

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

Theunis G Smit¹, Nicolette J Taylor^{1*} and Stephanie JE Midgley²

¹*Department of Plant and Soil Sciences, University of Pretoria, Private Bag X20, Pretoria 0028,
South Africa*

²*Department of Horticultural Science, Stellenbosch University,
Private Bag XI, Matieland 7602, South Africa*

*Corresponding author E-mail: nicolette.taylor@up.ac.za, tel +27 12 420 3666

Highlights

- Macadamias have fairly low net CO₂ assimilation rates.
- There are both stomatal and non-stomatal limitations to photosynthesis.
- Macadamias are predominantly isohydric.
- High sink periods associated with variable response of stomatal conductance to VPD.
- A hydraulic limitation exists within the stem to leaf pathway.

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 CO₂ assimilation rates (A_{\max}) ($8.34 \pm 1.21 \mu\text{mol CO}_2$

$\text{m}^{-2} \text{s}^{-1}$). 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 ($\psi_{\text{leaf}} = -1.16 \pm 0.43 \text{ MPa}$) 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} ($10.27 \pm 2.23 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) compared to non-fruiting periods ($A_{\text{max}} = 6.58 \pm 2.00 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). 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 are proposed to deviate from a purely isohydric water management strategy.

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

1. 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 (CO₂) 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 schlerophylly 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 favorable 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 behavior (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 tradeoff 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.

2. Materials and Methods

2.1 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 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 m² 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-fruited phenological stages were visually determined. Fruiting (F) stages (November to April) were regarded as periods after premature nut drop and before harvest, whilst non-fruited 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).

2.2 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 1.

Table 1: Mean weather variables, including air temperature (T_{air}), air vapour pressure deficit (VPD_{air}), solar radiation, 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. N is the number of replicate gas exchange measurements.

Measurement Date	N	T_{air} (°C)	VPD_{air} (kPa)	Solar Radiation (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 gas exchange parameters measured included net light-saturated CO₂ assimilation rate (A_{max}), stomatal conductance (g_s), and intercellular CO₂ 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 CO₂ concentration was maintained at 400 $\mu\text{mol mol}^{-1}$, the flow rate was 400 $\mu\text{mol s}^{-1}$, PAR inside the chamber was maintained between 1500 – 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (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.

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 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and CO_2 concentration (400, 300, 200, 150, 100, 50, 0, 400, 600, 700, 1000, 2000 $\mu\text{mol mol}^{-1}$) within the chamber. For light response curves the CO_2 concentration was controlled at 400 $\mu\text{mol mol}^{-1}$, whilst PAR was set at 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and a rate of change per minute less than 0.1 $\mu\text{mol m}^{-2} \text{s}^{-1}$. 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 (I) 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).

2.3 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 PTFE 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. Conducting sapwood was determined by injecting a weak solution of safranin stain into the stem and determining the width of stained tissue by extracting a core of wood using a stem corer. 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 Cohen and Naor (2002), where k was separated into the pathway from the soil to the stem ($k_{soil-stem}$) and from the stem to the leaves ($k_{stem-leaf}$). Daily values of k were calculated as a mean of daytime hourly values. The root-stem interface was calculated using Equation 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 2, with the fraction of sunlit canopy leaf area (α) 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 3, with leaf specific hydraulic conductance (k_L) calculated using Equation 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.

$$k_{\text{soil-stem}} = J / (\psi_{\text{soil}} - \psi_{\text{stem}}) \quad (1)$$

$$k_{\text{stem-leaf}} = J / (\psi_{\text{stem}} - (\alpha\psi_{\text{sun leaf}} + (1-\alpha)\psi_{\text{shade leaf}})) \quad (2)$$

$$k_{\text{soil-leaf}} = J / (\psi_{\text{soil}} - (\alpha\psi_{\text{sun leaf}} + (1-\alpha)\psi_{\text{shade leaf}})) \quad (3)$$

$$k_L = g_s / ((\psi_{\text{soil}} - \psi_{\text{sun leaf}}) / \text{VPD}_{\text{air}}) \quad (4)$$

2.4 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 $p \leq 0.05$.

3. Results

3.1 Seasonal Weather and crop phenology

Mean air temperature throughout the study period was 20.1°C, with a mean daily maximum of 26.4°C and a mean daily minimum of 12.2°C (Figure 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.

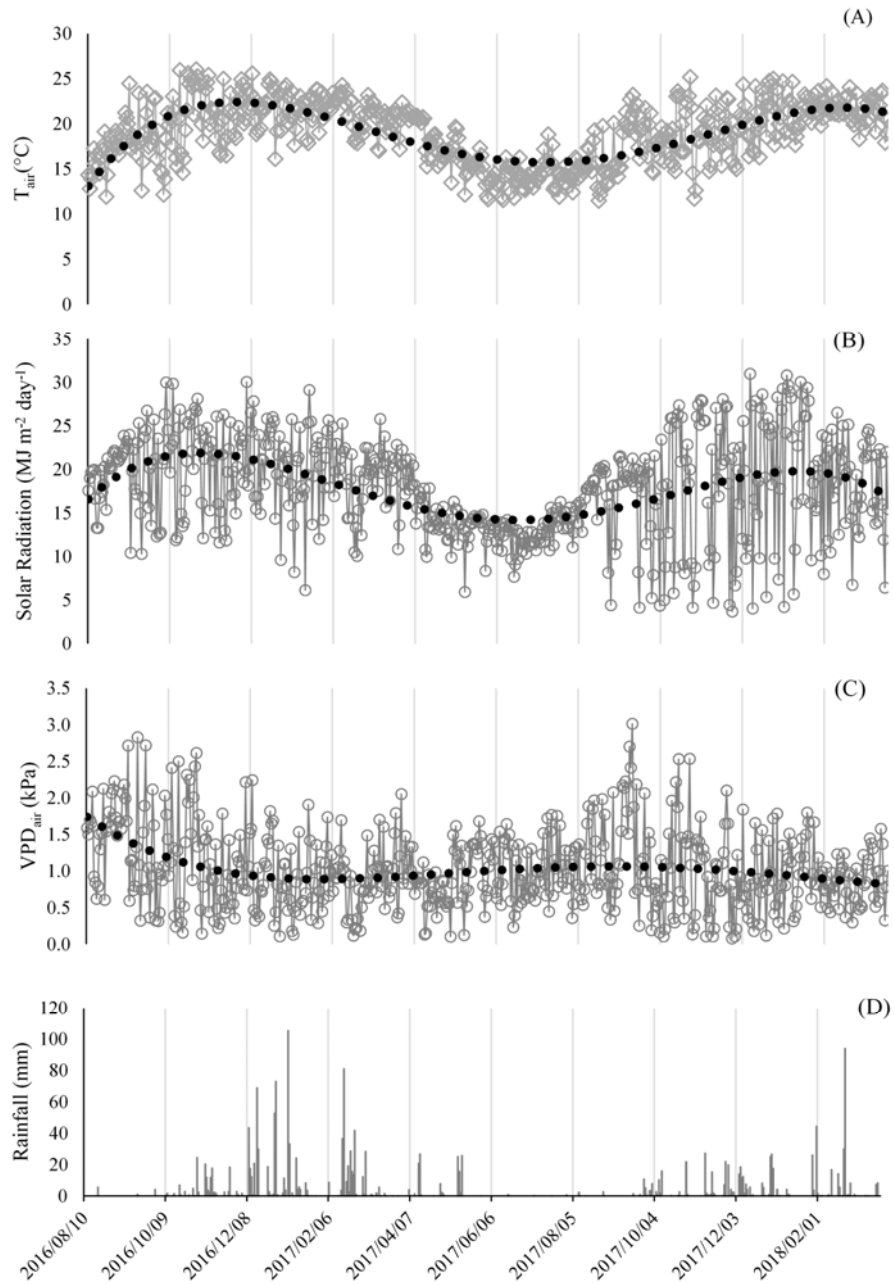


Figure 1: Daily weather variables recorded at the study site including (A) air temperature (T_{air}), (B) Solar radiation, (C) vapour pressure deficit (VPD_{air}) and (D) Rainfall. Dotted lines are polynomial trend lines fitted to daily data.

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 13 September 2017) 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 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 2 according to phenological stages, using the results of Stephenson et al. (2003). 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.

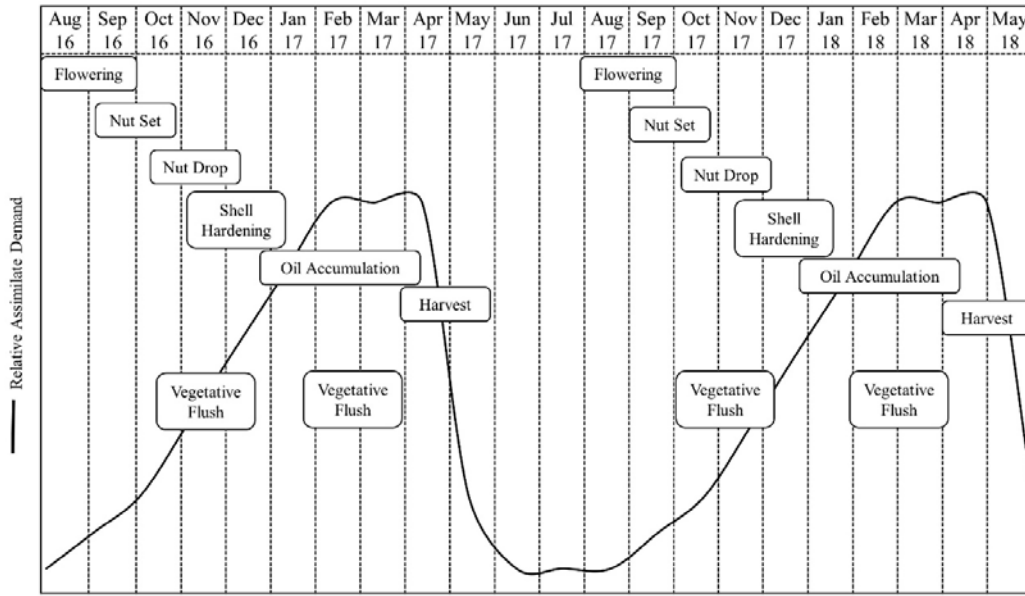


Figure 2: Major phenological events and harvest time of macadamia trees from August 2016 to May 2018 based on visual observations. Increases and decreases in relative assimilate demand throughout this period were estimated based on results by Stephenson et al. (1989) and approximately coincide with the presence or absence of fruit and especially the oil accumulation period.

3.2 Leaf gas exchange

3.2.1 Responses to environmental variables

Light-saturated rate of net CO₂ 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 3A). In response to increasing VPD_{leaf} , A_{max} reached a maximum between 1.0 – 2.5 kPa (Figure 3B), 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 3C). The relationship between A_{max} and g_s (Figure 3D) was non-saturating under the measured values of g_s .

In response to increasing C_i , A increased linearly up to approximately 180 $\mu\text{mol mol}^{-1}$, after which the response flattened out during the shift from the CO₂-limited region to the RuBP-limited region (Figure

3E). At C_i concentrations above $400 \mu\text{mol mol}^{-1}$ the mean A was approximately three times higher ($17.16 \mu\text{mol m}^{-2} \text{s}^{-1}$) than the mean value of A^1 (A at ambient C_i when $C_a = 400 \mu\text{mol mol}^{-1}$), which was approximately $6.64 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 3E). Stomatal limitation as calculated from these response curves amounted to approximately 33% of all limitations. Macadamias reached A_{max} at PAR levels of $>900 \mu\text{mol m}^{-2} \text{s}^{-1}$ (examples of photosynthetic light response curves are given in Figure 3F). Mean A_{max} obtained from light response curves ($8.77 \pm 2.49 \mu\text{mol m}^{-2} \text{s}^{-1}$) was comparable to mean seasonal spot measurements of A_{max} ($8.34 \pm 1.21 \mu\text{mol m}^{-2} \text{s}^{-1}$) made at PAR levels of $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$.

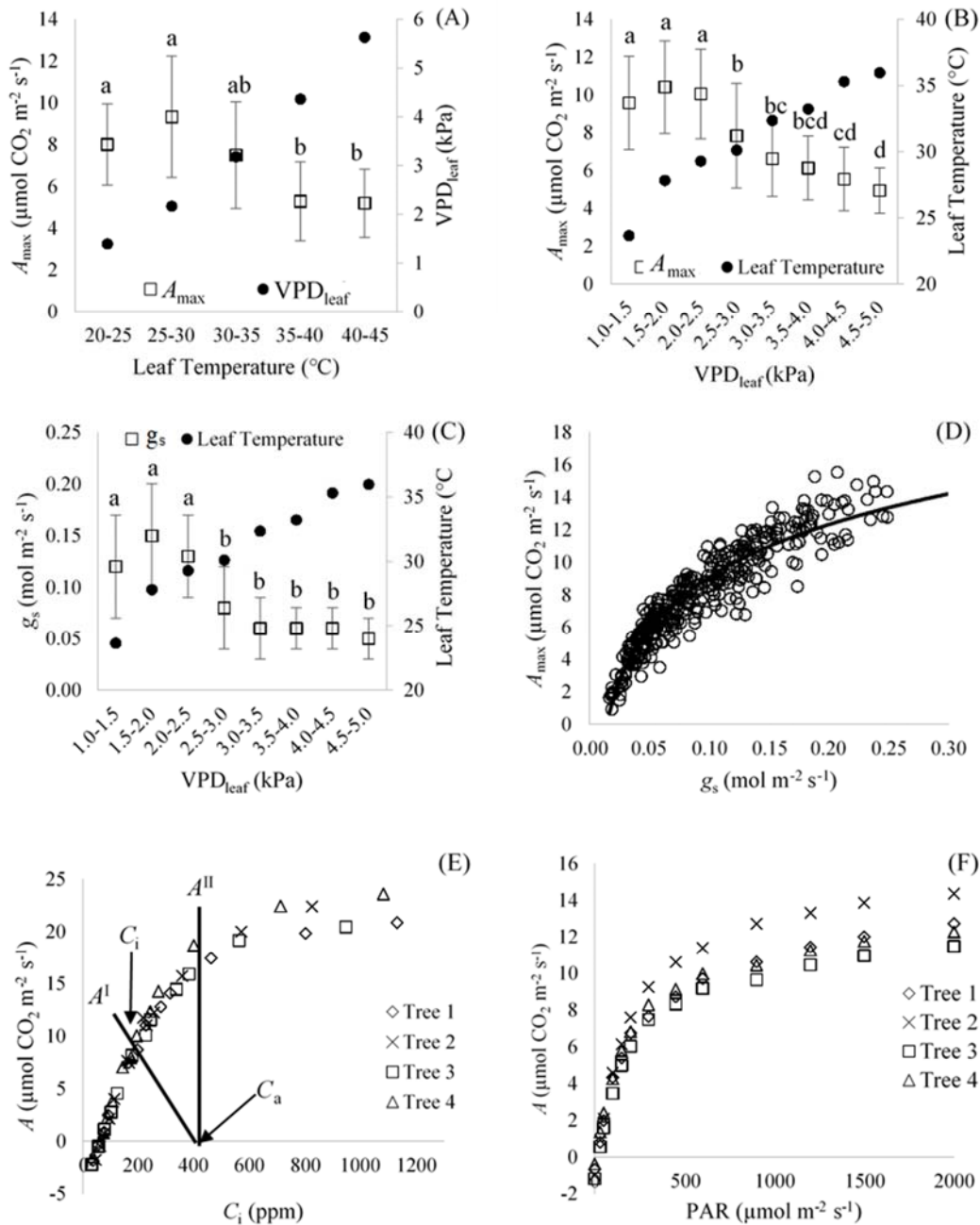


Figure 3: Response of A_{\max} to increasing (A) leaf temperature (T_{leaf}) ($N=500$) and (B) leaf to air vapour pressure deficit (VPD_{leaf}) ($N=500$), (C) the response of stomatal conductance (g_s) to VPD_{leaf} ($N=500$), and (D) the response of light-saturated net CO₂ assimilation rates A_{\max} to g_s ($N=500$). (E) Representative responses of net assimilation (A) to intercellular CO₂ concentration (C_i) showing the method used to calculate stomatal limitations ($I = (A^{II} - A^I)/A^{II}$) as outlined by Long and Bernacchi (2003) of four experimental trees ($N=51$) measured on 7 December 2017. (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.

3.2.2 Influence of fruit on leaf gas exchange

Mean A_{\max} varied throughout the data collection period between a minimum of $6.03 \mu\text{mol m}^{-2} \text{s}^{-1}$ (9 August 2016) and a maximum of $11.21 \mu\text{mol m}^{-2} \text{s}^{-1}$ (8 December 2017) (Table 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 9 August 2016 and 23 March 2017, 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 2).

Table 2: Mean light saturated net CO₂ 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 shown in bold face. Means in columns were separated by LSD at 5% when $P \leq 0.05$ according to repeated measures ANOVA.

Date	<i>N</i>	A_{\max} ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	g_s ($\text{mol m}^{-2} \text{ s}^{-1}$)	VPD_{leaf} (kPa)	T_{leaf} (°C)	VPD_{air} (kPa)	T_{air} (°C)
9 August 2016	140	6.03 c	0.05 c	3.2 ab	31.0 b	1.7 b	21.9 c
13 October 2016	23	7.88 bc	0.11 b	1.4 c	22.2 d	0.6 d	17.4 d
23 March 2017	26	8.64 b	0.12 b	3.5 a	34.6 a	2.3 a	27.9 a
11 May 2017	21	8.43 b	0.10 b	2.6 b	32.3 b	2.3 a	25.2 b
8 December 2017	38	11.21 a	0.15 a	1.7 c	26.8 c	1.2 c	21.6 c
Mean		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 4A). 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 4B). 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 4C). The majority of higher A_{\max} and corresponding g_s values were obtained during the F period (Figure 4D). Higher A values

were achieved for similar C_i levels during F periods compared to NF periods (Figure 4E). However, responses of A to increases in PAR (Figure 4E) 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 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 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 3. Estimates of J_{\max} , and TPU were higher during F periods compared to NF periods (Table 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 3).

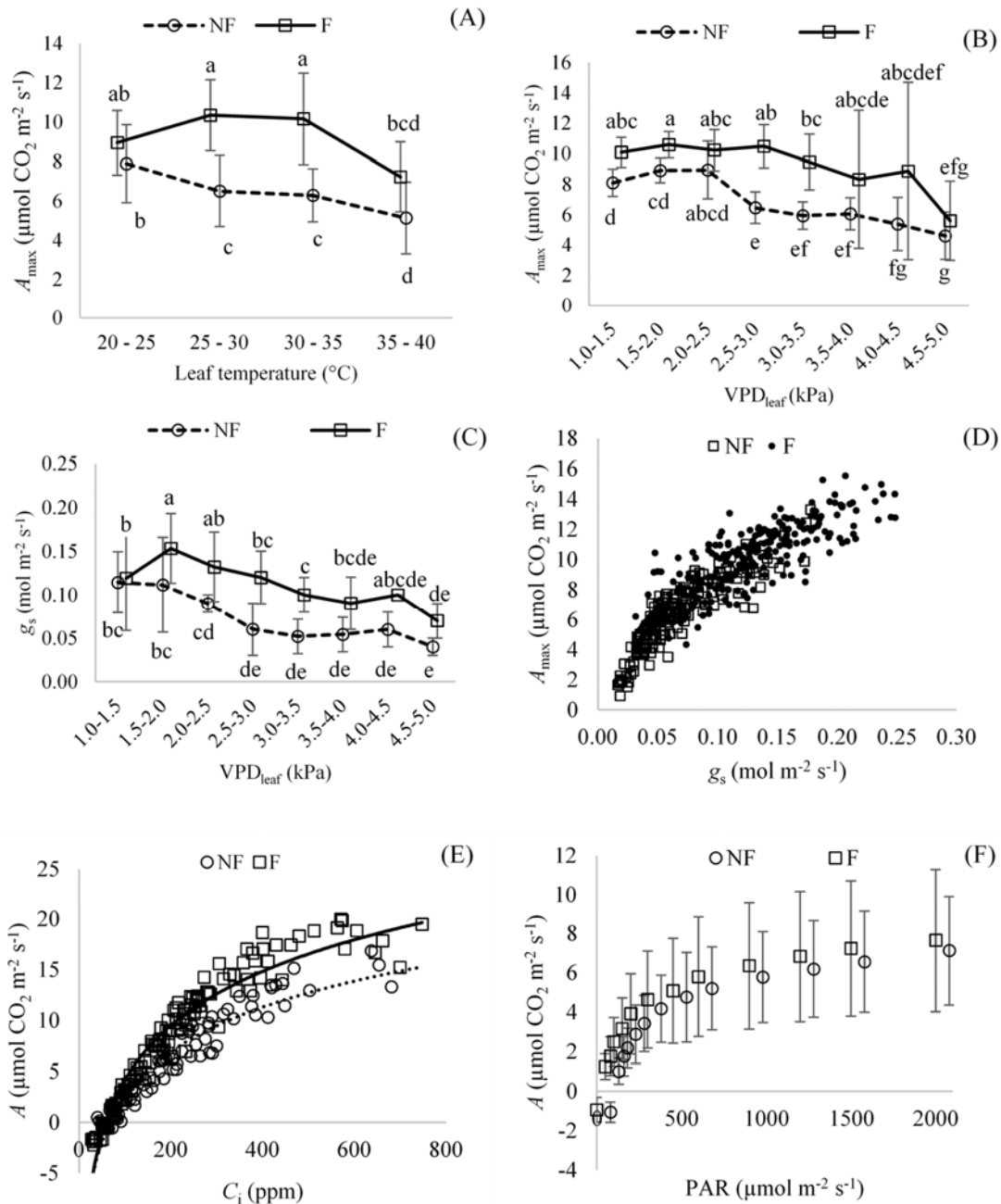


Figure 4: Response of light-saturated net CO₂ assimilation rates (A_{max}) to increasing (A) leaf temperature and (B) air to leaf vapour pressure deficit (VPD_{leaf}). (C) The response of stomatal conductance (g_s) to VPD_{leaf} , and (D) the response of A_{max} to g_s during non-fruited (N= 261) and fruited (N= 239) stages. (E) Responses of net assimilation (A) to intercellular CO₂ concentration (C_i) during non-fruited stages (N= 78) and fruited stages (N= 138) stages. (F) Response of A to PAR in fully sun-exposed leaves of all experimental trees during non-fruited stages (N= 368) and fruited stages (N= 272) stages. Means were separated by LSD at 5% when $P \leq 0.05$ according to repeated measures ANOVA.

Table 3: Comparison of mean (\pm standard deviation) weather conditions and photosynthetic parameters during fruiting and non-fruiting stages. Weather variables are means of daytime (6:00 am – 6:00 pm) air temperature and air vapour pressure deficit (VPD_{air}). Photosynthetic parameters obtained from spot measurements of leaf gas exchange include leaf temperature (T_{leaf}), leaf-to-air vapour pressure deficit (VPD_{Leaf}), light saturated net CO_2 assimilation rate (A_{max}) and 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), stomatal limitation (l), light compensation point (LCP), apparent quantum efficiency (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. Means were separated by LSD at 5% when $P \leq 0.05$. N is the number of measurements.

	N	Fruiting (Mean \pm SD)	N	Non-Fruiting (Mean \pm SD)
<u>Spot Measurements</u>				
A_{max} ($\mu\text{mol CO}_2\ \text{m}^{-2}\ \text{s}^{-1}$)	228	10.27 \pm 2.23 ^a	196	6.58 \pm 2.00 ^b
g_s ($\text{mol m}^{-2}\ \text{s}^{-1}$)	228	0.13 \pm 0.05 ^a	196	0.07 \pm 0.03 ^b
T_{leaf} (°C)	228	28.66 \pm 2.27 ^b	196	30.28 \pm 3.45 ^a
T_{air} (°C)		23.58 \pm 1.36 ^a		19.93 \pm 2.59 ^b
VPD_{leaf} (kPa)	228	1.91 \pm 0.61 ^b	196	2.97 \pm 0.80 ^a
VPD_{air} (kPa)		1.04 \pm 0.40		1.37 \pm 0.62
<u>A/C_i Response Curve Analysis</u>				
$V_{c\ max}$ ($\mu\text{mol m}^{-2}\ \text{s}^{-1}$)	9	72.80 \pm 14.31	13	55.92 \pm 25.51
J_{max} ($\mu\text{mol m}^{-2}\ \text{s}^{-1}$)	9	90.24 \pm 10.39 ^a	13	73.35 \pm 12.86 ^b
TPU ($\mu\text{mol m}^{-2}\ \text{s}^{-1}$)	9	6.53 \pm 1.05 ^a	13	4.77 \pm 0.78 ^b
R_d ($\mu\text{mol m}^{-2}\ \text{s}^{-1}$)	9	0.35 \pm 0.25 ^b	13	1.01 \pm 0.45 ^a
l (%)	9	35.23 \pm 8.96	13	29.24 \pm 9.32
<u>$A/$PAR Response Curve Analysis</u>				
LCP ($\mu\text{mol PAR m}^{-2}\ \text{s}^{-1}$)	7	7.73 \pm 5.83	13	11.01 \pm 9.81
AQE (mol mol^{-1})	7	0.05 \pm 0.01	13	0.03 \pm 0.02
Day respiration rate ($\mu\text{mol m}^{-2}\ \text{s}^{-1}$)	7	0.37 \pm 0.24	13	0.33 \pm 0.30

3.3 Water relations

Pre-dawn leaf water potentials (ψ_{pd}) throughout the duration of the trial revealed only minimal variation, with a mean value of -0.13 ± 0.04 MPa (data not shown). During the course of a day (selected days shown in Figure 5), VPD_{air} increased to a maximum around 13:00 in summer (8 December 2016), between 12:00 and 13:00 in autumn (5 April 2017, 18 April 2018) and around 12:00 in winter (14 July 2017) (Figure 5A). 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 5B). 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 5A and 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 5C). 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 6A). This corresponded to a VPD_{leaf} of approximately 2.1 – 2.9 kPa (Figure 6B).

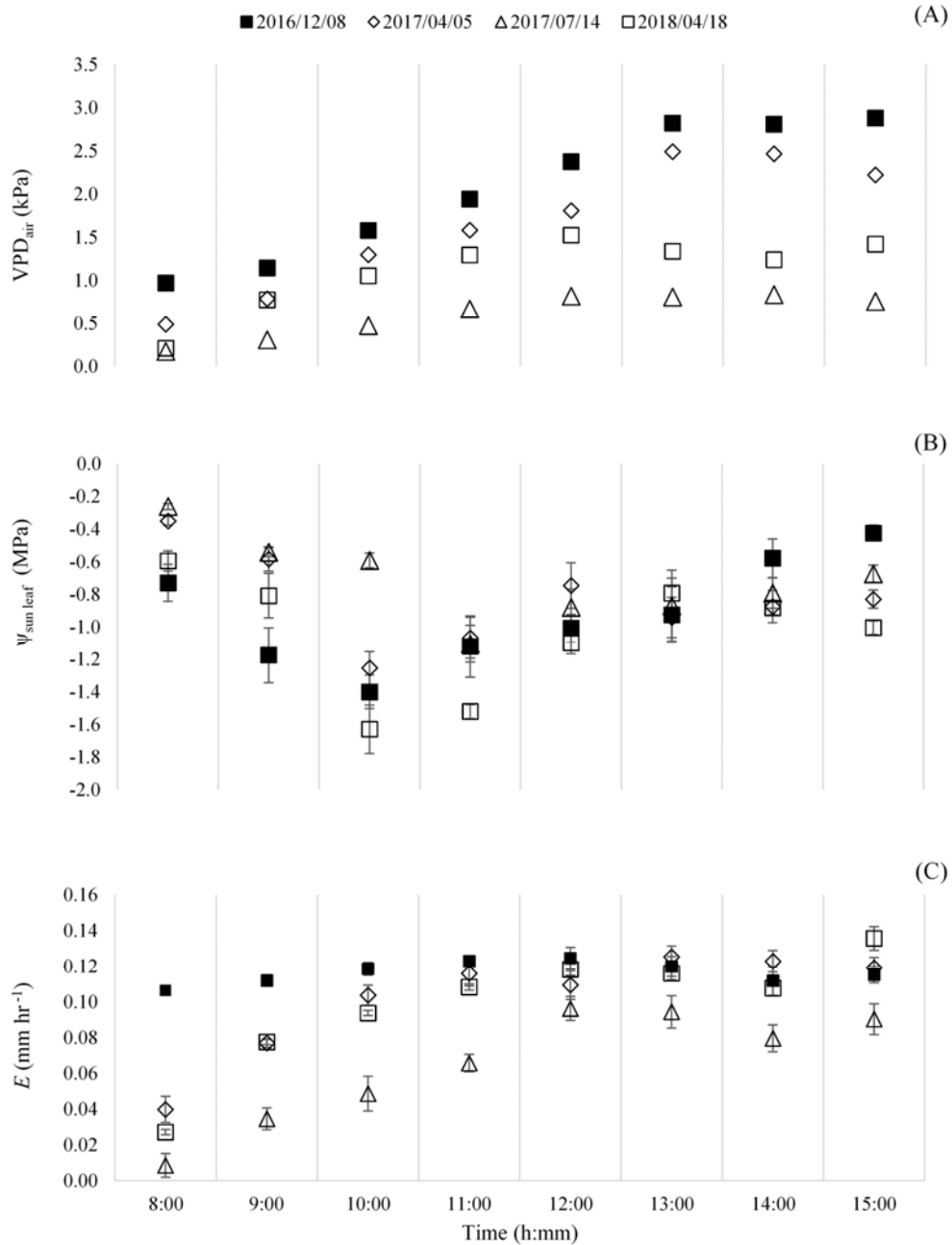


Figure 5: Diurnal progression of (A) air vapour pressure deficit (VPD_{air}), (B) sun exposed leaf water potential ($\psi_{sun\ leaf}$) obtained from random canopy sampling, and (C) whole tree transpiration rate (E) for four measurement days with contrasting weather conditions. Measurements of $\psi_{sun\ leaf}$ ($N = 384$) and E ($N = 128$) are means of four trees (\pm standard deviation).

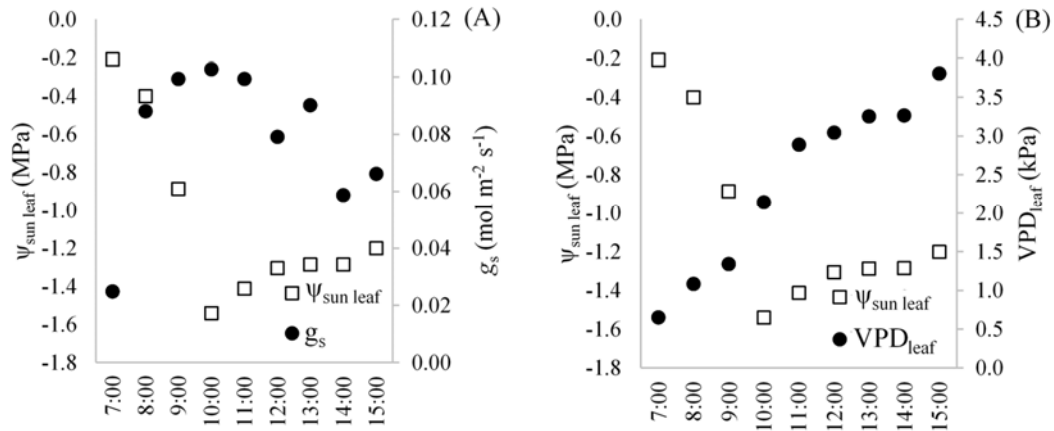


Figure 6: The diurnal trends in (A) sun exposed leaf water potential ($\psi_{\text{sun leaf}}$) and stomatal conductance (g_s) and (B) $\psi_{\text{sun leaf}}$ and air to leaf vapour pressure deficit (VPD_{leaf}), measured on the same leaf (13 July 2017).

The seasonal mean of whole tree hydraulic conductance ($k_{\text{soil-leaf}}$) was estimated at 3.44 ± 2.13 $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$, with a mean conductance at the stem-leaf interface ($k_{\text{stem-leaf}}$) of 7.43 ± 5.05 $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$, and a mean conductance at the soil-stem interface ($k_{\text{soil-stem}}$) of 7.85 ± 4.48 $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$. However, large variation in hydraulic conductance was found, with estimates varying between individual trees and between consecutive days of measurements (Figure 7A). An analysis of the diurnal variation of the various components of the hydraulic pathway revealed that both $k_{\text{soil-leaf}}$ and $k_{\text{soil-stem}}$ increased slowly until 10:00, where after the increase was more rapid (especially $k_{\text{soil-stem}}$), before slowly decreasing after 15:00 (Figure 7B). In contrast, $k_{\text{stem-leaf}}$ increased rapidly until 08:00, then decreased rapidly from 8:00 – 10:00, before increasing from 11:00 onwards until 14:00 (Figure 7B). Maximum g_s was reached between 8:00 – 11:00, after which g_s decreased rapidly. The mean $k_{\text{soil-leaf}}$, obtained from the inverse of the slope of the linear relationship (Figure 7C) between whole tree transpiration rate (E) and $\psi_{\text{sun leaf}}$ was slightly higher (3.95 ± 2.24 $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$) than that determined from Equation 3 (3.44 ± 2.13 $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$). There was a positive linear relationship between leaf specific conductance (k_L) and g_s (Figure 7D).

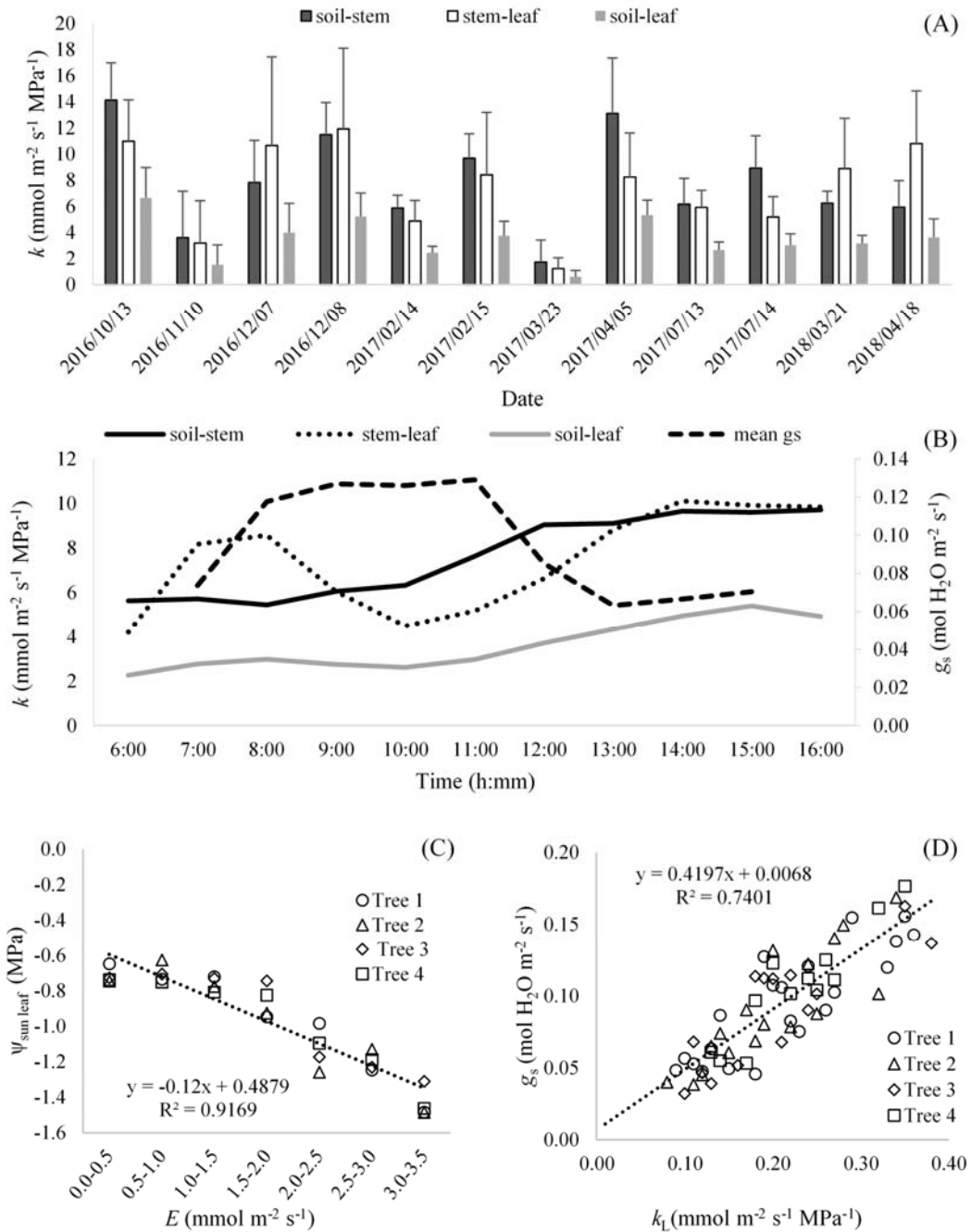


Figure 7: Means (\pm standard deviation) of (A) daily seasonal and (B) diurnal progression of individual components of hydraulic conductance, including soil to stem ($k_{\text{soil-stem}}$), stem to leaves ($k_{\text{stem-leaf}}$), whole tree hydraulic conductance ($k_{\text{soil-leaf}}$) of four study 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 exposed leaf water potential ($\psi_{\text{sun leaf}}$) 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) g_s for four study trees measured on 13 July 2017.

4. Discussion

This study has reaffirmed that macadamias have low rates of carbon assimilation, 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 $8.34 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, which is similar to values reported by Huett (2004) (8-10 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). 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 $15 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (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 $1.1 \mu\text{mol m}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$, which was lower than that of sclerophyllous citrus leaves ($1.7 - 2.2 \mu\text{mol m}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$) and approximately half of that estimated for peach ($3.1 - 4.0 \mu\text{mol m}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$). 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 $180 \mu\text{mol mol}^{-1}$, led to a linear increase in A , with values greater than $17 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ being recorded at these levels (Figure 3E). Intercellular CO_2 concentrations greater than $400 \mu\text{mol mol}^{-1}$ 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 2007) 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 $>900 \mu\text{mol m}^{-2} \text{s}^{-1}$ 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 during measurements and non-limiting soil water conditions (Figure 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 probably reflects different techniques for estimating $k_{\text{soil-leaf}}$. The values from this study ($k_{\text{soil-leaf}} = 3.44 \pm 2.13 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$), using whole tree transpiration, are more comparable with other fruit tree species, such as apple ($4.4 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$, Cohen and Naor 2002) and kiwifruit ($2.44\text{-}3.83 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$, Clearwater et al. 2004), which suggests that macadamia whole tree hydraulic conductance is comparable to other fruit trees. 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 7B), 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 7D), 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 4B & C). Both A_{max} and g_s were significantly higher during fruit bearing periods compared to periods when the tree lacked fruit (Table 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 4B & C). These results suggest that macadamias have the capability to maintain g_s for longer under high evaporative demands during fruit bearing periods.

Given the significant assimilate demand by the oil storing fruit of macadamia 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 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 CO₂ assimilation rates of macadamias over a production season are fairly low, which is 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.

5. Acknowledgements

We are grateful to Johan Lindeque and Mayo Estates for allowing us access to commercial macadamia orchards and to Mr AH Smit for assisting in the data collection and processing.

6. Funding

This is a directed, funded and managed project by South Africa's Water Research Commission (Project K5/2552//4, Water use of avocado and macadamia orchards), with co-funding from Macadamias South Africa NPC.

7. References

Burgess SS, Adams MA, Turner NC, Beverly CR, Ong CK, Khan AA, Bleby TM (2001) An improved heat pulse method to measure low and reverse rates of sap flow in woody plants. *Tree Physiol* 21:589-598.

- Causton D, Dale MP (1990) The monomolecular and rectangular hyperbola as empirical models of the response of photosynthetic rate to photon flux density, with applications to three *Veronica* species. *Ann Bot* 65:389-394.
- Cherbiy-Hoffmann SU, Searles PS, Hall AJ, Rousseaux MC (2012) Influence of light environment on yield determinants and components in large olive hedgerows following mechanical pruning in the subtropics of the Southern Hemisphere. *Sci Hort* 137:36-42.
- Clearwater M, Lowe R, Hofstee B, Barclay C, Mandemaker A, Blattmann P. 2004. Hydraulic conductance and rootstock effects in grafted vines of kiwifruit. *Journal of Experimental Botany* 55: 1371-1382.
- Cohen S, Naor A (2002) The effect of three rootstocks on water use, canopy conductance and hydraulic parameters of apple trees and predicting canopy from hydraulic conductance. *Plant Cell Environ* 25:17-28.
- Farquhar GD, Sharkey TD (1982) Stomatal conductance and photosynthesis. *Annu Rev Plant Biol* 33:317-345.
- Flexas J, Ribas-Carbó M, Diaz-Espejo A, Galm ESJ, Medrano H (2008) Mesophyll conductance to CO₂: current knowledge and future prospects. *Plant Cell Environ* 31:602-621.
- Flore J, Lakso AN. 1989. Environmental and physiological regulation of photosynthesis in fruit crops. *Horticultural Reviews* 11: 111-157.
- Gindaba J, Wand S. 2007a. Climate ameliorating measures influence photosynthetic gas exchange of apple leaves. *Annals of applied biology* 150: 75-80.
- Gindaba J, Wand SJ. 2007b. Do fruit sunburn control measures affect leaf photosynthetic rate and stomatal conductance in 'Royal Gala' apple? *Environmental and Experimental Botany* 59: 160-165.
- Gleason SM, Westoby M, Jansen S, Choat B, Hacke UG, Pratt RB, Bhaskar R, Brodribb TJ, Bucci SJ, Cao KF (2016) Weak tradeoff between xylem safety and xylem-specific hydraulic efficiency across the world's woody plant species. *New Phytol* 209:123-136.
- Hardner CM, Peace C, Lowe AJ, Neal J, Pisanu P, Powell M, Schmidt A, Spain C, Williams K (2009) Genetic resources and domestication of *Macadamia*. *Hortic Rev* 35:1-125.

- Higgins S, Larsen F, Bendel R, Rademaker G, Bassman J, Bidlake W, Al Wir A (1992) Comparative gas exchange characteristics of potted, glasshouse-grown almond, apple, fig, grape, olive, peach and Asian pear. *Sci Hort* 52:313-329.
- Hölttä T, Lintunen A, Chan T, Mäkelä A, Nikinmaa E (2017) A steady-state stomatal model of balanced leaf gas exchange, hydraulics and maximal source–sink flux. *Tree Physiol* 37:851-868.
- Hubbard RM, Ryan M, Stiller V, Sperry J (2001) Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. *Plant Cell Environ* 24:113-121.
- Huett D (2004) *Macadamia* physiology review: a canopy light response study and literature review. *Crop Pasture Sci* 55:609-624.
- Jifon JL, Syvertsen JP (2003) Moderate shade can increase net gas exchange and reduce photoinhibition in citrus leaves. *Tree Physiol* 23:119-127.
- Jordan GJ, Dillon RA, Weston PH (2005) Solar radiation as a factor in the evolution of scleromorphic leaf anatomy in Proteaceae. *Am J Bot* 92:789-796.
- Klein T (2014) The variability of stomatal sensitivity to leaf water potential across tree species indicates a continuum between isohydric and anisohydric behaviours. *Funct Ecol* 28:1313-1320.
- Liang J, Zhang J, Wong M (1997) Can stomatal closure caused by xylem ABA explain the inhibition of leaf photosynthesis under soil drying? *Photosynth Res* 51:149-159.
- Lloyd J (1991) Modelling stomatal responses to environment in *Macadamia integrifolia*. *Funct Plant Biol* 18:649-660.
- Lloyd J, Howie H (1989) Salinity, Stomatal Responses and Whole-Tree Hydraulic Conductivity of Orchard 'Washington Navel' Orange, *Citrus sinensis* (L.) Osbeck. *Funct Plant Biol* 16:169-179.
- Lloyd J, Syvertsen J, Kriedemann P, Farquhar G (1992) Low conductances for CO₂ diffusion from stomata to the sites of carboxylation in leaves of woody species. *Plant Cell Environ* 15:873-899.
- Lloyd J, Trochoulias T, Ensbey R (1991) Stomatal responses and whole-tree hydraulic conductivity of orchard *Macadamia integrifolia* under irrigated and non-irrigated conditions. *Funct Plant Biol* 18:661-671.

- Long S, Bernacchi C (2003) Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. *J Exp Bot* 54:2393-2401.
- Marchi S, Tognetti R, Minnocci A, Borghi M, Sebastiani L (2008) Variation in mesophyll anatomy and photosynthetic capacity during leaf development in a deciduous mesophyte fruit tree (*Prunus persica*) and an evergreen sclerophyllous Mediterranean shrub (*Olea europaea*). *Trees* 22:559.
- McDowell NG, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytol* 178:719-739.
- Medina CL, Souza RP, Machado EC, Ribeiro RV, Silva JA (2002) Photosynthetic response of citrus grown under reflective aluminized polypropylene shading nets. *Sci Hort* 96:115-125.
- Mitchell PJ, McAdam SA, Pinkard EA, Brodribb TJ (2016) Significant contribution from foliage-derived ABA in regulating gas exchange in *Pinus radiata*. *Tree Physiol* 37:236-245.
- Moreschet S, Cohen Y, Green GC, Fuc M (1990) The partitioning of hydraulic conductances within mature orange trees. *J Exp Bot* 41:833-839.
- Naor A (2001) Irrigation and Crop Load Influence Fruit Size and Water Relations in Field-grown Spadona Pear. *J Am Soc Hortic Sci* 126:252-255.
- Nardini A, Salleo S (2000) Limitation of stomatal conductance by hydraulic traits: sensing or preventing xylem cavitation? *Trees* 15:14-24.
- Niinemets Ü, Sack L (2006) Structural determinants of leaf light-harvesting capacity and photosynthetic potentials. *Progress in Botany*. Springer, pp 385-419.
- Niinemets Ü, Wright IJ, Evans JR (2009) Leaf mesophyll diffusion conductance in 35 Australian sclerophylls covering a broad range of foliage structural and physiological variation. *J Exp Bot* 60:2433-2449.
- Pantin F, Monnet F, Jannaud D, Costa JM, Renaud J, Muller B, Simonneau T, Genty B. 2013. The dual effect of abscisic acid on stomata. *New Phytologist* 197: 65-72.
- Pretorius JJ, Wand SJ (2003) Late-season stomatal sensitivity to microclimate is influenced by sink strength and soil moisture stress in 'Braestar' apple trees in South Africa. *Sci Hort* 98:157-171.

- Read J, Sanson GD, de Garine-Wichatitsky M, Jaffré T (2006) Sclerophylly in two contrasting tropical environments: low nutrients vs. low rainfall. *Am J Bot* 93:1601-1614.
- Sade N, Moshelion M (2014) The dynamic isohydric–aniso-hydric behavior of plants upon fruit development: taking a risk for the next generation. *Tree Physiol* 34:1199-1202..
- Schultz HR (2003) Differences in hydraulic architecture account for near isohydric and aniso-hydric behaviour of two field grown *Vitis vinifera* L. cultivars during drought. *Plant Cell Environ* 26:1393-1405.
- Sharkey TD, Bernacchi CJ, Farquhar GD, Singsaas EL (2007) Fitting photosynthetic carbon dioxide response curves for C₃ leaves. *Plant Cell Environ* 30:1035-1040.
- Silber A, Israeli Y, Levi M, Keinan A, Chudi G, Golan A, Noy M, Levkovitch I, Narkis K, Naor A (2013) The roles of fruit sink in the regulation of gas exchange and water uptake: a case study for avocado. *Agric Water Manag* 116:21-28.
- Sofo A, Dichio B, Montanaro G, Xiloyannis C (2009) Photosynthetic performance and light response of two olive cultivars under different water and light regimes. *Photosynthetica* 47:602-608.
- Sperry JS (2000) Hydraulic constraints on plant gas exchange. *Agric For Meteorol* 104:13-23.
- Sperry J, Alder N, Eastlack S (1993) The effect of reduced hydraulic conductance on stomatal conductance and xylem cavitation. *J Exp Bot* 44:1075-1082.
- Stephenson R (2005) Macadamia: Domestication and commercialization. *Chron Horticult* 45:11-15.
- Stephenson R, Gallagher E, Rasmussen T. 1989. Effects of growth manipulation on carbohydrate reserves of macadamia trees. *Scientia Horticulturae* 40: 227-235.
- Stephenson R, Gallagher E, Doogan V. 2003. Macadamia responses to mild water stress at different phenological stages. *Crop and Pasture Sci* 54: 67-75.
- Stephenson R, Cull B, Mayer D. 1986. Effects of site, climate, cultivar, flushing, and soil and leaf nutrient status on yields of macadamia in south east Queensland. *Scientia Horticulturae* 30: 227-235.
- Stephenson R, Trochoulis T. 1994. Macadamia. In *Handbook of environmental physiology of fruit crops Volume II: Sub-Tropical and Tropical Crops*, Eds. Schaffer, B. and Anderson, PC. CRC Press, Inc, pp 147-164.

- Syvertsen J, Goñi C, Otero A. 2003. Fruit load and canopy shading affect leaf characteristics and net gas exchange of 'Spring' navel orange trees. *Tree Physiology* 23: 899-906.
- Syvertsen J, Lloyd J, McConchie C, Kriedemann P, Farquhar G (1995) On the relationship between leaf anatomy and CO₂ diffusion through the mesophyll of hypostomatous leaves. *Plant Cell Environ* 18:149-157.
- Tardieu F, Simonneau T (1998) Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours. *J Exp Bot*:419-432.
- Taylor N, Mahohoma W, Vahrmeijer J, Gush MB, Allen RG, Annandale JG (2015) Crop coefficient approaches based on fixed estimates of leaf resistance are not appropriate for estimating water use of citrus. *Irrigation Sci* 33:153-166.
- Wilkie J, Sedgley M, Olesen T. 2009. A model of vegetative flush development and its potential use managing macadamia (*Macadamia integrifolia*) tree canopies. *Crop and Pasture Sci* 60: 420-426
- Wullschleger SD (1993) Biochemical limitations to carbon assimilation in C₃ plants - a retrospective analysis of the A/Ci curves from 109 species. *J Exp Bot* 44:907-920.