal. Furthermore, it was hypothesized that the leaf level increase in g_s, would translate into subsequent increases in canopy transpiration (E_c). The study firstly aimed to establish that long distance transport of assimilates occurs in macadamias by using branch isolation methods on both vegetative and reproductive branches, whilst secondly aiming to determine if a leaf level upregulation of g_s occurs in the presence of fruit, and if so would this lead to an increase in E_c.

5.3 Materials and Methods

5.3.1 Orchard description, weather variables and fruit growth

The trial was conducted in the mature bearing (MB) orchard described in Chapter 3, Section 3.1.2. This orchard of irrigated macadamia trees (cv. HAES 695, "Beaumont", *M. tetraphylla* x *M. integrifolia*) was planted in 2005 at an 8 x 4 m spacing. Weather data, including air temperature (T_{air}), air vapour pressure deficit (VPD_{air}), solar radiation (R_s), wind speed and direction, air relative humidity (RH) and rainfall, was collected using methods and quality control measures outlined in Section 3.1.2. Reference evapotranspiration (ET_o) was calculated using measured weather variables and the FAO Penman-Monteith equation for a short grass reference surface, as described by Allen et al. (1998) and Pereira et al. (2015). Furthermore, canopy dimensions including height, width, breadth, and leaf area index (LAI) were measured throughout the trial. Methods used in the measurement of these components are outlined in Chapter 3, Section 3.1.3.

Fruit growth was monitored over two consecutive seasons, including the season before the trial (2016 – 2017) and during the trial (2017 – 2018). During both seasons, 20 nuts were randomly harvested across the orchard on a weekly basis from 1-week post anthesis up to 2017/02/27 and 2018/02/23 (approximately 19-weeks post anthesis) and eight weeks before harvest which occurred on 2017/04/30 and 2018/04/27. Measurements of fruit included nut in husk (NIH) wet mass and NIH diameter. The data was used to establish when fruits became significant sinks. It was assumed that the nuts become a significant non-functional sink (i.e. non-photosynthetic or structural sink) as soon as individual nut mass increased in a linear fashion, in accordance with Trueman (2013).

5.3.2 Sink manipulation trial - leaf gas exchange

Ten Macadamia trees close to the centre of the MB orchard (Described in Chapter 3) and within the same planting row, were selected for treatments. Four treatments, each consisting of twenty replicates, were randomly allocated to the ten selected trees. The treatments included phloem-girdled non-fruiting branches (GNF), non-fruiting branches (NF), phloem-girdled fruiting branches (GF) and fruiting branches (F). All branches were at least 0.5 m long. Non-fruiting branches bore no fruits or racemes (i.e. only vegetative growth) and 15 to 20 leaves per branch. Fruiting branches bore two or more nuts per branch and 15 to 20 leaves per branch. Girdling was administered approximately 8 weeks post anthesis (2017/12/12), and post premature nut drop (4 weeks post anthesis), by carefully removing a strip of bark (approximately 4.0 cm wide) with a pair of pliers, from the base of each branch without damaging underlying xylem tissue. The selected branches for each treatment where situated on the outside of the canopy, ensuring that leaves had historically been grown in sun-exposed environment. Furthermore, in order to determine the true influence of sink strength on leaf gas exchange, nuts on branches of the GF treatment were removed completely, approximately two months (2018/02/03) after the commencement of the trial.

Measurements of leaf gas exchange commenced on 2017/12/12 and were obtained using a photosynthesis system (Model: LI-6400 XT, LI-COR, Lincoln, Nebraska, USA). The measured parameters included, amongst others, net CO_2 assimilation rate (A), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i). Sensors inside the leaf cuvette monitored photosynthetically active radiation (PAR) and leaf temperature. Leaf-to-air vapour pressure deficit (VPD_{leaf}) was calculated from measured parameters. For all spot measurements, the CO_2 concentration was maintained at 400 µmol mol^{-1}, the flow rate was 400 µmol s^{-1}, PAR was maintained between 1500 – 2000 µmol m^{-2} s^{-1} (LI-6400 XT LED light source) and relative humidity was maintained at more than 50% (to prevent stomatal oscillations). Spot measurements made under these conditions (termed A_{max}) were typically recorded as soon as the A stabilized (total time in chamber less than 2 minutes). All leaves measured during the spot measurement campaign were fully sun-exposed (PAR > 1000 µmol m^{-2} s^{-1}) immediately before measurements of leaf gas exchange.

Leaf gas exchange measurements were made during seven data collection campaigns from 2017/12/12 (early fruiting period) to 2018/06/30 (after harvest). During the week in early February 2018 when the GF treatment was defruited, gas exchange was measured before defruiting (2018/02/03), and for two days after defruiting (2018/02/04 and 2018/02/05) to assess the rapidity of responses at leaf level. Measurements were made on randomly selected mature, hardened-off leaves, typically situated on the outside of the canopy, within 2 m from the soil surface. Measurements were made between 09:00 h and 16:00 h on the sun-exposed face of the canopy, on either the western or eastern side of the row. Selected daytime (08:00 h – 17:00 h) weather variables during each of the measurement days, together with the presence or absence of fruit on each date, are provided in Table 5.1.

5.3.3 Canopy Transpiration

Sap flow measurements were conducted across two seasons from 2016/08/10 to 2018/08/08, on four trees in the centre of the MB orchard. Details of sap flow measurements, as well as the calculation of canopy transpiration rate (E_c) is described in detail in Chapter 3, Section 3.1.5. Canopy transpiration (mm) was calculated using the ground area allocated to each experimental tree within the experimental orchard (i.e. 32 m^2). Average E_c as reported in this study consisted of hourly averaged E_c for each of the individually measured (N=4) trees outlined in Chapter 3, Section 3.1.5.

It should be noted that the four trees used for sap flow measurements were not used in the sink manipulation trial and the data are not linked to sink manipulation treatments. The influence of sink strength on E_c was thus assessed in relation to the normal phenologically-determined presence or absence of fruit on the tree during the time of measurement. For transpiration measurements, the non-fruiting period in both seasons was taken as 1 May – 31 October and the fruiting period was taken as 1 November – 30 April. Nuts were harvested by hand upon maturity on 2017/04/30 and 2018/04/27.

Table 5.1: Average (± standard deviation) daytime (8:00 – 17:00 h) weather variables including air temperature (T_{air}), air vapour pressure deficit (VPD_{air}) and total solar radiation (R_s) for each of the leaf gas exchange measurement days. *N* is the number of leaf gas exchange measurements per treatment. The presence (yes) or absence (no) of fruit during each of the seven leaf gas exchange measurement dates is also shown.

Measurement Date	Treatment	*N*	Fruits Present	T_{air} (°C)	VPD_{air} (kPa)	R_s (MJ m^{-2} day^{-1})
2017/12/12	GNF	5	No	27.9 ± 6.5	2.09 ± 1.1	27.86
	GF	5	Yes			
	NF	5	No			
	F	5	Yes			
2018/02/03	GNF	26	No	23.3 ± 1.5	0.69 ± 0.2	11.82
	GF	16	Yes			
	NF	23	No			
	F	21	Yes			
2018/02/04	GNF	16	No	23.9 ± 4.4	1.31 ± 0.6	22.17
	GF	15	No			
	NF	30	No			
	F	22	Yes			
2018/02/05	GNF	16	No	22.3 ± 2.7	0.93 ± 0.8	10.47
	GF	10	No			
	NF	18	No			
	F	17	Yes			
2018/03/19	GNF	25	No	24.1 ± 3.6	0.94 ± 0.4	15.47
	GF	21	No			
	NF	23	No			
	F	23	Yes			
2018/04/18	GNF	19	No	22.0 ± 3.8	1.56 ± 0.7	10.06
	GF	16	No			
	NF	18	No			
	F	20	Yes			
2018/06/30	GNF	8	No	20.6 ± 6.7	1.89 ± 1.0	11.50
	GF	11	No			
	NF	16	No			
	F	14	No			

GNF - Phloem-girdled non-fruiting branches GF - Phloem-girdled fruiting branches
NF - Non-fruiting branches F - Fruiting branches

Measurements of pre-dawn leaf water potential (ψ_{pd}) were made on sap-flow measurement trees using a Scholander pressure chamber (Model 600, PMS Instrument Company, Albany, OR, USA). These measurements were made in order to assess the water status of trees and to eliminate water stress as a confounding factor in any of the results. A total of 60 ψ_{pd} measurements were made throughout the trial. Water stress is believed to occur at ψ_{pd} exceeding -0.5 MPa based on research by Stephenson et al. (2003).

5.3.4 Statistical analysis

To analyse the influence of sink treatments on A_{max} and g_s, data from each of the measurement dates and treatments was compared using repeated measures ANOVA with Restricted Maximum Likelihood estimation (REML) in the Variance Estimation, Precision & Comparison methodology (VEPAC) of Statistica (TIBCO Software Inc. Version 13.3). The individual tree replicates was a random variable so that N=10. Using LSD multiple comparisons, the treatment means were regarded as different if $p \leq 0.05$.

Following the initial analysis of between treatment differences, it was determined that there were no statistically significant differences between NF and F treatments throughout the trial. As a result, all measurements of A_{max} and g_s for these treatments were combined into a single group labelled as non-girdled treatments. The non-girdled set of measurements was further subdivided into a pre-harvest period (PreHNG), which included all measurements on trees bearing fruit (from December to April) and an after-harvest (AHNG) period (June), which included all measurements on trees not bearing fruit. More details regarding these groupings can be found in Table 5.2. To determine if any differences in A_{max} and g_s in response to VPD_{leaf} existed between PreHNG and AHNG groups, data from all measurement dates linked to each of the aforementioned groups was grouped into four categories of VPD_{leaf} spanning 0.5 kPa each. Differences between groups were assessed by means of repeated measures ANOVA with REML in the VEPAC of Statistica (TIBCO Software Inc. Version 13.3).

Furthermore, to analyse the response of E_c to T_{air}, VPD_{air}, R_s and ET_o during fruiting and non-fruiting periods, data from all measurement dates were grouped into six T_{air}

categories spanning 5°C, eight categories of VPD_{air} spanning 0.5 kPa, four categories of R_s spanning 1.0 MJ m^{-2} h^{-1} and five categories of ET_o spanning 0.1 mm h^{-1}. Using repeated measures ANOVA with ANOVA with REML in the VEPAC of Statistica (TIBCO Software Inc. Version 13.3), a test for differences in E_c during fruiting and non-fruting periods was conducted. The individual tree replicate was a random variable so that *N*=4. Using LSD multiple comparisons, the treatment means were regarded as different if $p \leq 0.05$.

Table 5.2: Grouping of treatments post statistical analysis, including pre-harvest non-girdling (PreHNG) and after harvest non-girdling (AHNG). *N* is the number of leaf gas exchange measurements per group.

Group	Treatments Included	Date(s) Included	*N*
PreHNG	NF F	2017/12/12 2018/02/03 2018/02/04 2018/02/05 2018/03/19 2018/04/18	225
AHNG	NF F	2018/06/30	30

5.4 Results

5.4.1 Weather and fruit growth

Average daily air temperature throughout the trial was 19.0 °C, with the highest (21.7 °C) and lowest (13.9 °C) monthly average values measured in January and June 2018, respectively (Figure 5.1 A). Daily maximum air temperature never exceeded 35°C, with the highest daily maximum (34.2°C) coinciding with the highest daily average temperature on 25 December 2018. Total rainfall during the assessment period (2017/10/08 – 2018/06/30) was 504 mm, with most of the rainfall occurring in December 2017 (116 mm) and February 2018 (175 mm) (Figure 5.1 A). Total ET_o for the duration of the trial was 654 mm and exceeded total rainfall by 150 mm, while an average daily ET_o of 3.2 mm was measured during the same period (Figure 5.1 B). Total rainfall and irrigation was 600 mm and total ET_o exceeded this amount by 54 mm. However, measurements of predawn leaf water potential throughout the trial (data not shown) revealed that trees remained unstressed, even though ET_o exceeded total rainfall and irrigation. Daily average air vapour pressure deficit (VPD_{air}) was 0.92 kPa, and rarely exceeded 1.8 kPa (Figure 5.1 B).

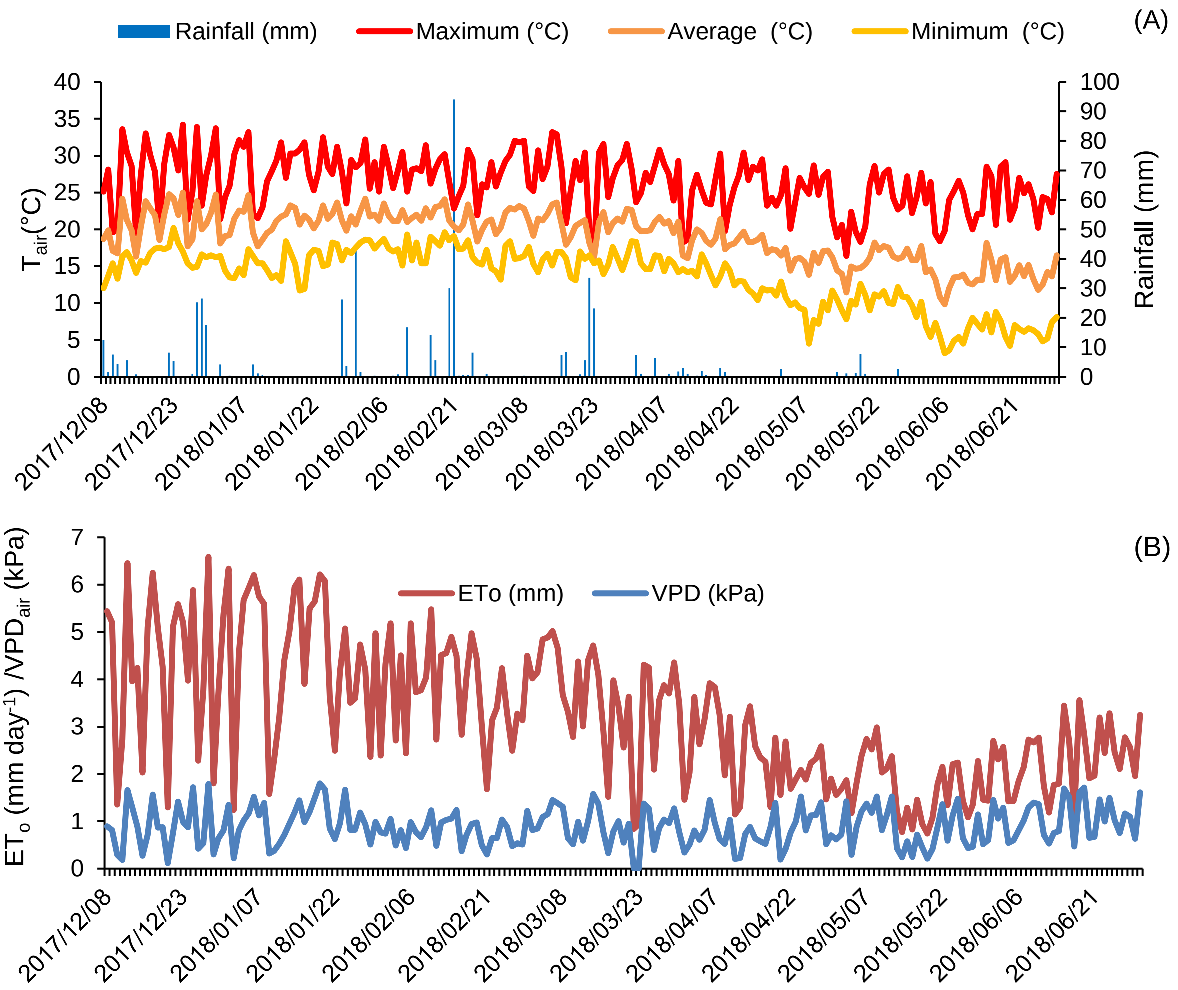

Figure 5.1: (A) Daily average, maximum, and minimum air temperature and rainfall, and (B) daily average reference evapotranspiration (ET_o) and air vapour pressure deficit (VPD_{air}) for the duration of the trial (2017/10/08 – 2018/06/30).

Throughout two growing seasons an approximate linear increase in NIH diameter was observed up to 10 weeks post anthesis. Thereafter the NIH diameter increased at a slower rate, and the final average diameter was 30.3 mm in both seasons (Figure 5.2 A). Individual NIH wet mass followed a sigmoidal pattern, increasing exponentially from 0 to 13 weeks post anthesis, where after only a very slight increase was observed for the remainder of fruit development (Figure 5.2 B). Average individual NIH wet mass 13 weeks post anthesis was 1.6 g, and only reached an average recorded final wet mass of 1.7 g approximately 20 weeks post anthesis. Individual NIH diameter and mass did not differ between seasons (Figure 5.2 A & B). Based on these measurements and existing knowledge of macadamia fruit development (Trueman 2013), it was estimated that the nuts became a significant non-functional sink when fruit growth entered the linear phase of the sigmoidal growth curve.

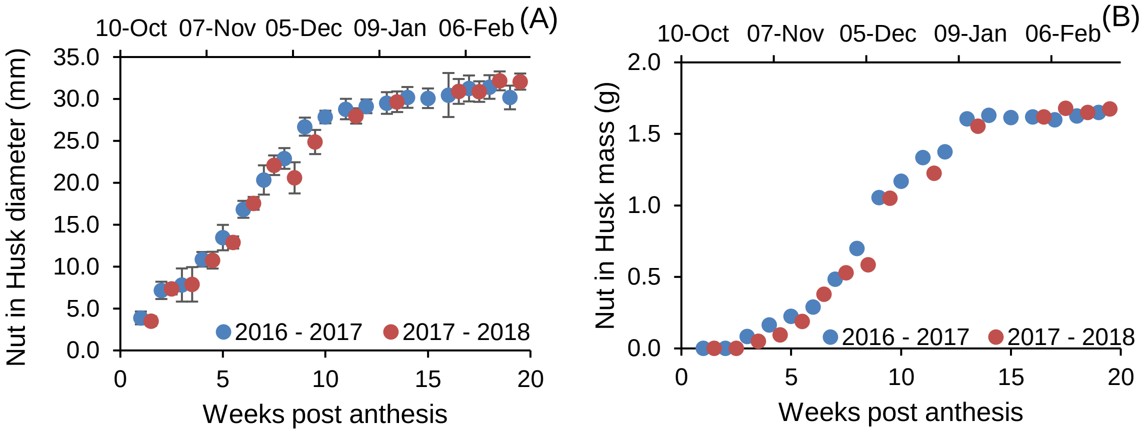

Figure 5.2: Average (± standard deviation) nut in husk diameter (A) and average nut in husk mass (B) of 20 randomly collected macadamia fruits from one weak post anthesis to 20 weeks post anthesis in the 2016-2017 and 2017-2018 production seasons.

In this study, the linear increase in mass commenced at approximately 5 weeks post anthesis, which roughly corresponded with the first week of November (Figure 5.2). This linear increase in nut mass continued until approximately 13 weeks post anthesis, which roughly corresponded to the start of January. From early January onwards, fruit diameter and mass only increased slightly until the end of the measurement period on 2017/02/27 and 2018/02/23. Given that the linear increase in fruit growth commenced approximately 5 weeks post anthesis, all periods between November and end-April (harvest) were designated as high sink strength periods, and periods from May to October as low sink strength periods.

5.4.2 Sink manipulation effects on leaf gas exchange

Light-saturated net CO_2 assimilation rate (A_{max}) at the start of the trial (2017/12/12) was not significantly different between the treatments, averaging 10.6 µmol CO_2 m^{-2} s^{-1}. There was also no significant difference in A_{max} between NF and F treatments throughout the trial (Figure 5.3A). A_{max} of the phloem girdled non-fruiting (GNF) treatment showed a strong reduction (to 2.7 µmol m^{-2} s^{-1}) relative to all other treatments when measured on 2018/02/03, eight weeks after girdling. Values remained significantly lower than those of the non-girdled treatments for the remainder of the season. Girdling induced an average seasonal reduction in A_{max} of 84.4% in the GNF treatment compared to pre-girdling levels, and an 82.8% reduction compared to NF and F treatments from 2018/02/03 to 2018/06/30.

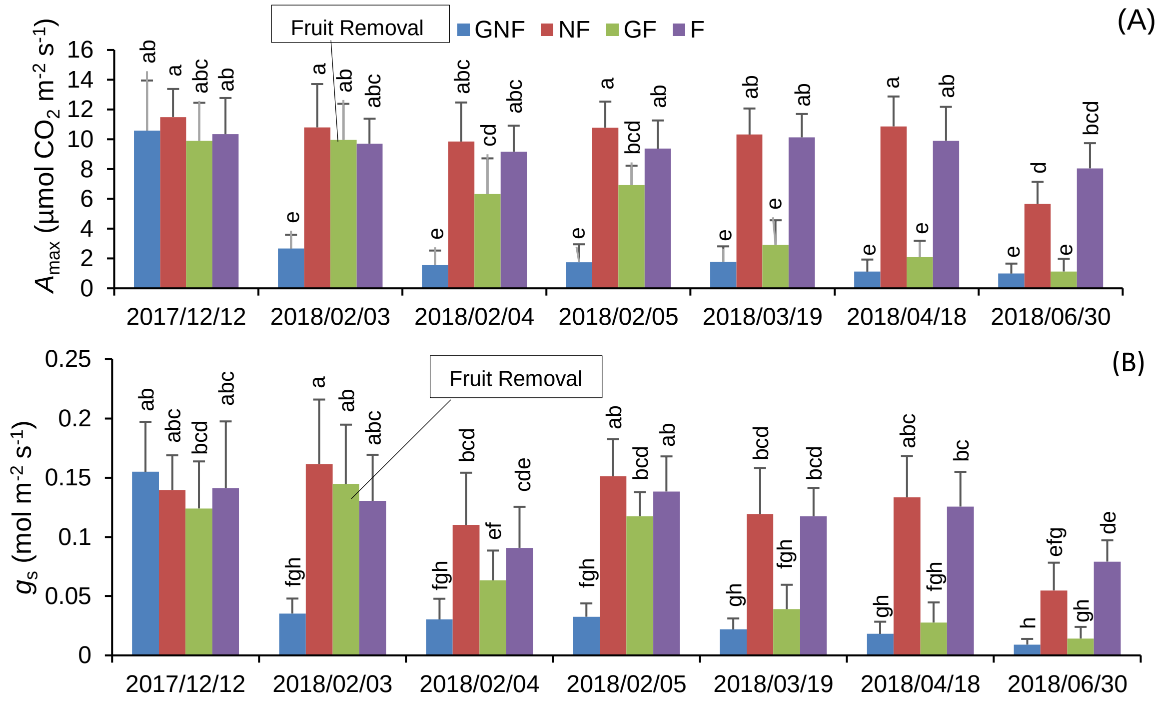

Figure 5.3: (A) Average (± standard deviation) light-saturated net CO$_2$ assimilation rate (A_{max}) and (B) stomatal conductance (g_s), measured on seven days during the trial period. Treatments included phloem-girdled non-fruiting branches (GNF), non-fruiting branches (NF), phloem-girdled fruiting branches (GF) and fruiting branches (F). GF branches were defruited on 3 February 2018. Means followed by the same letter are not significantly different (p = 0.05) as analysed using repeated measures ANOVA.

Phloem girdled fruit bearing (GF) treatments showed similar A_{max} values as NF and F treatments before fruit removal on 3 February 2018. One day after fruit removal, A_{max} of the GF treatment was lower, but not significantly so, compared to the NF and F treatments. On 2018/02/05, two days after fruit removal, A_{max} was significantly lower in the GF treatment than in the NF treatment, but was not significantly different to the F treatment. From 2018/03/19 (approximately six weeks after fruit removal) until the end of the trial on 2018/06/30, the GF treatment showed significantly lower A_{max} values compared to both the NF and F treatments, but similar values to the GNF treatment. Defruiting at harvest (2018/04/27) in the F treatment resulted in a smaller decrease in

A_{max} over an eight-week period compared to the NF treatment, which showed a stronger decrease. However, the final values on 2018/06/30 were not significantly different.

Similar to A_{max}, g_s was not significantly different between treatments at the start of the trial, averaging 0.13 mol m^{-2} s^{-1} (Figure 5.3 B). Again, no significant difference was observed between the NF and F treatments for the duration of the trial. On 2018/02/03, eight weeks after girdling, a significant reduction in g_s was measured in the GNF treatment compared to the other treatments. Girdling induced on average an 84.4% reduction in g_s in the GNF treatment over the remainder of the trial compared to the pre-girdling GNF value, and an 82.8% lower g_s compared to the NF and F treatments. A continuous reduction of g_s in the GNF treatment was observed from the time of girdling onwards, and final value of g_s on 30 June 2018 was close to zero (0.01 mol m^{-2} s^{-1}).

A significant reduction of g_s in the GF treatment compared to the NF treatment was observed one day after fruit removal (2018/02/04). It should be noted that values of g_s on 2018/02/04 were also lower than on 2018/02/03 for the NF (significantly) and F (not significantly) treatments, with all three treatments returning to similar values on 2018/02/05 as for 2018/02/03. The reduction in g_s on 2018/02/04 was likely due to sunny weather, with a high evaporative demand. Average hourly values of VPD$_{air}$ during leaf gas exchange measurements exceeded 1.4 kPa, compared to 0.69 kPa on 2018/02/03 and 0.93 kPa on 2018/02/05 (see Table 5.1). Similarly, R$_s$ on 2018/02/04 was 22 MJ m^{-2} day^{-1} compared to 12 MJ m^{-2} day^{-1} on 2018/02/03 and 10 MJ m^{-2} day^{-1} on 2018/02/05 (see Table 5.1). However, on 2018/03/19, six weeks after fruit removal in the GF treatment, g_s in this treatment was significantly lower compared to the NF and F treatments, remaining lower on 2018/04/18. On both days, g_s values were similar for the GF and GNF treatments. On 2018/06/30, after the commercial harvest (2018/04/27), g_s in the GF treatment did not differ significantly from the values for the NF and GNF treatments, but was lower compared to the F treatment.

Responses of A_{max} to VPD$_{leaf}$ in various categories revealed no significant differences for non-girdled treatments before and after harvest within the 1.00 – 2.50 kPa range

(Figure 5.4 A). The average A_{max} values in this range was 9.94 ± 2.25 µmol CO_2 m^{-2} s^{-1}. A significant difference was found for A_{max} of non-girdled treatments between the before and after harvest periods within the VPD_{leaf} range of 2.51 – 4.00 kPa. Pre-harvest A_{max} was on average 9.90 ± 1.67 µmol CO_2 m^{-2} s^{-1} compared to 6.59 ± 1.89 µmol CO_2 m^{-2} s^{-1} during the after harvest period. Non-girdled treatments showed differences in the responses of g_s to VPD_{leaf} in the pre- and after harvest periods (Figure 5.4 B). Values of g_s were significantly higher within the 1.00 – 1.50 kPa range during the after harvest period (g_s = 0.14 ± 0.03 mol m^{-2} s^{-1}) compared to the pre-harvest period (g_s = 0.10 ± 0.04 mol m^{-2} s^{-1}). At VPD_{leaf} ranges of 1.51 – 4.0 kPa, pre-harvest values of g_s were significantly higher than values for the after harvest period.

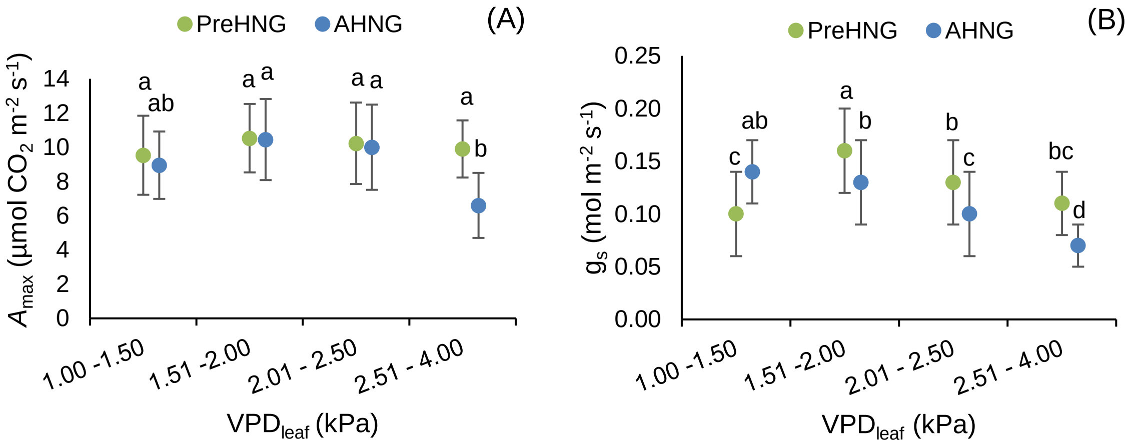

Figure 5.4: (A) Average (± standard deviation) net maximum CO_2 assimilation rate (A_{max}) and (B) stomatal conductance (g_s) of non-girdled branches pre-harvest (PreHNG) (N=225) and after harvest (AHNG) (N=30). Means followed by the same letter are not significantly different (p = 0.05) as analysed using repeated measures ANOVA.

5.4.3 The influence of fruit on canopy transpiration responses to atmospheric drivers

There were small differences in E_c in response to air temperature during fruiting and non-fruiting periods (Figure 5.5 A). Within the lower range of 15 – 20°C, E_c was significantly higher during the non-fruiting period (0.05 mm h^{-1}) compared to the fruiting period (0.03 mm h^{-1}), whilst in the higher range of 25 – 30°C, E_c was significantly higher during fruiting periods (0.11 mm h^{-1}) compared to non-fruiting periods (0.097 mm h^{-1}). Differences between fruiting and non-fruiting periods in the response of E_c to VPD_{air} were more distinct. E_c during fruiting periods was, on average, 0.02 ± 0.01 mm h^{-1} higher than during non-fruiting periods across all VPD_{air} ranges (Figure 5.5 B). Within the 0.0 – 3.0 kPa range, E_c during fruiting periods was significantly higher compared to non-fruiting periods, whilst no differences between the two periods were found at VPD_{air} above 3.0 kPa.

Within the 0.0 – 3.0 MJ m^{-2} h^{-1} solar radiation (R_s) range, E_c was significantly higher (by on average 0.01 ± 0.001 mm h^{-1}) during the fruiting period compared to the non-fruiting period. At R_s above 3.0 MJ m^{-2} h^{-1} no significant differences in E_c were found between fruiting and non-fruiting periods (Figure 5.5 C). The response of E_c to categories of ET_o were similar, with E_c being significantly higher (by on average 0.02 ± 0.009 mm h^{-1}) during fruiting compared to non-fruiting periods within the 0.0 – 0.3 mm h^{-1} range of ET_o (Figure 5.5 D). When ET_o exceeded 0.3 mm h^{-1} no significant differences in E_c were found between fruiting and non-fruiting periods.

Given that significant changes in canopy size across the full study period could act as a confounding factor for the observed responses of E_c to weather variables, average diurnal responses of weather variables and canopy transpiration during 1-30 April 2018 (fruiting) and 1-31 May 2018 (non-fruiting) were compared (Figure 5.6), with commercial harvest occurring on 2018/04/27. During these periods there were no measured differences in canopy size, with average LAI measured on 2018/04/18 being 4.6 m^2 m^{-2} and declining to 4.3 m^2 m^{-2} on 2018/06/03 following hand pruning, which commenced on 2018/06/16. Differences in measurements of canopy dimensions and volume made on 2018/01/17 and 2018/05/01 were also small, with trees showing only a slight increase (4 m^3) in canopy volume during this period. Given these minor differences in canopy size, it is proposed that changes in canopy size can be ignored as a confounding factor in the results presented.

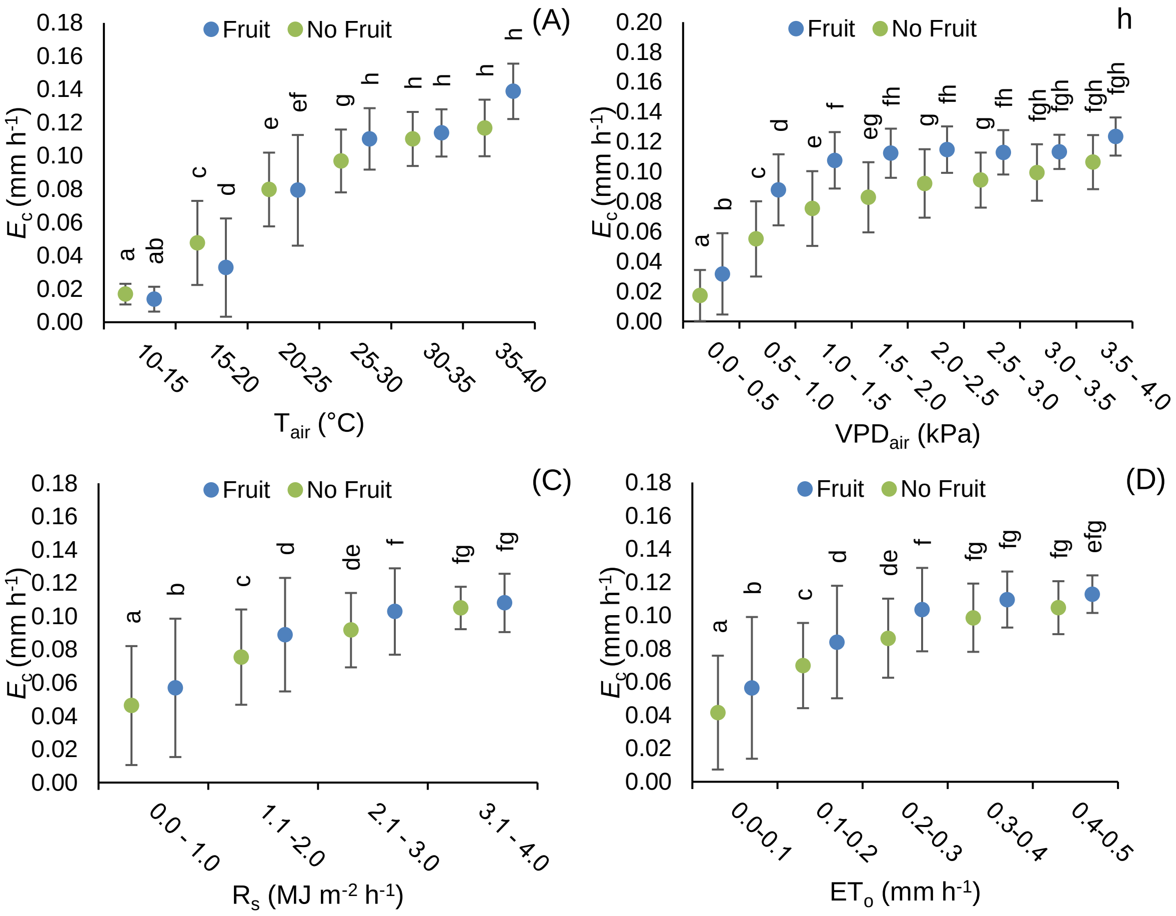

Figure 5.5: Response of average daytime (0600h – 1800h) hourly canopy transpiration (*E_c*) to (A) air temperature (T_{air}), (B) air vapour pressure deficit (VPD_{air}), (C) solar radiation (R_s) and (D) reference evapotranspiration (ET_o) during fruiting (*N*=8688) and non-fruiting (*N*=8717) periods over two consecutive seasons from 10 August 2016 to 8 August 2018. Means followed by the same letter are not significantly different (*p* = 0.05) as analysed using repeated measures ANOVA.

Given the large variances in most of the weather variables, there were no statistically significant differences (p=0.05) in T_{air} (Fig. 5.6 A), R_s (Fig. 5.6 B), VPD_{air} (Fig. 5.6 C) and ET_o (Fig. 5.6 D) during the fruiting and non-fruiting months. There were, however, differences in daytime E_c between the fruiting and non-fruiting months. During the fruiting month E_c was significantly higher at the following times: 09:00, 10:00, 12:00, 14:00, 15:00, and 16:00 (Figure 5.6 E). E_c at these times was on average 0.03 ± 0.01 mm h^{-1} higher during the fruiting month compared to the non-fruiting month.

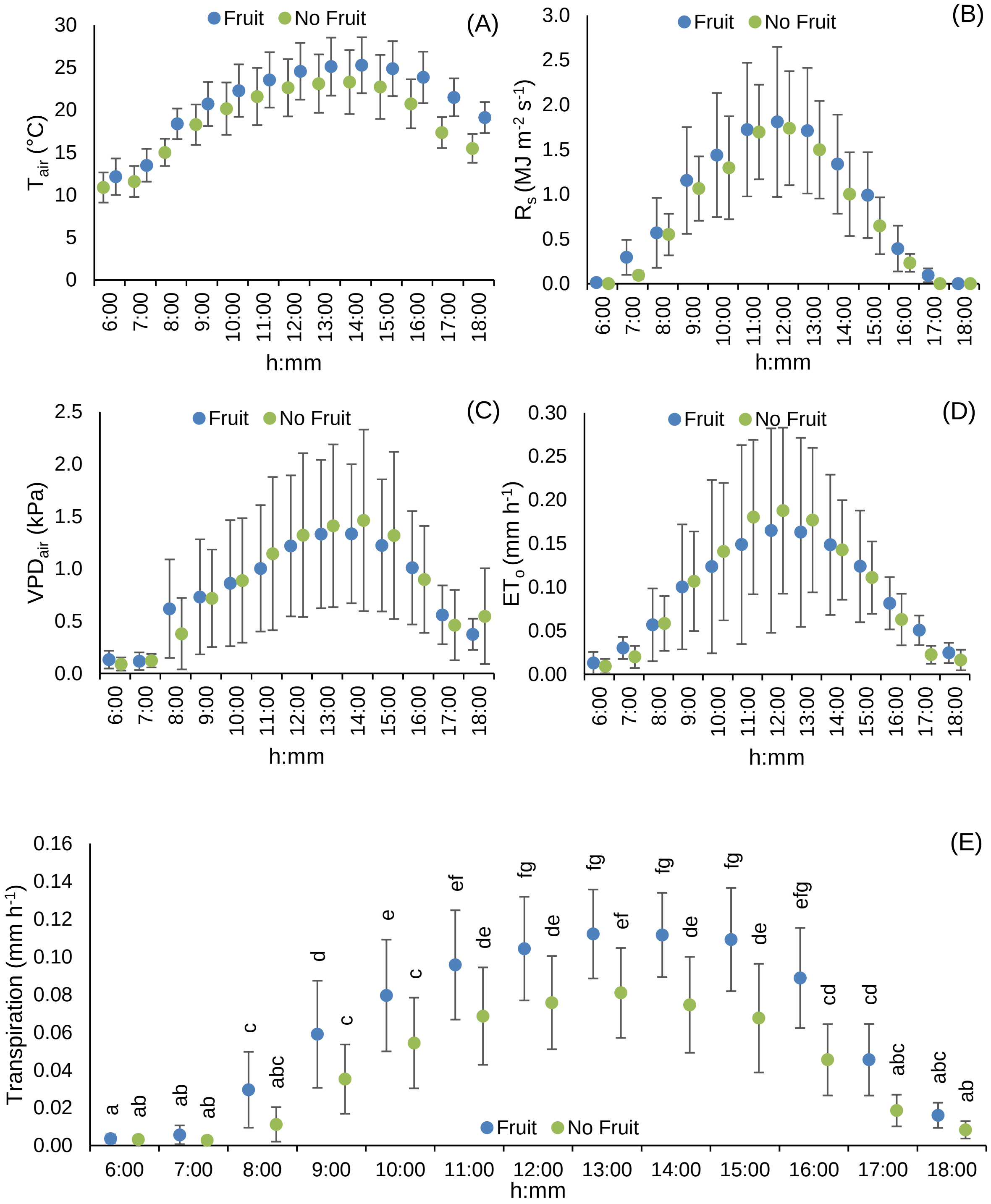

Figure 5.6: Average diurnal progression of (A) air temperature (T_{air}), (B) solar radiation (R_s), (C) air vapour pressure deficit (VPD_{air}), (D) reference evapotranspiration (ET_o) and (E) canopy transpiration during fruiting (*N*=403) and non-fruiting (*N*=390) months of April and May 2018, respectively. Means followed by the same letter are not significantly different (p = 0.05) as analysed using repeated measures ANOVA.

When the area under each curve in Figure 5.6 E was integrated, the average daytime transpiration during the fruiting month (April 2018) was 0.85 mm day^{-1} which was higher than the 0.55 mm day^{-1} calculated for the non-fruiting month (May 2018). Total monthly daytime transpiration for April 2018 (fruiting) was 27.5 mm compared to 18.3 mm for May 2018 (non-fruiting), whilst total ET_o for April 2018 was 80 mm and May 2018 was 76 mm.

5.5 Discussion

Upregulation in of A_{max}, g_s and E_c in the presence of fruit is a common phenomenon and has been demonstrated in a range of fruit tree crops, including apple (Wünsche et al., 2000), avocado (Silber et al. 2013), date palm (Zhen et al. 2019) and olive (Bustan et al., 2016). The physiological upregulation of E_c during fruiting periods is critical for irrigation scheduling and water use modelling, as soil water stress during fruiting periods negatively impact yield and quality of oil storing macadamia and olive (Stephenson et al. 2003; Casanova et al. 2017; Gucci et al. 2019). The effect of developing macadamia fruit, a strong non-functional sink (i.e. non-photosynthetic or structural sink), on leaf gas exchange and E_c has, however, not been examined, although results discussed in Chapter 4 have suggested that the presence of fruit could have a significant impact on leaf gas exchange.

Acknowledging the complexity of whole tree source-sink dynamics, and difficulties in scaling leaf gas exchange measurements to canopy level, the study used phloem girdled and non-girdled branches, with and without fruit, to examine what effect fruit has on leaf gas exchange of macadamias. No significant differences in A_{max} and g_s were observed between girdled fruiting (GF) branches and non-girdled branches (F & NF) approximately two months after girdling, whilst A_{max} and g_s of girdled non-fruiting (GNF) branches were significantly reduced during the same period. Before fruit removal, A_{max} and g_s of GF branches were approximately 3.8 times higher than that of GNF branches. Fruit removal on GF branches, however, resulted in decreased A_{max} and g_s compared to that of F and NF treatments approximately four weeks after fruit removal. This observation suggests that the presence of oil storing macadamia fruit, leads to the maintenance of high A_{max} and g_s in isolated branches.

Branch girdling is known to decrease both A_{max} and g_s in a range of horticultural crops including apple (Zhou and Quebedeaux 2003), grape (Harrell and Williams 1987; Roper and Williams 1989) and mango (Lu and Chacko 1998; Urban et al. 2004). However, in the presence of a strong sink, such as developing fruit, and especially in the case of oil storing fruit, such as olive and cashew, both A_{max} and g_s showed no decrease after girdling of fruit bearing branches (Proietti and Tombesi 1990; Schaper and Chacko 1993). Paul and Eastmond (2020), suggested that the conversion of simple sugars to lipids, as is the case in oil storing fruit, could mitigate the signals that would normally down-regulate A_{max} and g_s through end-product accumulation, meaning that photosynthesis is “blind” to carbon accumulation and can carry on unimpeded whilst carbon accumulates. This could partly explain why A_{max} and g_s of GF branches were no different from NF and F branches before fruit removal, but were significantly lower than non-girdled treatments more than a month after fruit removal.

Although the induction of a sink limitation by fruit removal in girdled branches has been shown to down-regulate A_{max} and g_s, it is unclear if the presence or absence of a sink would have any effect on A_{max} and g_s of non-girdled macadamia branches. In this study there were no significant differences in A_{max} and g_s between NF and F branches throughout the duration of the trial. The maintenance of high A_{max} and g_s, during fruiting periods was therefore not limited to branches containing the sink, but also occurred in branches containing no direct sink (i.e. non-fruiting branches). In coffee, Cannell (1971) demonstrated that there are large transfers of assimilates throughout the tree, which buffer the increased whole tree assimilate demand brought about by developing fruit. Based on evidence from this study, it would be reasonable to expect that similar mechanisms are present in macadamia.

The concept that fruit would draw assimilates from distal source leaves, stems from the basis of long distance assimilate transport in plants. The Münch (1930) hypothesis of long distance transport states that high assimilate concentrations within the photosynthetic cells results in diffusion of said assimilates into the phloem through plasmodesmata, and in the sieve tube elements the hydrostatic pressure created by the solute molecules motivates mass flow toward the sinks (Turgeon 2010b). The ability of assimilates to be transported over long distances, albeit to a range of sinks and not limited to developing fruit, would imply that an upregulation of leaf gas exchange, in the presence of a strong sink, would not be limited to leaves or branches in close proximity to fruit. Furthermore, it should be noted that the continuous transport of assimilates away from source leaves is essential for the maintenance of high levels of A_{max} (Turgeon 2010a; Turgeon 2010b), and it would therefore be reasonable to assume that in the absence of long distance transport in macadamia, A_{max} of NF branches would be appropriately downregulated so that carbon supply meets demand.

Considering that a degree of branch dependence, implying long distance transport of assimilates, is present in macadamias, fruit removal at the canopy level, resulted in a significant reduction of g_{s} in both F and NF branches, compared to g_{s} measured before commercial harvest, despite similar weather conditions during both measurements. Although no girdling was performed, similar responses have been found in apple (Wünsche et al., 2000) and date palm (Zhen et al. 2019), with g_{s} on fruiting trees being substantially higher than non-fruiting trees. This raises the question of whether or not the lack of a sink, in the form of developing fruit, would lead to the downregulation of long distance transport and subsequent accumulation of assimilates in photosynthetic tissues leading to downregulation of A_{max} and g_{s} as a result of feedback inhibition? Interestingly only A_{max} of NF branches was significantly lower after commercial harvest compared to before harvest. The non-significant reduction in A_{max} of F branches after harvest could stem from the need to replenish depleted assimilates stored within the branch itself as a result of reallocation to fruit during a possible source-sink imbalance, as has been shown in avocado (Van Vuuren et al. 1997), mango (Davie et al. 1999) and grape (Candolfi-Vasconcelos et al. 1994). The reliance of macadamia fruit development on stored assimilates has, however, not been shown but would provide valuable insights into the complex field of assimilate partitioning.

Nevertheless, in light of branch dependence demonstrated in this study, measurements of A_{max} and g_{s} for NF and F branches were combined into pre-harvest non-girdled (PreHNG) and after harvest non-girdled (AHNG) groups. The greatest differences between the PreHNG and AHNG measurements were found in g_{s}, which was significantly higher for the PreHNG group compared to the AHNG group, when $\mathrm{VPD}_{\mathrm{leaf}}$ exceeded 1.50 kPa. The same analysis, however, only showed a significant difference in A_{max} when VPD_{leaf} exceeded 2.50 kPa, being higher for the PreHNG group compared to the AHNG group. These results suggest that, firstly, macadamias are able to maintain A_{max} at a lower g_s (i.e. higher intrinsic water use efficiency calculated as A_{max}/g_s) at VPD_{leaf} greater than 1.50 kPa during the after harvest period, but at low VPD_{leaf} (<1.50 kPa) intrinsic water use efficiency would be greater during the pre-harvest/fruit bearing period. Increased intrinsic water use efficiency at VPD_{leaf} <1.50 kPa during fruit bearing periods would imply that there is a significant reduction in non-stomatal limitations of A_{max} during fruit bearing periods (see Chapter 4), considering that stomatal limitations of A_{max} were unlikely to have occurred at low VPD_{leaf}. Secondly, it would seem as if macadamias deviate from the premise that g_s optimizes carbon uptake relative to water loss in transpiration (Ball et al. 1987; Leuning 1995), when VPD_{leaf} exceeds 1.50 kPa during fruit bearing periods.

It is not clear why increases in macadamia g_s within the 1.50 – 2.50 kPa range of VPD_{leaf} during fruit bearing periods occurs without significant increases in A_{max}, seeing that this leads to reductions in intrinsic water use efficiency compared to non-fruiting periods. Lawson and Vialet-Chabrand (2019), have suggested that slow stomatal closure could cause non-synchronous behaviour between A and g_s, which can lead to lower than optimal intrinsic water use efficiency. Although, stomatal kinetics were not measured in this study, an array of recent studies have shown that sucrose, which is one of the most commonly produced assimilates, plays a significant role in stomatal opening or closure (Mansfield 2012; Lawson and Vialet-Chabrand 2019; Lawson and Matthews 2020). Lawson et al. (2008) and Lawson and Blatt (2014), suggested that an increase in stomatal kinetics of transgenic tabaco was observed when the regeneration of ribulose1,5-bisphosphate (RuBP) was suppressed. Regeneration of RuBP is often suppressed as a result of end product accumulation and reduced triose-phosphate utilization (TPU) (Mott et al. 1986; Heyneke and Fernie 2018; Sharkey 2019), which has been shown in macadamias in the absence of fruit/non-functional sink (see Chapter 4). It is therefore reasonable to assume that increased stomatal kinetics in the absence of fruit would lead to less of a disconnect between A and g_s and subsequently to increased intrinsic water use efficiency. Similarly, increased TPU during fruiting periods could result in sluggish stomatal responses, especially slower stomatal closure, thereby leading to increased transpiration without significant increases in carbon gain (Barral 2019; Lawson and Vialet-Chabrand 2019).

The upregulation of g_s in both F and NF branches during fruit bearing periods, and the prospect of significant long distance transport of assimilates, which aids this upregulation, was proposed to result in increases in E_c. Macadamia E_c followed a similar trend to that of g_s, with E_c responses to increases in VPD_{air}, R_s and ET_o, over two consecutive seasons, all being higher during fruiting compared to non-fruiting periods. The most distinct differences in E_c between the two periods was seen in the response to increases in VPD_{air}, with E_c within the 0.0 – 3.0 kPa VPD_{air} range being approximately 0.02 ± 0.009 mm h^{-1} greater during fruit bearing periods compared to periods containing no fruit. Variable responses of g_s to increases in VPD_{leaf} during high and low fruit load/sink strength periods were demonstrated in Chapter 4. The aforementioned discussions regarding long distance transport of assimilates and its effects on stomatal kinetics, would suggest that increases in g_s as a result of the presence of fruit could be scaled to a canopy level. Results in this study tend to suggest that there is an upregulation in E_c during the fruiting period as a result of significantly higher g_s at VPD_{leaf} > 1.5 kPa, which is subsequently observed in the significantly higher E_c rates to predominantly VPD_{air} within the 0.0 – 3.0 kPa range.

The major constraint to establishing causality between the upregulation of E_c and increased fruit load or sink strength across multiple seasons, is the dominating effect of changes in weather and canopy size, which would significantly impact canopy E_c. As a result, the diurnal course of a range of weather variables and transpiration was examined in April and May 2018, which was approximately one month before and one month after commercial harvest, during which changes in canopy size were negligible. Although weather variables, with the exception of air temperature, which was slightly higher in April 2018 compared to May 2018, were fairly similar, average diurnal E_c during April 2018 remained higher from 700h to 1800h compared to May 2018, with the distinct difference being the presence or absence of fruit. To account for possible variations in weather variables, which could lead to confounding, transpiration crop coefficients (K_t), which normalizes weather conditions, was calculated by dividing E_c by ET_o in a similar approach to that proposed by Villalobos et al. (2013). This analysis proved that increases in E_c observed in April 2018 were unrelated to weather variations, with average K_t during the fruit bearing April period being 0.34 compared to 0.28 in the fruitless May period (data not shown).

Studies on apples (Lenz 1986), avocado (Silber et al. 2013) and olive (Bustan et al. 2016) have shown that E_c of fruit bearing trees were significantly higher than that of non-fruiting trees under the same set of environmental conditions thereby implying that fruiting influences E_c of a range of fruit tree crops. Lenz (1986), showed that higher E_c in fruit bearing apple trees was not associated with higher leaf area. Silber et al. (2013), reported that in avocado, increased E_c during fruiting periods was attributed to higher g_s, which could be plausible for the apple study by Lenz (1986) considering the results by Pretorius and Wand (2003). By comparing macadamia E_c before and after commercial harvest, the study showed that E_c was ~20% higher during fruiting compared to non-fruiting periods. At VPD_{leaf} levels exceeding 1.50 kPa, g_s was approximately 25% higher before harvest compared to the after harvest period, which would explain the large differences observed in E_c. Evidence presented in this study would therefore suggest that the observed differences in E_c is as a result of variable leaf level responses of g_s to increasing VPD_{leaf}.

Given the observational basis of this study, it is suggested that future studies aim to quantify and track assimilate production, export and storage within sink and source leaves and tissues to try and establish when and how changes in leaf gas exchange occur within macadamias. Furthermore, given the significance of observed results in this study, it would be of great value to both researchers and irrigators if crop phenological and physiological parameters can be accounted for in water use models, to improve water management during especially fruit bearing periods.

CHAPTER 6: TRANSPIRATION OF MACADAMIA ORCHARDS

6.1 Abstract

Very few studies have focussed on the measurement and control of transpiration (E_c) in field grown macadamia. As a result, there have also been few published studies investigating and identifying the driving variables of macadamia E_c. Besides environmental factors driving E_c, physiological canopy traits and phenological factors may also have a profound impact on E_c. This study has, therefore, attempted to examine the response of macadamia E_c to variations in environmental conditions, whilst also attempting to account for the effect of physiological and phenological attributes on seasonal variation in E_c. Measurements of E_c, canopy dimensions, and phenology were made in two commercial bearing macadamia orchards varying in canopy size. These measurements commenced in August 2016 and concluded in August 2019, stretching across three consecutive cropping seasons. Transpiration of the mature bearing (MB) orchard, which had a ~50% larger canopy, than the immature bearing (IB) orchard, was ~60% higher than that of the IB orchard. In both orchards, E_c increased linearly with air vapour pressure deficit (VPD_{air}) and reference evapotranspiration (ET_o) at low atmospheric evaporative demands ($VPD_{air} < 0.8$ kPa and $ET_o < 0.13$ mm day^{-1}), but failed to increase at a similar rate during higher atmospheric evaporative demands, suggesting that macadamia E_c is a water supply controlled system. This supply controlled system revealed that E_c reached a nearly constant maximum ($E_{c\ max}$) at ET_o rates exceeding 3.5 mm day^{-1}, with the magnitude of $E_{c\ max}$ largely dictated by canopy size. Although the effect of inter-seasonal variations in canopy size was small, seasonal variations in macadamia transpiration crop coefficients (K_t) were observed in this study. These variations were thought to be largely driven by physiological changes as a result of crop phenology, and more specifically developing fruit. This study demonstrated that although macadamia E_c is driven by ET_o and canopy size, the physiological and phenological attributes of the crop could significantly impact E_c responses to environmental conditions.

Keywords: Canopy size, Phenology, Transpiration crop coefficients, Atmospheric evaporative demand

6.2 Introduction

Transpiration (E_c) is the productive use of water by the crop in response to its immediate environment. Although this does not define crop water needs or total water use (i.e. including evaporation or salt leaching components), it provides researchers, irrigators and growers with a detailed insight into how the crop reacts to a set of specific environmental variables in combination with physical attributes of the crop. Measurements of E_c enable researchers to isolate the beneficial component of water loss from the non-beneficial evaporation component of crop water use (Kool et al. 2014). This isolation of E_c, further allows researchers to manipulate vegetative growth through controlled deficit irrigation strategies, which in a vegetatively biased tree, such as macadamia (Stephenson et al. 1989b; Olesen 2005; Wilkie et al. 2009a; Olesen et al. 2011), could increase yield and subsequently water use efficiency (García et al. 2012; Van Halsema and Vincent 2012).

Although some research has been conducted to measure total water use of macadamia (Stephenson et al. 2003), only one study conducted by Gush and Taylor (2014) in White River, Mpumalanga, South Africa, has focussed on measurements of E_c in field grown macadamias. As a result, there are few published studies investigating and identifying the driving variables of macadamia E_c. Nevertheless, studies on environmental control of E_c in sclerophyllous citrus and olive (Moriana et al. 2002; Nicolás et al. 2008) have demonstrated that strong stomatal control over E_c exists, especially in response to changes in vapour pressure deficit (VPD) (Oguntunde et al. 2007; Nicolás et al. 2008), which was not dissimilar to stomatal responses in macadamias demonstrated in Chapter 4. Although stomatal control of E_c is well documented, it is still unclear how variation in canopy size and crop phenology would influence responses of macadamia E_c to environmental variables. This lack of information regarding macadamia water use has created uncertainty amongst irrigators, leading to a more risk-averse irrigation approach, where growers have aimed to replace reference evapotranspiration losses that would in all likelihood exceed total evapotranspiration (ET) of macadamia. This, according to Stephenson and Searle (2014), is a potentially wasteful water management approach for this crop. Furthermore, a lack of information has also led to the formulation of macadamia specific irrigation schedules based on in-field observations of changes in soil water content, which need to be validated and possibly refined, albeit through water use modelling, to aid irrigators in managing an already limited resource.

Although E_c estimates on their own cannot be used to schedule irrigation, the data could be used to explain some of the anomalies observed in orchard scale water use. One such anomaly, is the perceived increase in water use of macadamia during flowering (September), with recently conducted research suggesting that flowers contribute to increased canopy water loss via direct transpiration from flowers (Chirgwin 2016). Similarly, by observing trends in relative soil water content obtained from soil water monitoring equipment, macadamia growers have reduced irrigation volumes during post-harvest periods (May – August), without observing any decrease in yield and quality in the following season (Stephenson et al. 2003). This perceived decrease in water use could be due to various factors, one of which could be the onset of winter, whilst a reduction in canopy size through pruning occurs during this time, which might result in confounding observations. Both these observations highlight the fact that changes in the environment, as well as physiological and phenological attributes of the tree need to be taken into account when trying to explain E_c dynamics in a relatively new and understudied crop.

Even though the environment and physical attributes of a crop are key driving variables for E_c (Vose et al. 2003; O'Brien et al. 2004; Villalobos et al. 2013), physiological and phenological aspects of macadamia could be an important factor controlling E_c. Results from Chapters 4 and 5 have shown that macadamias employ strict leaf level stomatal control, a trait of isohydric plants, especially in response to increased atmospheric evaporative demand. The isohydric nature of the crop could also imply an upper limit to E_c, under conditions of high atmospheric evaporative demand. It is believed that the magnitude of maximum E_c will be determined largely by canopy size. The results from previous chapters have also shown that the responses of leaf gas exchange and subsequently canopy E_c varied between fruit bearing and non-fruit bearing phenological periods, which suggests the upper limit of E_c may be significantly influenced by crop phenology.

Accounting for changes in crop phenology is, however, rather challenging, considering the dominating effect of environmental drivers on transpiration. Fortunately, by calculating reference evapotranspiration (ET_o) (Allen et al. 1998), which represents nearly all effects of weather, and measuring E_c through sap flow techniques, transpiration crop coefficients (K_t), similar to those proposed by Villalobos et al. (2013), can be calculated, and used to study seasonal relationships between E_c and ET_o. Such K_t values, have not been reported for macadamias, but considering the similarities between macadamias and citrus, both being subtropical evergreen crops with sclerophyllous leaves and similar stomatal behaviour (Jifon and Syvertsen 2001; Ribeiro and Machado 2007), it would be reasonable to assume that trends in K_t values observed in citrus studies would be similar in macadamias. Allen and Pereira (2009) suggested that average K_t for citrus remained fairly constant throughout the season. Given that large changes in canopy size are unlikely to occur in mature macadamia orchards over a single season, it is not unreasonable to assume that macadamias would have similarly constant K_t values across the season. It is, however, unclear what effect physiological and phenological changes would have on the relationship between E_c and ET_o, especially considering that variation in E_c, unrelated to physical changes in tree canopy size brought about through pruning and significant differences in weather, have been reported (see Chapter 5).

This study hypothesized that macadamia E_c is a supply controlled system, which would result in an upper limit of E_c under conditions of high evaporative demand, with its magnitude dictated by physical orchard attributes (i.e. canopy size). Given the hypothesized upper limit of E_c, the study further hypothesized that variations in seasonal values of K_t can be expected in macadamias, given that the rate of E_c is unlikely to increase at the same rate of increase in ET_o and given the significant effect of physiology and phenology on leaf gas exchange and E_c. This study therefore aimed to examine the response of field grown macadamia E_c to variations in environmental conditions and canopy size, whilst also attempting to account for the effect of physiological and phenological attributes on seasonal variations in E_c.

6.3 Materials and Methods

6.3.1 Site description, weather variables, canopy measurements, and phenology

Site and orchard specific information, as well as details of the measurement of weather variables are outlined in Chapter 3. Missing solar radiation data, as a result of equipment failure during February – March 2018, was estimated using measurements of minimum and maximum air temperature. Measurements of weather variables and E_c were made over three cropping seasons outlined in Table 6.1.

Table 6.1: Start and end date of each cropping season assessed throughout the trial

Season	Start Date	End Date	Duration (days)
2016/17	2016/08/10	2017/08/09	364
2017/18	2017/08/10	2018/08/06	361
2018/19	2018/08/07	2019/08/05	363

These measurements were made in two macadamia orchards, consisting of a mature bearing (MB) and an immature bearing (IB) orchard, which differed largely based on canopy size. More details regarding the orchards used in this study can be found in Chapter 3, Table 3.1. Furthermore, canopy dimensions, including height, width, and breadth, as well as other canopy specific parameters including leaf area index (LAI) and fractional interception of photosynthetically active radiation (FI), were measured throughout the trial in both orchards, with methods outlined in Chapter 3.

Tree phenology and important management practices were recorded for each orchard. With exception of the onset of oil accumulation, all phenological periods and the duration of each, were determined by means of visual inspection of both orchards (Table 6.2). Oil accumulation was assumed to commence as soon as nut growth ceased to increase, as outlined in the description of nut growth measurements described in Chapter 5, and in line with reports from Stephenson et al. (1989).

Table 6.2: Phenology and important management practices recorded in the mature bearing (MB) and immature bearing (IB) macadamia orchards across three consecutive cropping seasons. The duration of each phenological period was an approximation based on visual inspection.

	Season								
	2016/17			2017/18			2018/19		
Phenological Stage	**Start Date**	**End Date**	**Duration (days)**	**Start Date**	**End Date**	**Duration (days)**	**Start Date**	**End Date**	**Duration (days)**
Pre-flowering	2016/08/10	2016/08/30	20	2017/07/16	2017/08/23	38	2018/07/16	2018/08/14	29
Flowering	2016/08/31	2016/09/20	20	2017/08/24	2017/09/14	21	2018/08/15	2018/09/12	28
Nut Set	2016/09/21	2016/10/15	24	2017/09/15	2017/10/07	22	2018/09/13	2018/10/20	37
Spring Flush	2016/10/16	2016/11/05	20	2017/10/08	2017/10/30	22	2018/10/21	2018/11/09	19
Premature Nut Drop	2016/11/06	2016/11/30	24	2017/10/31	2017/11/26	26	2018/11/10	2018/11/30	20
Nut Growth	2016/12/01	2017/01/15	45	2017/11/27	2018/01/20	54	2018/12/01	2019/01/23	53
Summer Flush	2017/01/16	2017/02/15	30	2018/01/21	2018/02/18	28	2019/01/24	2019/02/12	19
Oil Accumulation	2017/02/16	2017/04/19	62	2018/02/19	2018/04/25	65	2019/02/13	2019/04/20	66
Harvest	2017/04/20	2017/05/15	25	2018/04/26	2018/05/19	23	2019/04/21	2019/05/10	19
Flower Initiation	2017/05/16	2017/06/15	30	2018/05/20	2018/06/15	26	2019/05/11	2019/06/04	24
Pruning & Winter Rest	2017/06/16	2017/07/15	29	2018/06/16	2018/07/15	29	2019/06/05	2019/07/14	39

6.3.2 Transpiration Measurements

Sap flow measurements were performed in each of the respective orchards described in Chapter 3. E_c was measured in the MB during the 2016/17 and 2017/18 seasons, whilst E_c of the IB orchard was measured during the 2017/18 and 2018/19 seasons. Given that E_c was measured in this study using a sap flow technique, transpiration coefficient (K_t) values, as suggested by Villalobos et al. (2013), were calculated to normalize water loss for environmental conditions and to enable direct comparisons between the MB and IB orchards, seeing that the largest contributing variable would be the difference in canopy size between orchards. K_t was determined as:

$$K_t = \frac{E_c}{ET_o} \qquad \textbf{Equation 6.1}$$

To ensure that a direct comparison of transpiration and K_t could be made between the two orchards, external factors, with specific reference to soil water limitations, needed to be excluded from the trial, especially considering that total rainfall and irrigation was less than total calculated ET_o during the 2017/18 and 2018/19 seasons in both the orchards (Table 6.3). For this reason, regular measurements of pre-dawn leaf water potential (ψ_{pd}) were made for the duration of the trial in both orchards using a Scholander pressure chamber (Model 600, PMS Instrument Company, Albany, USA), to ensure that trees were not water stressed. Pre-dawn water potential measurements in both orchards (Figure 6.1) were well above the accepted stress threshold (-0.5 MPa) for macadamias (Stephenson et al. 2003), and water stress was therefore unlikely in these orchards, and transpiration was therefore unlikely to bet limited by soil water availability.

Table 6.3: Total seasonal reference evapotranspiration (ET_o), rainfall and irrigation of the mature bearing (MB) and intermediate bearing (IB) macadamia orchards. ND – Not determined.

Season	Total ET_o (mm)	Total Rainfall (mm)	MB Total Irrigation (mm)	IB Total Irrigation (mm)	MB Total Irrigation + Rainfall (mm)	IB Total Irrigation + Rainfall (mm)
2016/2017	1230	1170	305	ND	1475	ND
2017/2018	1195	760	190	110	950	870
2018/2019	1130	774	ND	97	ND	871

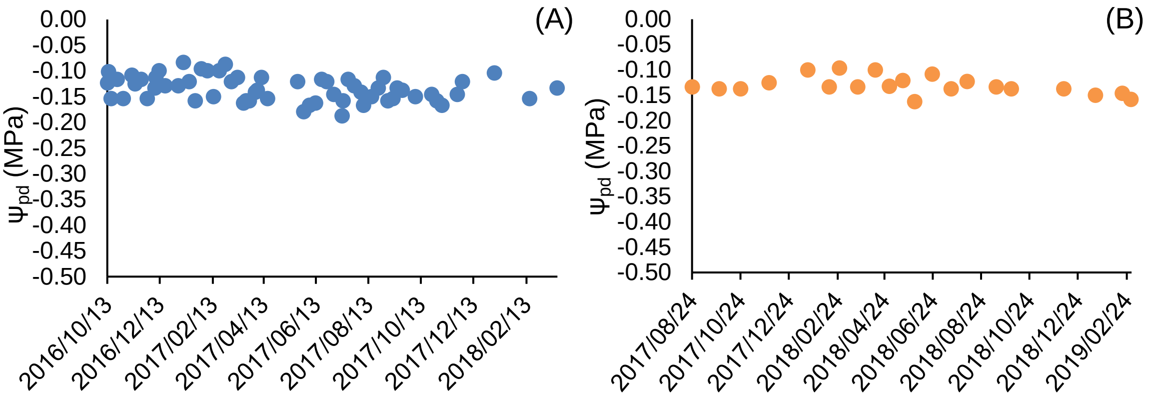

Figure 6.1: Pre-dawn leaf water potential (ψ_{pd}) measured in (A) the mature bearing macadamia orchard (*N*=60) and (B) intermediate bearing orchard (*N*=21) throughout the trial. Stress is believed to occur at pre-dawn leaf water potentials exceeding -0.5 MPa based on research from Stephenson (2003).

In order to determine whether E_c of macadamias is indeed supply controlled it was hypothesized that E_c would have an upper limit or maximum E_c ($E_{c\,max}$) under conditions of high evaporative demand. The $E_{c\,max}$ in each of the respective orchards, was calculated as the absolute maximum E_c measured within predetermined ranges of environmental variables as outlined in Table 6.4. Data from all measurement dates were grouped into five T_{air} categories spanning 5°C, five categories of VPD_{air} spanning 0.5 kPa, six categories of R_s spanning 5.0 MJ m^{-2} day^{-1} and twelve categories of ET_o spanning 0.5 mm day^{-1}. Similarly, absolute minimum E_c was determined in each of these groups to determine the lowest possible daily transpiration rate under a set of environmental variables. The grouping of variables into categories was done to change the data set from continuous to discrete, which allows for convenient means of summarizing or analysing such data.

Table 6.4: Standard deviation and number of measurements (*N*) of daily transpiration in mature bearing (MB) and immature bearing (IB) macadamia orchards for each of the environmental grouping variables including daytime (6:00 – 18:00) vapour pressure deficit ($\mathrm{VPD_{air}}$), air temperature ($\mathrm{T_{air}}$), solar radiation ($\mathrm{R_s}$), and reference evapotranspiration ($\mathrm{ET_o}$) used in the determination of the maximum transpiration rate ($E_{\mathrm{c\ max}}$).

		MB		IB	
	Range	*N*	Standard Deviation (mm $\mathrm{day^{-1}}$)	*N*	Standard Deviation (mm $\mathrm{day^{-1}}$)
$\mathrm{VPD_{air}}$ (kPa)	0.0-0.5	118	0.23	118	0.15
	0.5-1.0	281	0.22	261	0.15
	1.0-1.5	196	0.23	165	0.17
	1.5-2.0	89	0.20	77	0.15
	2.0-2.5	25	0.13	34	0.18
$\mathrm{T_{air}}$ (°C)	5-10	5	0.05	5	0.05
	10-15	148	0.15	115	0.11
	15-20	293	0.22	274	0.15
	20-25	265	0.18	269	0.14
	25-30	6	0.12	14	0.14
$\mathrm{R_s}$ (MJ $\mathrm{m^{-2}\ day^{-1}}$)	0-5	50	0.18	55	0.13
	5-10	87	0.18	87	0.13
	10-15	162	0.21	244	0.13
	15-20	188	0.16	164	0.14
	20-25	95	0.17	91	0.15
	25-30	8	0.11	5	0.08
$\mathrm{ET_o}$ (mm $\mathrm{day^{-1}}$)	0.0-0.5	25	0.24	25	0.14
	0.5-1.0	71	0.21	47	0.17
	1.0-1.5	83	0.14	73	0.13
	1.5-2.0	92	0.19	83	0.12
	2.0-2.5	91	0.17	91	0.13
	3.0-3.5	67	0.23	82	0.13
	3.5-4.0	92	0.19	95	0.14
	4.0-4.5	68	0.16	65	0.15
	4.5-5.0	59	0.14	49	0.14
	5.0-5.5	38	0.15	37	0.16
	5.5-6.0	24	0.13	22	0.14
	6.0-6.5	8	0.09	6	0.12

6.4 Results

6.4.1 Seasonal weather variables

Average temperature was fairly similar over the three production seasons with the mean temperature being 18.4, 18.0 and 19.1 °C during the 2016/17, 2017/18 and 2018/19 seasons respectively. During all three seasons of measurement, the highest average temperatures were recorded from December to March and were approximately 3.0 – 4.0 °C higher than the respective mean annual temperature. Mean daily solar radiation was slightly higher during the 2018/19 season (17.1 MJ m^{-2} day^{-1}) compared to the 2017/18 (16.6 MJ m^{-2} day^{-1}) and 2016/17 (16.1 MJ m^{-2} day^{-1}) seasons. Highest daily solar radiation coincided with the highest mean daily temperatures, occurring from December to March in both seasons (Figure 6.2). Mean annual rainfall was also significantly higher during the 2016/17 season (1170 mm), compared to the 2017/18 (760 mm) and 2018/19 (774 mm) seasons. Mean annual rainfall during both the 2017/18 and 2018/19 seasons were slightly lower than the long term mean annual rainfall for the Nelspruit region of 854 mm (Schulze 1997).

Average air vapour pressure deficit (VPD_{air}) was similar during both the 2017/18 and 2018/19 (1.0 kPa) seasons, compared to the 2016/17 season (1.1 kPa) (Figure 6.2). Highest monthly VPD values were observed from August to October in all three seasons. Total reference evapotranspiration (ET_o) was negligibly higher during the 2016/17 season (1196 mm), compared to both the 2017/18 (1189 mm) and 2018/19 (1195 mm) seasons, with average daily ET_o being higher during the 2016/17 season (3.32 mm day^{-1}), compared to the 2017/18 (3.25 mm day^{-1}) and 2018/19 (3.30 mm day^{-1}) seasons. The highest average daily ET_o was observed during the September and October periods of all three seasons, with average daily ET_o during this period being 3.9 mm day^{-1}. Maximum daily ET_o coincided with the highest average daily ET_o during the aforementioned period, with values greater than 6.0 mm day^{-1} being reached on selected occasions in both September and October of each season.

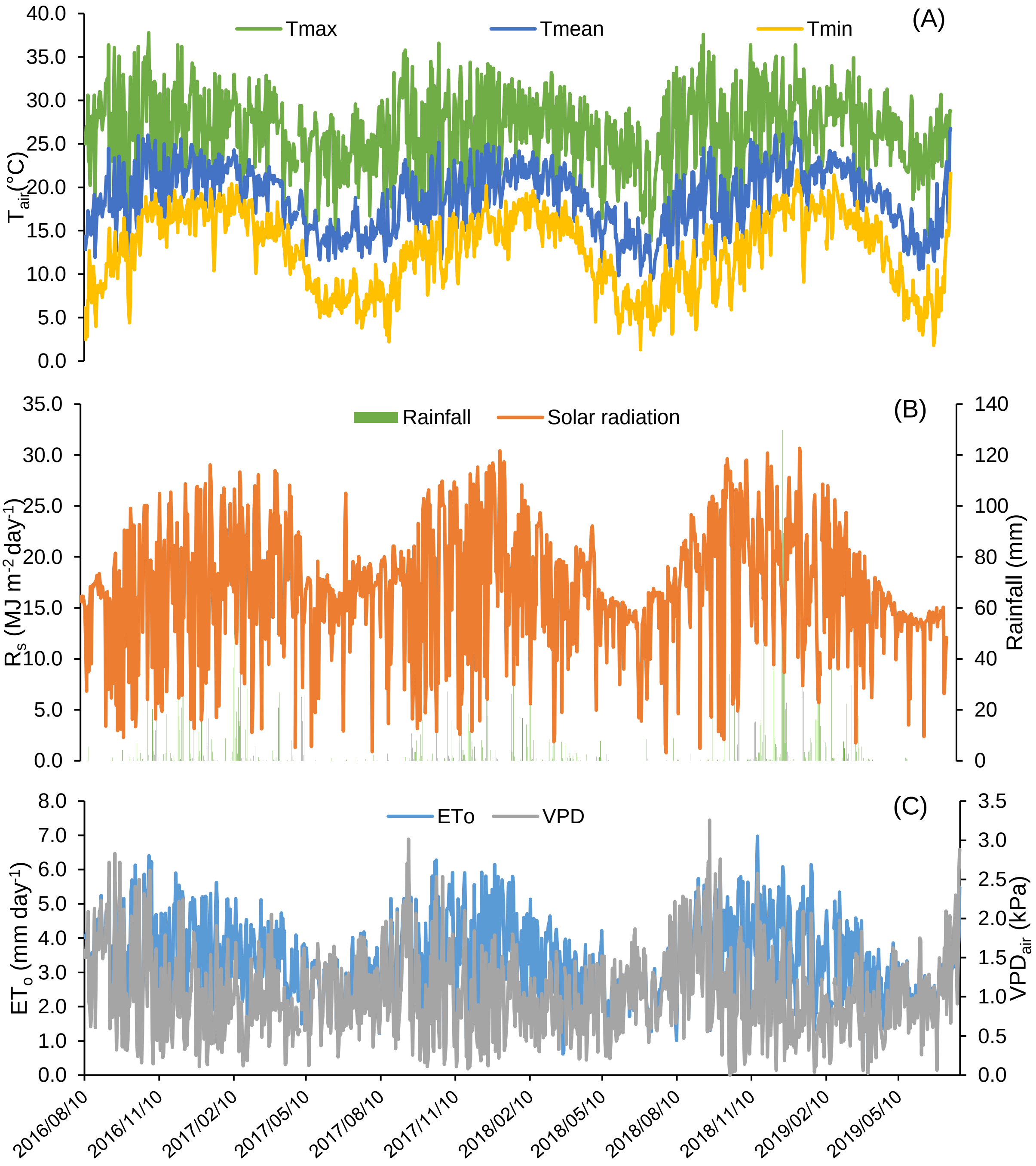

Figure 6.2: (A) Maximum, mean and minimum, daily air temperature (T_{air}), (B) total daily rainfall and solar radiation (R_s) and (C) reference evapotranspiration (ET_o) and air vapour pressure deficit (VPD_{air}) obtained from the automated weather station located close to the mature bearing macadamia orchard throughout three seasons (5 August 2016 to 5 August 2019).

6.4.2 Canopy Measurements

Trees in the MB orchard had a slightly lower volume of 71 m^3 in the 2016/17 season compared to 77 m^3 in the 2017/18 season, whilst average seasonal canopy volume in the IB remained relatively constant (39 m^3) throughout the 2017/18 and 2018/19 seasons (Figure 6.3). Trees in the IB orchards were on average approximately half the volume of trees in the MB orchard. In both orchards, trees reached a maximum volume during the late summer period and typically decreased in autumn/winter periods following pruning, as outlined in Table 6.2.

Average LAI in the MB orchard was slightly higher during the 2016/17 season (5.1 m^2 m^{-2}) compared to the 2017/18 season (4.8 m^2 m^{-2}), and was most likely due to the substantial reduction in leaf area brought about by pruning, which removed two large limbs out of each experimental tree, in an attempt to reduce tree height and increase radiation interception. Trees in the IB orchard showed an increase in LAI from 1.8 m^2 m^{-2} during the 2017/18 season to 2.3 m^2 m^{-2} during the 2018/19 season. The increase in LAI in the IB orchard was due to the limited amount of pruning of these orchards during the 2017/18 season, with only vertical, non-branching shoots being removed, which was in accordance with industry practice. A reduction in LAI is, however, seen following a heavier pruning regime in the 2018/19 season (Figure 6.3 D). Nevertheless, on an average LAI basis, trees in the IB orchard were approximately 60% smaller than those in the MB orchard during the 2017/18 season, during which measurements of transpiration were made in both orchards.

Even though seasonal variation of FI was observed in both orchards, FI remained between 0.55 and 0.80 for the MB orchard and between 0.20 and 0.35 for the IB orchard. A reduction in canopy volume and LAI following pruning in the IB orchard during the 2018/19 season, resulted in a slight, but negligible decrease in FI which is attributed to the dense nature of macadamia canopies.

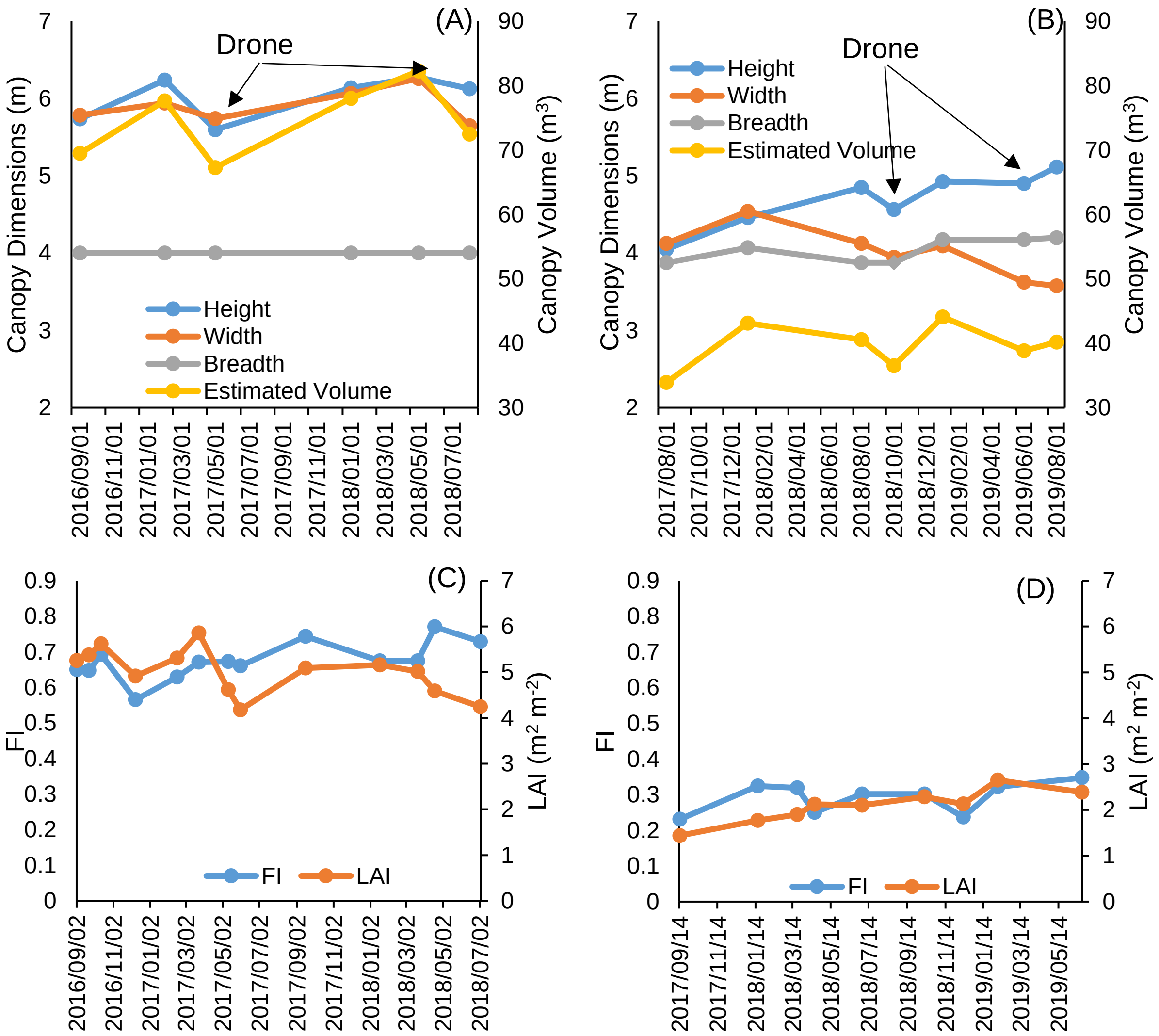

Figure 6.3: Average seasonal variation in canopy dimensions of the same four trees used in transpiration measurements in the (A) mature bearing and (B) immature bearing macadamia orchards. Seasonal average measurements of fractional interception of PAR (FI) and leaf area index (LAI) of the same four trees used in transpiration measurements in the (C) mature bearing and (D) immature bearing macadamia orchards. Measurements of canopy dimensions made using drone technology is indicated on both graphs by arrows.

6.4.3 Transpiration rates of macadamia orchards

Transpiration measurements for the MB macadamia orchard commenced on 2016/08/10 and were concluded on 2018/08/08. During the measurement campaign total transpiration ranged from 316 – 340 mm per annum, being higher during the 2016/17 season compared to the 2017/18 season (Table 6.5). The lower transpiration rates during the 2017/18 season were most likely attributed to slightly lower ET_o and smaller canopy size of the MB orchard during the same period compared to the

2016/17 season. The lowest and highest daily average transpiration rates in the MB orchard were recorded in June (0.7 mm day^{-1}) and January (1.1 mm day^{-1}) of each season, with daily maximum and minimum values of 1.4 mm day^{-1} and 0.2 mm day^{-1} being recorded over the two seasons of measurement (Figure 6.4).

Measurements of E_c in the IB macadamia orchard commenced on 2017/08/16 and were concluded on 2019/08/05. Total E_c during the 2017/18 season (195 mm) was higher than that measured during the 2018/19 season (167 mm) (Table 6.5). The lowest and highest daily average transpiration rates for the IB orchards were recorded in July (0.3 mm day^{-1}) and January (0.7 mm day^{-1}) of each season. Daily maximum and minimum values of 0.95 mm day^{-1} and 0.06 mm day^{-1} were recorded over the two seasons of measurement (Figure 6.4).

Table 6.5: Average daily transpiration rates (mm day^{-1}) across multiple seasons in the mature bearing (MB) and immature bearing (IB) macadamia orchards.

Orchard	Season	2016/17	2017/18	2018/19
MB	Spring	1.0	0.9	
	Summer	1.2	1.1	
	Autmn	1.1	0.8	
	Winter	0.9	0.7	
	Average	**1.1**	**0.9**	
IB	Spring		0.6	0.4
	Summer		0.7	0.5
	Autmn		0.5	0.4
	Winter		0.4	0.3
	Average		**0.5**	**0.4**

Given that the major difference between the MB and IB orchards is the size of the canopies, it is not surprising that trees in the MB orchard with an estimated canopy volume of 71 m^3, approximately double that of trees in the IB orchard, transpired 121 mm (~60%) more during the 2017/18 season than the IB orchard. Comparisons of E_c measurements between the two differently sized orchards, during the same measurement period, revealed that a strong linear relationship (R^2= 0.82) existed between total daily E_c of the two orchards (Figure 6.4).

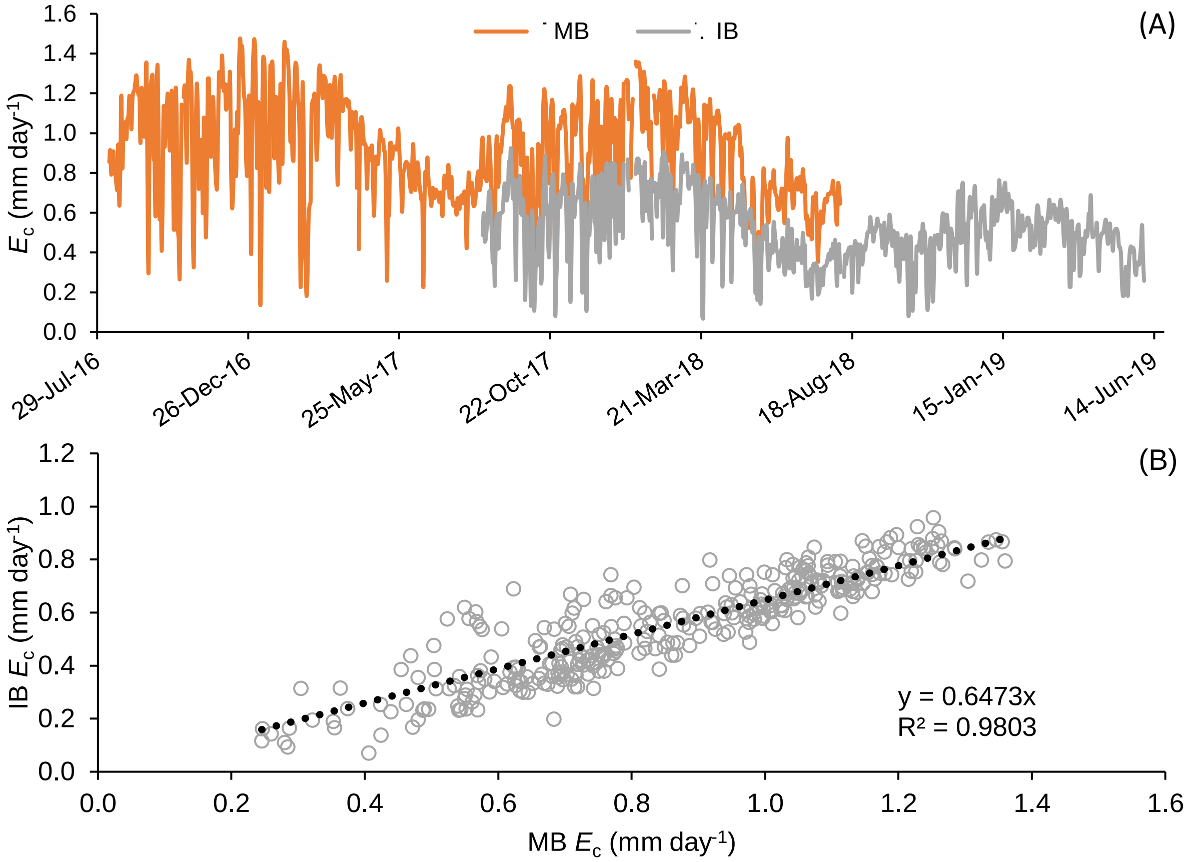

Figure 6.4: Daily transpiration (E_c) for (A) mature bearing (MB) and intermediate bearing (IB) macadamia orchards across three seasons. (B) Linear relationship between daily transpiration of MB and IB orchards from 16 August 2017 to 6 August 2018.

6.4.4 Transpiration response to environmental variables

Results from this study show that macadamia E_c increased in a non-linear fashion with weather variables, including VPD_{air}, ET_o, R_s and T_{air} (Figure 6.5). Given that soil water content was not limiting in this study, the reduction in the rate of E_c in response to increases in these environmental factors is an indication of a supply controlled system, and is evident in both the MB and IB orchards. Although a poor correlation was found for the response of transpiration to VPD_{air} in both orchards, it would seem as if the rate of transpiration decreases as VPD_{air} increases above 0.7 - 0.8 kPa in both orchards. This contributes to the supply controlled system which is most likely driven by strict leaf level stomatal control and subsequently reduced canopy conductance in response to increases in VPD_{air}. The distinct response of E_c to VPD_{air} is also supported by leaf-gas exchange measurements made in the MB orchard outlined in Chapter 4. The transpiration rate in both orchards also failed to increase substantially when R_s

exceeded 0.3 MJ m^{-2} h^{-1} (Figure 6.5 B). The response of E_c to ET_o, was similar to the solar radiation response, with E_c in both orchards failing to increase above 0.13 mm h^{-1} in the MB orchard and 0.07 mm h^{-1} in the IB orchard when ET_o increased above 0.05 mm h^{--1} (Figure 6.5 A).

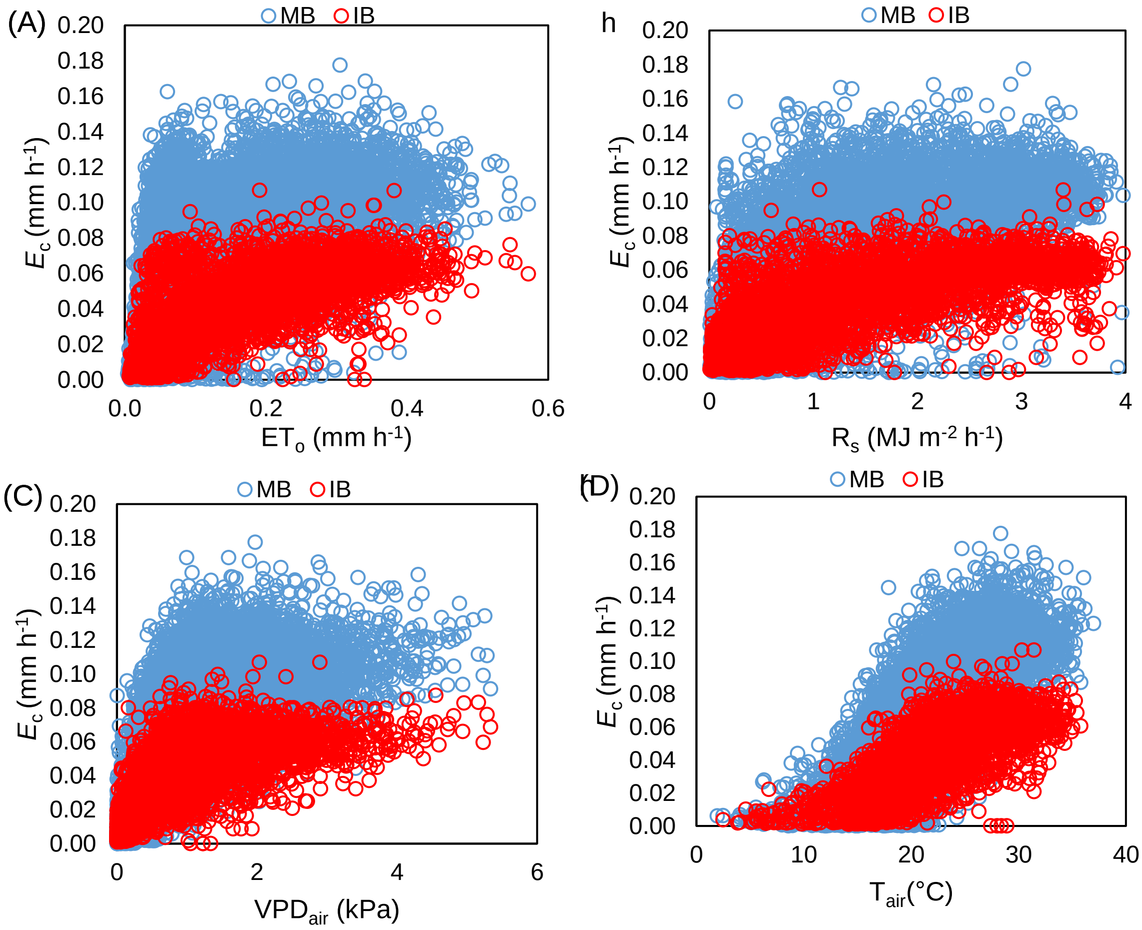

Figure 6.5: Relationship between average daytime hourly transpiration (E_c) and (A) reference evapotranspiration (ET_o), (B) solar radiation (R_s), (C) air vapour pressure deficit (VPD_{air}) and (D) air temperature (T_{air}) for mature bearing (MB) and immature bearing (IB) macadamia trees.

Diurnal trends in transpiration for both orchards on days with contrasting environmental variables, show that there seems to be an upper limit to E_c, with VPD_{air} and T_{air} appearing to be the major controlling variables of macadamia E_c (Figure 6.6). Average diurnal R_s on 2017/09/11 was fairly similar to that measured on 2018/03/01, whilst both T_{air} and VPD_{air} were higher during the former as compared to the latter day, yet there were no marked differences between the trends in diurnal E_c rates on these two days. The higher VPD_{air} on 2017/09/11 was largely attributed to higher T_{air} and lower relative humidity compared to measurements made on 2018/03/01. Nevertheless, comparing both these days to conditions on 2017/10/01, where VPD_{air} was approximately half of that measured on 2018/03/01, E_c in both the intermediate and mature bearing orchards, was considerably lower than that of the days with higher VPD_{air}.

A maximum rate of E_c is once again evident when examining the trends in diurnal E_c of macadamias during days with moderate and high VPD_{air} and ET_o (Figure 6.6). Measurements of E_c made on 2017/09/11 and 2018/03/01, days with distinctly different VPDs, revealed that E_c increased linearly as VPD increased until approximately 10:00 h, where after E_c remained fairly constant regardless of increases in VPD until late afternoon (15:00 – 16:00). E_c then tended to decline in the late afternoon as VPD decreased, but this decrease in E_c was most likely due to the decrease in incident solar radiation. The decrease in E_c of the IB orchard typically occurred later in the afternoon compared to the MB orchard, due to the fact that trees in the IB orchard had not formed a complete hedgerow and distribution of R_s throughout the canopy in these orchards was superior to that of the MB orchard, which had formed a complete hedgerow.

Furthermore, E_c responses to VPD_{air}, ET_o, R_s, and T_{air} demonstrated that maximum E_c ($E_{c\ max}$) in both MB and IB orchards increased and decreased at varying rates in response to increases in the respective environmental parameters (Figure 6.7 & Figure 6.8). In both orchards, $E_{c\ max}$ was highest within the 1.0 – 1.5 kPa VPD_{air} range, reaching transpiration rates of 1.5 mm day^{-1} and 0.9 mm day^{-1} in the MB (Figure 6.6) and IB orchard (Figure 6.7) respectively. VPD_{air} levels greater than 1.5 kPa resulted in a slightly decreased $E_{c\ max}$ in both orchards. The response of $E_{c\ max}$ to increases in R_s was also fairly similar between the two orchards, with the highest rates of $E_{c\ max}$ being observed once R_s exceeded 15 MJ m^{-2} day^{-1}. $E_{c\ max}$ of MB orchards declined slightly as R_s exceeded 25 MJ m^{-2} day^{-1}, which is most likely due to the associated increases in T_{air}, decreases in relative humidity and subsequent increases in VPD_{air}. This is substantiated by the decrease of $E_{c\ max}$ at T_{air} exceeding 25°C (Figure 6.7 & Figure 6.8). $E_{c\ max}$ of the IB orchard, however, showed no decrease in response to R_s exceeding 25 MJ m^{-2} day^{-1}, but the response of $E_{c\ max}$ to T_{air} exceeding 25°C was similar between the two orchards.

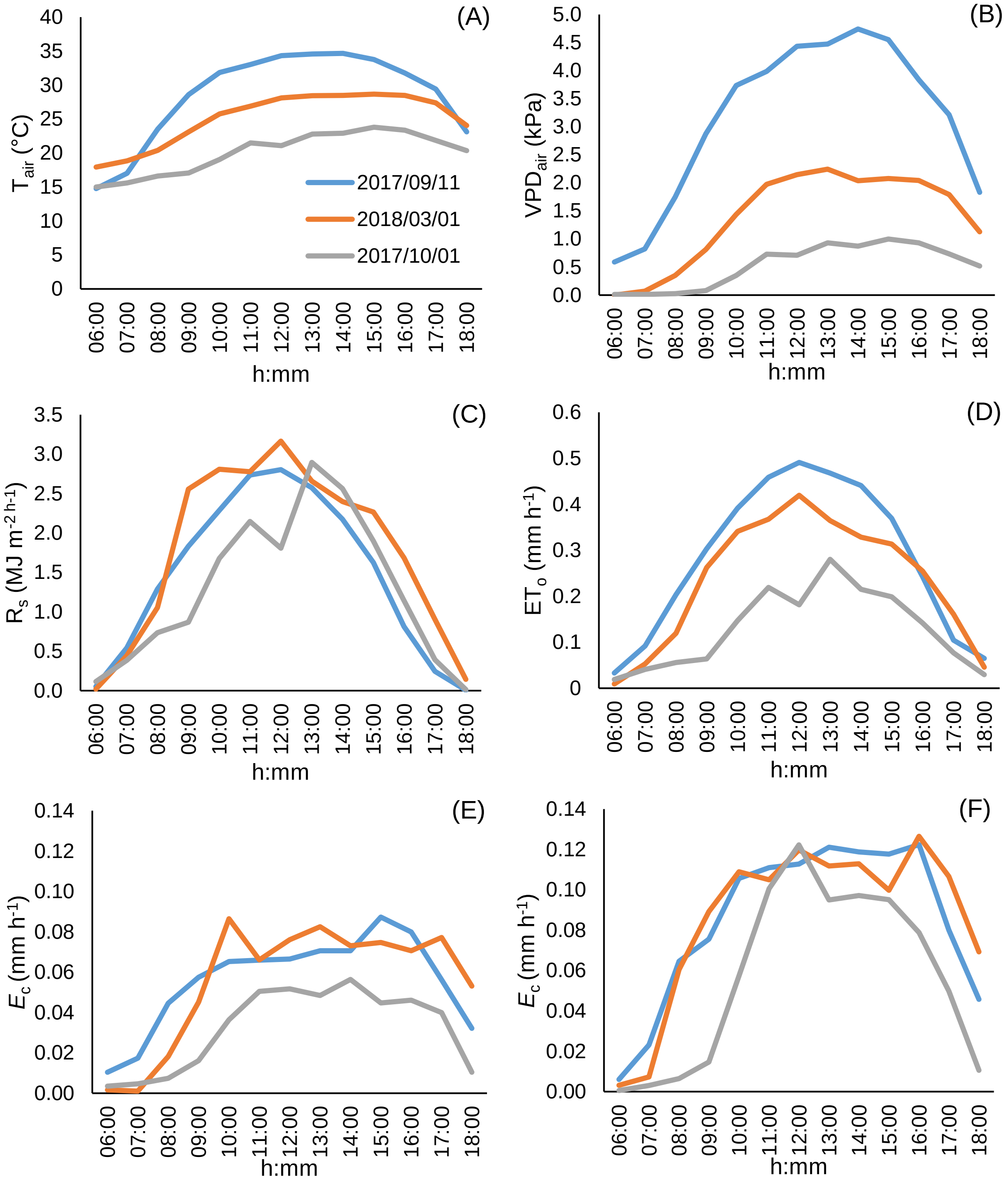

Figure 6.6: Diurnal measurements of (A) air temperature (T_{air}), (B) vapour pressure deficit (VPD_{air}), (C) solar radiation (R_s), (D) reference evapotranspiration (ET_o), (E) intermediate bearing macadamia transpiration (E_c) and (F) mature bearing macadamia transpiration (E_c) on three climatically distinct days 11 September 2017, 1 October 2017, and 1 March 2018.

Another striking similarity between the two differently sized orchards is the response of $E_{c\ max}$ to increases in ET_o. The $E_{c\ max}$ achieved under ET_o rates exceeding 3.5 mm day^{-1} was substantially different between the MB and IB orchard, with the MB orchard reaching average $E_{c\ max}$ rates of 1.4 mm day^{-1} compared to the IB orchard which reached average $E_{c\ max}$ rates of 0.9 mm day^{-1} at these ET_o rates. $E_{c\ max}$ rates under conditions where ET_o rates exceeded 3.5 mm day^{-1} was therefore approximately 60% higher in the MB orchard, which were ~60% larger, on a LAI basis, than trees in the IB orchard.

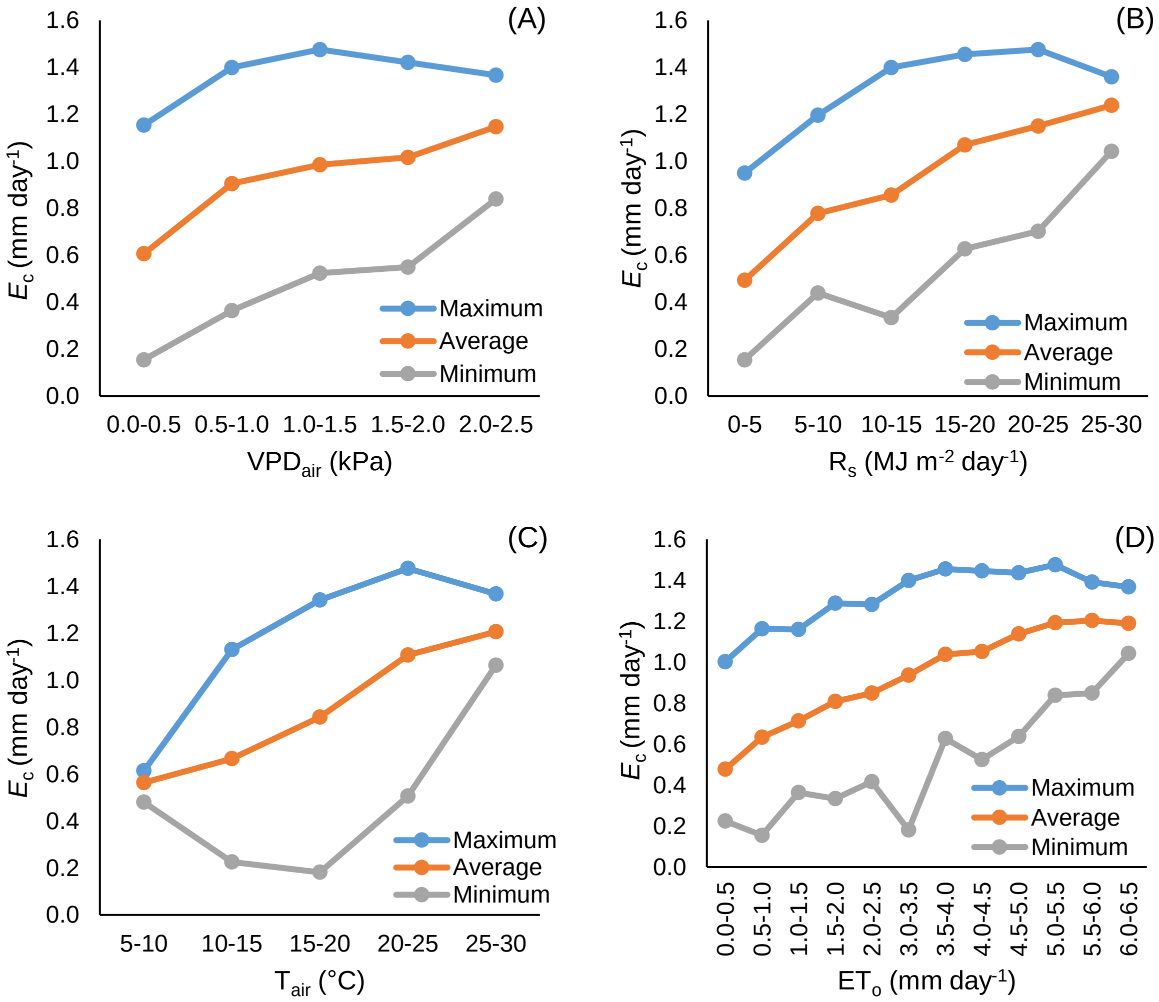

Figure 6.7: Average, maximum and minimum transpiration (E_c) of mature bearing macadamias in response to (A) vapour pressure deficit (VPD_{air}), (B) solar radiation (R_s), (C) air temperature (T_{air}), and (D) reference evapotranspiration (ET_o) across two cropping seasons stretching from 10 August 2016 to 6 August 2018.

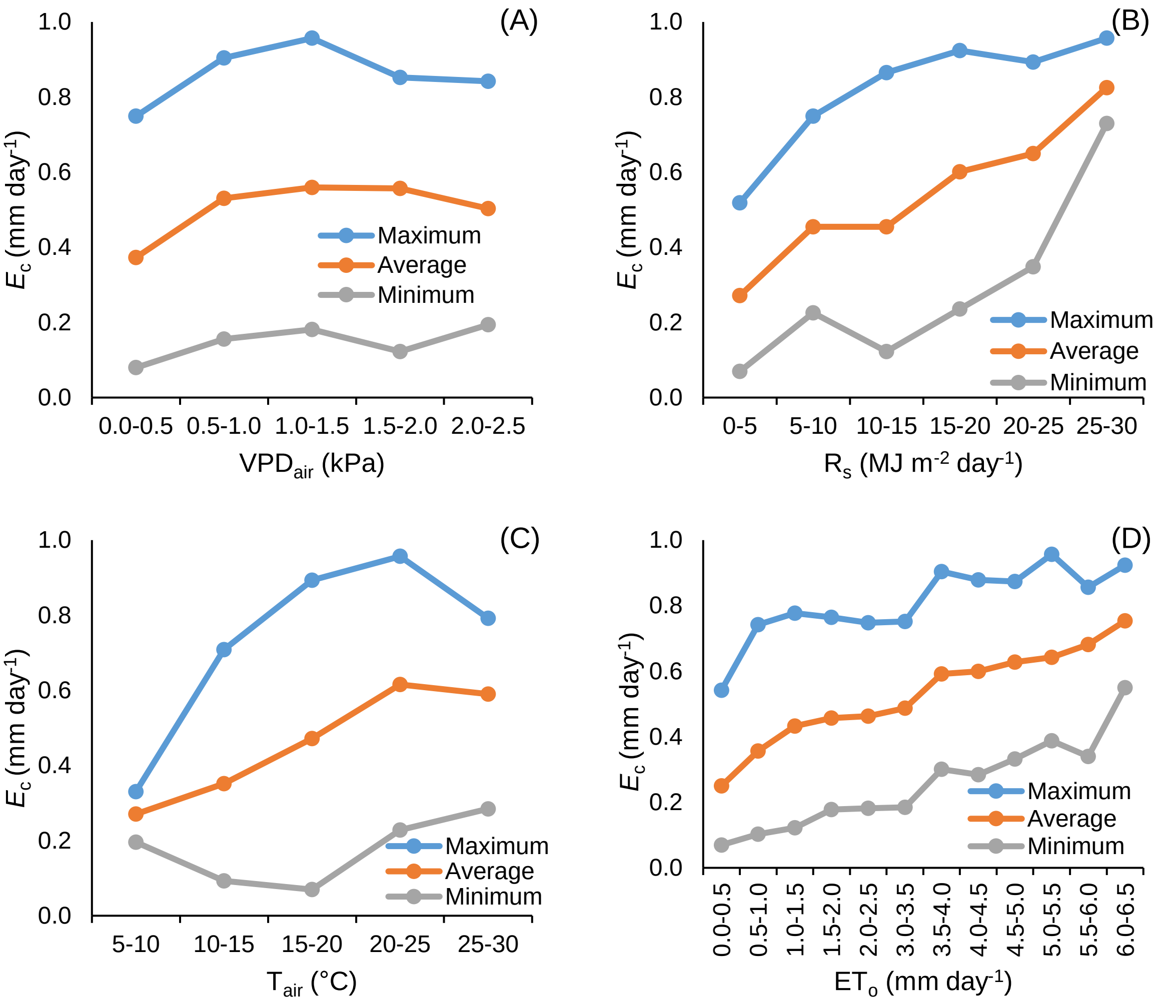

Figure 6.8: Average, maximum and minimum transpiration (E_c) of immature bearing macadamias in response to (A) vapour pressure deficit (VPD$_{air}$), (B) solar radiation (R$_s$), (C) air temperature (T$_{air}$), and (D) reference evapotranspiration (ET$_o$) across two cropping seasons stretching from 16 August 2017 to 5 August 2019.

6.4.5 Transpiration during different phenological stages

Although prevailing weather conditions remain the major factor controlling E_c, results from Chapter 5 have suggested that E_c varies during different phenological periods, especially during periods of high and low sink strength. Throughout the study period, E_c in both the MB and IB orchards, increased from a minimum at flower initiation to a maximum at flowering (Table 6.6). Daily E_c then decreased slightly during the nut set period, whereafter E_c rates continually increased in both orchards to the seasonal maxima throughout the nut growth to the summer flush period.

During the summer flush period, trees transpired on average 1.1 mm day^{-1} for the MB orchard and 0.6 mm day^{-1} for the IB orchard. These rates were approximately 0.2 mm day^{-1} higher than the average spring E_c of 0.9 mm day^{-1} for the MB orchard and 0.1 mm day^{-1} higher than the average seasonal E_c of 0.5 mm day^{-1} in the IB orchard. The increase during the summer vegetative flush period could be attributed to the increase in ET_o and canopy size, but could also be partially attributed to the combined sink strength of developing fruit and vegetative growth, as outlined in Chapter 5. There was, however, no difference in daily ET_o during spring and the summer vegetative flush period. Although increases in canopy size of each orchard cannot be eliminated as a factor causing increases in E_c during the summer flush period, transpiration rates during both the flowering and nut set period (Table 6.6) were not significantly lower than E_c rates measured during the spring and summer vegetative flush periods, although average LAI was greater during the flush periods (Figure 6.9).

Table 6.6: Average (± standard deviation) daily transpiration rates for the mature bearing (MB) and immature bearing (IB) macadamia orchards during different phenological and management periods across consecutive cropping seasons, as outlined in Tables 6.1 – 6.2. Means followed by the same letter are not significantly different (*p* = 0.05) as analysed using one-way repeated measures ANOVA, in each of the orchards.

Phenological Stage	Transpiration MB (mm day^{-1})	Transpiration IB (mm day^{-1})
Pre-flowering	0.75 ± 0.24 ^{a}	0.37 ± 0.15 ^{a}
Flowering	1.01 ± 0.23 ^{b}	0.51 ± 0.12 ^{b}
Nut Set	0.87 ± 0.27 ^{abc}	0.45 ± 0.15 ^{abc}
Spring Flush	0.91 ± 0.28 ^{bc}	0.46 ± 0.17 ^{abc}
Premature Nut Drop	0.95 ± 0.25 ^{bc}	0.55 ± 0.15 ^{b}
Nut Growth	1.03 ± 0.15 ^{b}	0.62 ± 0.10 ^{b}
Summer Flush	1.12 ± 0.24 ^{b}	0.63 ± 0.16 ^{b}
Oil Accumulation	0.99 ± 0.12 ^{b}	0.57 ± 0.15 ^{b}
Harvest	0.74 ± 0.30 ^{ac}	0.50 ± 0.12 ^{bc}
Flower Initiation	0.77 ± 0.12 ^{ac}	0.38 ± 0.10 ^{ac}
Pruning & Winter Rest	0.68 ± 0.18 ^{a}	0.33 ± 0.08 ^{a}

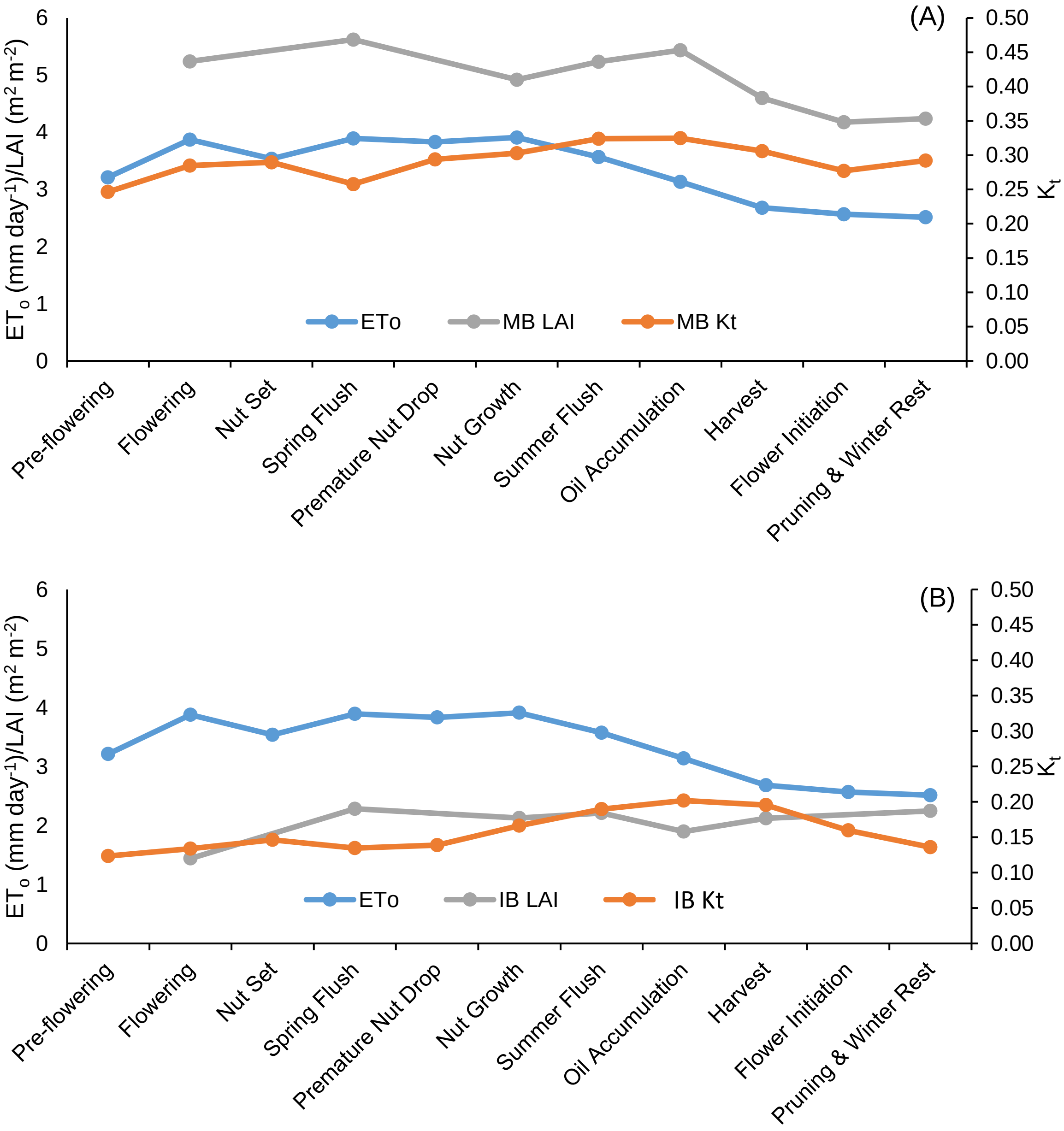

Figure 6.9: Average daily reference evapotranspiration (ET$_o$), leaf area index (LAI), and transpiration crop coefficients (K$_t$) for (A) mature bearing (MB) and (B) immature bearing (IB) macadamia orchards measured during different phenological periods.

The results from this study compliments anecdotal evidence from growers, who have observed an increase in water use during flowering (Table 6.6). It is commonly believed that this increase in E_c is as a result of transpiration by the flowers themselves. Although this cannot be ruled out at the moment, it is more likely that the increase in E_c at this time is a response to increased R_s, VPD_{air}, and ET$_o$ (Figure 6.1) during the onset of spring. What is, however, surprising, is that during the flowering period, ET$_o$

was similar to that measured during the spring flush period (Table 6.6), whilst LAI of both MB and IB orchards were approximately 10% less during the flowering period, yet average K_t during the flowering period was 10% higher in the MB orchard and remained constant in the IB orchard compared to that of the spring flush period. This observation would suggest that given the fact that average ET_o was similar between the two aforementioned periods and that increases in canopy size did not occur, increased K_t during flowering could possibly be attributed to direct water loss from flowers, which ultimately increased measured E_c.

During the harvesting to pre-flowering periods, average daily E_c rates were approximately 0.2 – 0.4 mm day^{-1} lower compared to average spring and summer E_c rates (Table 6.5) in both MB and IB orchards. This decrease in E_c from the summer flush period to the harvesting and post-harvest period occurred in conjunction with a decrease in both canopy size and seasonal decreases in ET_o (Figure 6.9). During the autumn and winter period, ET_o was on average 2.2 mm day^{-1} and was approximately 1.5 mm day^{-1} less than the average ET_o during the summer period (Figure 6.10). The weather variable leading to the greatest decrease in ET_o is the reduction in total daily R_s (Figure 6.10), which was approximately 30% lower compared to the seasonal average of 16.5 MJ m^{-2} day^{-1}. Reduced total daily R_s was as a result of shorter day lengths during winter and autumn, which subsequently lead to reduced total daily E_c.

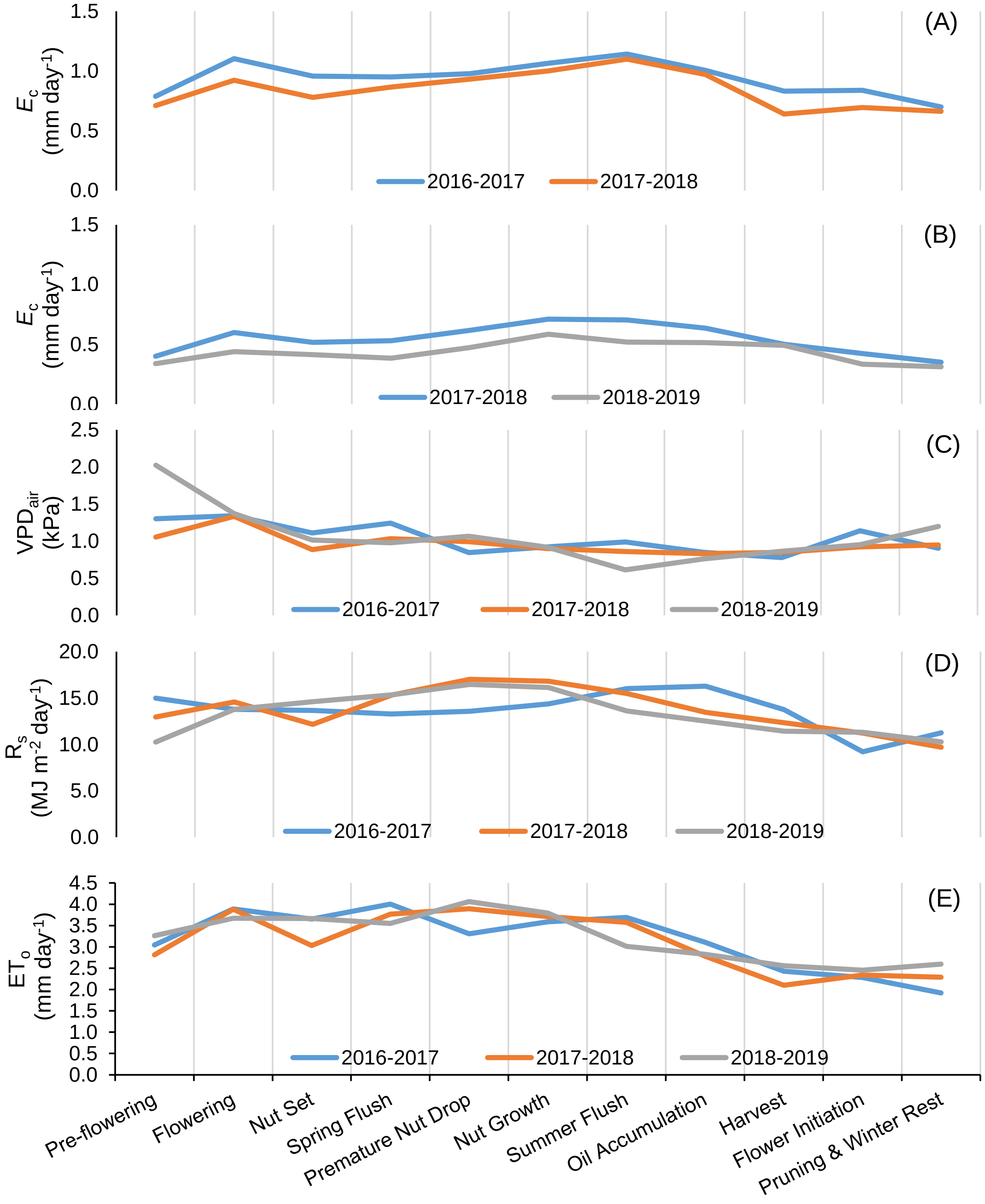

Figure 6.10: Daily averages of transpiration (E_c) in the (A) mature bearing (MB) and (B) immature bearing (IB) macadamia orchards and accompanying daily average (C) air vapour pressure deficit (VPD$_{air}$), (D) solar radiation (R$_s$), and (E) reference evapotranspiration (ET$_o$) during each phenological stage across three consecutive cropping seasons.

6.4.6 Transpiration crop coefficients

Average transpiration crop coefficients (K_t) of the MB macadamia orchard (0.34), determined over two consecutive seasons, was approximately double that of the IB orchard (0.18). The large difference between the K_t values of the two orchards is mostly attributed to the large differences in canopy size, with trees in the IB orchard being approximately 60% smaller than that of trees in MB orchard (Figure 6.3). A positive linear relationship ($R^2 = 0.71$) was also apparent between the daily K_t values of the MB and IB orchards obtained during the 2017/18 season (Figure 6.11). The slope of the linear regression equation can therefore be used as an indication of the fraction of E_c attributed to physical or physiological attributes of the crop. The slope of the relationship, being 0.62, would suggest that E_c of IB orchards is 62% that of the MB orchard due to differences in physical attributes of canopies.

Transpiration crop coefficients from both orchards varied substantially throughout the study period (Figure 6.11). In the MB orchard, K_t reached a maximum in March and April in the 2016/17 and 2017/18 seasons, with an average maximum K_t of 0.46. This was approximately double that of maximum K_t from the IB orchard during April of both the 2017/18 and 2018/19 seasons. In both orchards, K_t increased in a near linear fashion from a low in September to a seasonal maximum in April before again declining to a minimum in late August/beginning September of the following season. Considering that K_t normalizes for environmental conditions, increases in K_t from September to April during each season would suggest that increases in K_t follow increases in canopy size, given the fact that during this period, two vegetative flushes would have occurred. Similarly, the decrease in K_t from April to September would have been as a result of decreases in canopy size as a result of pruning. Although this cannot be ruled out in both these orchards, it should be noted that pruning only commenced in June of all of the seasons, yet substantial decreases in K_t were already evident from the end of April (Figure 6.11). These observed decreases in K_t also indicates that increases in E_c is not as rapid as the increases in ET_o, further adding to the fact that E_c in macadamias is more supply controlled than demand limited under conditions of high ET_o. Supply controlled E_c implies that the rate of water supply to the leaves is substantially lower than the atmospheric evaporative demand.

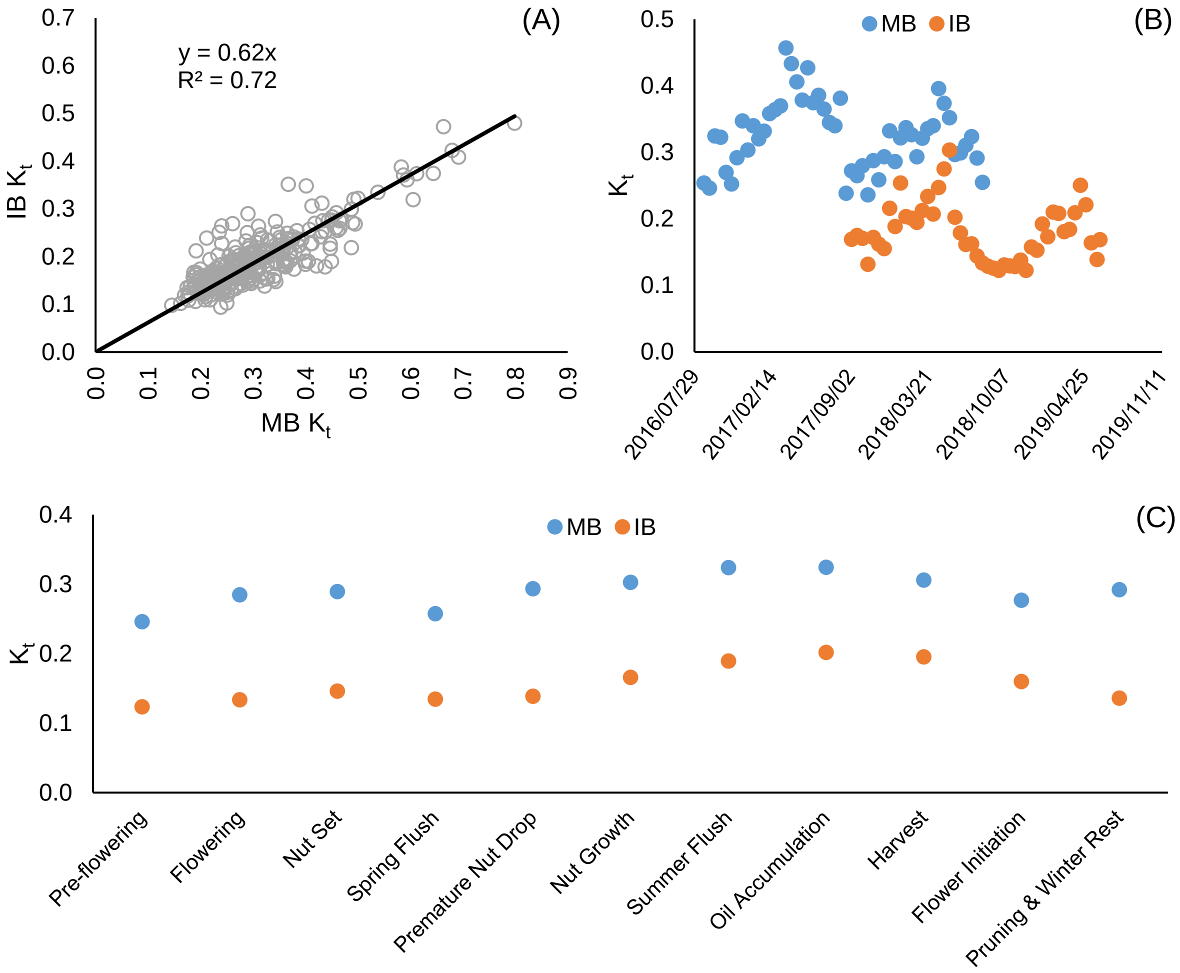

Figure 6.11: (A) Linear relationship between daily transpiration coefficients (K_t) of mature bearing (MB) and immature bearing (IB) macadamias determined during the 2017/2018 season. (B) Fortnightly averaged transpiration coefficients (K_t) for mature bearing (MB) and immature bearing (IB) macadamia orchards measured throughout the duration of the trial. (C) Average transpiration crop coefficients (K_t) for mature bearing (MB) and immature bearing (IB) macadamia orchards determined during the respective phenological periods recorded throughout the duration of the trial.

The increases in K_t from September to April of each season also roughly coincides with increases in nut growth and development, with increases in K_t tending to reach a maximum just before harvest (April), before decreasing again directly after harvest. The increase in K_t observed in both orchards during the fruit development phase (periods post premature nut drop to harvest), occurred without substantial increases in LAI (Figure 6.9) and throughout the continuous decline in ET_o, which would suggest that E_c increased more during these periods relative to increases in ET_o. These increases in E_c could therefore be mediated by increased stomatal and therefore also canopy conductance in the presence of developing fruit. Results from Chapter 5 have demonstrated a rapid decline in gas exchange and subsequently E_c after fruit removal, which could also provide some explanation for the rapid decline in K_t directly after harvest, which typically commenced from 20 April in each of the respective orchards and seasons (Table 6.2). These decreases in K_t occurred before any physical reductions in canopy size were made. Directly following the harvest period (i.e. the flower initiation period), average K_t over two consecutive seasons was 10% less in the MB orchard and 40% less in the IB orchard compared to K_t estimated during the harvest period, yet there was only a small difference in ET_o (3% lower during flower initiation) and LAI (2% lower during flower initiation) compared to the harvest period (Figure 6.9). This sudden reduction in K_t without significant reductions in canopy size could be indicative of physiological control over E_c during different phenological stages.

6.5 Discussion

Transpiration is the most important beneficial component to water loss from crops, and increasing E_c should invariably lead to increased dry matter production. Gaining a clear understanding of the variables driving E_c is therefore critical, not only to the field of water use and irrigation research, but also for new orchard developments where site selection is critical. There have, however, been very few published studies examining driving variables of macadamia E_c, and as a result much uncertainty pertaining to the effective water management of the crop exists. It is also unclear if current water management strategies, derived from anecdotal evidence by macadamia growers, is justified. This study has, therefore, aimed to determine how macadamia E_c is regulated by both environmental conditions and physical attributes (i.e. canopy size) of the trees, whilst also attempting to determine how physiological and phenological factors would impact E_c, to better explain E_c of macadamia orchards.

This study has shown that canopy size is a major determinant of E_c in macadamias, which is consistent with observations in a range of fruit tree crops including apple (Li et al. 2002; Li et al. 2003; Auzmendi et al. 2011), citrus (Villalobos et al. 2009; Marin and Angelocci 2011; Villalobos et al. 2013) and olive (Orgaz et al. 2006; Orgaz et al. 2007; Paço et al. 2014). Trees within the MB orchard, which had a LAI 60% higher than trees in the IB orchard, transpired approximately 60% more than the IB macadamia orchard under the same set of environmental conditions. Although variations in canopy size would have a profound impact on E_c, the average daily E_c rates for macadamias in this study was fairly low compared to previously published macadamia studies, with Gush and Taylor (2014) reporting average seasonal E_c rates of 1.2 mm day^{-1} in MB macadamia trees with a seasonal average LAI of ~ 5.0 m^{2} m^{-2}. Average daily E_c rates obtained from this study showed that the MB orchard (average LAI of ~ 5.0 m^{2} m^{-2}) transpired approximately 0.9 mm day^{-1}, whilst the smaller IB orchard transpired 0.5 mm day^{-1} (average LAI of ~ 2.0 m^{2} m^{-2}). The transpiration rates for macadamias measured in this study, were also significantly lower than those measured in mature citrus trees (~4.0 mm day^{-1}) (Rana et al. 2005) using similar sapflow measurements. This is rather surprising considering that the average LAI in the study by Rana et al. (2005) was 2.1 m^{2} m^{-2} which was less than half of that reported for the MB orchard in this study, yet E_c in citrus was more than four times greater than that of macadamia. Similarly, Ramos and Santos (2009) reported that E_c for olive with a LAI of 1.1 m^{2} m^{-2}, which was almost five times lower than that of MB macadamia trees in this study, was approximately 2.5 mm day^{-1} which is significantly higher than that reported for macadamias in this study. These values of E_c reported for both citrus and olive, are also substantially higher than that reported by Gush and Taylor (2014), which in combination with the results in this study could suggest that macadamias are rather conservative water users, a trait which could stem from strict stomatal control in this predominantly isohydric crop.

Comparisons between E_c measured in the MB and IB orchards, exposed to the same set of environmental conditions, have shown that E_c can be scaled linearly with canopy size. The strong linear relationship ($R^2 > 0.8$) between E_c measured in the MB and IB orchard would suggest that the slope of the relationship can be used to estimate E_c of orchards, with varying canopy size, that are exposed to the same set of environmental conditions. The slope, which is a unitless factor of 0.65, clearly indicates that E_c of trees in the IB orchard, which are half (on a canopy volume basis) that of the MB trees, would transpire 60% less than MB trees. Although the robustness of scaling E_c based only on canopy size is questionable, it is believed that given the similar response of E_c to environmental factors between the MB and IB orchards, this approach could prove to be fairly robust. This approach could therefore be used by growers who wish to extrapolate measurements of E_c, under the same set of environmental conditions, from one orchard to other orchards of varying canopy size.

One of the distinct responses in macadamia E_c is the non-linear fashion with which E_c increases in response to T_{air}, R_s, VPD_{air}, and ET_o. This reduction in the rate of E_c, under conditions of non-limiting soil water conditions, especially in response to VPD_{air} and ET_o, would suggest that macadamia E_c is a supply controlled system implying that the rate of water supply to the leaves is substantially lower than the atmospheric evaporative demand. Ibraimo (2018), also demonstrated a non-linear increase in macadamia E_c to increasing VPD_{air}, ET_o and similar responses of E_c to both R_s and T_{air}. Nevertheless, the similarity between the response of transpiration to increases in VPD_{air} and ET_o, imply that macadamia transpiration is most likely regulated by strict stomatal control in response to increases in VPD_{air}, which would also suggest canopy conductance is fairly low. Low stomatal and canopy conductance in response to increasing evaporative demand most likely stems from the hydraulic limitations within the conducting tissues of macadamias, which has been discussed in Chapter 4. This non-linear response of E_c to VPD_{air} has also been shown in subtropical crops such as citrus (Oguntunde et al. 2007). In citrus this response is believed to stem from a water supply limitation from the roots to the stems and leaves of citrus, as a result of low hydraulic conductance in citrus roots (Cohen et al. 1983; Zekri and Parsons 1989; Rieger 1995; Rodríguez-Gamir et al. 2010). Results, as presented in Chapter 4, in combination with the results from Lloyd et al. (1991) which reported high hydraulic conductance of macadamia trees relative to most other fruit trees, would suggest that the observed supply limitation, especially at high rates of atmospheric evaporative demand, is not be attributed to hydraulic limitations within the root to stem interface, but would most likely be attributed to hydraulic limitations within the stem to leaf interface as discussed at length in Chapter 4.

These hydraulic limitations typically lead to strict stomatal control under periods of high evaporative demand (Prado and Maurel 2013; Martínez-Vilalta and Garcia-Forner 2017), and would imply that macadamias have an upper limit to E_c. This study has confirmed that this is indeed the case in macadamias, with maximum daily E_c rates (E_c

$_{max}$) in both orchards being reached when ET_o rates exceeded 3.5 mm day^{-1}. Increases in ET_o above 3.5 mm day^{-1} led to no further increase in E_c. The similarity in this response between the two orchards, with contrasting sizes, could suggest that this is either a species or cultivar specific trait, seeing that studies on citrus (Villalobos et al. 2009) and olive (Gucci et al. 2000; Tognetti et al. 2002) have shown within species differences in canopy conductance, and therefore also E_c, grown under the same set of environmental conditions. Although this study has not investigated such potential differences, determining these thresholds for a range of macadamia cultivars could prove to be of great value to the macadamia industry by improving site selection based on environmental suitability of certain cultivars, whilst also providing more insight into the differences in water use and dry matter accumulation between cultivars.

No differences in the response of $E_{c\ max}$ to ET_o were observed in the two differently sized orchards, the magnitude of $E_{c\ max}$, however, differed substantially between the two orchards. It would, therefore, seem that although macadamia E_c is controlled in a manner which complements the hydraulic capabilities of the tree, the increase in canopy volume and subsequently conducting surface leads to increases in E_c. An interesting prospect of these findings is that given the results discussed in Chapter 4, which suggest that the major hydraulic constraint is within the stem to leaf interface, the increase in leaf area would not lead to macadamias exceeding the hydraulic conductivity within the soil to stem interface and it is therefore plausible that macadamia transpiration can be scaled linearly with canopy size. Scaling transpiration linearly with canopy size has been reported in eucalyptus (Hatton et al. 1998; Forrester et al. 2010), but such results have not been published for macadamias.

Nevertheless, the study also attempted to examine seasonal variations in E_c, especially in response to phenological changes to validate anecdotal evidence of increases and decreases in macadamia water use as observed by growers. Increases in water use, as observed by macadamias growers, during the flowering period seems to be relative to water use observed during the pre-flowering period. During the pre-flowering period ET_o was lower compared to the flowering period, which largely explains the 25% increase in daily E_c rates in both MB and IB orchards from the pre-flowering to the flowering period. This 25% increase in daily E_c rates would undoubtedly cause observable increases in water use as reported by growers, which would be exacerbated by higher levels of soil evaporation (not measured in this study), due to increased solar radiation reaching the orchard floor following winter pruning and the lack of substantial increases in canopy size before the onset of spring.

Furthermore, results from this study complement currently recommended industry management practices, where growers reduce irrigation volumes during the harvesting period until the pre-flowering period. During these periods, average daily E_c rates were lower than average seasonal daily E_c rates. This significant decrease in E_c during the post-harvest period is in part due to slight decreases in canopy size as a result of applications of exogenous ethylene (Ethephon) during harvest, which is known to cause leaf drop followed by maintenance pruning directly after harvest. The combination of these two practices resulted in 5-10% reduction in canopy size in the MB and IB orchards. These reductions in canopy size coincided with observed decreases in E_c, but it is highly unlikely that these slight reductions in canopy size alone would be responsible for the observed decreases in $E_{c.}$ A reduction in canopy size, achieved through pruning, aims to increase radiation penetration into the tree canopy and decrease canopy height. This should theoretically lead to increased solar radiation interception by previously shaded leaves, which will subsequently lead to increased leaf gas exchange (Huett 2004) and therefore compensate for some of the loss in canopy size and subsequently have a smaller impact on E_c. It is therefore more likely that the observed decreases in E_c during the post-harvest period is attributed to decreased atmospheric evaporative demand during late autumn and winter. During these periods, ET_o was on average 40% less than that of average ET_o during the summer period which led to a 20-30% decrease in average daily E_c in both orchards over multiple seasons.

Not only do these reduced E_c rates during the post-harvest period complement current management practices, it also provides some more insight into the results of Stephenson (2003), which showed that during the floral initiation period, which roughly corresponds with the harvest and pruning/winter rest period in this study, mild water stress had no significant effect on yield and quality of macadamias. This could be due to the relatively low E_c during this period, which even under conditions of mild water stress and slight stomatal closure, would have been sufficient to sustain whole tree photo-assimilate requirements during this period. In combination with the results from Stephenson (2003), the results from this study would suggest that growers could potentially reduce irrigation by 20-30% from the summer norm during the post-harvest period.

Seasonal variation in E_c is not unusual, the study, however, attempted to establish if increases in E_c were independent of increases in ET_o. Addressing the physiological and phenological aspect of changes in E_c is rather difficult considering the variation in environmental conditions across seasons. Results from both orchards in this study have shown there is a large amount of variation in K_t throughout the season and although the increases and decreases in K_t could be as a result of increases and decreases in canopy size, these responses were not consistent. Transpiration crop coefficients in both orchards remained fairly constant between the pre-flowering to premature nut drop period, before increasing in an approximate linear fashion from premature nut drop to oil accumulation, after which it declined up to the harvest period before increasing slightly towards the pruning and winter rest period (Figure 6.11 C).

Although a decrease in canopy size, brought about by pruning, is expected to decrease E_c and subsequently K_t during the winter rest period, there was no reduction in canopy size within the April to June period of each season, yet K_t declined substantially at the end of April, which coincided with the period following harvest. During the post-harvest period both ET_o and LAI was only slightly lower in both orchards compared to that in the pre-harvest periods, yet average K_t over two consecutive seasons was 10% less in the MB orchard and 40% less in the IB orchard. Given results presented in Chapters 4 and 5, it is believed that these decreases in K_t following harvest were as a result of the removal of developing and maturing fruit. The presence of developing fruit (periods post premature nut drop to harvest) resulted in higher E_c relative to that of ET_o, which seemed to decrease throughout the fruit development period. During the fruit development period significant variations in canopy size, observed as small variations in LAI, were ruled out as a possible confounding factor for the observed variation in K_t in both orchards. Although large variations in canopy size cannot be ruled out, it is clear that variations in macadamia K_t occur without significant changes in canopy size. The changes in K_t across a range of phenological periods could be used to provide growers and irrigators with more insight regarding crop E_c in relation to ET_o. Results also suggest that given these variations, a single crop coefficient approach, could possibly provide reasonable estimate of seasonal E_c, but it is highly unlikely that a crop coefficient approach would provide accurate estimations of E_c for shorter periods in macadamias. Given the observed limit to E_c under conditions of high evaporative demand, which is believed to stem from strict stomatal control, it is believed that a stomatal conductance model, similar to that originally proposed by Jarvis (1976), would be able to provide reasonable estimate of macadamia E_c.

CHAPTER 7: MODELLING MACADAMIA TRANSPIRATION

7.1 Abstract

The macadamia industry consists of a range of orchards, varying in size, row orientation, and most importantly environmental conditions, all of which contribute to distinctly different transpiration (E_c) rates. Fortunately crop water use models have allowed researchers to estimate crop specific E_c for a range of orchards and environments. No such models have, however, been successfully parameterized and validated for macadamias, with the nett result being that any measurements of E_c are very site specific. This study has therefore attempted to parameterize and validate three E_c models, including the widely used FAO-56 dual crop coefficient model, the Jarvis-Steward g_c model in conjunction with the Penman-Monteith equation and E_c model, using measurement of E_c in two macadamia orchards of varying canopy size. The study hypothesized that mechanistic modelling approaches would provide more accurate estimates of E_c in macadamias, especially on a daily basis where high degrees of environmental variation exist, compared to an empirical model based on the premise of demand limited E_c. Furthermore, the study hypothesized that macadamias are highly coupled to the atmosphere, and the high degree of coupling would allow for direct estimation of E_c using a E_c model. The study showed, that a poor estimation of daily transpiration crop coefficients (K_t) and E_c was obtained using the FAO-56 dual crop coefficient model, which was largely attributed to overestimation of K_t and E_c at daily reference evapotranspiration (ET_o) rates exceeding 4.0 mm day^{-1}, and an underestimation of K_t and E_c when ET_o < 2.0 mm day^{-1}. The model, however, provided reasonable estimates of K_t and E_c on a monthly or seasonal basis, with only slight discrepancies being observed between measured and simulated K_t and E_c from January to April in each season, which was attributed to physiological upregulation of E_c in the presence of fruit. The g_c model in conjunction with the Penman-Monteith equation, provided more accurate estimates of daily E_c in both the MB and IB orchards, compared to the empirical FAO-56 dual crop coefficient model, but was particularly sensitive to seasonal changes in leaf area index (LAI), with adjustments of maximum canopy conductance ($g_{c\ max}$) being required to achieve accurate estimates of E_c. Adjustments for variations in LAI, however, failed to provide improved estimates of E_c during the January to April period, reaffirming the phenological and physiological influence of fruit on g_c and E_c during this period. Measurements of macadamia g_c in this study was rather low (0.3 – 0.7 mm s^{-1}) in relation to g_a (37 - 75 mm s^{-1}), suggesting that macadamias will be well coupled to the atmosphere. The high degree of coupling in macadamia implies that changes in g_c would lead to direct changes in E_c, which contributed to the success of the use of a E_c model. This model provided reasonable estimates of daily E_c without multiple adjustments for canopy size, using the effective fraction of ground cover ($f_{c\ \mathrm{eff}}$), being needed within each of the orchards. The E_c model, similar to the other models tested, however, failed to provide reasonable estimates of E_c during the January to April period.

Keywords: Transpiration crop coefficients, Canopy conductance, Coupling, Mechanistic model

7.2 Introduction

The rapid expansion of the world macadamia industry has seen the crop being cultivated in a range of subtropical areas. The majority of these areas are in semi-arid countries, such as South Africa and Australia, which are characterized by highly erratic and unevenly distributed rainfall (Malherbe et al. 2016; Baudoin et al. 2017; Deo et al. 2017). As a result, the demand on fresh water suitable for irrigation has increased substantially, and in some cases fresh water extraction could potentially exceed the capacity of the local water catchment (Van Heerden 2004). To prevent this from happening, local authorities make use of micrometeorological measurements and water use models to not only determine crop water needs, but more importantly to issue water use licences (Van Heerden et al. 2009). The most common modelling approach used, not only by researchers but also by farmers, is the relatively simple FAO-56 crop coefficient (K_c) model (Equation 7.1) (Allen et al. 1998), where crop water use (ET_c) is calculated by multiplying reference evapotranspiration (ET_o) by a crop specific K_c value, as follows

$$ET_c = ET_o \times K_c$$

Equation 7.1

Although the approach is widely adopted and has proven to be a reliable and trustworthy indication of crop water requirements in many crops (Lazzara and Rana 2010; Guerra et al. 2016), its accuracy is dependent on the accuracy and robustness of the K_c values. Various K_c values have been determined, under a range of environmental conditions, for subtropical crops such as citrus (Boman 1994; Petillo and Castel 2007; Snyder and O'Connell 2007; Villalobos et al. 2009) and olive (Villalobos et al. 2000; Orgaz et al. 2006; Cammalleri et al. 2013; Paço et al. 2014), which has added to the reliability and accuracy of this approach in these crops. To date, very few published studies have reported K_c values for macadamias (Gush and Taylor 2015), which largely stems from the lack of ET measurements in macadamia orchards. Nevertheless, Carr (2013) has reported that a K_c value of 0.65 for macadamias has been used in other studies (Hancock and Banda 1991), but the accuracy of this value has not been tested. It is, however, highly unlikely that a single K_c factor would be suitable for all macadamias due to the typical variability of K_c values measured between different locations and within orchards at the same locations, as has been found in a variety of fruit tree crops (Wang et al. 2007; Villalobos et al. 2013; Marsal et al. 2014).

Although the lack of trialled and tested crop coefficients limit the applicability and practicality of this approach in macadamia, it is unclear if this model would provide reasonable estimates of macadamia water use. The simplicity of the model, however, makes it appealing, and the validation of such a model could be of great value to irrigators. The major limitation of this approach, especially to macadamias, is that the model assumes that ET_c is a demand limited process. Responses of macadamia E_c to ET_o have, however, shown that E_c is a supply limited process in macadamia orchards. Given that, on average, E_c accounts for more than 60% of total ET on an ecosystem scale (Schlesinger and Jasechko 2014) and could be even greater in orchards where conditions of full canopy cover are present (Kool et al. 2014), it is highly unlikely that Equation 7.1 would provide reasonable estimates of macadamia ET across a season. Furthermore, the dominance of E_c in relation to total ET would necessitate a modelling approach which separates ET into beneficial water loss (i.e. E_c) and non-beneficial water loss occurring as a result of soil evaporation (E_s). Fortunately, the dual crop coefficient model (Allen et al., 1998) allows for the separation of K_c into a basal crop coefficient (K_{cb}), consisting of mainly E_c and small amount of E_s occurring by diffusion in the surface layer when the soil is dry, and soil evaporation coefficient (K_e). This separation allows for the modelling of E_c separately from E_s, which allows the assessment of the ability of a demand limited model to estimate E_c of a supply controlled crop. Furthermore, the successful parameterization of the dual crop coefficient model to obtain estimates of E_c, albeit on a monthly basis, would provide irrigators and water licencing authorities with a strategic water management tool, which at this stage is still lacking in macadamias.

The dynamic, but strict stomatal control and subsequently supply controlled E_c observed in macadamias would suggest that a more mechanistic modelling approach would provide more accurate estimates of macadamia E_c. Models which are able to predict stomatal conductance (g_s) and subsequently canopy conductance (g_c) would most likely be able to predict E_c of macadamias more accurately than the FAO-56 model. Stomatal conductance models are typically applied on either a leaf or canopy scale, depending on the scope of the study. When trying to obtain accurate estimates of whole tree E_c, models applied at a leaf level typically provide less accurate estimates of whole tree E_c, mainly as a result of variations in solar radiation interception and leaf morphological and physiological changes within a canopy (Wirtz 2000; Niinemets et al. 2015; Wu et al. 2017). Most studies have therefore aimed at modelling g_c in order to obtain more accurate estimate of whole tree E_c. Nonetheless, the well-known Jarvis-Stewart model (Jarvis, 1976) has been used extensively to model g_s and g_c. This response weights maximum stomatal conductance ($g_{s\ max}$) according to environmental variables driving stomatal conductance. In its earliest form (Equation 7.2) the multiplicative model proposed by Jarvis (1976) incorporated solar radiation (S_R), ambient carbon dioxide concentration (C_a), air vapour pressure difference (VPD_{air}), air temperature (T_{air}) and leaf water status (Ψ_{leaf}) as weighting factors for maximum stomatal conductance ($g_{s\ max}$).

$$g_s = g_{s\ max} f(S_R) f(C_a) f(VPD_{air}) f(T_{air}) f(\Psi_{leaf}) \qquad \textbf{Equation 7.2}$$

This rather simplistic model captures the complexity of stomatal behaviour and becomes increasingly complicated once scaled to a canopy or orchard level. Variations of this model, have been used with varying degrees of success to model g_c, by assuming that the tree canopy and subsequently the entire orchard can be viewed as a single "big leaf". Once the model is successfully parameterized, an estimate of g_c can be obtained and could then be used in conjunction with the Penman-Monteith equation (Monteith 1965; Monteith and Unsworth 1990) to obtain estimates of E_c, whilst some studies have also obtained reasonable estimates of ET using this method (Rana et al. 2005; Consoli and Papa 2013). These so-called "big leaf" models (Rana et al., 2005, Oguntunde et al., 2007), have one major assumption being that entire crop fields or orchards are treated as a single surface with uniform characteristics. Most orchards, however, consist of an intricate combination of covered and exposed soil surfaces as well as varying wetting patterns depending on irrigation systems and management practices. Regardless of this shortcoming, these models have been used with great success in a range of crops (Rana et al. 2005; Oguntunde et al., 2007; Consoli and Papa 2013).

The use of g_c in models of E_c can prove to be fairly complex due to the large variation of g_c over a short time period and in response to the range of environmental variables outlined in Equation 7.2. Similarly, the Penman-Monteith equation (Monteith 1965; Monteith and Unsworth 1990) requires either measurements or reliable estimates of aerodynamic conductance (g_a) to obtain reasonable estimates of E_c. One of the largest limitations to the widespread implementation of the Jarvis-Stewart model (Jarvis, 1976), in combination with the Penman-Monteith equation, is therefore linked to the difficulty in obtaining model parameters. Seeing that no published studies have attempted to parameterize the model for macadamias, it is unclear if parameters obtained from the Jarvis-Stewart model, in combination with the Penman-Monteith equation, would be applicable for determining E_c of a wide range of orchards, differing in canopy size and in different climatic regions.

The lack of published values for g_c and g_a in macadamias, however, also creates an opportunity to model macadamia E_c with a less parameter intensive model. One such model has been proposed by Whitley et al. (2009) and is a simplified version of the Jarvis steward model, which estimates E_c without the use of g_c or g_a and has shown to provide reasonable estimates of E_c in native Australian forest. The model follows a similar form to that proposed by Jarvis-Stewart model (Jarvis, 1976) and is as follows:

$$E_c = E_{c\,max} f(S_R) f(VPD_{air}) f(\theta) \qquad \textbf{Equation 7.3}$$

where $E_{c\,max}$ is the maximum E_c recorded and is scaled as a function of radiant flux density (S_R), air vapour pressure deficit (VPD_{air}) and available volumetric soil moisture content (θ). The simplicity of the model makes it rather intriguing and given the proposed $E_{c\,max}$ observed in macadamias, as discussed in Chapter 6, the model could prove to provide reasonable estimates of E_c in macadamias.

Although, all these models have potential shortfalls, which may limit the accuracy of E_c estimations, no published studies have attempted to validate any these models for macadamias. The study hypothesized that mechanistic modelling approaches would provide more accurate estimates of E_c in macadamias, especially on a daily basis where high degrees of environmental variation exist, compared to an empirical model based on the premise of demand limited E_c. Furthermore, the study hypothesized that macadamias are highly coupled to the atmosphere, and the high degree of coupling would allow for direct estimation of E_c using a E_c model. This chapter therefore aimed to validate the accuracy of both the FAO-56 dual crop coefficient model and a Jarvis-Steward type canopy conductance model, used in conjunction with the Penman-Monteith equation, to obtain reasonable estimates of E_c in two macadamia orchards of varying canopy size. It further aimed to validate the use of a simplified Jarvis-Steward model, as proposed by Whitley et al. (2009), to estimate E_c directly in orchards of varying canopy size, without the use of g_c.

7.3 Materials and Methods

7.3.1 Site description, weather variables, canopy measurements, and transpiration

Site and orchard specific information, as well as details surrounding the measurement of weather variables and transpiration measurements are outlined in Chapter 3, Section 3.1. Transpiration measurements used in the model validation and parameterization of this study were made in two macadamia orchards, consisting of a mature bearing (MB) and an immature bearing (IB) orchard, which differed largely based on canopy size. More details regarding the orchards used in this study can be found in Chapter 3, Table 3.1. Furthermore, canopy dimensions including height, width, and breadth, as well as other canopy specific parameters including leaf area index (LAI) and fractional interception of PAR (FI) were measured throughout the trial in both orchards, with methods used in measurements of these components being outlined in Chapter 3, Section 3.1.3.

7.3.2 Modelling transpiration using the dual crop coefficient model

The strict definition of a basal crop coefficient (K_{cb}) includes some evaporation when the soil surface is dry (Allen et al. 1998) and as direct measurements of E_c were made using a sap flow method in this trial, transpiration crop coefficients (K_t) were derived instead of K_{cb}, as proposed by Villalobos et al. (2013). Daily K_t values were calculated by dividing measurements of E_c by daily ET_o as follows:

$$K_t = \frac{E_c}{ET_o}$$ **Equation 7.4**

Estimates of K_t were calculated according to the procedure outlined by Allen and Pereira (2009), where K_t during conditions of nearly full ground cover ($K_{t\ full}$) is multiplied with a density coefficient (K_d), which is linked to the abundance of vegetation present, and is presented as follows:

$$K_t = K_{t\ full} \times K_d$$ **Equation 7.5**

Where daily values of K_d were calculated in accordance with Allen and Pereira (2009) as:

$$K_d = \min\left(1, M_L f_{c\ eff}, f_{c\ eff}^{\left(\frac{1}{1+h}\right)}\right)$$ **Equation 7.6**

where $f_{c\ eff}$ is the effective fraction of ground covered or shaded by vegetation [0.01-1] near solar noon, M_L is a multiplier on $f_{c\ eff}$ describing the effect of canopy density on shading and on maximum relative evapotranspiration per fraction of ground shaded [1.5-2.0], with a value of 1.5 recommended for citrus (Allen and Pereira, 2009) and subsequently selected for both macadamia orchards in this study and h is tree height.

The effective fraction of ground covered ($f_{c\ \mathrm{eff}}$) was calculated as the ratio of tree canopy width to inter-row spacing or the ratio of ground shaded area by the crop at solar noon to the total area available to the tree, following Allen et al. (1998) in the MB macadamia orchard with a north-south orientation. In the IB orchard, which is orientated in an approximate east west direction, $f_{c\ \mathrm{eff}}$ was calculated according to Allen et al. (1998) as follows:

$$f_{c\ \mathrm{eff}} = \frac{f_c}{\sin(\beta)} \leq 1$$ **Equation 7.7**

where f_c is the observed fraction of soil surface that is covered by vegetation as seen from directly overhead. $f_{c\ \mathrm{eff}}$ is usually calculated at solar noon, such that β (mean elevation angle of the sun above the horizon during the period of maximum evapotranspiration) can be calculated as:

$$\beta = \arcsin\left[\sin(\varphi)\sin(\delta)+\cos(\varphi)\cos(\delta)\right]$$ **Equation 7.8**

where φ is latitude and δ is solar declination in radians. The average $f_{c\ \mathrm{eff}}$ values determined during the measurement period were 0.73 for the MB orchard and 0.48 for the IB orchard (Table 7.1).

Furthermore, in accordance with Allen and Pereira (2009), $K_{t\ \mathrm{full}}$ can be approximated, for large stand size (greater than about 500 m^2), as a function of mean plant height (h, m) (Table 7.1) and adjusted for climate using wind speed (u_2, m s^{-1}), percentage minimum relative humidity (RH_{min}), and the degree of stomatal control on E_c relative to most agricultural crops (F_r, unitless), as follows:

$$K_{t\ \mathrm{full}} = F_r\left(\min(1.0+0.1h,\ 1.20)+[0.04(u_2-2)-0.004(RH_{min}-45)]\left(\frac{h}{3}\right)^{0.3}\right)$$ **Equation 7.9**

where F_r [0-1] is a relative adjustment factor for stomatal control and was calculated as follows:

$$F_r \approx \frac{\Delta+\gamma(1+0.34u_2)}{\Delta+\gamma\left(1+0.34u_2\frac{r_{leaf}}{100}\right)}$$ **Equation 7.10**

where r_{leaf} is the mean leaf resistance (s m^{-1}); Δ is the slope of the saturation vapour pressure versus air temperature curve (kPa $°C^{-1}$) and γ is the psychrometric constant (kPa $°C^{-1}$). r_{leaf} for most agricultural crops under full cover conditions (when the LAI exceeds 3.0 m^2 m^{-2}) is 100 s m^{-1} (Allen and Pereira 2009). No published studies have suggested any r_{leaf} values for macadamias and therefore average values of r_{leaf} for macadamias in the MB was calculated during multiple periods (Table 7.2) of the growing season. Given that the average LAI for the IB orchard was less than 3.0 m^2 m^{-2} the term $r_{leaf}/100$ in Equation 7.10 was replaced with $r_s/50$, where r_s is estimated bulk canopy resistance, as suggested by Allen and Pereira (2009). Both r_{leaf} and r_s values for each orchard were estimated by inverting Equation 7.10, after solving for F_r by inverting Equation 7.9, using known daily values of $K_{t\ full}$. $K_{t\ full}$ values were calculated using measured daily K_t and K_d estimated from measured data. The same r_{leaf} and r_s values, which can be found in Table 7.2, were subsequently used to estimate F_r for independent seasons of measurements using Equation 7.10 in order to estimate K_t and E_c values for model validation purposes.

Table 7.1: Measured and calculated canopy parameters for the mature bearing (MB) and immature bearing (IB) macadamia orchards used as input parameters in the FAO-56 dual crop coefficient model.

Orchard	Mature Bearing	Immature Bearing
Between Row Width (m)	8.0	8.0
Canopy Width (m)[a]	5.9	4.0
Canopy Height (m)[a]	6.0	4.7
$f_{c\ eff}$	0.73	0.48

[a]Mean seasonal measurements

Taylor et al. (2015), demonstrated that the use of a single value of r_{leaf} in the estimation of crop coefficients was not appropriate for estimating water use of citrus and suggested that the use of monthly estimates of r_{leaf} might provide more accurate estimations of water use in citrus. Given the lack of information regarding r_{leaf} and r_s in macadamias, it was decided to determine if the accuracy of the FAO-56 dual crop coefficient model would be increased by using monthly r_{leaf} values as opposed to four (quarterly averaged values), two (initial to midseason and end of the season values) or a single (seasonally averaged value) estimate. Both r_{leaf} and r_s values used in this evaluation can be found in Table 7.2.

Table 7.2: Average calculated leaf resistance using Equation 7.9 and 7.10 (r_{leaf}) for the mature bearing (MB) macadamias orchard and canopy resistance (r_s) for the immature bearing (IB) macadamia orchard during different time periods within the parameterization phase of the FAO-56 dual crop coefficient model.

	MB			IB		
	Date Range	*N*	r_{leaf} (s m^{-1})	Date Range	*N*	r_s (s m^{-1})
Single	2016/08/10 - 2017/07/31	394	2332	2017/08/16 - 2018/07/31	334	1995
Two	2016/08/10 - 2017/01/31	174	2301	2017/08/16 - 2018/01/31	158	1995
	2017/02/01 - 2017/07/31	175	2369	2018/02/01 - 2018/07/31	176	1994
Quarterly	2016/08/10 - 2016/10/31	82	2348	2017/08/16 - 2017/10/31	76	1977
	2016/11/01 - 2017/01/31	92	2255	2017/11/01 - 2018/01/31	82	2014
	2017/02/01 - 2017/04/30	84	2566	2018/02/01 - 2018/04/30	85	1833
	2017/05/01 - 2017/07/31	91	2219	2018/05/01 - 2018/07/31	91	2155
Monthly	2016/08/10 - 2016/08/31	22	2448	2017/08/16 - 2017/08/31	16	1797
	2016/09/01 - 2016/09/30	30	2093	2017/09/01 - 2017/09/30	30	1903
	2016/10/01 - 2016/10/31	30	2503	2017/10/01 - 2017/10/31	30	2231
	2016/11/01 - 2016/11/30	30	2093	2017/11/01 - 2017/11/30	29	2140
	2016/12/01 - 2016/12/31	31	2278	2017/12/01 - 2017/12/31	29	1939
	2017/01/01 - 2017/01/31	31	2393	2018/01/01 - 2018/01/31	24	1964
	2017/02/01 - 2017/02/28	24	3035	2018/02/01 - 2018/02/28	27	1749
	2017/03/01 - 2017/03/31	31	2168	2018/03/01 - 2018/03/31	30	1872
	2017/04/01 - 2017/04/30	29	1994	2018/04/01 - 2018/04/30	28	1880
	2017/05/01 - 2017/05/31	30	2495	2018/05/01 - 2018/05/31	30	2043
	2017/06/01 - 2017/06/30	30	2062	2018/06/01 - 2018/06/30	30	2057
	2017/07/01 - 2017/07/31	31	2600	2018/07/01 - 2018/07/31	31	2366

7.3.2.1 Model parameterization and validation

The FAO-56 dual crop coefficient model was parameterized in the MB orchard by using daily measured K_t and E_c from 2016/08/10 to 2017/07/31 and validated using data from 2017/08/01 – 2018/07/05. The FAO-56 dual crop coefficient model was parameterized for the IB orchard using data from 2017/08/10 to 2018/07/31 and model validation was done from 2018/08/01 to 2019/07/23.

7.3.3 Modelling transpiration using a canopy conductance model in conjunction with the Penman-Monteith equation

7.3.3.1 Calculation of canopy conductance

Canopy conductance (g_c) was calculated using hourly transpiration measurements obtained from the MB orchard from 2016/09/15 – 2017/01/15, by inverting the Penman-Monteith equation (Monteith and Unsworth, 1990) as follows:

Equation 7.11

$$g_c = \frac{\lambda E_c \gamma g_a}{\Delta(R_n - G) + \rho_a C_p g_a VPD - \lambda E_c(\Delta - \gamma)}$$

where λ is the latent heat of vaporization of water (J kg^{-1}), E_c is canopy transpiration (kg m^{-2} s^{-1}), Δ is the slope of the vapour pressure curve (kPa K^{-1}), R_n is net radiation at the crop surface (W m^{-2}), G is soil heat flux (W m^{-2}) taken as 10 % of R_n, ρ_a is the density of dry air (kg m^{-3}), C_p is the specific heat capacity of the air (J kg^{-1} K^{-1}), VPD is saturation vapour pressure deficit (kPa), γ is the psychrometric constant (kPa K^{-1}), g_a is the aerodynamic conductance (m s^{-1}) and g_c is the canopy conductance (m s^{-1}). R_n was estimated from shortwave radiation measured at the automatic weather station according to Allen et al. (1998) using measurements of macadamia albedo (0.13) obtained from the four-component net radiometer on the eddy covariance system outlined in Chapter 3, Section 3.3.

Aerodynamic conductance (g_a) was calculated as suggested by Rana et al (2005):

Equation 7.12

$$g_a = \frac{k^2 u_z}{\ln((z-d)/z_0)\ln((z-d)/(h_c-d))}$$

where k is the von Karman’s constant equal to 0.4, u_z is the wind speed (m s^{-1}) at the z wind measurement height (m), d is the zero plane displacement estimated as d = $0.67h_c$, z_o is the roughness length taken as $0.1h_c$ and h_c is the mean orchard height (Table 7.1). Windspeed above the canopy (6 m) was calculated using Equation 7.13, by calculating the attenuation coefficient (*a*) for macadamias, which was obtained by inverting Equation 7.13, by using measured values of wind speed from the automatic weather station at 2 m above the ground and above canopy windspeed (measured during a window period in the MB orchard) at a height of 6 m above the ground. The equation was used in accordance to that described by Campbell and Norman (2012) as:

$$u_h = u_z \exp\left[a\left(\frac{z}{h} - 1\right)\right] \quad \textbf{Equation 7.13}$$

where u_z is the wind speed (m s^{-1}) at the z wind measurement height (m) and u_h is the wind speed (m s^{-1}) at the top of the canopy (h) height (m), which in this study was 6 m for the MB orchard and 4.7 m for the IB orchard (Table 7.1). The attenuation coefficient (*a*) calculated for macadamias in this study was 0.68.

7.3.3.2 Modelling Canopy Conductance

Canopy conductance was modelled using a Jarvis-type model (Jarvis 1976), similar to the one used by Oguntunde et al. (2007), on an hourly basis with weather data as follows:

$$g_{c,j} = g_{c\,max} f(S_R) f(VPD_{air}) f(T_{air}) \quad \textbf{Equation 7.14}$$

where $g_{c,j}$ is the canopy conductance predicted by the Jarvis model, $g_{c\ max}$ is the maximum canopy conductance (m s^{-1}), $f(S_R)$ is a function of solar radiation, $f(VPD_{air})$ is a function of vapour pressure deficit and $f(T_{air})$ is a function of air temperature. The functions have values ranging between 0 and 1. A response function for soil water content has been included in the Jarvis-type model in some studies, particularly native forests (e.g. Whitley et al. 2008), but as the orchards in this study were well-irrigated this function was set to one. The control functions of temperature and solar radiation were similar to those of Oguntunde et al. (2007) and took the following forms:

$$f(S_R) = \frac{S_R}{R_m}\left(\frac{R_m + k_R}{S_R + k_R}\right) \quad \textbf{Equation 7.15}$$

$$f(T_{air}) = \frac{(T_a - T_L)(T_H - T_a)^t}{(k_T - T_L)(T_H - k_T)} \quad \textbf{Equation 7.16}$$

$$t=\frac{T_H-k_T}{k_T-T_L}$$ **Equation 7.17**

where k_R and k_T are model parameters for the respective functions in which they are used, T_L and T_H are the lower and upper temperature limit to transpiration fixed at 0 and 45 ^oC, respectively (Oguntunde et al. 2007). R_m is an arbitrary radiation constant, often fixed at 1000 W m^{-2} (e.g. Sommer et al., 2002; Wright et al., 1995). For the control function for vapour pressure deficit the equation derived by Zhang et al. (1997) was used. The equation is stated as:

$$f(VPD_{air})=\frac{1+k_{D1}VPD}{1-k_{D2}VPD}$$ **Equation 7.18**

where k_{D1} and k_{D2} are modelled parameters.

7.3.3.3 Model Parameterization

Parameters $g_{c\,max}$, k_R, k_T, k_{D1} and k_{D2} were optimised by minimising the sum of squares of the residuals of the day-time (0800 h to 1700h) measured and modelled canopy conductance as:

$$S^2(k)=\sum_{i=1}^{n}\left(g_{c,i}-g_{c,j}(k,x_i)\right)$$ **Equation 7.19**

where $g_{c,i}$ is the i^{th} value of canopy conductance calculated using Equation 7.11 using measured transpiration data, $g_{c,j}$ is the corresponding canopy conductance value predicted by the Jarvis model, k represents the model parameters (k_R, k_T, k_{D1} and k_{D2}) and x_i is the input variables of the i^{th} model value. Minimisation of S^2 was carried out by optimising k using the solver function in Microsoft Excel.

7.3.3.4 Model Validation

Validation of the model was performed by calculating g_c using the optimised parameters of the Jarvis model and subsequently using these values in the Penman-Monteith equation to estimate hourly E_c. Only E_c values for the day-time (0800 h to 1700h) period were used to evaluate the performance of the model. These values were compared to the day-time E_c measured using the sap flow measurements in the MB and IB orchard. Model validation in the MB orchard stretched from 2017/08/16 – 2018/08/06, whilst the model was validated over two consecutive seasons in the IB orchard, with the first validation phase stretching from 2017/09/30 – 2018/07/31 and the second validation stretching from 2018/08/10 – 2019/04/30.

7.3.4 Modelling transpiration using a modified Jarvis Steward type model

The E_c model proposed by Whitley et al. (2009) (Equation 7.3) was modified for this study, by excluding the volumetric soil water content (θ) function from the equation given that both orchards were irrigated throughout the duration of the trial and soil water content was unlikely to have placed a limitation on $E_{c\ max}$ in this study. Measurements of pre-dawn leaf water potential throughout the course of the trial emphasized the absence of water stress in measurement trees. This study did, however, include air temperature (T_{air}) as a modulating factor for $E_{c\ max}$ following results from Chapter 6, and the model took the following form:

$$E_c = E_{c\ max} f(S_R) f(VPD_{air}) f(T_{air}) \qquad \textbf{Equation 7.20}$$

Both the S_R and T_{air} response functions took the same form as that presented in Equations 7.15 – 7.17, with T_L, T_H and R_m fixed at 0 °C, 45 °C and 1000 W m^{-2} respectively. The response function of VPD_{air} was, however, different to that used in the g_c model and took the following form as proposed by Whitley et al. (2009):

$$f(VPD) = k_{e1} VPD_{air} \exp(-k_{e2} VPD_{air}) \qquad \textbf{Equation 7.21}$$

where, parameters k_{e1} and k_{e2} describe the rate of change at low and high VPD_{air} and were generated as part of the model parameterization phase.

Similar to the parameterization of the g_c model, parameters $E_{c\ max}$, k_R, k_T, k_{e1} and k_{e2} were optimised by minimising the sum of squares of the residuals of the measured and modelled E_c (Equation 7.19). Parameters for Equation 7.20 were obtained using daily measured E_c obtained from sap flow measurements in the MB orchard from 2016/09/15 – 2017/02/15

7.3.4.1 Model Validation

Validation of the model was performed by simulating E_c using the optimised parameters of the Whitley et al. (2009) model and comparing these values to measured E_c for the day-time (0800 h to 1700h) period using the sap flow measurements in the MB and IB orchard. Model validation in the MB orchard stretched from 2017/08/16 – 2018/08/06, whilst the model was validated over two consecutive seasons in the IB orchard, with the first validation phase stretching from 2017/09/30 – 2018/07/31 and the second validation stretching from 2018/08/10 – 2019/04/30.

7.3.5 Scaling $g_{c\ max}$ and $E_{c\ max}$ for orchards with varying canopy size.

The study attempted to model E_c in two differently sized macadamia orchards located in close proximity of one another, and as a result adjustments for variations in canopy size needed to be made given that the larger of the two orchards was used to paramaterize both the g_c and E_c models. It was decided that the g_c model (Equation 7.14) would need scaling on the $g_{c\ max}$ term, and was subsequently scaled using measurements of LAI. Scaling was done by dividing $g_{c\ max}$ obtained during the model paramaterization phase by the average LAI of the MB orchard during the same period. By dividing $g_{c\ max}$ with LAI, a leaf area specific $g_{c\ max\ LAI}$ (mm m^2 s^{-1} m^{-2}) could be obtained and substituted back into Equation 7.14 so that canopy adjusted g_c was obtained as:

$$g_{c\ mod} = \mathrm{LAI}\ g_{c\ max\ adj} f(\mathrm{S_R}) f(\mathrm{VPD_{air}}) f(\mathrm{T_{air}}) \qquad \textbf{Equation 7.22}$$

Similarly, adjustments for canopy size needed to be made for the $E_{c\ max}$ term of Equation 7.20. However, given that the study aimed to keep the input parameters of the model easily obtainable, the LAI adjustment used in the g_c model was replaced by an adjustment for canopy size using $f_{c\ eff}$ as proposed by Allen and Pereira (2009). The $E_{c\ max}$ obtained during the model parameterization phase of the MB orchard was divided by the $f_{c\ eff}$ value of the orchard to obtain $E_{c\ max\ f c\ eff}$ (mm h^{-1}). This term was substituted into Equation 7.20 so that $E_{c\ mod}$ was obtained as:

$$E_{\mathrm{c\ mod}} = f_{\mathrm{c\ eff}}\ E_{\mathrm{c\ max\ adj}}\ f(\mathrm{S_R}) f(\mathrm{VPD_{air}}) f(\mathrm{T_{air}}) \qquad \textbf{Equation 7.23}$$

7.3.6 Statistical analysis

The evaluation of model performance throughout this study was done with the aid of statistical parameters, including coefficient of determination (R^2), mean absolute percentage diffrence (MAPD), root of the mean square error (RMSE) and index of agreement (D) of Willmott (1982). Model performance was considered satisfactory when RMSE< half the standard deviation of measured values, $R^2 > 0.8$, MAE < 20% and D > 0.8 (de Jager 1994).

7.4 Results and discussion

7.4.1 Estimates of K_t and E_c derived from the FAO-56 dual crop coefficient model

Comparisons between measured and simulated daily K_t values obtained during the model parameterization and validation periods, using single r_{leaf} and r_s values (Table 7.2) are displayed in Figure 7.1 for the MB orchard and Figure 7.2 for the IB orchard. Poor parameterization and validation of daily K_t derived from the FAO-56 dual crop coefficient model was observed in both the MB and IB orchard.

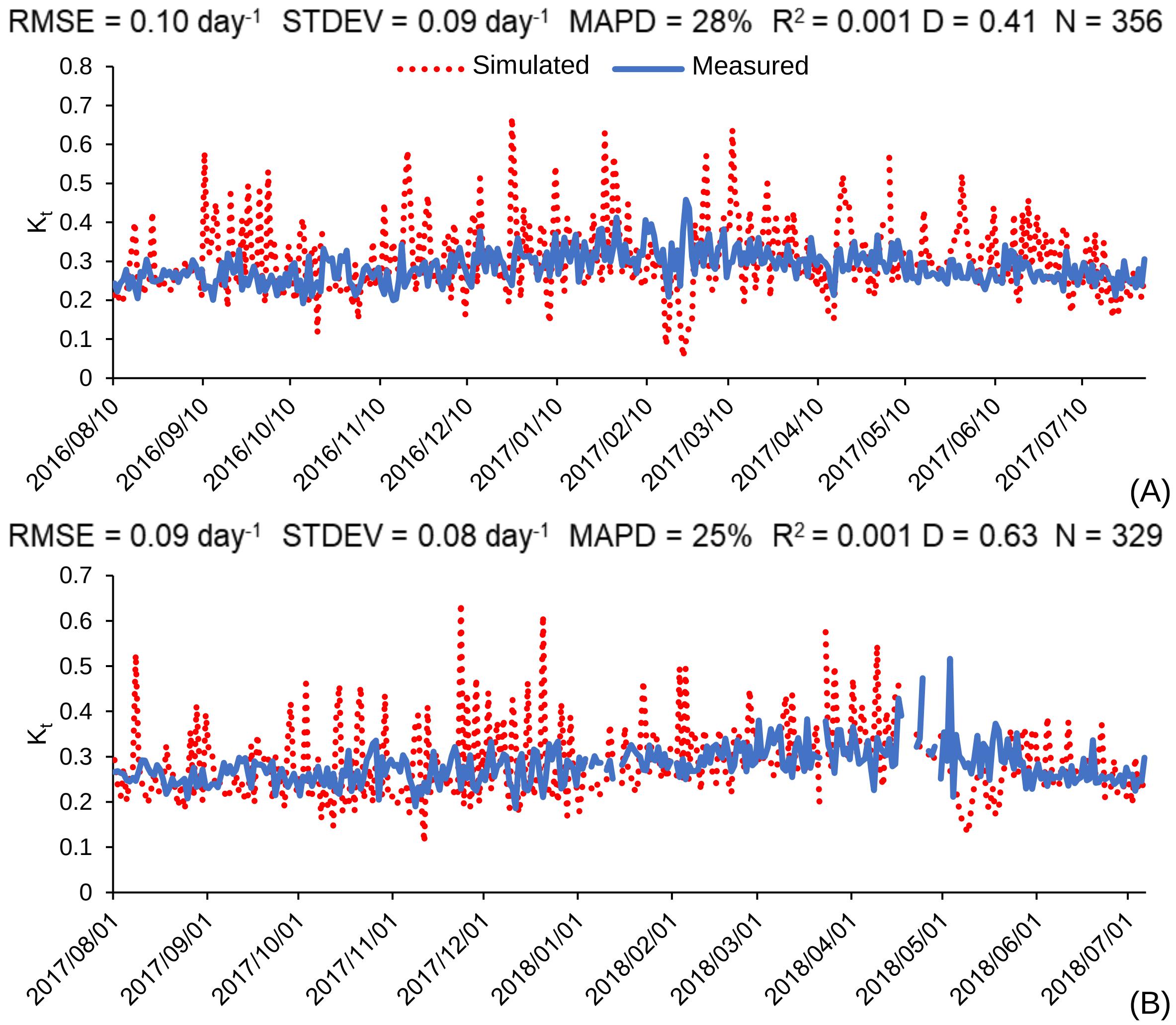

Figure 7.1: Comparison between daily measured and simulated transpiration crop coefficients (K_t) for the mature bearing (MB) orchard during the (A) parameterization and (B) validation of the FAO-56 dual crop coefficient model using a single r_{leaf} value. Missing data is due to missing solar radiation data as a result of equipment failure.

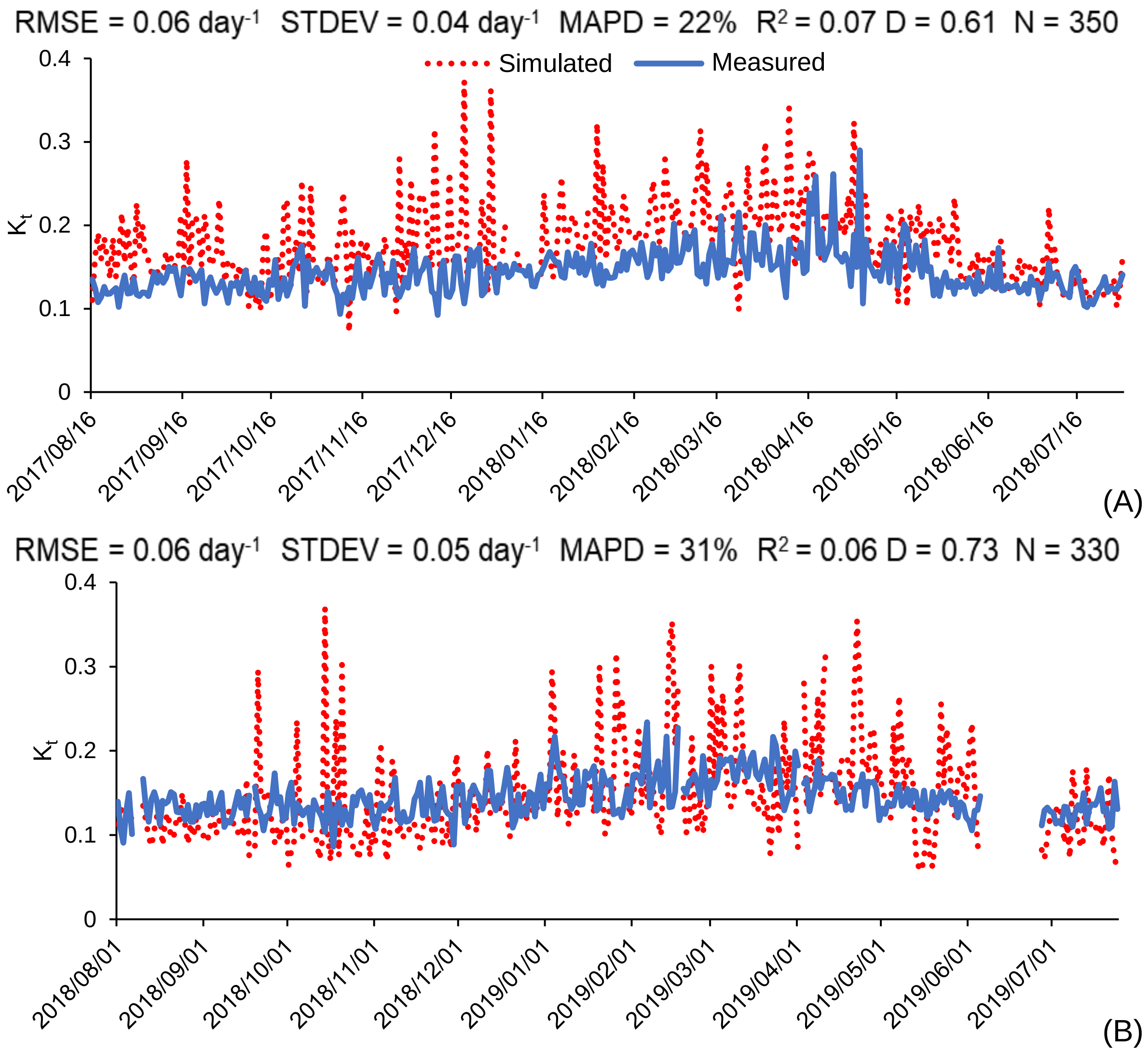

Figure 7.2: Comparison between daily measured and simulated transpiration crop coefficients (K_t) for the immature bearing (IB) orchard during the (A) parameterization and (B) validation of the FAO-56 dual crop coefficient model using a single r_s value. Missing data is due to missing solar radiation data as a result of equipment failure.

In both orchards RMSE, R^2, MAPE and D observed between measured and simulated daily K_t were outside the criteria of acceptability for model validation and parameterization (RMSE > half the standard deviation of the measured data, MAPE >20% and R^2 and D below 0.8). As expected, the poor parameterization of the FAO-56 dual crop coefficient model to simulate daily K_t, whilst using a single seasonal r_{leaf} and r_s value, resulted in equally poor parameterization and validation of the model to simulate daily E_c in the MB (Figure 7.3) and IB (Figure 7.4) macadamia orchards.

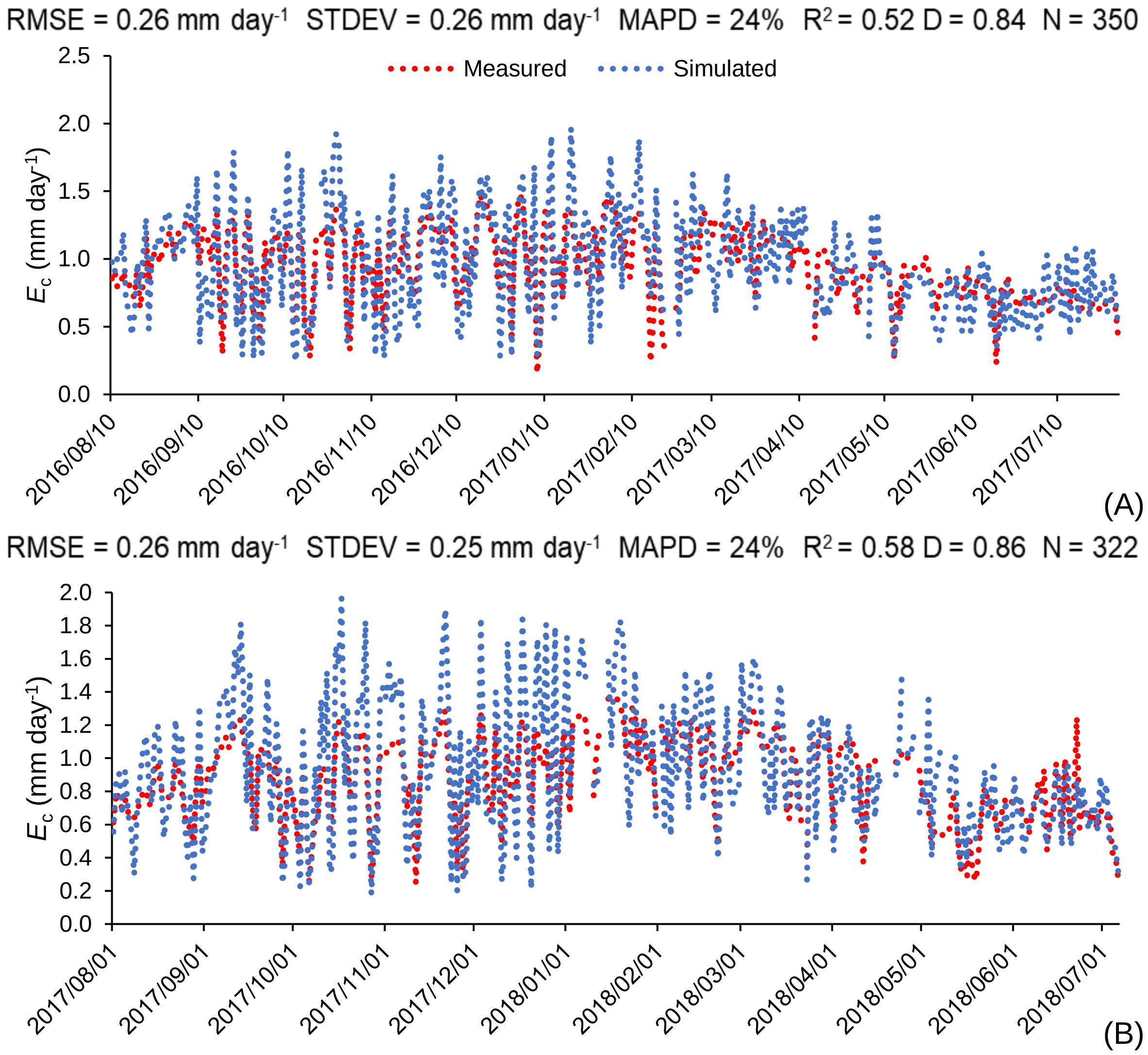

Figure 7.3: Comparison between daily measured and simulated transpiration (*E_c*) for the mature bearing (MB) orchard during the (A) parameterization and (B) validation of the FAO-56 dual crop coefficient model using a single r_{leaf} value. Missing data is due to missing solar radiation data as a result of equipment failure.

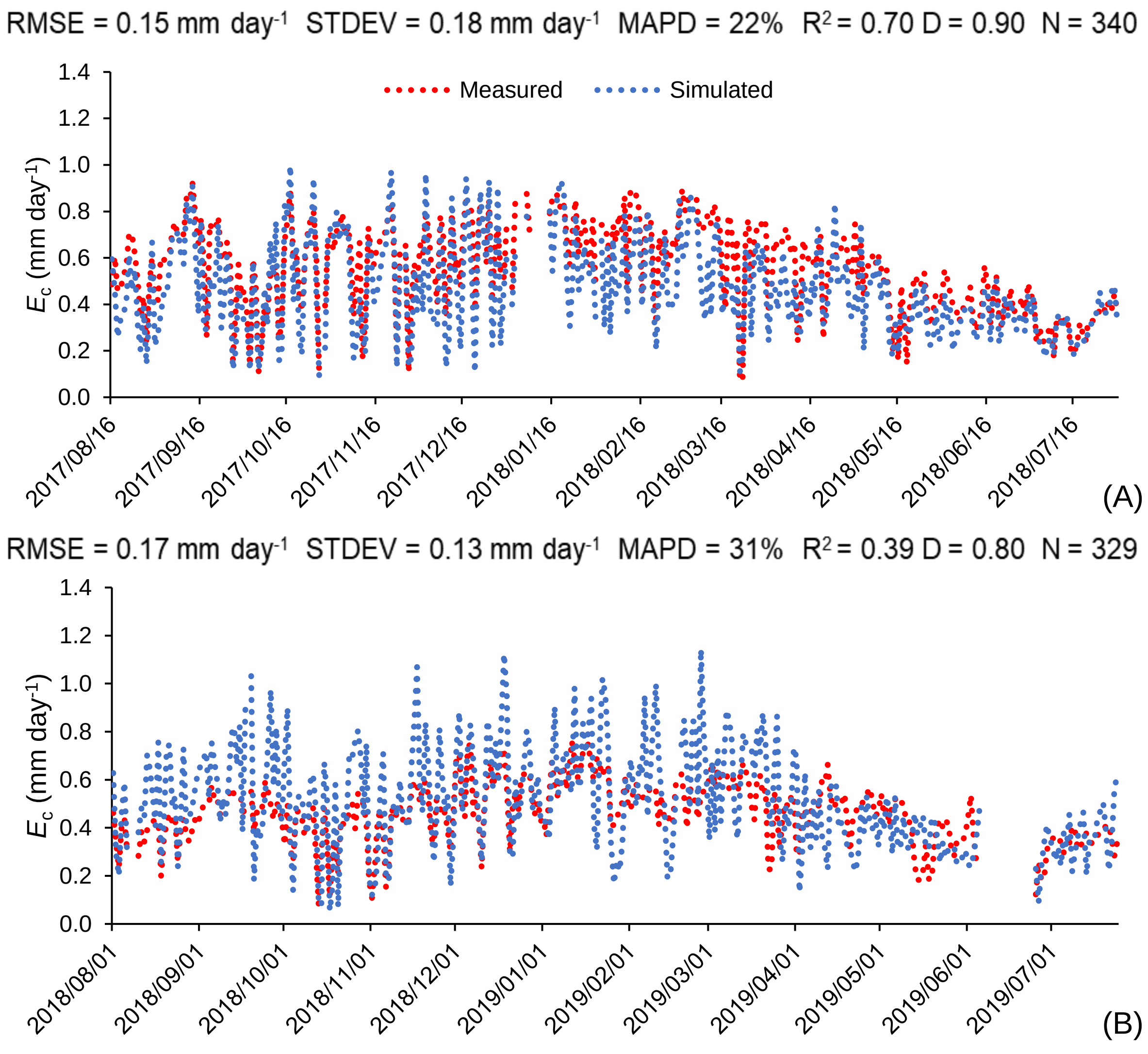

Figure 7.4: Comparison between daily measured and simulated transpiration (E_c) for the immature bearing (IB) orchard during the (A) parameterization and (B) validation of the FAO-56 dual crop coefficient model using a single r_s value. Missing data is due to missing solar radiation data as a result of equipment failure.

In both orchards, model criteria when using a single r_{leaf} and r_s value, were not met for comparisons between measured and simulated daily E_c during both the model parameterization and validation phase. RMSE was outside the stipulated criteria during both the parameterization and validation phase, which suggest that standard deviation between the measured and simulated values of daily E_c in each of the data sets used was rather high. The largest discrepancies between the measured and simulated daily K_t and E_c largely stem from the underestimation of both daily K_t and E_c at low ET_o (<2.5 mm day^{-1}) and overestimation of K_t and E_c at high ET_o (>4.0 mm day^{-1} in the MB orchard and >4.5 mm day^{-1} in the IB orchard) (Figure 7.5 A-D).

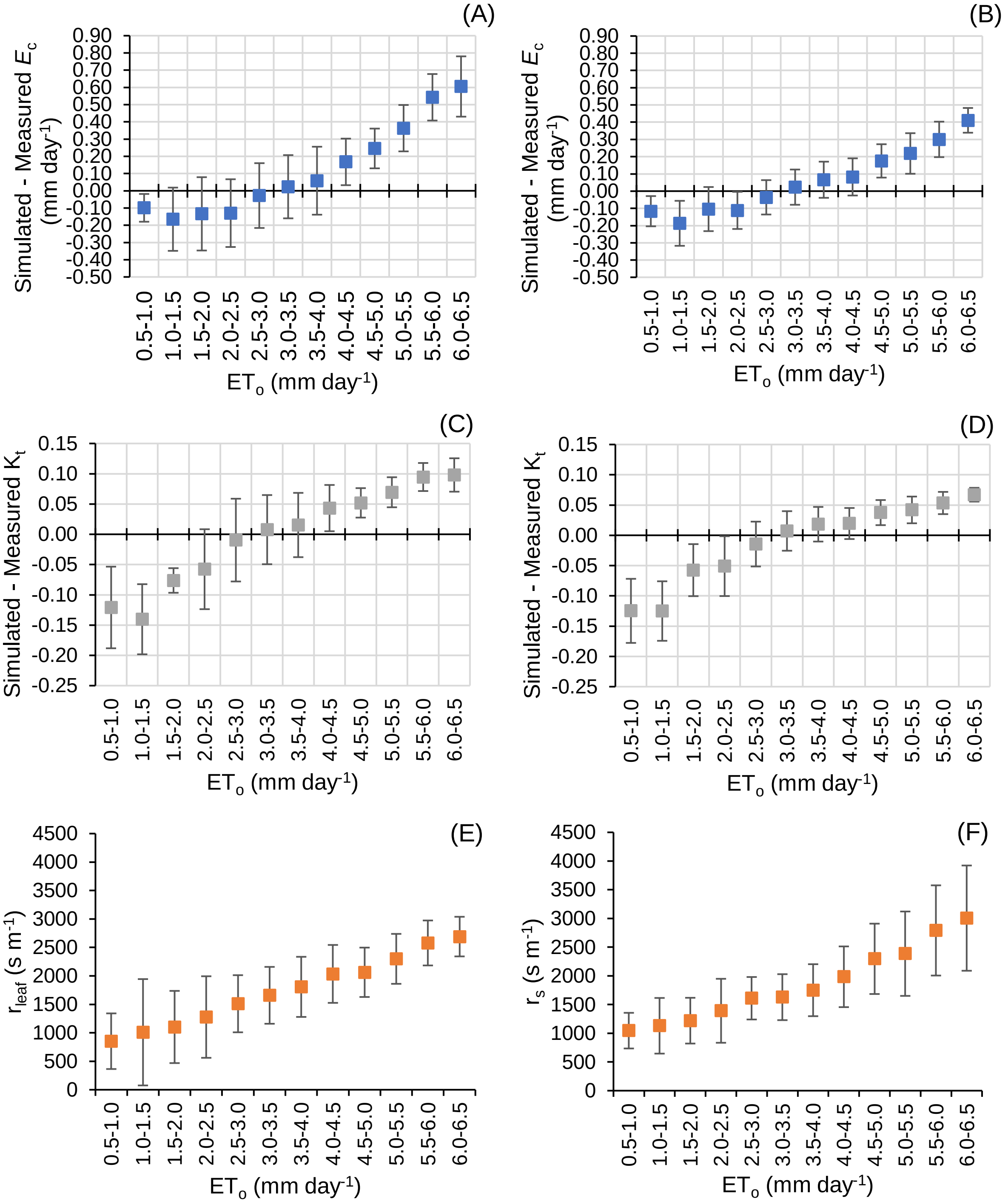

Figure 7.5: Average (± standard deviation) difference between simulated and measured daily transpiration (E_c) and daily transpiration crop coefficients (K_t) throughout a range of daily reference evapotranspiration (ET_o) rates for the mature bearing (MB) (A, C) and immature bearing (IB) (B, D) orchards during model validation of the FAO-56 dual crop coefficient model. Average (± standard deviation) of calculated (E) leaf resistance (r_{leaf}) for the mature bearing (MB) orchard and (F) surface resistance (r_s) using Equation 7.9 and 7.10 for the immature bearing (IB) orchard in response to a range of ET_o.

Poor model performance on a daily basis was, however, expected seeing that the FAO-56 model is based on the premise that E_c is a demand limited process, whereby a plateau in macadamia E_c is reached when ET_o exceeds 3.5 mm day^{-1}. Therefore, on days where ET_o exceeded 3.5 mm day^{-1}, daily simulated macadamia E_c would be greatly overestimated, whilst measured E_c, would have remained either constant or declined slightly. Similarly, on days where ET_o was less than 2.5 mm day^{-1}, simulated macadamia E_c was slightly underestimated in relation to measured E_c, implying that macadamias E_c is higher in relation to ET_o (i.e. larger K_t) at lower demands. This was confirmed in both macadamia orchards (Figure 7.5 C & D), with large differences observed between simulated and measured K_t at ET_o <2.5 mm day^{-1}.

Although the discrepancies between measured and simulated daily K_t and E_c could be due to a range of factors, this is most likely due to the use of a single average value of r_{leaf} and r_s for the entire validation period. Calculated r_{leaf} and r_s for macadamias in this study revealed that although an approximate linear relationship exists between ET_o and either calculated r_{leaf} or r_s, a large amount of variability in calculated r_{leaf} and r_s is observed throughout the range of daily ET_o (Figure 7.5 E-F). A substantial amount of variability is therefore lost in the averaging of r_{leaf} values across a season, as is the case when using a single seasonal value. Multiple estimates of r_{leaf} were therefore tested in an attempt to improve model accuracy. The results from this analysis are presented in Tables 7.3 and 7.4.

In both MB and IB orchards the use of multiple r_{leaf} and r_s values failed to improve the accuracy of the FAO-56 dual crop coefficient model to simulate daily values of both K_t and E_c. Although multiple values tended to decrease MAPE and increase R^2, RMSE and D remained similar and were unsatisfactory for modelling purposes. The high RMSE in the model parameterization and validation of K_t and E_c, in both the MB and IB orchards, tend to suggest that data points obtained from the model simulation were not well concentrated around the line of best fit. Nevertheless, results presented would suggest that the FAO-56 dual crop coefficient model could not be successfully parameterized and would subsequently lead to poor estimations of daily values of both K_t and E_c of independent data sets.

Table 7.3: Statistical evaluation of the FAO-56 dual crop coefficient model during model parameterization and validation of daily transpiration coefficients (K_t) and daily transpiration (E_c) using single and multiple values of leaf resistance (r_{leaf}) calculated for mature bearing (MB) macadamia orchard (Table 7.2). Statistical parameters include the number of observations used (*N*), root mean square error (RMSE), mean absolute percentage error (MAPE) index of agreement (D) of Willmott (1982), and coefficient of determination (R^2).

	Mature Bearing Macadamia Orchard									
	Parameterization K_t					Validation K_t				
	N	RMSE	MAPE (%)	D	R^2	*N*	RMSE	MAPE (%)	D	R^2
Single	353	0.10	28	0.47	0.001	329	0.09	25	0.64	0.001
Two	353	0.10	28	0.47	0.001	329	0.09	25	0.63	0.001
Quarterly	353	0.10	27	0.47	0.001	329	0.09	25	0.61	0.01
Monthly	353	0.10	26	0.53	0.03	329	0.10	27	0.60	0.01
	Parameterization E_c					Validation E_c				
	N	RMSE (mm day^{-1})	MAPE (%)	D	R^2	*N*	RMSE (mm day^{-1})	MAPE (%)	D	R^2
Single	350	0.26	24	0.84	0.52	322	0.26	24	0.86	0.59
Two	350	0.26	24	0.84	0.53	322	0.27	24	0.86	0.58
Quarterly	350	0.26	24	0.84	0.50	322	0.27	25	0.85	0.55
Monthly	350	0.25	23	0.85	0.54	322	0.29	26	0.84	0.52

Table 7.4: Statistical evaluation of the FAO-56 dual crop coefficient model during model parameterization and validation of daily transpiration coefficients (K_t) and daily transpiration (E_c) using single and multiple values of bulk canopy resistance (r_s) calculated for immature bearing (IB) macadamia orchard (Table 7.2). Statistical parameters include the number of observations used (N), root mean square error (RMSE), mean absolute percentage error (MAPE), index of agreement (D) of Willmott (1982), and coefficient of determination (R^2).

	Immature Bearing Macadamia Orchard									
	Parameterization K_t					Validation K_t				
	N	RMSE	MAPE (%)	D	R^2	*N*	RMSE	MAPE (%)	D	R^2
Single	350	0.07	22	0.60	0.07	330	0.06	32	0.72	0.04
Two	350	0.07	22	0.60	0.07	330	0.06	32	0.72	0.04
Quarterly	350	0.06	22	0.60	0.07	330	0.06	32	0.73	0.06
Monthly	350	0.06	22	0.61	0.07	330	0.06	31	0.74	0.06
	Parameterization E_c					Validation E_c				
	N	RMSE (mm day^{-1})	MAPE (%)	D	R^2	*N*	RMSE (mm day^{-1})	MAPE (%)	D	R^2
Single	340	0.14	22	0.90	0.70	329	0.17	32	0.81	0.35
Two	340	0.13	22	0.90	0.70	329	0.17	32	0.81	0.35
Quarterly	340	0.14	22	0.90	0.70	329	0.17	32	0.80	0.37
Monthly	340	0.15	22	0.90	0.70	329	0.18	32	0.81	0.39

From the results, it is clear that the large daily variation in r_{leaf} and $r_{s, \text{in relation to}}$ ET_o, leads to poor performance of the FAO-56 dual crop coefficient model to simulate daily K_t and E_c. Seeing the poor model performance was observed on a daily time step, the study attempted to evaluate the accuracy of the FAO-56 dual crop coefficient model using greater time steps (i.e. monthly or seasonally). Accurate estimates of monthly or seasonal K_t and E_c would allow for the FAO-56 dual crop coefficient model to be used as a strategic irrigation planning and water licencing tool in macadamias. The study therefore used a single value of r_{leaf} and r_s (Table 7.2) in conjunction with monthly and seasonally averaged ET_o and RH_{min} to determine monthly and seasonal estimates of K_t. Monthly and seasonal E_c was estimated by using monthly and seasonal totals of ET_o and multiplying these totals with derived K_t values for each of the respective periods. The comparisons between monthly and seasonally measured and simulated K_t and E_c, using a single value of r_{leaf} and r_s, is presented in Figure 7.6 for the MB and Figure 7.7 for the IB orchard.

Monthly and seasonally measured and simulated K_t and E_c showed a substantial increase in accuracy compared to simulations on a daily basis. In both the MB and IB orchard all statistical criteria were met, and differences between measured and simulated K_t and E_c were relatively small. The substantial increases in accuracy observed at this time scale, is largely attributed to compensatory errors, which ultimately mask any under or overestimations observed on a daily basis. For example, average ET_o throughout the entire measurement period ranged from 2.5 mm day^{-1} in winter to 4.0 mm day^{-1} summer, which is within the ET_o range where the model simulated K_t and E_c most accurately (Figure 7.5).

Regardless of increased model accuracy, the FAO-56 dual crop coefficient model slightly underestimated both K_t and E_c on a monthly and seasonal basis in the MB orchard. In contrast, the model slightly overestimated K_t and E_c in the IB orchard from July to December and slightly underestimated both K_t and E_c from January to May during the period of model validation. In both the MB and IB orchards K_t and E_c were consistently underestimated during autumn (March-April), a period which coincides with nut maturation and oil accumulation, which has been shown to cause an upregulation in E_c unrelated to increases in ET_o. In this study the model underestimated

E_c during the autumn period by 4.0 mm in the MB orchard and 2.5 mm in the IB orchard. Although a single value of r_{leaf} and r_s was used in these assessments, it should be noted that both r_{leaf} and r_s was substantially lower in April (Table 7.2) compared to the single r_{leaf} and r_s used in the assessment. Substituting only r_{leaf} and r_s in April, with lower values (Table 7.2) resulted in less of an underestimation in both E_c and K_t (data not shown). Lower r_{leaf} and r_s values during April would lead to increases in F_r, $K_{t\ full}$ and subsequently K_t and E_c. Decreases in r_{leaf} and r_s in April implies that an upregulation in in stomatal conductance occurs during this period, which has been reported in this study (see results in Chapters 4 and 5).

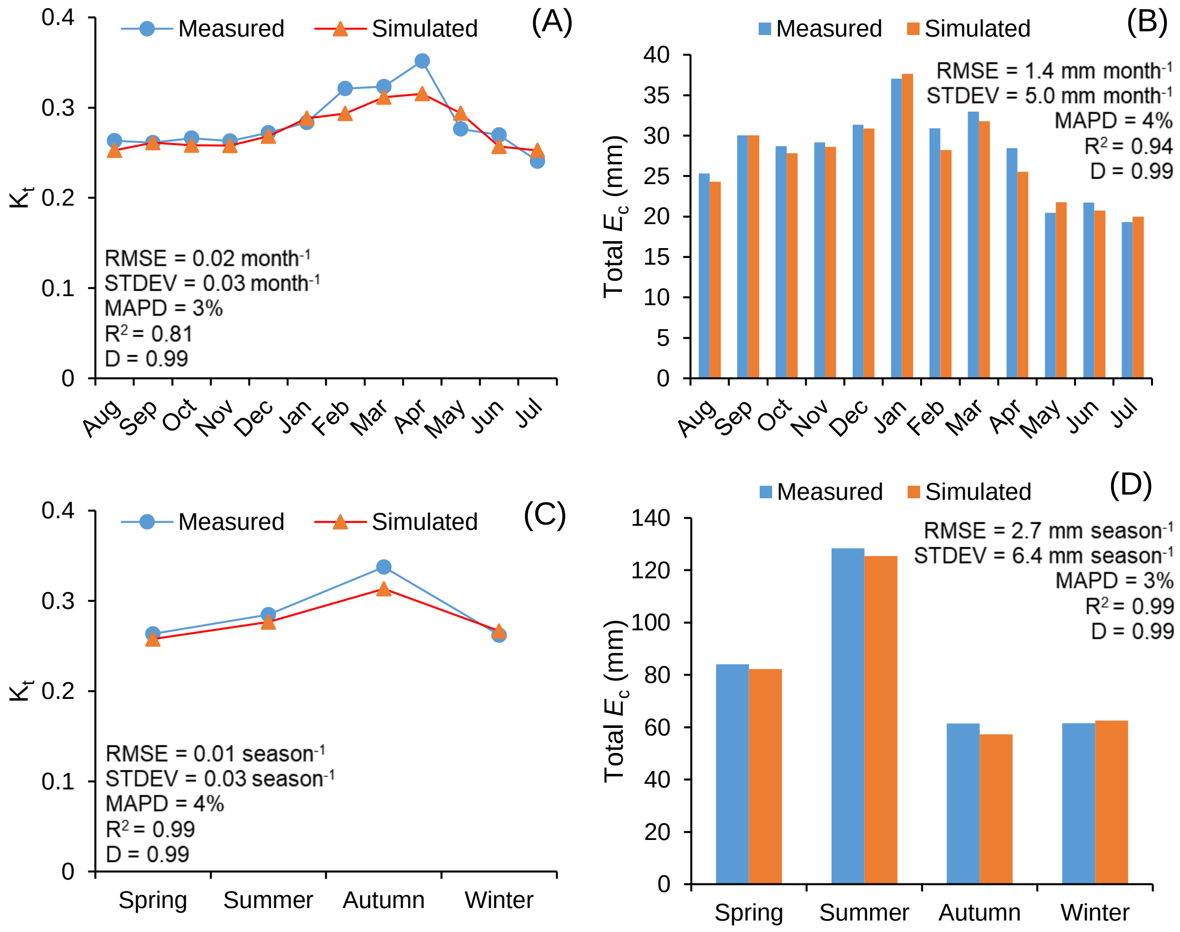

Figure 7.6: Comparison between monthly (A, B) and seasonally (C, D) measured and simulated transpiration crop coefficients (K_t) and transpiration (E_c) for the mature bearing (MB) orchard during the model validation phase (2017/08/01 – 2018/07/31) of the FAO-56 dual crop coefficient model using a single r_{leaf} value.

Regardless of the slight variances between measured and simulated values of K_t and E_c, the FAO-56 dual crop coefficient model yielded fairly accurate estimates of both K_t and E_c on a monthly or seasonal basis. Total E_c measured in the MB orchard during the period of model validation (2017/08/01 – 2018/07/31) was 335 mm and was only 8 mm more than that simulated using the FAO-56 dual crop coefficient model (327 mm). In the IB orchard the total measured seasonal E_c differed by 1 mm from that of the simulated value, being 170 mm measured compared to the 171 mm simulated by the model. This analysis of the FAO-56 dual crop coefficient model would therefore suggest that poor model performance is to be expected if the model is to be used for daily estimates, but model performance is satisfactory if used for estimates of monthly or seasonal K_t and E_c.

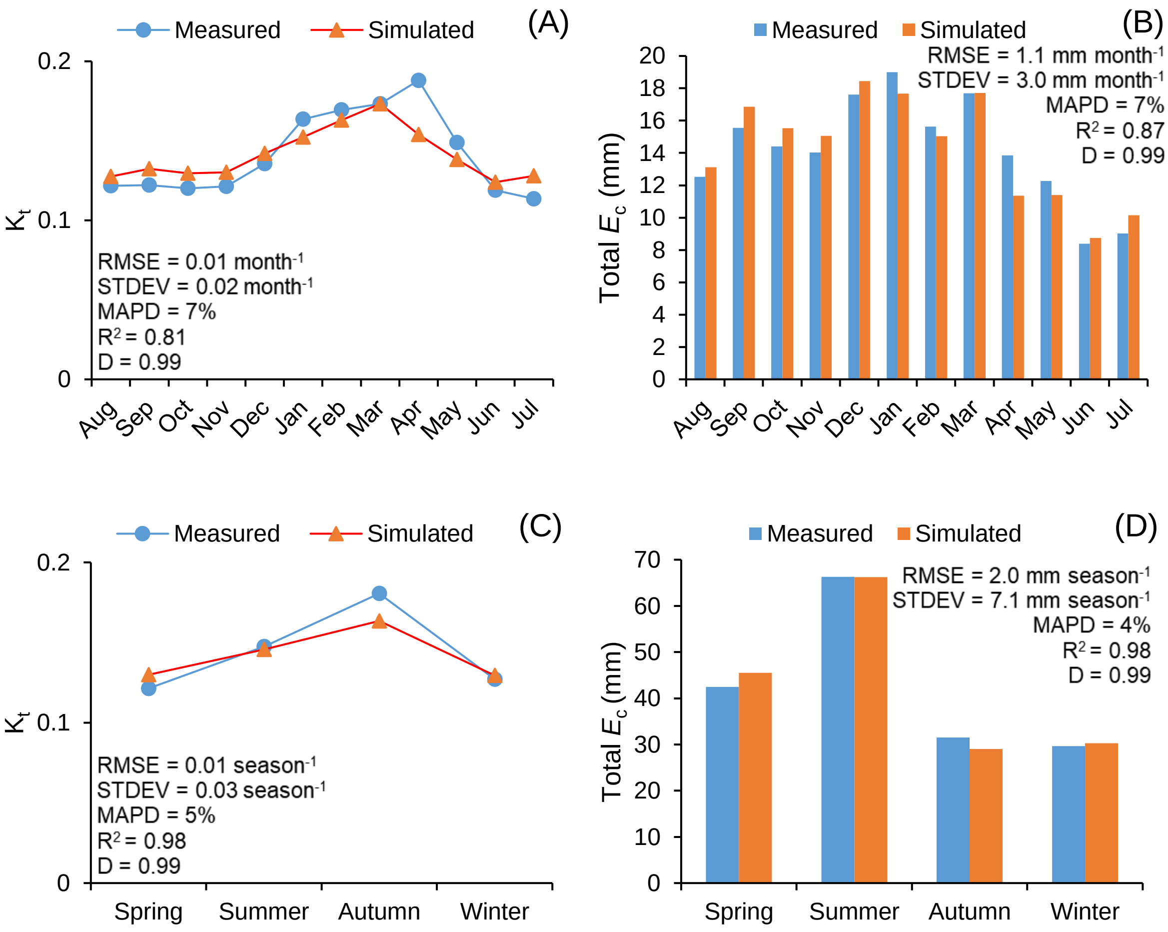

Figure 7.7: Comparison between monthly (A, B) and seasonally (C, D) measured and simulated transpiration crop coefficients (K_t) and transpiration (E_c) for the immature bearing (IB) orchard during the model validation phase (2018/08/01 – 2019/07/31) of the FAO-56 dual crop coefficient model using a single r_s value.

7.4.2 Estimates of E_c using a canopy conductance model in conjunction with the Penman-Monteith equation

The successful use of the Penman-Monteith equation to estimate E_c requires reliable estimates or measurements of both g_a and g_c. Calculated g_a throughout the duration of the trial, yielded a daytime (0800 h to 1700 h) average value of 75 ± 31 mm s^{-1} in the MB orchard and 37 ± 19 mm s^{-1} in the IB orchard. These values, although high, were similar to values of g_a measured (average 68 mm s^{-1}) during a window period of eddy covariance measurements in the MB orchard when using windspeed above the canopy and friction velocity determined using the 3D sonic anemometer, as described by Kumagai et al. (2004). Average daytime (0800 h to 1700 h) calculations of g_c, made during the model parameterization period (2016/09/15 – 2017/01/15), were rather low (0.7 mm s^{-1} in the MB orchard), with the absolute maximum calculated g_c obtained during the same period being 1.2 mm s^{-1} in the MB orchard. Following the successful parameterization of the Jarvis-type g_c model (Equation 7.14), it was determined that maximum g_c ($g_{c\ max}$) in the MB orchard was 1.2 mm s^{-1} (Table 7.5). Maximum calculated and parameterized values of g_c were similar, implying that $g_{c\ max}$ obtained through least squares regression analysis was a fair measure of maximum g_c. Both maximum and average calculated g_c values were substantially lower than those reported by Villalobos et al. (2013) for citrus and olive (1.6 – 2.2 mm s^{-1}) and a range of deciduous tree crops (5.4 – 8.1 mm s^{-1}). These low g_c values calculated for macadamia mostly stems from the low E_c rates recorded in both MB and IB orchards in this study.

Table 7.5: Optimised parameters for Equation 7.14 – 7.18 used to model canopy conductance (g_c). Parameters were generated through non-linear least squares regression analysis using data from the mature bearing (MB) macadamia orchard.

Parameter	Value
$g_{c\ max}$ (mm s^{-1})	1.2076
k_{D1} (kPa)	-0.1377
k_{D2} (kPa)	-0.3178
k_T (^oC)	22.54
k_R (W m^{-2})	17.83
R^2	0.83

Measured (calculated from the inverted Penman-Montieth equation) daytime average g_c across the study period was 0.7 mm s^{-1} in the MB orchard, and 0.3 mm s^{-1} in the IB orchard, and were similar to average modelled g_c. Although the Jarvis-Steward g_c (Equation 7.14) model provided satisfactory estimates of g_c during the model parameterization phase (Figure 7.8 A), model acceptability criteria were not met during the validation phase in both orchards (Figure 7.8 B & C). The discrepancies between simulated and measured g_c, largely stems from the observed seasonal variation of measured g_c (Figure 7.8 D & E). Measured g_c reached a maximum during summer, whereafter g_c declined gradually from the end of February until reaching a minimum in winter (Figure 7.8 D & E). The seasonal variation in measured g_c is, however, expected given that both R_s and T_{air} are lower, and VPD_{air} is slightly higher during the winter months compared to summer months (Figure 7.8 F).

Modelled g_c tended to follow a similar seasonal trend, but some discrepancies between measured and modelled g_c were evident throughout the trial. In the MB orchard, there was a substantial overestimation of g_c from May to October, which coincides with significant changes in canopy size, as a result of pruning, as well as physiological changes brought about by fruit removal. In April, a period where E_c has been shown to be significantly higher without significant increases in canopy size and atmospheric evaporative demand (see Chapter 5), g_c was underestimated by ~20%. Similar results were observed in the IB orchard during the April period. The model, however, tended to underestimate g_c from March – May and October – December, whilst overestimating g_c in the July-August period. In the IB orchard, the discrepancies between measured and modelled g_c was largely attributed to increases and decreases in canopy size. These observations imply that g_c is sensitive to changes in LAI, and $g_{c\ max}$ would need adjustment throughout the season in order to obtain accurate estimates of g_c in macadamias.

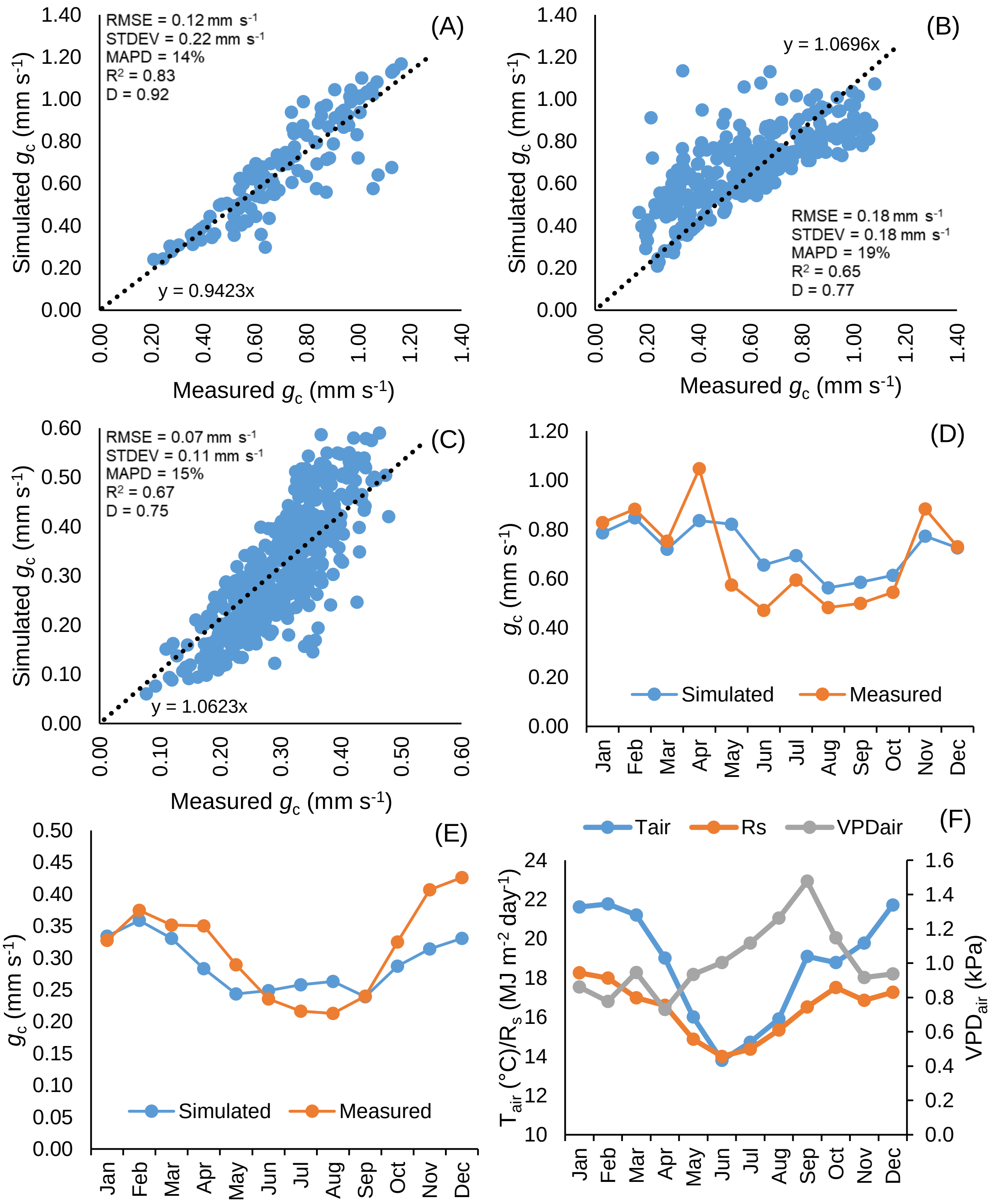

Figure 7.8: Hourly measured (calculated by inverting the penman-montieth equation) and simulated (using Equation 7.14) daily averaged canopy conductance (*g_c*) for the mature bearing (MB) orchard during the (A) model parameterization phase (2016/09/15 – 2017/01/15), (B) validation phase (2017/08/16 – 2018/08/06) and (C) during the model validation phase (2017/09/30 – 2019/04/30) in the immature bearing (IB) orchard. Average monthly measured and simulated *g_c* in the (D) MB and (E) IB orchard and (F) weather variables including air temperature (T_{air}), solar radiation (R_s) and air vapour pressure deficit (VPD_{air}).

Nevertheless, due to the low g_c measured for macadamias in this study, especially in relation to the high estimated g_a, the average calculated decoupling coefficient (Ω) (Jarvis & McNaughton 1986) for both orchards in this study equalled 0.08. This would imply that macadamia canopies are well coupled to the atmosphere. The strong degree of coupling observed in this study, is characteristic of tall, rough crops where windspeed is sufficient to cause sufficient mixing of the atmosphere resulting in high g_a (Jarvis & McNaughton 1986). This has been shown in a range subtropical fruit tree crops, with Ω <0.15 in citrus (Marin and Angelocci 2011; Marin et al. 2016) and olive (Tognetti et al. 2009). Similar results have also been for macadamia, with Ω reported by Ibraimo (2018) being <0.25. Well coupled canopies would suggest that g_c responds to bulk atmospheric conditions, which is evident from Figure 7.8, and would imply that changes in g_c would result in changes in E_c. The response of E_c to varying atmospheric conditions, should therefore be similar to the response of g_c to the same set of atmospheric conditions.

Failing to account for the effect of LAI on g_c, would therefore lead to decreased accuracy in simulations of E_c. This was evident in this study, with estimated total daily E_c using unadjusted $g_{c\ max}$ in combination with the Penman-Monteith equation (Equation 7.11), yielding a good correlation between measured and simulated E_c in the MB orchard during the model parameterization phase (Figure 7.9 A). However, when $g_{c\ max}$ was left unadjusted during the model validation phase (2017/08/16 – 2018/08/06), the model failed to produce acceptable estimations of E_c in the MB orchard (Figure 7.9 B). Poor model performance was largely as a result of overestimations of E_c during spring (August – September 2017) and winter periods (June – July 2018).

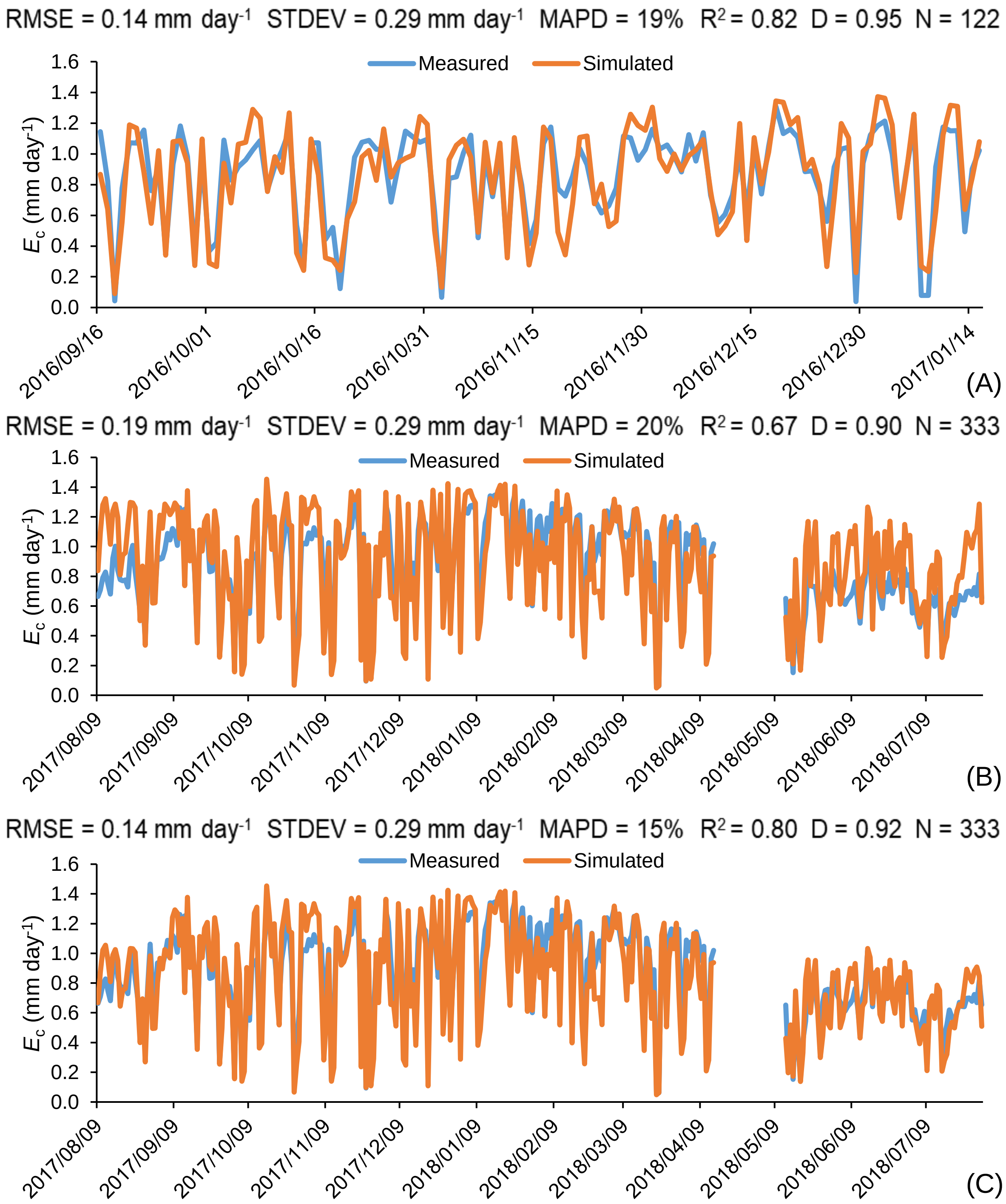

Figure 7.9: Comparison between hourly measured and simulated, transpiration (E_c) totalled on a daily basis, for the mature bearing (MB) orchard during the (A) parameterization phase (2016/09/15 – 2017/01/15) and validation (2017/08/16 – 2018/08/06) phase of the canopy conductance model using an (B) unadjusted and (C) adjusted maximum canopy conductance ($g_{c\ max}$). Missing data is due to missing solar radiation data as a result of equipment failure.

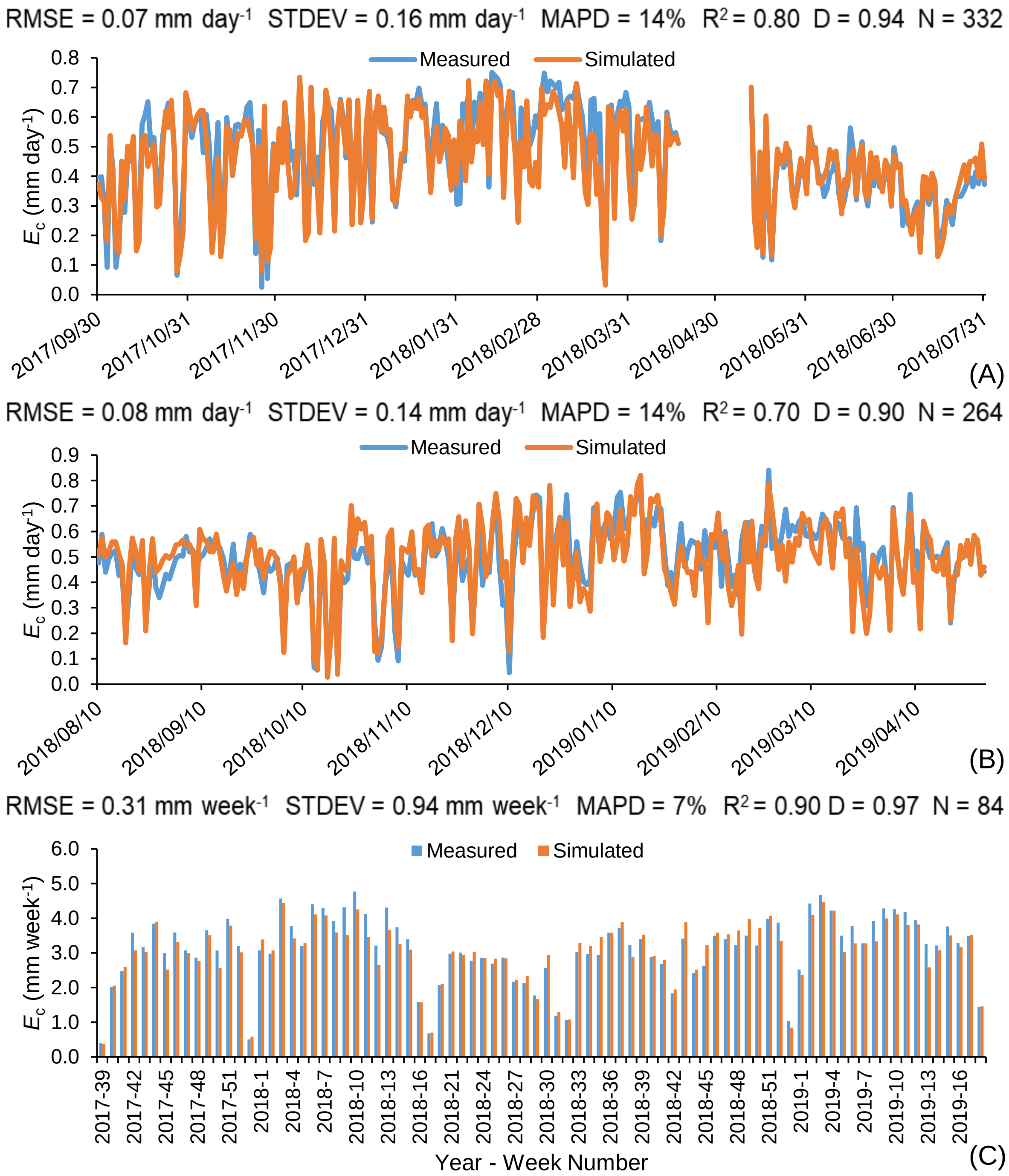

Figure 7.10: Comparison between hourly measured and simulated, transpiration (*E*$_c$) totalled on a daily basis, for the immature bearing (IB) orchard during the (A) first validation (2017/09/30 – 2018/07/31) and (B) second validation (2018/08/10 – 2019/04/30) phase and (C) hourly measured and simulated weekly totalled transpiration (*E*$_c$) throughout both validation periods of the canopy conductance model using adjusted maximum canopy conductance ($g_{c\ max}$). Missing data is due to missing solar radiation data as a result of equipment failure.

These overestimations in the MB orchard most likely stem from the overestimation of $g_{c\ max}$ for these periods, which in turn was linked to a decrease in LAI compared to that used during the model parameterization phase. Results from Chapter 6, Section 6.4.2, shows that average LAI during the model parameterization phase was 5.4 $m^2\ m^{-2}$ compared to 4.3 $m^2\ m^{-2}$ before spring of 2017, and 4.4 $m^2\ m^{-2}$ during the winter of 2018. The decrease in LAI observed in the MB orchard was as a result of pruning, which attempted to increase radiation penetration into canopies, whilst also reducing tree height. Nevertheless, parameterization, of especially $g_{c\ max}$, during the period when LAI was higher could therefore have resulted in a higher $g_{c\ max}$, as a result of the higher E_c linked to the higher LAI, thereby leading to an overestimation during spring and winter in the MB orchard. Seasonal variations in $g_{c\ max}$, as a result of variations in leaf area have also been observed in olive (Testi et al. 2006). The importance of accounting for canopy size in the $g_{c\ max}$ estimate was confirmed, as adjusting $g_{c\ max}$ using LAI (Equation 7.21) lead to increased model accuracy (Figure 7.10 C), with RMSE being 0.14 mm day^{-1}, $R^2>0.8$ and $D>0.9$ which were all well within the modelling acceptability criteria.

The IB orchard had LAI of 1.8 $m^2\ m^{-2}$ during the 2017 - 2018 season and an average LAI of 2.2 $m^2\ m^{-2}$ during the 2018 - 2019 season and following downward adjustment of $g_{c\ max}$, which equalled the LAI ratio between the MB and IB orchard, the model provided reasonable estimates of E_c for the IB orchard over the two model validation periods (Figure 7.10 A & B). During the first validation phase (2017/09/30 – 2018/07/31), all statistical criteria for acceptable model accuracy were met, with RMSE being 0.07 mm day^{-1} and both R^2 and D exceeding 0.8. During the second validation phase (2018/08/10 – 2019/04/30) RMSE and R^2 were, however, outside of the specified criteria. The largest variation between simulated and measured E_c in the IB orchard was observed from the start of January 2019 to the end of April 2019. Although no noticeable changes in LAI occurred at this time, the period coincided with the oil filling and nut maturation. Variable stomatal and transpirational responses to environmental variables have been demonstrated in this study (see Chapters 4 and 5), which would imply that model performance would be influenced by physiological changes unaccounted for by model parameters. The removal of this specific period from the model validation phase in the IB orchard led to a substantial increase in statistical parameters, with RMSE decreasing to 0.07 mm day^{-1} and both R^2 and D exceeding 0.8.

Seeing that similar discrepancies between measured and simulated E_c were found during the January 2018 – March 2018 period in both the MB (Figure 7.9) and IB (Figure 7.10) orchards, it was proposed that the removal of these periods from the model validation period would lead to increased model performance. The removal of these periods, only led to a slight improvement in statistical parameters in both the MB and IB orchard (data not shown), which was largely as a result of missing hourly solar radiation data during April 2018, which is a period during which the model tends to significantly underestimate g_c (Figure 7.8). Given that improvements in model accuracy were achieved by removing the January - April periods from analysis, the error in estimated E_c during this period is most probably linked to poor estimates of g_c, which was attributed to physiological upregulation of g_s which was not accounted for during parameterisation of the g_c model.

The accuracy of the g_c model in the IB orchard was also improved once hourly estimated E_c values were totalled on a weekly basis, with RMSE being 0.31 mm week^{-1}, and both R^2 and D exceeding 0.9. Accurate estimates of E_c over a weekly period provide irrigators with enough information to assess irrigation practices retrospectively and make adjustments accordingly in the following week. Although accurate estimates of E_c could be achieved using the Penman-Monteith (Monteith and Unsworth, 1990) equation, it required accurate estimates of both g_c and g_a. In this study reasonable estimates of g_c could be obtained in two orchards of varying canopy size using the Jarvis (1976) model, provided that $g_{c\ max}$ was adjusted for canopy size, using measurements of LAI (Equation 7.22). Although estimates of g_a were fairly high in this study, they were not unreasonably high compared to average g_a estimated in arrange of forest canopies (Mallick et al. 2016; de Aguiar et al. 2017). It is, however, not always fair to compare estimates of g_a between studies, as a number of factors, including tree height and prevailing windspeed, influence these estimates. Nevertheless, McNaughton and Jarvis (1983) suggested that in the case of well-ventilated canopies, such as orchards, the role of g_a is far less critical than g_c in determining E_c. Studies on olive by Villalobos et al. (2000) and Orgaz et al. (2007) have reaffirmed this statement, by showing that estimates of E_c, in well coupled olive orchards, were not sensitive to changes in g_a. Orgaz et al. (2007), however, noted that the sensitivity of E_c to changes in g_a would increase substantially in orchards which are decoupled from the atmosphere, a phenomenon which commonly occurs at low winspeed, largely because boundary layer conductance has a significant effect on g_c and small changes in g_a would have a substantial effect on E_c in these crops.

Given that this study is the first to successfully parameterize the g_c Jarvis model (Jarvis 1976) for macadamias, it is unclear if the model parameters (Table 7.5) used in this study would be transferable to other orchards and environments. Even if these parameters are transferable, the difficulty in obtaining accurate measurements of LAI used to scale $g_{c\ max}$ makes this modelling approach less favourable compared to the model by Whitley et al. (2009), which only requires an estimate of $E_{c\ max}$. The accuracy of the Whitley et al. (2009) model has, however, not been established in macadamias, but if proven to be accurate could be a simplified alternative for the combined approach using the Jarvis (1976) model and Penman-Monteith (Monteith and Unsworth, 1990) equation.

7.4.3 Estimates of E_c using a using a modified Jarvis steward type model as proposed by Whitley et al. (2009)

The simplified model suggested by Whitley et al. (2009) requires similar parameterization to that of the canopy conductance model. This model was successfully parameterized, with model variables used in the simulation of E_c presented in Table 7.6. Parameterization through non-linear least squares regression analysis yielded a maximum E_c ($E_{c\ max}$) rate of 0.17 mm h^{-1} and was exactly the same as maximum hourly measured E_c in the MB orchard. There was also no difference between the daily maximum E_c measured (1.5 mm day^{-1}) in the MB orchard and that simulated by the model. Daily maximum E_c measured in this study was similar to that found in olive (1.1 – 2.3 mm day^{-1}) (Santos et al. 2007; Rousseaux et al. 2009; Masmoudi et al. 2010), implying that E_c measurements in this study were reasonable.

Table 7.6: Optimised parameters for Equation 7.20 used to model transpiration (E_c). Parameters were generated through non-linear least squares regression analysis using data from the mature bearing (MB) macadamia orchard.

Parameter	Value
$E_{c\ max}$ (mm h^{-1})	0.174
K_{e1} (kPa)	1.672
K_{e2} (kPa)	0.618
k_T (°C)	44.36
k_R (W m^{-2})	12.46
R^2	0.82

During the parameterization phase (2016/09/16 – 2017/02/15) of the Whitley et al. (2009) model, the model simulated daily E_c in the MB orchard with a high degree of accuracy, as all the statistical criteria were met during this phase (Figure 7.11 A). The model also provided reasonable estimates of daily E_c in the MB orchard during the model validation phase (2017/08/09 – 2018/07/31) (Figure 7.11 B), only underestimating total seasonal transpiration by 5 mm, whilst meeting all the statistical criteria for acceptable model accuracy. Seeing that $f_{c\ eff}$ varied little between the model parameterization and validation period, no adjustment in $E_{c\ max}$ was needed in the MB orchard to achieve reasonable estimates of E_c.

Adjustments in $E_{c\ max}$ were, however, required in the IB orchard to achieve reasonable estimates of daily E_c given the large changes in $f_{c\ eff}$ during the measurement period. The Whitley et al. (2009) model meeting model accuracy criteria during the first model validation phase in the IB orchard (Figure 7.12 A). During this phase, RMSE was 0.07 mm day^{-1} and both R^2 and D exceeded 0.8. Model performance was similar during the second validation phase in the IB orchard (Figure 7.12 B), with the exception of R^2 which was lower ($R^2 = 0.65$). Similar to the g_c model, the Whitley et al. (2009) model was most inaccurate from the start of January to the end of March, when E_c was underestimating on a daily basis. This period corresponds to the nut maturation and oil filling period in macadamias, during which increases in E_c have been reported without corresponding increases in atmospheric evaporative demand. Removal of this period from the model validation phase resulted in substantial improvements in statistical parameters, with RMSE decreasing to 0.05 mm day^{-1} and R^2 exceeding 0.8, whilst D was greater than 0.95.

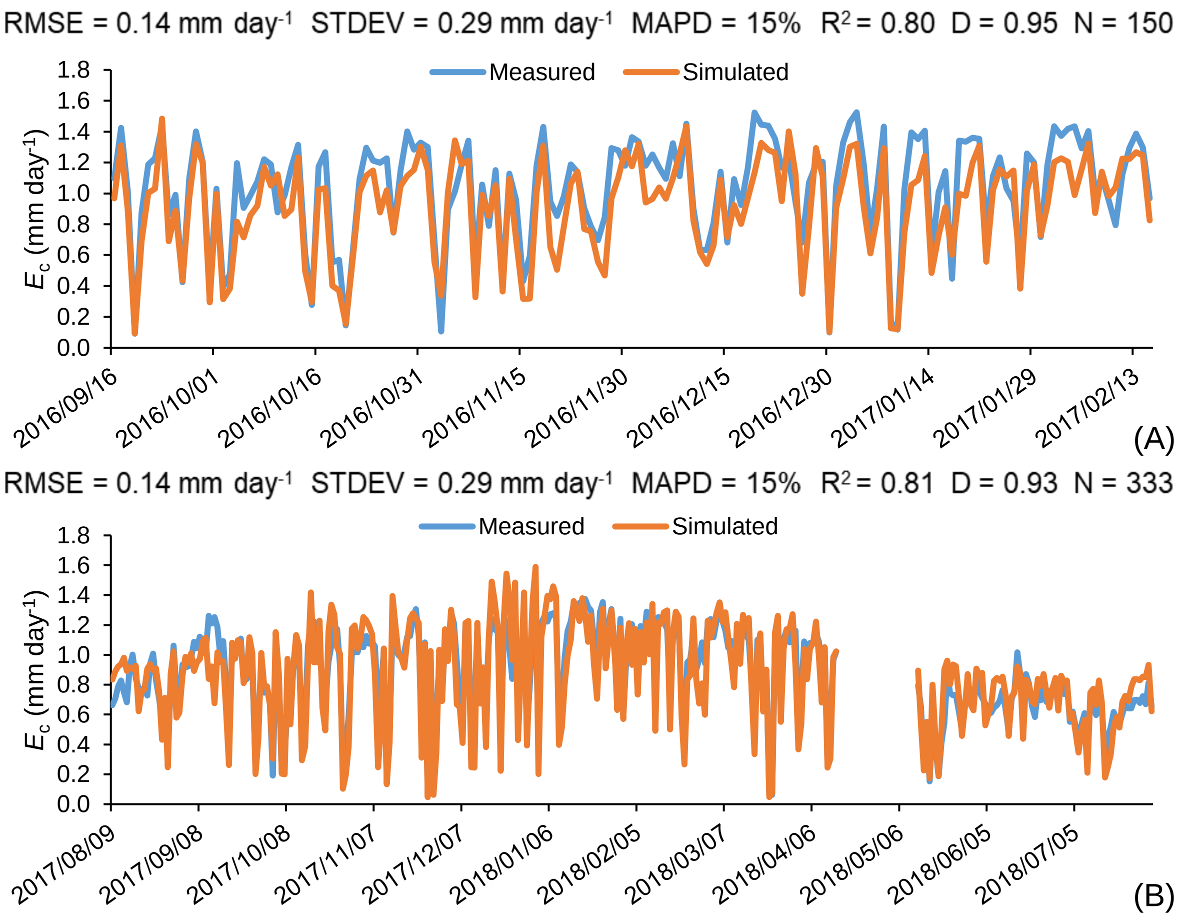

Figure 7.11: Comparison between hourly measured and simulated transpiration (E_c) totalled on a daily basis for the mature bearing (MB) orchard during the (A) parameterization (2016/09/16 – 2017/02/15) and (B) validation (2017/08/16 – 2018/08/06) of the Whitley et al. (2009) model. Missing data is due to missing solar radiation data as a result of equipment failure.

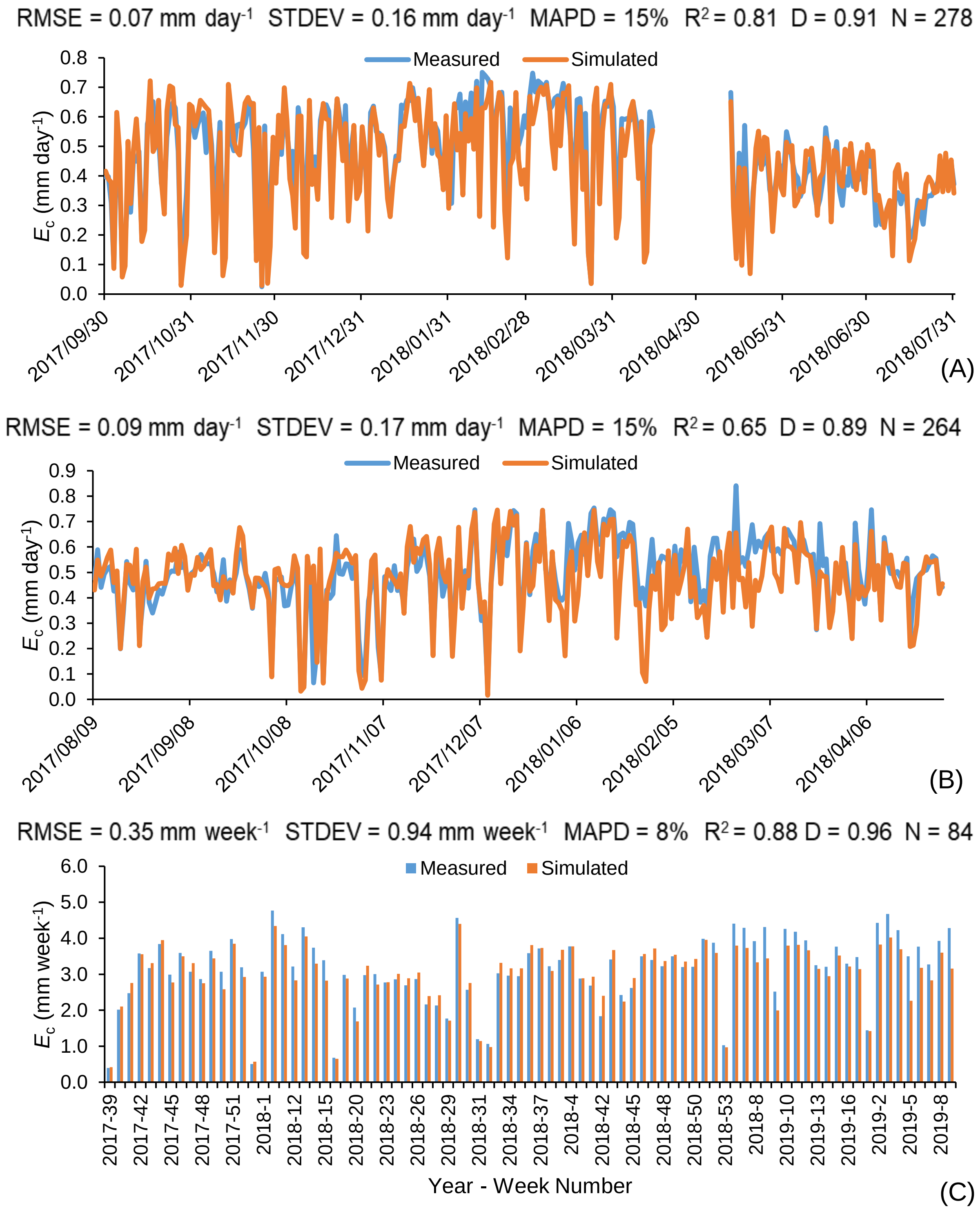

Figure 7.12: Comparison between hourly measured and simulated transpiration (E_c) totalled on a daily basis for the immature bearing (IB) orchard during the (A) first validation (2017/09/30 – 2018/07/31) and (B) second validation (2018/08/10 – 2019/04/30) phase and (C) hourly measured and simulated weekly totalled transpiration (E_c) throughout both validation periods of the Whitley et al. (2009) model. Missing data is due to missing solar radiation data as a result of equipment failure.

The Whitley et al. (2009) model also provided reasonable estimates for hourly simulations of E_c totalled on a weekly basis (Figure 7.12 C), with RMSE being 0.36 mm week^{-1} and both R^2 and D exceeding 0.8. The model, however, slightly underestimated total E_c over a close to two year period by 10 mm, which given the fact that the model requires only a few easily measurable parameters, with the exception of $E_{c\,max}$, is rather exceptional. The successful parameterization and validation of the model in this study provides an alternative approach to the parameter intensive g_c model, but given that an independent data set in a different climatic region was not available for evaluating this model, the approach should be used with caution.

The model by Whitley et al. (2009) should, however, be well suited to crops which are well coupled to the atmosphere and exert strong stomatal control over transpiration. The model assumes that there is a maximum transpiration rate which would not be exceeded, and is only achieved once optimal environmental conditions are present. The premise of the model is therefore in line with the behaviour of an isohydric crop, where leaf water potential prevented from dropping below a critical level, by strictly controlling water loss through stomatal control, which would imply that the maximum rate of E_c would not be exceeded, irrespective of atmospheric evaporative demand.

7.5 Conclusion

A number of models are available for estimation of transpiration, which differ greatly in complexity. One of the most commonly used models to estimate E_c is the FAO-56 dual crop coefficient approach which is based on the premise that E_c is a demand limited process. Macadamia E_c is, however, supply controlled, and a canopy conductance (g_c) modelling approach should be better suited to estimate macadamia E_c. These models, however, require reliable measurements or estimates of g_c and aerodynamic conductance (g_a) to obtain accurate estimates of E_c, which are often difficult to determine, and are site/orchard specific. As a result, there is an opportunity to evaluate the accuracy of less parameter intensive models to estimate macadamia E_c, which would be more readily applied by a number of end users.

Given that macadamia E_c is a supply controlled system, arising from strict stomatal control in response to increases in atmospheric evaporative demand, it was not surprising that the empirical FAO-56 dual crop coefficient model provided poor estimates of daily K_t and E_c. The discrepancies between measured and modelled K_t and E_c, stem from an overestimation of K_t and E_c at ET_o rates > 4.0 mm day^{-1}, and an underestimation of K_t and E_c when ET_o < 2.0 mm day^{-1}. These over and under estimations of K_t and E_c were largely due to the linear relationship between r_{leaf} and r_s and ET_o. The model, however, provided reasonable estimates of monthly and seasonal K_t and E_c in both the MB and IB orchard, which is most likely due to compensatory errors over the longer period of estimation. The ability of the FAO-56 dual crop coefficient model to simulate K_t and E_c over longer periods of time makes this model a valuable tool for water licencing authorities and strategic irrigation planning and management.

On a daily time step, the Jarvis-Steward g_c model in conjunction with the Penman-Monteith equation, provided reasonable estimates of E_c, but was shown to be particularly sensitive to seasonal changes in LAI. Only after adjustments in $g_{c\ max}$ using LAI, did the model provide accurate estimates of E_c in both orchards. The model, however, failed to provide accurate estimates of both g_c and E_c, by consistently under estimating g_c and E_c during specifically April, a period during which the presence of fruit has been shown to cause a significant upregulation in stomatal conductance and E_c, further reiterating the significant impact of phenology and physiology on macadamia E_c. It also emphasizes the fact that although a model, such as the Jarvis-Steward g_c model, is able to account for significant variability in environmental conditions, the model failed to account for physiological and phenological changes which significantly impact g_c and subsequently E_c.

Regardless of the possible limitation of the Jarvis-Steward type g_c model, measurements of g_c in macadamia orchards are rather low (0.3 – 0.7 mm s^{-1}) in relation to g_a (37 - 75 mm s^{-1}), confirming that macadamias are well coupled to the atmosphere ($\Omega = 0.08$). The high degree of coupling in macadamia implies that changes in g_c would lead to direct changes in E_c, which contributed to the success of a simplified E_c model. This model provided reasonable estimates of daily E_c, without multiple adjustments for canopy size being needed within each of the orchards. The use of this simplified model, which performed comparably to the parameter intensive g_c model, provides both scientist and researchers with an alternative approach to estimate E_c in a range of macadamia orchards. Considering that this model provided accurate estimates of E_c on a daily or weekly basis, it could be used for irrigation scheduling, which would be of great value to the macadamia industry. It should, however, be emphasized that although this study has successfully parameterized three different E_c models, and alluded to the various possible limitations of these models, these models have not been tested in other orchards and environments. A large degree of uncertainty regarding the transferability of model parameters derived in this study, to other macadamia orchards therefore exists. For example, the use of the FAO-56 dual crop coefficient approach would be limited to environments where average ET_o is between 2.0 – 4.0 mm day^{-1}, whilst parameters such as $g_{c\ max}$ and $E_{c\ max}$ could vary between cultivars, thereby limiting widespread implementation of the mechanistic model evaluated in this study. Furthermore, before these models could be used to successfully schedule irrigation, soil evaporation needs to be accounted for in order to quantify total crop water use.

CHAPTER 8: GENERAL CONCLUSIONS AND RECOMMENDATIONS

8.1 Study Conclusions

Despite the rapid expansion of irrigated macadamia production, information regarding water use of the crop is lacking. In order to provide producers with water use guidelines which are applicable to a range of environments, this study examined the mechanisms and driving variables of macadamia water use to select and parameterize water use models. This study has therefore provided valuable insight into the seasonal water use dynamics of macadamias. Not only has it shown that macadamias have substantial stomatal and non-stomatal limitations to net CO_2 assimilation, it has shown that the crop is predominantly isohydric in nature, a trait which has a significant effect on the E_c of macadamias. Furthermore, it has revealed that macadamia phenology, in particular the presence of oil storing fruits, can have a significant effect on crop physiology which resulted seasonal variations of E_c, which were unaccounted for by variations in the major driving variables of macadamia E_c being canopy size and atmospheric evaporative demand. Lastly, this study has used measurements of leaf gas exchange and E_c, to successfully select, parameterize, and validate a range of E_c models in two field-grown macadamia orchards of differing canopy size

Fairly low net CO_2 assimilation (A) rates were obtained for macadamias in this study, especially compared to other subtropical evergreen crops, such as citrus and olive. These low A values could largely be explained by both stomatal and non-stomatal limitations to A, but could also relate to the hydraulic limitation identified in the leaf to stem interface. Non-stomatal limitations in macadamias, which include previously demonstrated low mesophyll conductance (g_m) and light limitations within the internal leaf space, is attributed to the sclerophyllous nature of macadamia leaves. Stomatal limitations to A accounted for approximately one third of the total limitation to carbon assimilation in macadamias. Understanding stomatal behaviour, and more specifically stomatal conductance (g_s), was therefore essential to understanding the relationship between carbon gain and water lost through transpiration. Macadamia g_s is carefully controlled in response to increasing leaf vapour pressure deficit (VPD_{leaf}), with a decline in g_s being observed when VPD_{leaf} exceeded 2.50 kPa. Strict stomatal control was accompanied with nearly constant midday leaf water potentials, which is typical of isohydric water management strategies in plants. Isohydric behaviour is often linked to an underlying hydraulic limitation, which necessitates strict leaf level control of g_s which could possibly reflect a need to avoid hydraulic failure hydraulic failure as a result of xylem embolism. An examination of hydraulic conductance within macadamias has showed that although whole tree hydraulic conductance is comparable with other tree crops, there is a significant hydraulic limitation within the stem to leaf interface. This hydraulic limitation most likely leads to decreases in relative water content within the leaf space, directly resulting in decreased g_s under conditions of high VPD_{leaf}.

The distinct stomatal behaviour shared between predominantly isohydric tree crops, creates an interesting scenario when examining whole tree transpiration (E_c). It raises the question that if macadamias are predominantly isohydric, and display strict leaf level control of g_s under conditions of high VPD_{leaf}, would E_c respond in a similar fashion? This study suggests that this is exactly what happens in macadamias. Under conditions of non-limiting soil water, macadamia E_c increased linearly with air vapour pressure deficit (VPD_{air}) and reference evapotranspiration (ET_o) at low atmospheric evaporative demands (VPD_{air} < 0.8 kPa and ET_o <0.13 mm day^{-1}), but at higher atmospheric evaporative demands the rate of increase in macadamia E_c decreased, suggesting that macadamia E_c is a water supply controlled system. Supply controlled E_c implies that the rate of water supply to the leaves is substantially lower than the atmospheric evaporative demand. It could be argued that a supply controlled system is synonymous with crops which follow a predominantly isohydric water management strategy, given that isohydricity largely stems from a hydraulic limitation within crops, which is managed through strict stomatal control of water loss. Nevertheless, responses of E_c to a range of weather variables were between the orchards examined in this study.

Differences in the magnitude of E_c were, however, observed between the two orchards examined. The distinguishing factor between the two orchards was canopy size, with trees in the mature bearing (MB) orchard being approximately double the size of trees, on a LAI basis, within the immature bearing (IB) orchard. Larger trees in the MB orchard transpired approximately 60% more than trees in the IB orchard exposed to the same set of weather conditions, confirming that E_c is significantly influenced by canopy size.

An examination of transpiration crop coefficients (K_t), which was used to study the variation of E_c in relation to ET_o, showed that there was a large degree of variation in seasonal K_t, which could largely be attributed to variations in canopy size. Although macadamia E_c is greatly influenced by canopy size, this study has shown that significant increases in E_c, and subsequently K_t, occurred during certain phenological periods, without any substantial increases in canopy size or ET_o. The most significant of these increases in K_t was observed during April, where both E_c and K_t were approximately 20% higher compared to that measured in May, with the only significant difference during these periods being the presence or absence of fruit. This would imply that macadamia E_c is also substantially influenced by physiological changes brought about by variation in crop phenology.

In order to determine causality between the upregulation of E_c and the presence of fruit, this study used measurements of leaf gas exchange on a combination of phloem girdled and un-girdled, fruiting and non-fruiting branches, to determine the physiological basis of increased E_c. Results from this study have shown that both A_{max} and g_s were significantly higher during fruiting compared to non-fruiting periods. The increases in A_{max} were mediated by increased rates of electron transport (J_{max}) and triose phosphate use (TPU), which is proposed to be as a result of increased transport of assimilates away from source leaves to oil accumulating fruit which act as substantial sinks. Although increased g_s during fruit bearing periods could be due to a range of factors, it is proposed that increased TPU during fruit bearing, which has been demonstrated in this study, results in a decreased rate of stomatal closure at VPD_{leaf} rates which would otherwise lead to a reduction in g_s. The increase in g_s in the presence of fruit, was not limited to fruit bearing branches, which is most likely due to the long distance transport of assimilates from source to sinks and not just from leaves subtending fruit. This also implies that physiological variations in g_s brought about by phenology can be scaled to a canopy level, and could influence E_c.

This study has demonstrated that macadamia E_c is under strict control of g_c, which was confirmed by the high degree of coupling (Ω = 0.08) in orchards. Well coupled macadamia canopies were attributed to low measured g_c (0.3 – 0.7 mm s^{-1}) in relation to g_a (37 - 75 mm s^{-1}). The high degree of coupling in macadamias has also added to the success of mechanistic modelling approaches used in this study, with the Jarvis-Steward type g_c model accurately estimating macadamia E_c on a daily or weekly basis when used in conjunction with the Penman-Monteith equation. This model was, however, parameter intensive and required reasonable estimates of both g_c and g_a, which can be difficult to obtain. Nevertheless, the study has demonstrated that although g_c could be estimated using a stomatal conductance model, multiple adjustments of the maximum canopy conductance ($g_{c\ max}$) model parameter was required to account for changes in leaf area index (LAI) over a season and as a result of pruning. The distinct relationship between g_c and E_c, as a result of the high degree of coupling, created an opportunity to estimate E_c directly using a simplified and less parameter intensive mechanistic model. The study therefore evaluated a model, similar to that proposed by Whitley et al. (2009), in macadamias. Not only did the model provide good estimates of E_c on both a daily and weekly basis, with comparable accuracy to the g_c model, it only required an adjustment for canopy size between orchards, using easily obtained measures of canopy dimensions including canopy width and breadth used in the calculation of the effective fraction of ground cover ($f_{c\ eff}$). It has therefore been shown that the model could be used in macadamia orchards varying in canopy size, it is, however, unclear if this model would provide accurate estimates of E_c in different orchards, especially those consisting of cultivars different to the one used in this study, seeing that $E_{c\ max}$ might differ substantially between cultivars.

Regardless of the observed success of mechanistic models to estimate E_c, this study also attempted to evaluate the ability of the widely used and accepted empirical FAO-56 dual crop coefficient model to estimate macadamia E_c, given that most farmers are familiar with the crop coefficient approach. As expected the model provided poor estimates of both K_t and E_c on a daily basis, which was largely attributed to an overestimation of K_t and E_c at daily ET_o rates > 4.0 mm day^{-1}, and an underestimation of K_t and E_c when ET_o < 2.0 mm day^{-1}. These over and under estimations of K_t and E_c was largely due to the linear relationship between r_{leaf} and r_s and ET_o, which stems from the fact that the model is based on the premise that E_c is a demand limited process, whilst macadamia E_c is a supply controlled system. The model, however, provided reasonable estimates of K_t and E_c on a monthly or seasonal basis, which is most likely due to the compensatory errors over the longer period of estimation. The FAO-56 dual crop coefficient model could therefore be used with a great degree of reliability by institutions that depend on crop coefficients to determine water allocations.

Regardless of the accuracy of both g_c and E_c models and the empirical FAO-56 dual crop coefficient model in estimating macadamia E_c, all these models showed discrepancies between measured and simulated E_c during especially April of each season, a period during which oil accumulation in fruits occur. The significant effect of crop phenology, in particular the presence of oil storing fruit, on crop physiology and subsequently g_s, g_c and E_c during the month of April, was not accounted for by any of the models evaluated in this study. This observation not only reiterates the significant effect of phenology and physiology on macadamia E_c observed in this study, but highlights another potential shortcoming of both mechanistic and empirical models evaluated. Although these models have been widely used, they fail to account for the physiological changes in g_s, g_c and subsequently E_c brought about by phenology. In this study, the failure to account for these changes led to an underestimation of E_c during the oil accumulation period, which could potentially lead to reduced fruit quality as a result of soil water stress, if these models where used for irrigation scheduling.

Even though the models evaluated in this study had certain shortcomings, this is the first study that has successfully parameterized and validated these models in macadamias. This has provided the macadamia industry with a strategic water and irrigation management tool in the form of the FAO-56 dual crop coefficient model, and an irrigation scheduling tool in the form of g_c and E_c models. Futhermore, the study has proposed methods to overcome some of the shortcomings of the models evaluated in this study, which could add significant value to the field of macadamia water use modeling. Caution should, however, be used before these models are fully implemented, largely due to the fact that the model parameters derived in this study have not been tested in different orchards and growing environments. Furthermore, it should be emphasized that the models evaluated in this study only provided estimates of E_c and estimates of soil evaporation would be required for the determination of the irrigation requirements of the crop.

8.2 Recommendations for future research

This study has focussed on the seasonal water use dynamics of macadamia orchards, and included a range of leaf gas exchange measurements, which were insightful from both a horticultural and ecological perspective. These measurements were, however, limited to one cultivar (HAES 695) which is a hybrid cross of *M. integrifolia* (Maiden & Betche) and *M. tetraphylla* (L.A.S. Johnson), and is the dominant cultivar planted in South Africa. This cultivar is, however, not as popular in other parts of the world, with most cultivars being descendants of *M. integrifolia*, a species which has evolved in a distinctly different environment to that of *M. tetraphylla*. It would therefore be of great interest to researchers to determine possible difference in leaf gas exchange between the various cultivars. Of particular interest would be to examine if differences in the response of A_{max} and g_s to increases in VPD_{leaf} exists between cultivars, and if these differences could help explain the cultivar performance in relation to growing environment.

Furthermore, the daily and seasonal total E_c reported in this study was unexpectedly low considering the size of trees used in this study. It would therefore be of great value to the macadamia industry to obtain additional measurements of E_c in a range of different orchards, consisting of different cultivars, to aid macadamia growers in increasing irrigation and water use efficiency. It is further proposed that additional measurements of E_c in macadamias is necessary to validate parameters generated in mechanistic models developed in this study. Of particular interest would be to determine if differences exist in both $g_{c\ max}$ and $E_{c\ max}$ between different cultivars, as these parameters could have a significant impact on model accuracy.

Lastly, a significant portion of this study has focused on the effect of sink strength on macadamia physiology. Most conclusions made in this study with regards to sink strength and assimilate transport in macadamias has, however, been on an observational basis. Future research focussing on experimental determination of source-sink relationships, assimilate partitioning and its effect on macadamia leaf gas exchange, would therefore be fundamental to our understanding of the relationship between E_c and carbon gain, at a leaf and canopy level. Furthermore, future research should attempt to incorporate physiological changes in leaf gas exchange, as brought about by changes in sink strength, into both empirical and mechanistic E_c models to improve the accuracy of these models.

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