The impact of water stress at different phenological stages on the yield and quality of *Macadamia* (F. Muell)

by

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Submitted in fulfilment of the requirements for the degree

M.Sc. (Agric.) Horticultural Science

In the Faculty of Natural & Agricultural Sciences

University of Pretoria

Pretoria

30 July 2021

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DECLARATION: PLAGIARISM

I, Armand Hendrik Smit declare that the thesis/dissertation, which I hereby submit for the degree M.Sc. (Agric.) Horticultural Science at theUniversity of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

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ABSTRACT

Irrigated agriculture is currently the single largest consumer of water on the planet, accounting for a near 22% of total freshwater consumption. Efficient water use strategies in the agricultural sector are therefore fundamental. Current macadamia irrigation quantities are, however, based on the physiology, phenology and morphology of other horticultural crops, which could be less tolerant to water stress and deficit irrigation. A lack of crop specific information with regards to water stress tolerances results in a large amount of uncertainty with regards to efficient water management practices, a problem that is exacerbated in macadamia given the inconsistent responses to irrigation reported previously. This study, therefore, attempted to determine the effect of water stress at different phenological stages on the yield and quality of macadamias. Seven water deficit treatments were imposed on 63 randomly selected, mature bearing macadamia trees (cv. HAES 695, 'Beaumont', *M. tetraphylla x M. integrifolia*, grafted) and respective yield, quality and physiological responses were compared to a well-watered control over a three year period. Mild water deficits were imposed at various phenological periods and was analyzed alongside longer term water deficit treatments. Tree based measurements such as stomatal conductance (g_s), light saturated net photosynthetic assimilation rate (A_{max}), pre-dawn leaf (Ψ_{pd}) and midday stem (Ψ_x) water potential, were used in conjunction with soil water potential measurements to establish different macadamia water deficit thresholds under orchard conditions. Results from this study suggest that macadamias are less sensitive to water deficit conditions than most other horticultural crops. Withholding water during key phenological stages had no impact on yield for one season of measurements relative to the control. Irrigating half the volume of the control or relying solely on rainfall also had no impact on yield over two seasons. Water deficits during flowering and nut set, and shell hardening phenological stages were detrimental to macadamia quality, significantly reducing the percentage total kernel recovery relative to the control. Severe water deficits at any phenological stage will likely have a negative impact on both yield and quality, however, in deep soils and under well distributed rainfall conditions, reaching such level of stress would be unlikely. Results from this study suggest that both Ψ_{pd} and Ψ_x can be used as indicators of water stress in macadamia orchards, with Ψ_{pd} <-0.6 MPa and Ψ_x <-0.9 MPa probably indicating the onset of stress that could impact both yield and quality.

ACKNOWLEDGMENTS

I would like to dedicate this document to my immediate family. Thank you all for the sacrifices that you have made throughout your lives to allow me to achieve these heights. I am undeservedly blessed to have you all in my life.

Special thanks to:

- My brother and best friend, Dr Theunis Smit. Theunis, I won’t be a fraction of the person that I am today if it was not for you. Thank you for your guidance and help throughout this journey. You are truly the best gift that this world could have given me and I will always be grateful to have you.
- My dearest wife and fellow researcher, Maike Smit. Thank you Maike for always being there, whether you were helping with field measurements, preparing food, providing a shoulder to cry on or some words of encouragement. Your help and love is truly appreciated and I can not explain how lucky I feel to have you.
- Dr Nicolette Taylor, thank you for all the help and guidance over the course of my study. Thank you for all the life lessons which made me a better person. You truly live by your life motto, "In a world where you can be anything, be kind". Thank you for everything.
- The owners of DuMak farm, Faan and his son Rohann Du Toit, thank you for allowing us to conduct our experiments on your farm. The trust you have put in us is truly appreciated and we will forever be grateful. Thank you for your labour and the lengths you went to, to help make this project successful.
- The project funders who made this project possible, the Water Research Commission (WRC), South African Macadamia Association (SAMAC), Agricultural Research Council (ARC), University of Pretoria and the University of Kwazulu-Natal.
- My mother, thank you for everything you have done to set me up in life. “Ons almal is baie lief vir jou”.

It is evident that this document is the result of many individuals’ hard work and sacrifice. Thank you all for the time, guidance and trust throughout this journey. I will forever be indebted to you all.

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LIST OF ABBREVIATIONS

- cv.	Cultivar
- DI	Deficit Irrigation
- ET	Evapotranspiration
- NIH	Nut In Husk
- PRD	Partial Root Zone Drying
- RDI	Regulated Deficit Irrigation
- TKR	Total Kernel Recovery
- VPD	Vapour Pressure Deficit
- WIS	Wet In Shell
- WU	Water Use
- WUE	Water Use Efficiency
- WUP	Water Use Productivity

LIST OF SYMBOLS

- A_{max}	Light saturated net photosynthetic assimilation rate
- A	Net photosynthetic assimilation rate
- ET_o	Reference Evapotranspiration
- g_s	Stomatal conductance
- m	Meter
- VPD_L	Leaf to air Vapour Pressure Deficit
- VPD_{air}	Air Vapour Pressure Deficit
- T	Transpiration rate
- T_{air}	Air temperature
- T_{leaf}	Leaf temperature
- S_{Rad}	Solar radiation
- Ψ	Plant water potential
- Ψ_L	Leaf water potential
- Ψ_x	Bagged midday stem water potential
- Ψ_{pd}	Pre-dawn leaf water potential

CHAPTER 1: INTRODUCTION

1.1 Rationale

Macadamia (F. Muell) spp. are long-lived evergreen trees with a subtropical and tropical origin, forming part of the Proteaceae family (Hardner et al., 2009). Since the late nineteenth century, macadamia has been extensively cultivated for its oil-rich, nutritious and therefore high valued kernel (Lloyd et al., 1991). Despite extensive cultivation in other parts of the world, macadamia was first introduced to South Africa in the 1960's (SAMAC, 2019). Currently, South Africa is amongst the leading producers in the global macadamia industry **(Table 1.1)**, competing exclusively with Australia in terms of largest total production (INC, 2019). It is also the fastest growing tree crop industry in South Africa, with an estimated growth of 6300 ha of new plantings in 2018 (SAMAC, 2019). The industry is largely export-driven, with approximately 97% of the annual production being exported. Almost 50% of the South African crop is exported as nut in shell (NIS) to Asia, and the remainder of the crop is processed to kernel. The United States of America are the largest kernel importer, with large portions of the imported product going to the ingredients market. Other markets include Europe, Japan, Southeast Asia and the Middle East (INC, 2019). The export nature of the crop has contributed significantly to growth in the South African economy (R4 billion in 2018), generating foreign exchange and creating an estimated 10 000 permanent and 9 000 seasonal employment opportunities.

Table 1.1 Total global macadamia kernel production for the year 2018 (INC, 2019)

Country	Kernel production (Metric Tons)
South Africa	16 965
Australia	14 800
Kenya	7 750
China	6 000
USA	4 239
Guatemala	2 150
Malawi	1 619
Brazil	1 550
Others	4 234
Total Production	**59 300**

Further growth in the South African macadamia industry is, however, associated with several constraints. Considering the availability of suitable land, uncertainty regarding future prices and possible market saturation, water availability is arguably the largest constraining factor to more rapid growth of the industry. Concerns regarding water availability are further aggravated when considering future and current climate change (Fereres and Evans, 2006). The majority of the South Africa macadamia industry is located in regions with seasonal and often unpredictable rainfall. This, combined with the fact that it is an evergreen perennial crop, often means that irrigation is necessary for sustainable crop production, which leads to the age-old question of when and how much water is required?

1.2 Justification

The South African macadamia industry is spread around all parts of semi-arid South Africa, with the main growing areas being Levubu and Tzaneen in the Limpopo province, Hazyview to Barberton in Mpumalanga, coastal KwaZulu-Natal and small parts of the Eastern and Western Cape provinces. Production areas are, however, associated with strong regional climatic variability **(Figure 1.1)**, enabling some areas (eg. parts of KwaZulu-Natal southern coast and Hazyview) to be predominantly rainfed, whilst the majority are dependant on irrigation in order to meet crop evapotranspiration (ET) requirements and minimise risks linked to water shortages at critical phenological stages (Fereres et al., 2012).

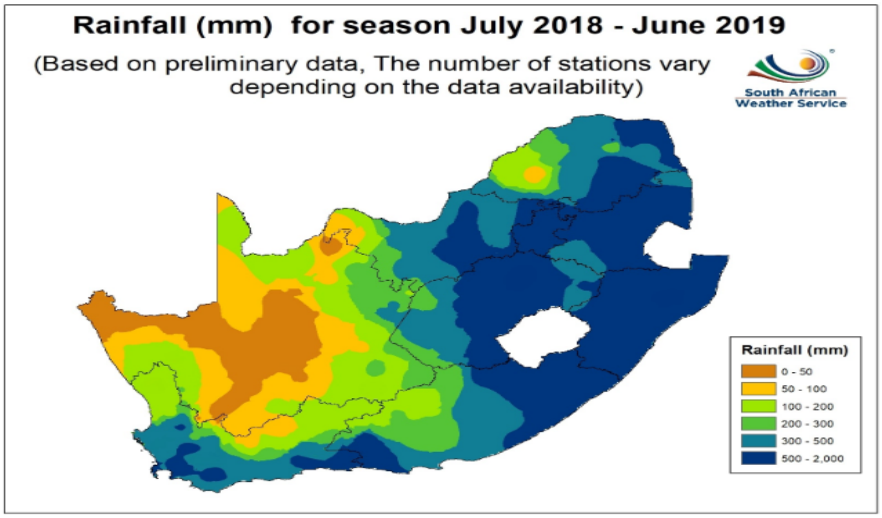


Figure 1.1 Schematic representation of South Africa's inherent regional rainfall variability for the period July 2018 to June 2019

Currently, there is a considerable lack of scientific research regarding the water use of macadamia orchards. Despite a previous study by Taylor and Gush (2014) and ongoing research focusing on the upper-limit for water use of macadamia (WRC Project K5/2552//4), there is very little information available on macadamia tree water use, irrigation requirements, the effect of water stress on physiological functioning, yield and nut quality, as well as the implications of water deficits for orchard management. Filling these knowledge gaps is essential for sustainable industry expansion, in particular, because of scarce surface and groundwater resources in the major macadamia producing areas of the world. Elevated social water awareness, a growing human population and increased competition for water between various industries have further highlighted the need for the global agricultural industry to search for alternative water management strategies to improve water use efficiency (WUE) in the face of possible future water restrictions (Fereres and Evans, 2006).

Previous studies by Stephenson et al. (1989b), Stephenson et al. (2003) and Lloyd (1991), have focussed on the effects of temperature, tree water status, soil water status, relative humidity and other environmental variables on macadamia growth and productivity. The study by Stephenson et al. (2003), focussed on assessing the effect of mild water stress during different phenological stages and came to the conclusion that water stress during the pre-flowering period had no effect on crop performance, whilst mild water stress during nut maturation significantly impacted crop performance. Although useful, the aforementioned studies were primarily conducted on potted trees, in draining lysimeters or under controlled conditions, which are significantly different from orchard conditions especially in terms of total tree root volume which could have greatly influenced results obtained in the study. The effects of limited water availability on macadamia productivity should therefore be reassessed under orchard conditions to eliminate confounding factors.

Considering that studies by Stephenson et al. (1989b), Stephenson et al. (2003) and Lloyd (1991), have suggested that macadamias have a certain degree of drought tolerance in combination with the proposed isohydric nature of macadamias (Smit et. al. 2020), it is critically important that water management practices should be reassessed to exploit the trees’ natural ability to grow and reproduce under limiting soil water conditions. Seeing that irrigated agriculture aims at minimizing the risk associated with limiting soil water, it also aims at increasing water use efficiency and acknowledges that water is a finite resource. Determining the minimum amount of water that an orchard needs to produce the maximum possible crop is a question which continues to allude macadamia growers and is exacerbated by the limited and slightly contradicting research in the field of macadamia water use. Studies by Trochoulias and Johns (1992) and Awada et al. (1967) for example, have shown no significant increase in macadamia yield in response to varying rates of irrigation in high rainfall areas over 8 and 3 years respectively. A study by Searle and Lu (2002) concluded that, where the rainfall to Class A pan evaporation ratio exceeded 0.5, there may be no benefit of irrigating macadamias. These findings, in combination with the drought tolerant traits of macadamia (Lloyd, 1991, Stephenson et al., 2003), would suggest that if a single fixed irrigation strategy is followed, a commonly used practice by macadamia producers (Searle and Lu, 2002, Stephenson and Searle, 2014), it might lead to over or under irrigation depending on the crop and growing environment. It would therefore be useful to assess the efficiency of current irrigation water use in commercial irrigated orchards and to reassess current recommended irrigation strategies. Furthermore, it would be useful to establish the viability of regulated deficit irrigation (RDI) practices in macadamia orchards and to identify critical phenological stages where a mild water deficit will have no significant effect on nut yield and quality. This will allow producers to optimise on-farm water use, minimise inputs and make informed decisions on irrigation scheduling and irrigation water management under semi-arid conditions, by restricting irrigation to yield- and quality- limiting stages.

The implementation of RDI practices in macadamia orchards, however, requires an understanding of the crop's physiological response to water deficits and a method to schedule irrigation and/or to detect and monitor the degree of water stress (Fereres and Evans, 2006). The practicality of leaf- (Ψ_L), pre-dawn leaf- (Ψ_{pd}) and bagged midday stem- (Ψ_x) water potentials as indicators of water deficits in macadamia orchards should therefore be assessed by correlating measurements of Ψ_L, Ψ_{pd}, and Ψ_x with measurements of gas exchange. This is particularly important since a study by Searle and Lu (2002), measuring midday Ψ_L in Australian orchards, have failed to show trees under stress (<-1.2 MPa), even after a prolonged period (81 days) without rain. This, in combination with the aforementioned discussions, highlights the need for this study to examine the effect of mild water stress on the physiology and productivity of field-grown macadamias.

1.3 Hypotheses

1. Prolonged deficit irrigation will have no significant effect on macadamia yield and quality.
2. Mild water deficits during flower initiation will have no effect on yield and quality.
3. Water deficits during flowering and nut set and premature nut abscission will reduce yield, as there will be a reduction in final nut set and an increase in premature nut abscission
4. Water deficits during nut maturation will be detrimental to both nut yield and quality.
5. Due to the isohydric characteristics of macadamia, midday leaf water potential measurements will not be significantly different under water deficit and well-watered conditions, but stomatal conductance and therefore maximum net photosynthetic assimilation rate and leaf temperature will be different. A decline in stomatal conductance, pre-dawn leaf and midday stem water potential will therefore serve as good indicators of the degree of crop water stress in macadamia orchards.

1.4 Aims

1. To determine the impact of water stress at different phenological stages on the yield and quality of macadamia orchards.
2. To assess the practicality of macadamia water deficit thresholds that triggers an irrigation event under orchard conditions.

1.5 Objectives

1. To implement a mild water deficit at different macadamia phenological stages
2. To determine yield and quality as a result of mild water deficit treatments during different phenological stages.
3. To apply a mild water deficit throughout the season to macadamia trees
4. To measure pre-dawn leaf and midday stem and leaf water potentials whilst implementing a mild water deficit.

5. To measure photosynthesis and stomatal conductance whilst implementing a mild water deficit.
6. To establish a relationship between pre-dawn leaf water potential and midday stem water potential measurements to be used as future indicators of crop water deficits and relate this to photosynthesis measurements.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Tree water use and gas exchange responses to water stress differ substantially between different, plant genera as well as between species within the same genera (Galmés et al., 2007, Corcuera et al., 2002, Ryan, 2011). These differences become more apparent when comparing evergreen and deciduous tree species and species with different photosynthetic pathways. Ecologically, evergreen species are commonly found where climatic seasonality is not distinct or where resources are difficult to obtain, whilst deciduous species favour sites where climatic seasonality results in distinct favourable and unfavourable periods for growth (Bai et al., 2015). Both tree types have, however, evolved to have physiological characteristics which allow for instances where more than one apparent strategy is adaptive to the same environmental variables.

Over decades, both evergreen and deciduous fruit tree crops have been selected and bred to express fruiting as well as physiological characteristics which allow for adaptability to a range of environmental variables. Yet, unlike other high-value horticultural crops, such as citrus, grapes and apples, macadamia is comparatively under-cultivated (Hardner et al., 2009). As macadamia is in the process of being domesticated, with cultivars only a few generations from the wild, macadamia germplasm is relatively underdeveloped and closely related to its Proteaceae origin (Hardner et al., 2009). This combined with the lack of scientific research may lead to uninformed assumptions with regards to the management of macadamia trees, based on the physiology of other, more cultivated, horticultural crops.

Managing irrigation water requires a substantial amount of knowledge about crop phenology, morphology and water use, as well as the influence of soil and environmental factors on the aforementioned. The underdeveloped and “wild” Proteaceae nature of commercial macadamias amplifies the possibility that these trees will respond substantially different to other horticultural crops when exposed to the same range of soil and climatic variables. This review, therefore, aims to combine currently available literature to understand macadamia phenology, in relation to water stress physiology and the subsequent impact on yield and quality.

2.2 Macadamia and its relation to members of the Proteaceae family

The family Proteaceae, consist of around 79 genera and over 1700 species and is made up of mainly shrubs, trees and some herbs, of which most members display distinct xerophytic characteristics (Nock et al., 2014). Species within the family are found in various parts of the world, including China, New Guinea, Tahiti, New Zealand and parts of South America (Chattaway, 1948, Weston and Crisp, 1996). The majority of the species are, however, native to parts of coastal Australia and South Africa, with each of these countries being home to over 1000 different species (Weston and Crisp, 1996). Most of these species grow in nutrient poor soils, but range from arid zones, to closed tropical rainforests, mediterranean climates and alpine areas. Currently, Proteaceae species are not well represented in ecosystems with abundant water, with most species being adapted to arid, fire-prone habitats (Johnson et al., 1963). Even though some representative members, such as *Cardwellia sublimis*, *Orites excelsa*, *Grevillea robusta* and *Protea cynaroides,* are used extensively in the furniture, timber and ornamental industry, the Proteaceae family contains few members of economic importance. However, in recent times *Macadamia* (F Muell) spp. have become a high value horticultural crop, contributing substantially to the international dried fruit and nut trade (INC, 2019, Hardner et al., 2009).

Macadamias are medium-sized evergreen trees, native to the coastal rainforests of eastern Australia. Of the numerous macadamia species (Hardner et al., 2009), only two are edible, being *Macadamia tetraphylla* (Johnson) (Trueman and Turnbull 1994) and *Macadamia integrifolia* (Maiden and Betche), with the latter and inter species hybrids (*M. tetraphylla x M. integrifolia*) dominating commercial production. Since *M. integrifolia* is most widely used in commercial production, most scientific studies have been performed on *M. integrifolia* cultivars. Physiological and phenological characteristics described in literature are therefore predominantly based on *M. integrifolia* and may be significantly different to that of *M. tetraphylla* and inter species hybrids. The two species are, however, easily distinguishable by means of distinct phenotypic/morphological differences, which include the colour of the inflorescence, colour of the new flush, number of leaves per node and the surface texture of the shell (Allan, 2007). Regardless of the aforementioned differences, *Macadamia* species, in general, have many overlapping morphological and physiological characteristics between the different cultivars and with other Proteacea species (Hardner et al., 2009, Johnson, 1954), which make them highly adaptable to periods of limited water availability.

2.2.1 Morphological and physiological drought adaptations of macadamia

Macadamia leaf characteristics

Like most members of the family Proteaceae, macadamia canopies consist of xeromorphic evergreen leaves, with characteristic sclerified bundle sheath tissue (Carr, 2013). Notwithstanding the fact that macadamia has its evolutionary origin in the fringes of the subtropical rainforests of Australia, evergreen sclerophyllous leaves are more regularly associated with arid habitats and are rather non-characteristic in plants originating from areas with abundant water (Schlesinger and Chabot, 1977). Despite sclerophylly being well documented (Salleo and Nardini, 2000, Jordan et al., 2005) and universally characterised by hard, stiff and leathery leaves, its adaptive significance has been questioned (Read et al., 2006). Oertli et al. (1990) describe sclerophyllous leaves to be an adaptation to periodic water deficits by increasing cell wall thickness and rigidity, thereby reducing the risk of cell collapse due to negative turgor pressures associated with water stress. The hard and stiff nature of the leaves can be attributed to an abundance of mechanical cells, including fibres and sclereids, that are orientated differently within the leaf mesophyll and depending on location, may have different functions (Salleo and Nardini, 2000). For example, mechanical tissues with large enough lumina may serve as water reservoirs during diurnal water deficits, while mechanical cells surrounding vascular bundles are hypothesized to promote leaf rehydration via capillary action following drought induced cavitation (Salleo et al., 1997). Sclerophyllous trees further tend to have thicker leaves due to the thickening of all leaf tissue and the leaf cuticle, which aids plants in limiting water loss from aerial tissue (Parkhurst, 1994).

Not only are evergreen sclerophyllous leaves hypothesized to be advantageous to plants for the survival of periodic droughts, sclerophylly may be a nonspecific response to a range of stresses (Salleo and Nardini, 2000, Read et al., 2006). In fact, Read et al. (2006) described evergreen sclerophyllous leaves to be an adaptation to, or a consequence of, low-nutrient conditions. Suggesting evergreen sclerophyllous leaves to be more nutrient use efficient, allowing trees to be more adapted to nutrient limited conditions, which commonly occur during occasional soil water deficits (Vietz, 1972, Hu and Schmidhalter, 2005). Sclerophylly is further suggested to provide possible resistance to frost damage (Larcher, 2005) or be a response to, or as a result of, excessive solar radiation (Jordan et al., 2005), which may all interdependently influence tree growth and survival. These leaves are especially beneficial for the conservation of minerals by regulating long-term photosynthetic efficiency and the rate of litter fall, limiting nutrient losses and the carbohydrate expenditure to regenerate a new flush (Baldocchi et al., 2010). In addition, the photosynthetic rate of sclerophyllous leaves tend to be lower, which with long lived leaves, may be necessary for evergreen sclerophyllous trees to somewhat endure environmental stresses, through maximizing returns on investment (Read et al., 2006).

Macadamias solely have stomata on the abaxial side of the leaves at densities of 360-500 mm^{-2} (Carr, 2013), which is similar to what is described for other evergreen horticultural crops such as citrus and olives (Stephenson et al., 1989b, Kriedemann, 1986, Chartzoulakis et al., 1999), both also having sclerophyllous leaves. Leaf stomatal density and stomatal conductance, inherently regulate maximum carbon uptake rates and the potential rate of water loss from plant leaves (de Boer et al., 2016). Stomatal aperture, therefore, plays a dynamic role in gas exchange, ensuring adequate CO_2 supply while avoiding transpiration rates in excess of leaf water supply rate (Drake et al., 2019). Consequently, hypostomatic leaves (leaves with stomata on only one surface) generally having lower stomatal densities than amphistomatous leaves (stomata on both surfaces), have an inherent constraint to losing excessive amounts of water over short periods (Drake et al., 2019). Moreover, distribution of stomata over only one surface increases leaf boundary layer resistance, further constraining excessive gas exchange, which is of particular importance during periods of high atmospheric evaporative demands in arid environments (Drake et al., 2019, Drake et al., 2013). Hypostomatous leaves with stomates on the abaxial side typically experience lower radiation and therefore lower evaporative demand, due to the lower leaf temperature when compared to leaves with stomates on the sunlit adaxial side (Schymanski et al., 2013). Hence, by locating the most photosynthetically active cells on the adaxial side of the leaf (opposite side of the water supply system to the stomata), hypostomatous macadamia leaves isolate sensitive photosynthetic tissue from large leaf-to-air vapour pressure deficits caused by the outside environment (Drake et al., 2019, Buckley, 2017) and allow for buffering of rapid fluctuations in water potentials during gas exchange (Drake et al., 2019). This hypostomatic leaf characteristic, therefore, contributes to macadamias evolutionary adaptability to inhabit a range of different, often fluctuating environments, by implementing strict control of tree water status and carbon gain to avoid desiccation.

Plant adaptability to arid environments is primarily determined by a plants ability to regulate water loss from aerial tissue (Baker and Procopiou, 1997). Baker (1974) and Whitecross and Armstrong (1972) have shown that the thickness of the leaf epicuticular wax layer is linearly related to environmental conditions, with an increase in thickness being observed with increases in ambient temperature and radiant flux and corresponding decreases in humidity, which is often the case in arid environments. The authors further found that the wax content of the cuticular layer may be directly related to the plants' drought adaptability. A detailed study by Baker and Procopiou (1997) showed that macadamia has a exceptionally thick adaxial leaf cuticular layer, which was similar to that of notoriously drought tolerant olive (Sofo et al., 2008, Gimenez et al., 1996). The adaxial leaf cuticular layer of macadamia exceeded 1 mg/cm^2 and contained large quantities of cuticular waxes in the form of long chain aliphatic constituents absorbed in the cutin matrix. Despite difficulties in the separation of the cuticular membrane, these authors showed that the abaxial side of macadamia leaves are also protected by a substantial cuticular layer, further highlighting the adaptation of macadamia to environmental stresses. Stephenson et al. (1989b) and Stephenson and Trochoulias (1994) showed that macadamia has no pubescence, a common leaf characteristic on other drought adapted species such as olives. Nonetheless, Baker (1974) revealed that thick, tightly absorbed crystalline waxes in the form of a amorphous crust, such as those found on both the adaxial and abaxial leaf surfaces of macadamia (Baker and Procopiou, 1997), aid pistachio and olive in the upkeep of turgidity of plant cells by dissipating large quantities of incident solar radiation. Baker (1974), indicated that plants tend to change the composition and quantity of these epicuticular waxes in response to stresses, raising concern of the potential counterproductive effects of irrigation on the normal mechanisms of drought tolerance in plants, as such traits could be valuable during water deficit periods.

Macadamia root characteristics

As for macadamia leaf characteristics above, a thorough understanding of the morphology, growth dynamics and distribution of a trees root system, together with its adaptations to seasonal water deficits, is required in order to assess the viability of different irrigation practices (Fereres and Soriano, 2007, Kirda, 2002, Li et al., 1989). Root characteristics tend to differ between different tree species, environmental factors and cropping systems (Fernández et al., 1992). For example, it is generally assumed that evergreen trees tend to have relatively deep root systems, allowing them to access water from deeper in the soil profile throughout rainless periods (Sobrado, 1997). Similarly, it is commonly assumed that tree crops under rainfed conditions produce deeper roots, which are critical for survival during prolonged water deficit periods.

These stereotypes, however, may not provide an accurate description of the root system of macadamias since a very detailed study by Firth et al. (2003b) described the macadamia root system to have neither of the typical assumed characteristics of an evergreen or a rainfed tree. Firth et al. (2003b), not distinguishing between irrigated and non-irrigated trees, described the macadamia root system as relatively shallow and spreading, with the taproot of an un-grafted seven-year-old seedling and a 12-year old grafted tree being 1.2 m and 0.6 m in length respectively, while a recent study showed macadamias to have an effective rooting depth of 0.8-1.6 m depending on soil type (Carr, 2013). Even though macadamia seedlings develop a taproot, it is invariably damaged during nursery and transplanting procedures and hence the nature of the root system changes (Stephenson, 2004). The roots of other horticultural crops, such as citrus have been recorded up to depths of 3.6 m, however, the highest abundance of roots was recorded within the top 0.6 m of the soil surface (Bevington and Castle, 1982).

Firth et al. (2003b) showed that the lateral root framework and associated fibrous roots of macadamia trees occur primarily within the upper 0.4 m of the soil surface, with the presence of dense clusters of proteoid roots at the same depth. Firth et al. (2003b) also recorded the highest density of both fibrous roots within 1.0 m of the trunk, which is consistent with that of deciduous tree crops, such as apples and pears (Atkinson, 1980). This abundance of fine fibrous and proteoid roots is, however, an integral part of the specialised root system of macadamias, as reviewed by Stephenson (2004).

Macadamias and other members of the Proteaceae family produce dense mats of fine proteoid roots (**Figure 2.1**), which increase water and nutrient acquisition by increasing the root surface area coming into contact with the soil (Dinkelaker et al., 1995). Once water and nutrients are optimally utilized in the rhizosphere, there is no additional advantage to justify carbohydrate allocation to proteoid roots, hence they are generally short lived. Proteoid root abundance is therefore largely related to the availability of nutrients (Watt and Evans, 1999), especially phosphate (P) (Keerthisinghe et al., 1998, Aitken et al., 1992, Dinkelaker et al., 1995), and soil water. Firth et al. (2003b) recorded the presence of macadamia proteoid roots at greater depths than what was previously recorded for Proteaceae species, and these roots appeared to be functional for over a year under relatively dry conditions. Despite macadamia roots occurring primarily within the upper 0.4 m of the soil surface, Lloyd et al. (1991) implied that macadamias may be able to extract and transport water from deep (>60 cm) within the soil profile and at fairly low soil water contents. This in combination with the presence of proteoid roots at greater depths may allow macadamias to maintain their plant water status (avoid stress) during prolonged droughts by extracting soil moisture at depth (Stephenson and Searle, 2014, Lloyd et al., 1991). In addition, the lateral root framework and associated fibrous roots appeared to retain their function for one and a half years before starting to decay upon the onset of the rain season and wet soil conditions, which are favourable for fungal growth. This raises a concern about the potential counterproductive effects of ineffective irrigation practices to root longevity. Under anaerobic conditions, as experienced under prolonged over-irrigated conditions, root longevity often decreases due to internal and external toxic compounds and the shortage of oxygen (Stephenson, 2004). In addition to a decrease in nutrient uptake, over-irrigation further increases the prevalence of microbial pathogens which may result in an increased potential for root diseases, as well as a reduction in root growth due to a decrease in soil temperature (Stephenson, 2004).

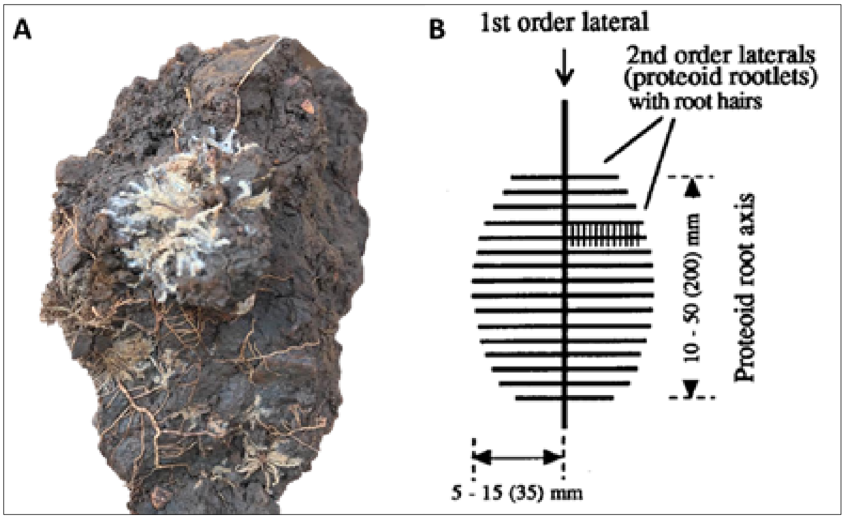


Figure 2.1 Visual (A) and schematic (B) representation of proteoid root characteristics (Dinkelaker et al., 1995)

Root longevity and efficiency in water and nutrient uptake are also related to the suberin levels in root cell walls. Suberin is a fatty acid derived compound that is impermeable to water. Older roots have a greater degree of suberisation giving them their characteristic brown colour (opposed to the white colour of young root tips) and making them less efficient in water uptake. The suberisation of roots, therefore, tends to be favourable under dry soil conditions, enabling roots to survive without desiccation. In some plants, roots suberise to the root tip under adverse conditions, but can resume growth upon the onset of favourable conditions, even after prolonged periods of drought. In fact, Firth et al. (2003b) noted a similar response in macadamia roots, finding that the fibrous roots of macadamia have the capacity to regenerate new growth from desiccated roots following a drought. This author further observed macadamia fibrous roots to be more hardened and non-fleshy when compared to other subtropical crops, such as avocado, suggesting macadamia roots may be better adapted and less likely to suffer tissue collapse under dry soil conditions.

Gas exchange and whole tree water relations

Certain adaptations in plants that aid them in surviving adverse conditions and allow for long term survival are not necessarily ideal agronomic characteristics. For example, evergreen sclerophyllous species tend to have lower photosynthetic rates and lower short-term productivity than deciduous species in order to maintain physiological functioning under adverse conditions (Read et al., 2006). This means that sclerophyllous species could have an agronomic competitive disadvantage under optimal conditions.

This is no different for sclerophyllous macadamia species. Macadamia leaves, once hardened-off, have a light saturated net photosynthetic assimilation rate (A_{max}) of approximately 14.0 µmol m^{-2} s^{-1} (Lloyd, 1991) which is similar to those reported for other evergreen crops such as citrus (13 µmol m^{-2} s^{-1}) (Assar et al., 2014, Ribeiro and Machado, 2007) and avocado (9.0 µmol m^{-2} s^{-1}) (Schaffer et al., 1991) but is substantially lower than that of deciduous almond trees (18 µmol m^{-2} s^{-1}) (Lakso, 1989). Net photosynthetic assimilation rate, however, is influenced by a number of factors which include stomatal conductance (g_s) which in turn are influenced by internal leaf CO_2 concentrations, leaf-to-air vapour pressure deficit (VPD_L), plant water potential (Ψ) and irradiance, to name a few (Nobel, 1999, Ribeiro and Machado, 2007, von Caemmerer and Baker, 2007). Drought stress on the other hand is known to influence a range of the aforementioned physiological processes such as g_s, VPD_L and Ψ (Ow et al., 2011). Due to the relationship between A_{max} and these physiological parameters, a change in A_{max} can be used as an indication of crop water stress, should water availability be the only limiting factor.

Nonetheless, plants strive to maintain Ψ within an optimal range to avoid xylem cavitation and retain plant turgor (Jones and Tardieu, 1998). This can, however, only be achieved by complete or partial stomatal closure in response to high evaporative demands, which comes at the cost of photosynthesis. According to Syvertsen and Lloyd (1994), variations in plant water relations is the principal plant response to their surroundings and changing environment. This seems to be particularly accurate for macadamias. When measuring diurnal g_s and leaf water potential (Ψ_L) between irrigated and non-irrigated macadamias, Lloyd et al. (1991) noted that g_s was lower in early mornings and reached a maximum at noon, while Ψ_L reached a minimum (-1.1 MPa for irrigated and -1.2 MPa for non-irrigated tree) just prior to noon (10:00), after which a decline in g_s was observed. Lloyd et al. (1991) also noted a partial reduction in g_s for both irrigated and non-irrigated trees when leaf temperatures reached a maximum of 30°C at 9:00, with similar minimum Ψ_L reached one hour prior to partial stomatal closure. On this hot day, an initial reduction in Ψ_L was noted from pre-dawn until 8:00, after which Ψ_L remained constant for both irrigated and non-irrigated trees before increasing after 16:00. Stomatal conductance was, however, substantially lower for non-irrigated trees than for irrigated trees on both occasions. In another attempt to quantify macadamia responses to water stress, Stephenson and Gallagher (1989) measured g_s and Ψ_L of one-year-old potted macadamia trees under controlled conditions. The authors recorded $g_s = 0$ at Ψ_L of -1.8 MPa to -2.0 MPa, while zero turgor occurred when Ψ_L reached −3.8 MPa and mature leaves were permanently damaged at −5.0 MPa. Soft young leaves wilted at Ψ_L of −2.4 MPa.

Similar observations to Lloyd et al. (1991) of stomatal closure in the absence of changes in Ψ_L have been made for a variety of species (Roman et al., 2015, Tardieu and Simonneau, 1998, Saliendra and Meinzer, 1989, Bates and Hall, 1981, Blackman and Davies, 1985). This common phenomenon described by Lloyd et al. (1991) for macadamias is typically referred to as an isohydric plant water management behaviour (Tardieu and Simonneau, 1998). In isohydric plants, midday Ψ_L remains relatively constant irrespective of fluctuations in pre-dawn water potentials (Ψ_{pd}) (therefore soil water content) and atmospheric evaporative demands (Campbell and Norman, 2012, Jones and Tardieu, 1998), primarily due to strong stomatal down regulation of g_s under water limited conditions. Consequently, this is viewed as a valuable physiological plant adaptation to periodic droughts (Franks et al., 2007). Recent work by Smit et al. (2020) has, however, suggested that macadamias may follow a more isohydrodynamic water management approach, as described by Franks et al. (2007), depending on crop sink demand. Following an isohydrodynamic approach can provide plants with an adaptive advantage under both semi-arid and arid conditions. A dynamic approach may enable plants to maintain a constant Ψ_L with periodic soil water content fluctuations, whilst still allowing plants to opportunistically maintain carbon assimilation under both water limitations and high vapour pressure deficit (VPD), permitting long term survival under water deficit conditions (McDowell et al., 2008, Alsina et al., 2007). Even though water deficits are not often experienced in irrigated agriculture, periods of high VPD are common during critical vegetative and reproductive growth stages. Hence, crops which follow a dynamic approach to somewhat maintain agricultural productivity during periods unfavourable to growth may be more productive in the long term.

2.2.2 Macadamia phenology

Evergreen gas exchange and whole tree water relations tend to be influenced by various environmental and internal plant stimuli, such as the crop sink demand. These stimuli, however, change with different stages of crop development and climatic seasonality. Macadamia gas exchange and whole tree water relations are therefore influenced by changes in the tree phenological cycle (Stephenson et al., 2003, Carr, 2013, Stephenson and Searle, 2014, Huett, 2004) (**Figure 2.2**).

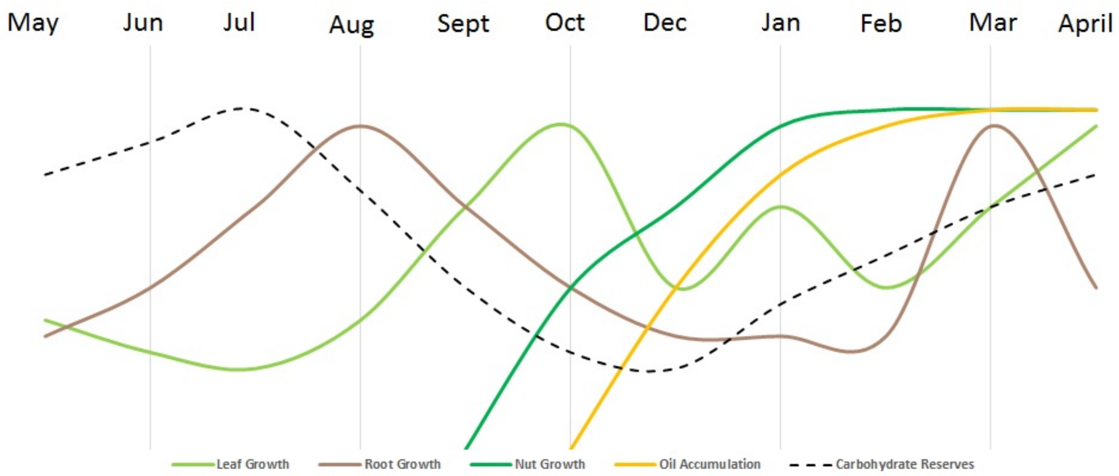


Figure 2.2 Schematic representation of macadamia phenology under Southern hemisphere conditions. Chart derived from Stephenson et al. (1989a)

Macadamia vegetative growth

Macadamia vegetative growth commonly occurs through periodic flushes (Olesen, 2005, Carr, 2013), followed by budding and stem elongation before a new vegetative flush occurs. Recently emerged macadamia flush leaves expand for an approximate 20 days before hardening off (Huett, 2004), which is similar to what was previously noted for avocado (Schaffer et al., 1991). Two major macadamia flushes generally occur during spring (August – September) and the end of summer (March – April) under South African conditions.

During the autumn and winter months preceding the first spring flush, the tree stores large amounts of carbohydrates in preparation for this event (Cormack and Bate, 1976). Even though the tree is actively photosynthesising during the spring flush period, stored carbohydrate reserves are reported to supplement fruit and shoot growth during peak demand (Huett, 2004). These reserves are therefore at their lowest during periods of peak vegetative and reproductive growth, as illustrated in **Figure 2.2** (Stephenson et al., 1989a). The spring vegetative flush further coincides with the onset of flowering and the start of nut growth, which further increases the need for readily available energy in the form of carbohydrates (Stephenson et al., 1989a). This is exacerbated by the fact that young macadamia flush leaves are initially net sinks of photosynthates, hence they have a negative net assimilation rate (A), before becoming the most productive part of the tree canopy (Huett, 2004).

Vegetative growth, however, is highly influenced by environmental factors including temperature and water availability, as well as various management practices such as pruning and the availability of nitrogen. These factors can influence both the timing and extent of the vegetative flush. Trochoulias and Lahav (1983) demonstrated temperatures between 20°C and 25°C to be optimal for vegetative growth. Although some growth still occurs at 15°C, winter temperatures lower than 10°C may delay the onset of the spring flush, until mean daily temperatures are consistently higher than this lower threshold (Stephenson et al., 1986). Similar inhibitory effects on vegetative growth have been recorded for trees subjected to water deficit conditions, with vegetative growth only commencing once favourable soil water content levels are attained (Stephenson et al., 2003).

Analogous to vegetative flushes, macadamias typically have two substantial root flushes within a single production season. These root flushes are believed to commence in the autumn or early winter period, with a second flush in spring, typically alternating with a vegetative flush. Due to competition between roots and shoots for carbohydrate reserves (Stephenson, 2004), smaller root flushes are expected during periods of peak vegetative flush, as observed in **Figure 2.2**. Similar observations have been made previously for both avocados and other deciduous crops (Bevington and Castle, 1985). In an above-ground rhizotron study in Florida, avocado shoot and root growth flushes appeared to be synchronised and alternated on 30 to 60-day cycles (Ploetz et al., 1993). Although tree carbohydrate dynamics have a distinct influence on the rate and extent of the root flush, factors such as temperature, water, soil chemistry and structure also have a noteworthy effect on root growth (Stephenson, 2004).

Trochoulias and Lahav (1983) found that optimal soil temperatures of approximately 15°C may promote root growth, whilst Allan (1972) showed that high soil temperatures (>20°C) can lead to a significant reduction in root growth. The reduction in root growth due to high temperatures can, however, not be explained by a single process, as it is a function of various complex interactions ranging from changes in root respiratory levels to the inhibition of functional enzymes (Pregitzer et al., 2000). High soil temperatures are often linked to higher soil evaporation rates (Monteith, 1981), and inhibition of topsoil root growth could therefore also be due to a reduction in soil water content.

Macadamia reproductive growth

Macadamia reproductive phenology is influenced by various environmental (Stephenson and Gallagher, 1986a, Stephenson et al., 2003) and tree specific factors, such as stem length and age (Wilkie et al., 2009). Characteristically, macadamia flower initiation occurs during the shortening days (day length 10 h 40–50 minutes) (Carr, 2013) in late autumn or winter when minimum temperature is between 11°C and 15 °C (Trueman, 2013), after which flower buds are dormant for two to three months depending on weather conditions. Macadamia flowering naturally commences with the onset of spring and a rise in temperatures, with flowering concentrated during August and September under South African conditions. Even though Stephenson and Gallagher (1986a) have shown that night temperatures may play a major part in the extent and timing of flower development, few studies have described the influence of other environmental variables, especially water availability, on the extent and timing of flower development. A study by Stephenson et al. (2003) found that the timing of water stress, as well as the severity of water stress, had no consistent effect on flowering intensity. Further studies investigating the effect of water stress on flower development are therefore required.

Nonetheless, nut set shortly follows flower anthesis, after which the embryo, ovule and endosperm start growing rapidly after fertilization (Walton et al., 2012, McConchie et al., 1996). Three periods of abscission occur at different stages after anthesis, where after only 0.3% of the initial set fruit per raceme may reach maturity (Nagao, 2011, Carr, 2013). Premature nut abscission (also known as November drop) occurs around eight weeks after anthesis and can significantly influence final yield. November drop is influenced particularly by high temperatures, low atmospheric humidity (Stephenson and Gallagher, 1986a) and water availability, which have been demonstrated to significantly increase premature nut abscission (Stephenson et al., 2003). Premature nut abscission is believed to be a natural crop load adjustment, with studies showing that increased leaf area during this stage decreased nut drop and therefore also increased final nut yield (Stephenson and Gallagher, 1986a). Factors influencing the build-up of tree carbohydrate reserves and current photosynthesis during this period may therefore have a significant impact on the severity of abscission (McFadyen et al., 2012).

Following the nut abscission period, the embryo develops in those nuts remaining on the tree and absorbs the entire endosperm (nut fill), until the embryo comes into contact with the inner integumentary membrane of the testa. The testa will harden and become lignified as the embryo matures (McConchie et al., 1996) which is commonly referred to as shell hardening. Macadamia kernel (embryo) takes approximately six months to mature and at this stage, the kernel contains at least 72% oil and has a specific gravity <1.0 g/cm^3 (Carr, 2013). Nut fill, shell hardening and oil accumulation all require significant carbohydrate supply (**Figure 2.2**) which, if not sufficiently available, may influence final kernel yield and quality. Hence, being a biological process, kernel mass and shell thickness have been found to be particularly influenced by temperature extremes and water limitations during the different phenological stages (Stephenson and Gallagher, 1986b, Stephenson et al., 2003).

2.3 Macadamia water relations

The terms yield potential, drought resistance and water use efficiency (WUE) are often used in agricultural environments without a thorough understanding of the meaning. Yield potential is generally defined as the maximum yield achievable under non-stressed conditions. Drought resistance on the other hand is defined in a physiological context by Blum (2005) as some degree of dehydration-avoidance and/or dehydration-tolerance expressed by a plant. In simplest terms, WUE is defined as crop yield (kg) per unit water use (WU) (the denominator). Water use is expressed as total crop evapotranspiration (ET) (ET = evaporation (E) + transpiration (T)). On a biological and agronomic level, WUE should, however, be defined as the amount of photoassimilates or carbohydrate equivalents formed per unit water used (Howell, 2001, Stephenson and Chapman, 2010). This is fundamental to the understanding of WUE, as otherwise oil producing crops would often be described as "underperforming" (if measured on a mass basis only) when compared to sugar producing crops. Knowing this, water allocations can also rather justified in terms of water-productivity (WP), expressed as income per unit water used (Fereres and Evans, 2006).

Due to complex genetic interactions and the long-term nature of breeding programs, increasing yield potential and inherent drought resistance in perennial crops is not always feasible in the short term. Improvements in crop WUE are therefore more often achieved through means of irrigation water management, for example by reducing E, since it is described as the largest, most controllable factor in terms of non-beneficial water use in irrigated horticultural crop production (Fereres and Evans, 2006). Reducing total water use through irrigation scheduling can, however, only be achieved by having (a) an estimation of crop water use; (b) an understanding of the crop response to water deficits and; (c) a method to schedule irrigation and/or to detect and monitor the degree of water stress (Fereres and Evans, 2006).

Conventionally, irrigation scheduling in fruit trees aims to avoid tree water deficits by irrigating to the full ET requirements of the crop during all growth stages. In recent times, however, WUE of horticultural crops have been improved by not only improving the efficiency of water application through improved irrigation technology, but also through irrigating less than the full crop ET requirements (Fereres and Soriano, 2007). This premeditated scheduling of irrigation water to meet less than crop ET requirements in order to reduce water use while maintaining or increasing farm profitability, is termed deficit irrigation (DI). Deficit irrigation strategies often include either regulated deficit irrigation (RDI) or partial root-zone drying (PRD) (Kang and Zhang, 2004) (**Figure 2.3**).

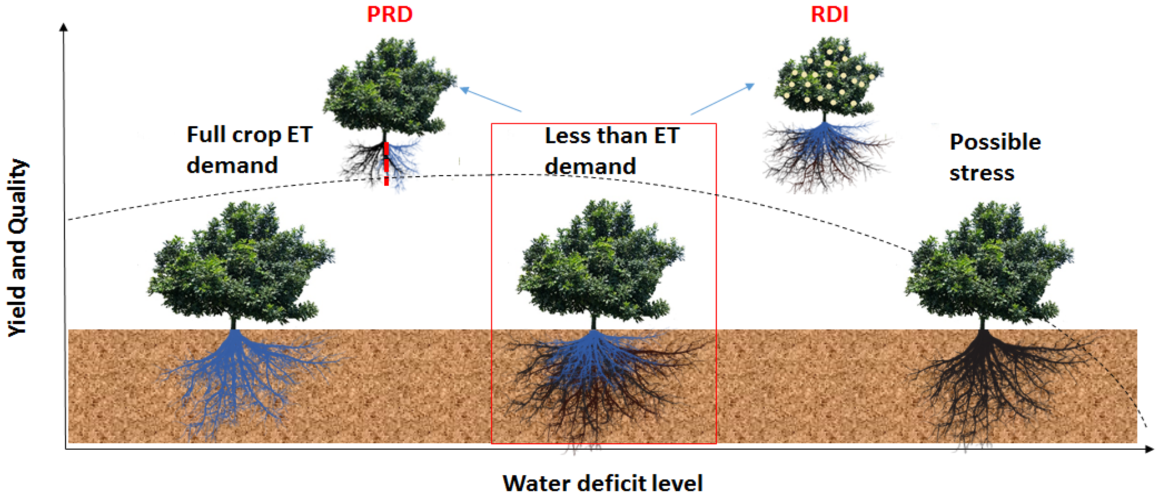


Figure 2.3 Schematic representation of deficit irrigation (DI) practices including two DI categories namely, partial root-zone drying (PRD) and regulated deficit irrigation (RDI). Blue shading of roots denotes well-watered and black indicates water deficit.

Under RDI, water application below crop ET requirements is imposed during crop developmental stages when tree yield and quality responses are not sensitive to water deficits (Boland et al., 2000, Ebel et al., 1995, Mitchell and Chalmers, 1982). Water savings of up to 23% and improvements in WUE between 14% and 27% using RDI in citrus have previously been recorded by Gasque et al. (2010). Hence, this strategy could be valuable during circumstances when seasonal water allocations do not meet crop ET. In addition, there are a significant number of reports indicating a minimal impact of DI practices on fruit yield and quality (Ballester et al., 2011, Hutton et al., 2007, Hutton and Loveys, 2011, Moriana et al., 2003). Effective RDI implementation does, however, require the identification of the critical phenological stages least sensitive to water deficits, as reported by Stephenson et al. (2003) in a macadamia field study, conducted on draining lysimeters.

Few studies have reported the water use and water relations of macadamia under field conditions. According to Stephenson et al. (2003), macadamia has an average ET requirement of 75 l tree^{-1} day^{-1}, ranging between 50 l tree^{-1} day^{-1} in winter and 80 l tree^{-1} day^{-1} in summer. It is further reported that the minimum annual rainfall required for successful macadamia production is approximately 1000 mm, while yields are improved by irrigation in areas where relative dry periods coincide with the flowering and early nut sizing stages (Carr, 2013). Stephenson et al. (2003) further reported that water stress at floral development, premature nut drop and nut maturation had a significant effect on the yield and quality of macadamias. Searle and Lu (2002) have, however, reported macadamias to have a daily ET requirement less than what is proposed by Stephenson et al. (2003). The uncertainty surrounding macadamia water requirements, in combination with large variations in irrigation reports, therefore allows for a number of opportunities to assess and research the feasibility of DI practices in macadamia production.

2.4 Current and proposed macadamia irrigation strategies

Irrigation, especially in high value horticultural crops, has shown to not only protect large investments against the risk of droughts, but also to increase and stabilize long term crop productivity. It has further been shown that efficient irrigation management practices not only maintains but improves harvested product quality and therefore any value added product thereafter (Fereres and Evans, 2006). Although this has been known for almost as long as the existence of commercial irrigated horticulture, it has taken many years of irrigation for humankind to realise that irrigation can have negative environmental (e.g. accumulation of salts, leaching of nutrients and erosion), yield and therefore financial consequences (Fereres and Evans, 2006, Shock and Wang, 2011).

Macadamias, no different to other horticultural crops, require a large initial investment in terms of land, purchase of grafted trees, machinery, infrastructure, soil preparation and in most areas, irrigation. In Australia, the installation of irrigation depends on the average annual rainfall, with areas exceeding an annual rainfall of 1140 mm being mainly rain-fed (Carr, 2013). Supplementary irrigation during the first four years after transplantation, beginning with 40 L week^{-1} tree^{-1} in year one is considered essential in both New South Wales and southern Queensland, Australia. Similar recommendations (20-30 L week^{-1} tree^{-1}) with regards to supplementary irrigation in the first production years are made under South African growing conditions. Most South African orchards are, however, irrigated and irrigation water quantities often exceed the recommended rate of 20-30 L week^{-1} tree^{-1}. In most South African regions, a weekly irrigation volume of up to 420 L week^{-1} tree^{-1} is recommended in mature (> 8-year-old) macadamia orchards (personal communication with industry consultants).

Weekly irrigation volumes are, however, far exceeding the recommended 420 L week^{-1} tree^{-1} under Australian conditions, with a recommended rate of 150 L week^{-1} tree^{-1} for two-year-old trees, increasing the maximum weekly rate by the same amount each year thereafter, up to year 10 (1500 L week^{-1} tree^{-1}) (Carr, 2013). No consistent and conclusive reports have, however, been made to justify the need for irrigation in macadamia orchards, especially in high rainfall regions (Awada et al., 1967, Trochoulias and Johns, 1992).

Nevertheless, South African macadamia orchards are currently typically irrigated using micro-sprinkler or drip irrigation systems, applying water at various rates depending on the respective emitter volume and system pressure. Likewise, newly developed ultra-low flow drippers (<1.0 l/hr emitter rate) provide growers with a large wetting pattern like that delivered by micro-sprinklers. With the higher efficiency, they are becoming more popular and a large number of orchards are changing over to this system. This change in systems is one of the most obvious approaches to improve WUE and nutrient use in macadamia orchards by reducing unproductive water losses through reducing or eliminating E and deep drainage (Fereres et al., 2003). Searle and Lu (2002) showed that simple strategies such as converting from micro-sprinklers to drip irrigation can reduce water use by 23-35% from flowering through to nut maturity without any adverse effects on yield or quality.

Installing any of the aforementioned irrigation systems, however, forms a substantial part of the large initial capital investment in macadamia production. The average fixed cost of irrigation installation ranges between R45 000 and R75 000 per hectare, depending on the amount of existing infrastructure and the level of automation within the irrigation design. Variable costs of irrigation are, however, estimated to be at R3.36/mm water when irrigated by a micro-sprinkler system (Bungay, 2018). Hence, with current recommended irrigation quantities of approximately 570 mm ha^{-1} year^{-1} (350 L/week), the average cost of irrigation would not exceed R2000 ha^{-1} year^{-1}. Although substantially lower than the cost of irrigation for sugarcane (± R8000 ha^{-1} year^{-1}) in the same production regions, at an average macadamia dry nut in shell (DIS) price of R71.63/kg (SAMAC, 2019), the cost of irrigation water may not be of any significant value if average yields of up to 3000 kg/ha DIS is achieved.

Due to the high crop value and the low cost of irrigation water, the use of DI practices, therefore, cannot be justified from a cost savings perspective and the real value possibly lies in the improvement of on farm WUE (kg produced per unit water used) and WUP (Rand generated per unit water used) (Stephenson and Searle, 2014). Using DI practices in macadamia orchards can therefore be justified through the large opportunity cost related to irrigating less, by allowing for expansion on previously deemed “unsuitable” land, expanding current planted areas, improving tree health by decreasing disease incidence and most importantly, maintaining orchard productivity and profitability during water limited periods, for example, droughts.

CHAPTER 3: MATERIALS AND METHODS

Two trial sites were used during the course of this study. Water deficits at different phenological stages were implemented in a mature orchard over three seasons, whilst a shorter trial to assess the physiological response of young macadamia trees (intermediate orchard) was conducted over a period of a month. Both orchards were located on the same farm, situated approximately 25 km west of Nelspruit town in the Schagen Valley, Mpumalanga.

Figure 3.1 Positioning of the mature and intermediate 'Beaumont' macadamia orchards on the Du Mak farm

3.1 Mature orchard

3.1.1 Trial site

The trial site was situated approximately 25 km west of Nelspruit town in the Schagen Valley, Mpumalanga (GPS-Coordinates: 25°23'43.3"S 30°46'47.1"E) on a macadamia orchard planted in 2003 (14 years old at the start of the trial) of roughly 2.9 ha (896 trees) in size. The orchard consisted of 18 rows of bearing, micro-sprinkler irrigated macadamia trees (cv. HAES 695, 'Beaumont', *M. tetraphylla x M. integrifolia*, grafted on 'Beaumont' rootstock) planted on an 8 x 4 m spacing, totalling 312 trees per hectare. Trees were planted in a North-South orientation with an average measured tree height at the start of the trial of 5.5 m and canopy width of 4.8 m, forming a complete hedgerow. Trees were pruned by selective limb removal on an annual basis to maximise light interception and orchard ventilation, removing no more than 30% of the tree canopy. The orchard consisted of predominantly 30% Westleigh, 30% Kroonstad, 16% Hutton, 16% Swartland, and 8% Shortlands soil types. Further information on the trial site soil characteristics can be found in **Annexure 1**. The orchard was not ridged, and trees were mulched, using pruned material, directly after harvest. Trees were irrigated by means of one micro-jet sprinkler (50 L h^{-1}) per tree, with a wetted diameter of 3.0 m, according to a cycle determined by readings from a capacitance probe (DFM Software Solutions CC, Stellenbosch, Western Cape, South Africa) installed at a 1.2 m depth between two macadamia trees in the orchard. Orchard details are provided in **Table 3.1**.

Table 3.1 'Beaumont' (6 95) orchard overview at Du Mak Farm in the Schagen Valley, Nelspruit

Cultivar	'Beaumont'
Rootstock	'Beaumont'
Age	14 years old (planted 2003)
Orchard block area	2.94 ha
Tree spacing	8 m x 4 m (312 trees ha^{-1}), no ridges
Row orientation	North-South
Irrigation	Micro-sprinklers, with a delivery rate of 50 L h^{-1} Wetted diameter = 3 m
Canopy dimension ($\bar{x}$ = 10 measurements)	Height 5.5 m, Width 4.8 m, Complete hedgerow
Canopy cover	0.71
Number of experimental trees	Three experimental blocks with seven treatments per block, each treatment consisting of three trees = 63 trees

3.1.2 Trial design

The response of macadamia to mild water deficit conditionswas measured on the basis of final nut set, nut drop, yield and quality. These measurements were made in conjunction with measurements of the physiological response of the trees to water deficit condition, which included leaf water potential and leaf gas exchange.

The experiment spanned over a period of three seasons from August 2017 to April 2020, where seven water deficit treatments were imposed on 63 randomly selected trees and respective yield, quality and physiological characteristics were evaluated. Yield and quality evaluations for the 2019/20 season were affected by the Covid-19 pandemic, as a result of harvest needing to occur during lockdown Level 5 when researchers did not have access to the orchard. The 2017/18 trial consisted of three replicate blocks consisting of three treatments (3 trees per treatment) (n = 9) at different water application rates namely: T1 = no-irrigation/rainfed; T2 = half irrigation; T3 = normal irrigation. Treatment T3 served as the experimental control. These treatments were implemented for three seasons. In addition to the aforementioned treatments, four water deficit treatments at different phenological stages (3 trees per treatment) namely: T4 = flowering and nut set; T5 = nut sizing and premature nut drop; T6 = shell hardening and T7 = oil accumulation, were applied to each replicate block during the 2018/19 and 2019/20 seasons. Treatments were assigned randomly to a completely randomized block design of which each treatment replicate occurred in every second row, separated by approximately 16 m to avoid any border effect interference (**Figure 3.2**). Treatments were applied to three consecutive trees within a row and each treatment was separated by approximately two trees or 8 m.

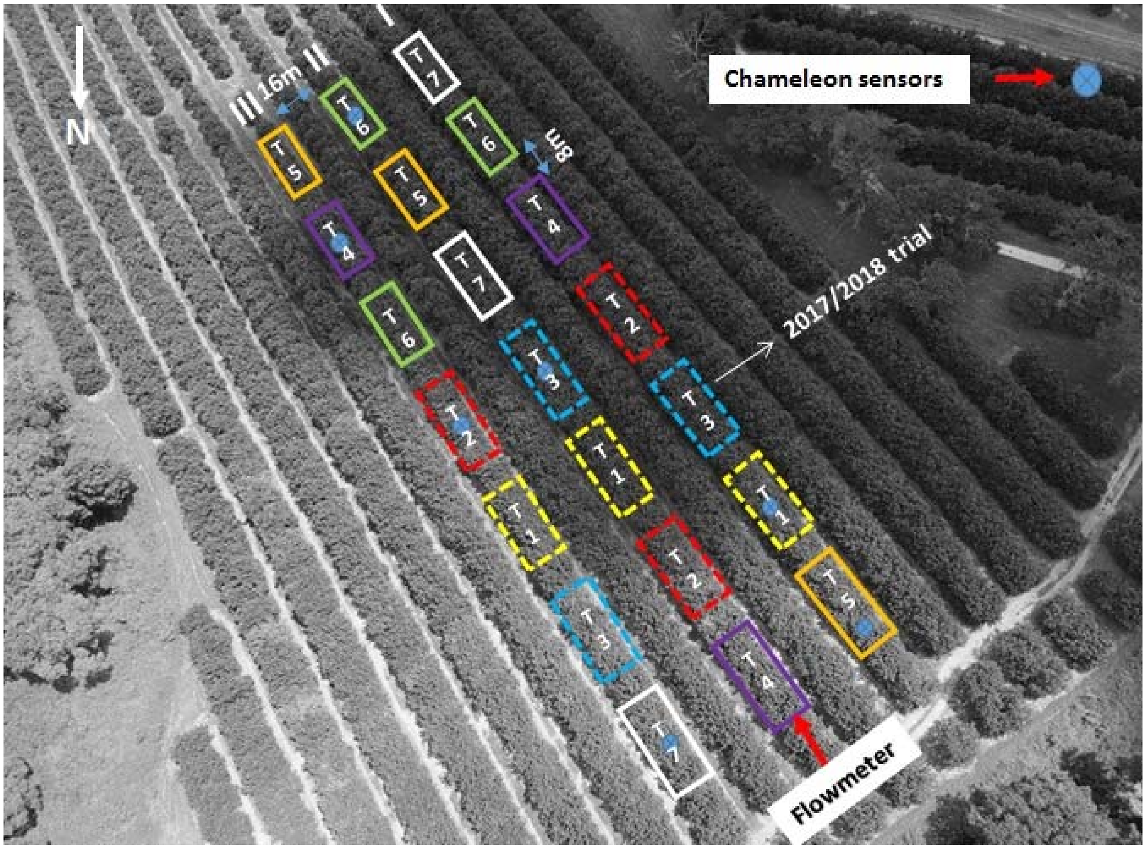


Figure 3.2 Orchard layout and completely randomized block design consisting of three replicated blocks used over a three season period. Dashed (--) blocks representing initial 2017/2018 trial blocks. T1- no-irrigation; T2- half irrigation; T3- normal irrigation (control); T4-T7 water deficit treatments at T4- flowering and nut set; T5- nut sizing and pre-mature drop; T6- shell hardening; T7- oil accumulation

3.1.3 Soil matric potential and water application rates

Measurements of soil water matric potential and irrigation volumes were collected throughout the duration of both the 2017/18 and 2018/19 seasons. Soil matric potential readings were performed over both 2017/18 and 2018/19 seasons for control, rainfed and half irrigation trees while matric potentials were only recorded over the 2018/19 seasons for trees stressed at different phenological stages. Soil water matric potential measurements were collected using seven Chameleon probes (VIA, 2019), each containing three sensors which were placed at three different depths (0.2 m, 0.4 m and 0.6 m) in one of each of the seven different treatment replicates (**Figure 3.2 & Figure 3.3**). Sensors were positioned strategically within the canopy drip line and placed within a 1 m radius of the tree stem. Chameleon probes measure soil matric potential and a colour is assigned to each matric potential range. These ranges included, Blue (0 to -

20 kPa); Green (-20 to -50 kPa); Red (>-50 kPa); and Grey (Unreadable). Measurements within the blue colour range represent conditions of sufficient water for plants, but leaching is likely to occur. Measurements within the green colour range represent conditions of sufficient water, but no leaching, while measurements within the red colour range represent dry soil conditions where stress is likely occurring. Grey colours presented in measurement figures are indicators of soil water depletion, with matric potential readings far exceeding -50 kPa, surpassing the measurement accuracy of the Chameleon sensors (VIA, 2019). Grey colours could also represent faulty instrumentation. Irrigation volumes were recorded by using a flowmeter plumbed into the start of the irrigation line of the middle replicate block (**Figure 3.2**).

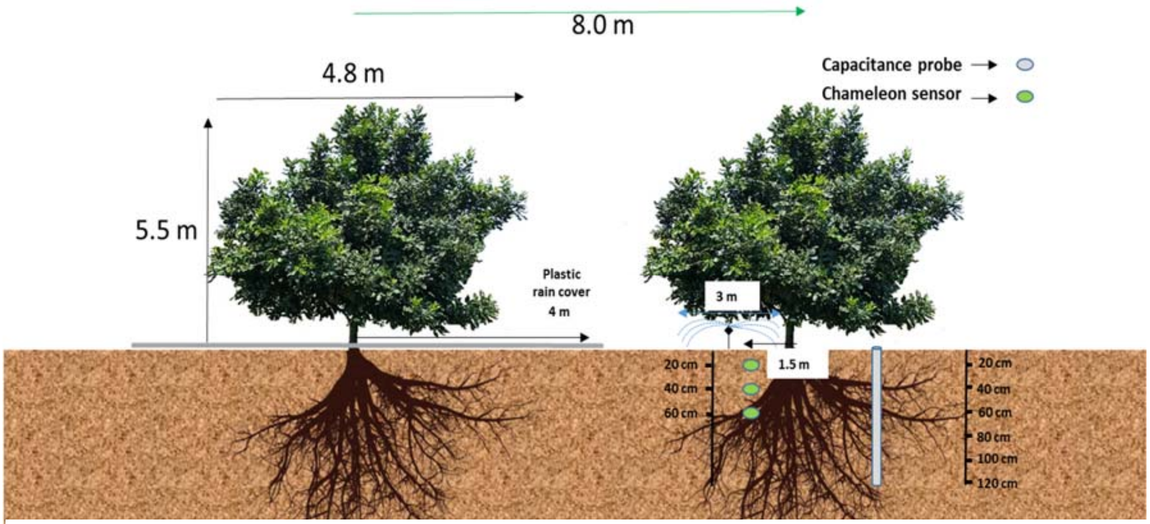


Figure 3.3 Placement of plastic rain covers and 7 chameleon water sensor probes in the macadamia orchard at Du Mak Farm in the Schagen Valley, Nelspruit

Irrigation quantities (expressed in mm) was calculated on a per canopy volume basis by dividing total applied litres (with given sprinkler diameter) by the total one dimensional canopy area which was assumed to be equal to the root area. This was done to assess the actual amount of water available to the tree within the rootzone.

3.1.4 Water deficit implementation at different phenological stages

In order to determine the effect of water deficits at different phenological stages, similar to those proposed by Stephenson et al. (2003), 200 µm clear plastic rain-covers were installed on each treatment replicate prior to the onset of a phenological stage (**Figure 3.4**). Plastic rain-covers were placed up to the tree stem and extended past the 32 m^2

soil surface allocated to each tree, eliminating unintentional water replenishment through the exclusion of rainfall. Valves were fitted to the micro-irrigation tube of each treatment tree in order to “turn-off” the micro-sprinkler and eliminate irrigation water during the respective phenological stages. The implementation of a no-irrigation/rainfed (T1) treatment was performed in a similar fashion, as irrigation water was eliminated with the use of valves fitted to the micro-irrigation tube of each tree. However, no plastic was placed under these trees. Half irrigation treatments were implemented by replacing 50 L h^{-1} sprinkler heads with 25 L h^{-1} sprinkler heads on each of the T2 treatment trees.

Figure 3.4 Implementation and placement of 200 µm clear plastic rain-covers beyond the 32m^2 allocated to each treatment tree in order to implement water deficits during different phenological stages by eliminating unwanted soil water replenishment

A preliminary water deficit trial to determine the level of water stress required to induce a mild macadamia water deficit, as well as the rate of decline in pre-dawn leaf water potential was performed on one no-irrigation/rainfed (T1) treatment. The preliminary trial commenced after the 2017/18 season harvest on 4 March 2018, where plastic rain-covers were installed on one of the three T1 treatment replicates. The preliminary trial was further used for the implementation of a mild water deficit during macadamia flower initiation. Based on the findings by Stephenson and Gallagher (1989), April-May is the best estimate of macadamia flower initiation and was therefore used to determine the effect of a mild water deficitduring the flower initiation period on flowering intensity, flower panicle size and nut set. Stephenson and Gallagher (1986a) further state that potential yield is determined at floral initiation, therefore understanding the impact of the environment on this important process was desirable. The preliminary trial was terminated on 7 May 2018, prior to the onset of flowering.

Water deficit implementation during flowering and nut set (T4) commenced on 13 July 2018 and 25 July 2019, where nine macadamia trees (3 trees per treatment replicate) were exposed to a mild water deficit during flowering and initial fruit set. Pre-dawn leaf water potential measurements were used as an indication of the degree of water stress experienced during the respective phenological stage. In previous studies soil water content was replenished if a pre-dawn leaf water potential reached -1.5 MPa (Stephenson et al., 1989b, Stephenson and Gallagher, 1987, Stephenson et al., 2003), but such low levels weren't reached during the course of this study. Replenishment of soil water occurred through the opening of valves on the micro-irrigation tubing, allowing for a 2-hour irrigation interval at the normal (50 L hr^{-1}) water application rate. At the end of the water deficit period, soil water content was replenished in order to re-establish a pre-dawn leaf water potential of -0.7 MPa, maintaining water stress without causing irreversible physiological damage. Plastic rain-covers were ultimately removed from T4 treatment trees after initial fruit set (2 October 2018 and 5 October 2019), where after trees were irrigated according to the control for the remainder of the season.

The implementation of a mild water deficitat nut sizing and premature nut drop (T5), shell hardening (T6) and oil accumulation (T7) during the 2018/19 season commenced on 1 October 2018, 3 December 2018 and 30 January 2019 respectively. Details of the implementation of the various treatments for the 2018/19 and 2019/20 seasons are presented in **Table 3.2**. Pre-dawn leaf water potential measurements and soil water replenishment occurred in a similar fashion for each of the deficit treatments, as described for the T4 treatment. Similarly, plastic rain-covers were removed from treatment trees upon the change of one phenological stage to another, where after trees were watered similar to the control for the remainder of the season.

Table 3.2 The implementation of water deficittreatments in the mature orchard at Du Mak Farm in the Schagen Valley, Nelspruit. Percentage (%) values represent the quantity of irrigation applied in relation to that of the treatment control, during a specific phenological stage

Treatment	Season	Fruit developmental Stage				
		Flower initiation	**Flowering and nut set**	**Nut sizing and premature nut drop**	**Shell hardening**	**Oil accumulation**
	2018/19	**4 Mar. – 7 May.**	**13 Jul. – 2 Oct.**	**1 Oct. – 12 Nov.**	**3 Dec. - 30 Jan.**	**30 Jan. – 21 Apr.**
	2019/20		**25 Jul. – 5 Oct.**	**5 Oct. – 12 Dec.**	**12 Dec. – 7 Feb.**	**7 Feb. – 17 Apr.**
T1		0%	0%	0%	0%	0%
T2		50%	50%	50%	50%	50%
T3		100%	100%	100%	100%	100%
T4		100%	0%	100%	100%	100%
T5		100%	100%	0%	100%	100%
T6		100%	100%	100%	0%	100%
T7		100%	100%	100%	100%	0%

3.2 Intermediate orchard

3.2.1 Trial site

The intermediate orchard trial site was situated on the same farm, Du Mak farm, in the Schagen Valley, Mpumalanga (GPS-Coordinates: 25°23'43.47"S, 30°46'59.24"E) in a 4-year-old macadamia orchard. Trees (cv. HAES 695, 'Beaumont', *M. tetraphylla x M. integrifolia*, grafted on 'Beaumont' rootstock) were planted on an 8 x 4 m spacing, totalling 312 trees per hectare. Trees were planted in a North-South orientation with an average measured tree height of 2.8 m and canopy width of 2.5 m. Trees had not yet, formed a complete hedgerow. Trees were pruned prior to the installation of equipment and treatment implementation, not removing more than 10% of the canopy volume. The orchard was ridged and consisted of predominantly sandy, well-drained soils. Trees were irrigated by means of one micro-jet sprinkler (25 L h^{-1}) per tree, with a wetted diameter of 1.5 m. Micro-jet sprinklers were placed close to the tree trunk, wetting only the immediate root zone within a 1 m radius of the tree base. Orchard details are provided in **Table 3.3**.

Table 3.3 'Beaumont' (695) intermediate orchard overview at Du Mak Farm in the Schagen Valley, Nelspruit

Cultivar	'Beaumont'
Rootstock	'Beaumont'
Age	4 years old (planted 2016)
Orchard block area	10 ha
Tree spacing	8 m x 4 m (312 trees ha^{-1}), Ridged
Row orientation	North-South
Irrigation	Micro-sprinklers, with a delivery rate of 25 L h^{-1} Wetted diameter = 1.5 m
Canopy dimension ($\bar{x}$ = 10 measurements)	Height 2.8 m, Width 2.5 m, not forming a complete hedgerow
Canopy cover	Approximately 20%
Number of experimental trees	Five experimental treatments with three trees per treatment = 15 trees

3.2.2 Trial design

The physiological response of macadamia trees to different levels of water deficit was determined by measuring leaf water potential and leaf gas exchange. The experiment spanned over a one month period from 25 May 2020 to 9 July 2020 where the aim was to implement different soil water deficit levels on 15 selected trees by withholding irrigation for different periods of time. The trial was made up of five treatments consisting of three replicates per treatment (3 trees per treatment) (n = 3). Five different water deficit levels were implemented on a 4-year-old macadamia orchard namely: I1 = 30 day deficit; I2 = 25 day deficit; I3 = 20 day deficit; I4 = 15 day deficit and I5 = 0 day deficit. Treatment I5 served as the experimental control. Treatments were assigned to three consecutive trees within a row, with each treatment separated by one non-treatment tree or approximately 4 m (**Figure 3.5**). Due to micro-jet sprinklers being placed close to the tree trunk, a 4 m buffer zone between treatments was sufficient and any border effect interference was avoided.

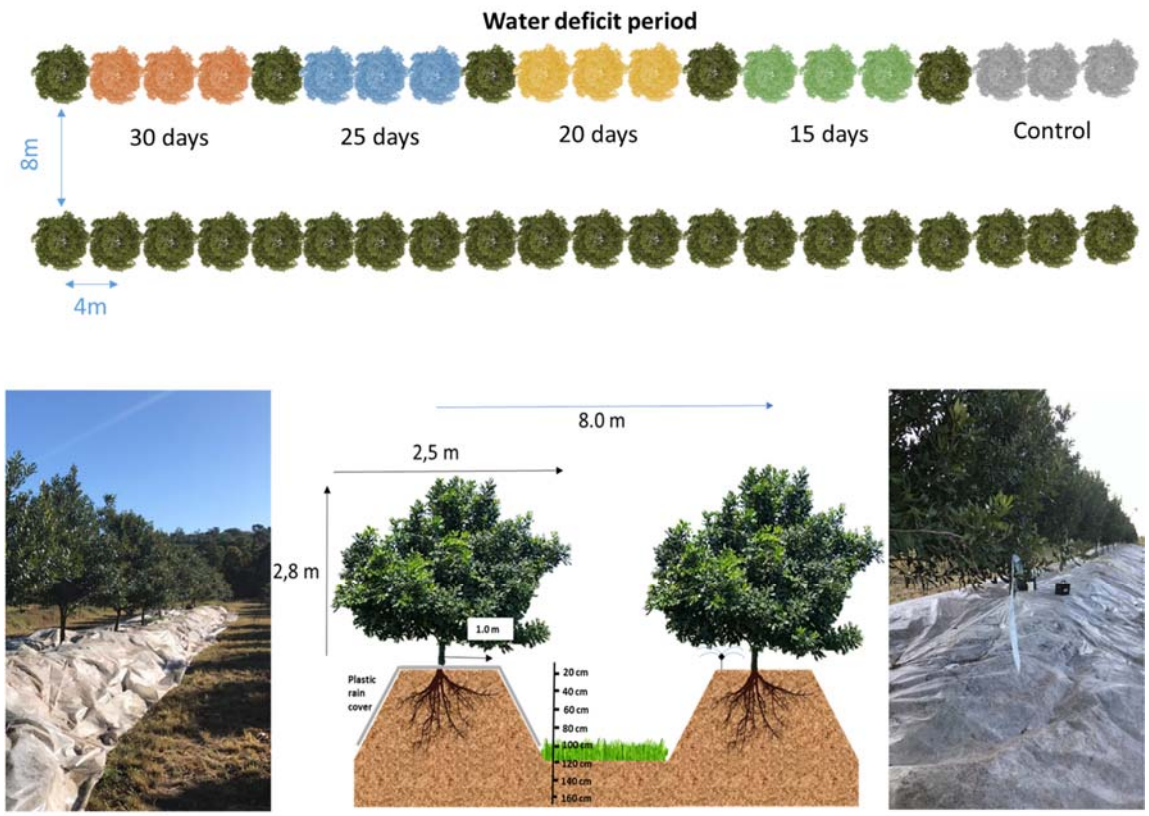


Figure 3.5 Intermediate orchard trial design and treatment implementation

3.2.3 Stress implementation and water deficit levels.

In order to determine the physiological response of macadamia to different levels of water deficit, 200 µm clear plastic rain-covers were installed on each treatment replicate on the day of stress implementation. Plastic rain-covers were placed up to the tree stem and extended to the edge of the ridged profile, covering the entire soil surface allocated to each tree (**Figure 3.5**). Valves were fitted to the micro-irrigation tube of each treatment tree in order to "turn-off" the micro-sprinkler and eliminate irrigation water during the respective treatment stages. A water deficit treatment was induced every 5 days, with the 30-day water deficit treatment starting on 25 May 2020. The 25, 20 and 15-day water deficit treatments commenced on 30 May, 4 June and 9 June 2020 respectively. Physiological measurements over all 5 treatments commenced 15 days later on 22 June 2020. The measurement period spanned a 3 day period from 22 June 2020 to 24 June 2020, after which all treatments (I2,3 and 4) other than the 30 day water deficit treatment (I1) and the treatment control (I5) were terminated. The I1 treatment was exposed to another 18 days without water replenishment (48 days cumulative water deficit) before the final physiological measurements were conducted on 9 July 2020. A summary of the implementation and termination of treatments in the intermediate orchard is presented in **Table 3.4**.

Table 3.4 Intermediate orchard treatment implementation and measurement overview at Du Mak Farm in the Schagen Valley, Nelspruit. * Indicates the treatment measurement dates, measuring leaf gas exchange

Measurement date	**Year**	**Treatment: Water deficit period**				
	2020	**48 days (I1) 25 May – 24 June**	**25 days (I2) 30 May – 24 June**	**20 days (I3) 4 June – 24 June**	**15 days (I4) 9 June – 24 June**	**0 days (i5) 24 June**
22 June		*	*	*	*	*
23 June		*	*	*	*	*
24 June		*	*	*	*	*
9 July		*				*

3.3 Meteorological measurements

To correlate measured tree variables with microclimatic conditions, weather data were obtained using a WS-GP1 Delta-T automatic weather station (Delta-T Devices Ltd, Cambridge, United Kingdom), which was installed within 7 km of the site. Weather data included solar radiation, wind speed and direction, temperature, relative humidity and rainfall data at 20-minute intervals. Daily reference evapotranspiration (ET_o) and vapour pressure deficit (VPD) were calculated using the procedure as described in FAO-56 (Allen et al., 1998) from weather data recorded by the automatic weather station.

3.4 Measurements of water stress under water deficit conditions

3.4.1 Water potential

Pre-dawn leaf (Ψ_{pd}) and midday stem (Ψ_x) and diurnal leaf (Ψ_L) water potentials were measured using a PMS system Scholander Pump Up Pressure chamber (PMS Instrument Company, Albany, USA) and a Scholander Pressure chamber (Model 600, PMS Instrument Company, Albany, OR, USA) on selected days. Pre-dawn leaf water potential measurements were made upon the implementation of a water deficit treatment and throughout the duration of the experiment on all stressed and non-stressed experimental trees in the mature orchard. Pre-dawn measurements in the intermediate orchard were only performed on four measurement days, 22-24 June 2020 and 9 July 2020. Mature, fully expanded leaves from the outside of the tree canopy were selected for pre-dawn measurement purposes. Pre-dawn measurement frequencies in the mature orchard depended on the water deficit level, with an increased frequency of measurements at higher water stress levels (<-0.5 MPa), as initial observations showed a rapid decline in Ψ_{pd} once a certain level of stress was reached. All Ψ_{pd} measurements were performed before 6:00.

Midday stem water potentials were measured by selecting fully expanded, mature leaves from within the tree canopy. Selected leaves were enclosed within aluminium covered plastic bags for a minimum of 30 min prior to measurement, in order to minimize the effect of external factors on measurements, stopping transpiration and enabling the leaf to come into equilibrium with the water potential of the stem (Begg and Turner, 1970). Midday stem water potential measurements took place between 11:30 and 13:00 on selected measurement days. In order to perform all Ψ_x measurements between 11:30 and 13:00 in the mature orchard, Ψ_x measurements were only performed on control and ongoing water deficit treatment trees (eg. Control & T4) for selected days. Diurnal Ψ_L were performed on an hourly basis between 7:00 and 17:00 during the different measuring campaigns. Diurnal Ψ_L was measured on the same mature, hardened-off leaves (the fourth leaf whorl) used for gas exchange measurements. Leaf water potential measurements were performed within 30 seconds after the respective gas exchange measurements were recorded.

3.4.2 Stomatal conductance and leaf gas exchange

Measurements of leaf gas exchange, including net photosynthetic assimilation rate (A), stomatal conductance (g_s), leaf temperature (T_{leaf}), leaf transpiration rate (T) and internal CO_2 concentration (C_i), were obtained using an infrared gas analyser (IRGA) (Model: LI-6400 XT, LI-COR, Lincoln, Nebraska, USA or Model: LI-6800, LI-COR, Lincoln, Nebraska, USA). 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 1200-1500 µmol $m^{-2}s^{-1}$ and relative humidity (RH) was maintained at more than 50%, as described by Smit et al. (2020). Spot measurements were, however, measured at ambient temperature and humidity inside the chamber and to minimize the effect that the chamber conditions will have on leaf gas exchange, measurements were made as soon as readings started stabilise.

Leaf gas exchange measurements were performed on mature, hardened-off leaves (the fourth leaf whorl), chosen from the outer Eastern and Western side of the tree canopy, which were exposed to the maximum photon flux density prevailing at the time. Three leaves per tree per treatment replicate (N = 9) were measured on an hourly basis in the mature orchard. Alternating between water deficitand control treatment replicates, diurnal leaf gas exchange measurements were performed on the day of termination of three of the stress treatments. Leaf gas exchange was measured over a two-day period on both the control and ongoing phenological water deficit treatment. Spot measurements were performed over three measurement campaigns during 2018/19 flower initiation, flower and nut set and shell hardening phenological stages in the mature orchard (**Table 3.5**).

Table 3.5 Date and frequency of leaf gas exchange measurements in the mature orchard at Du Mak Farm in the Schagen Valley, Nelspruit

Measurements	**Season**	**Fruit developmental Stage**		
		Flower initiation	**Flowering and nut set**	**Shell hardening**
	2018/19	**1 Jun. – 2 Jun**	**1 Oct. – 2 Oct.**	**30 Jan. – 31 Jan**
N		120	232	163

Similarly, leaf gas exchange measurements in the intermediate orchard were performed on mature, hardened-off leaves, chosen from the outer Eastern and Western side of the tree canopy, which were exposed to the maximum photon flux density prevailing at the time. Two leaves per tree per treatment replicate (N = 6) were measured on an hourly basis. Alternating between different treatment replicates, diurnal leaf gas exchange measurements were performed over a 3 day measurement period from 22 June 2020 to 24 June 2020. Additional leaf gas exchange measurements were performed on trees exposed to a 48-day water deficit on 9 July 2020 (**Table 3.6**).

Table 3.6 Date and frequency of leaf gas exchange measurements in the intermediate orchard at Du Mak Farm in the Schagen Valley, Nelspruit

Measurements	Season	Date			
	2019/20	**22 June**	**23 June**	**24 June**	**9 July**
N		264	256	219	100

3.5 Measuring the effect of a mild water deficit on growth, yield and quality

3.5.1 Nut drop

Immediately after nut set, the quantity of racemes and initial nut set was assessed on one branch of each of the 67 treatment trees, totalling 67 replicate measurements. Due to the variable and vigorous macadamia growth habit, branches were selected on the eastern side of all treatment trees based on the number of racemes per branch and measurement potential (set per raceme). Measurements to determine final nut set (January 2018/19 and 2019/20) were performed 4 months after initial nut set counts (October 2018/19 and 2019/20). Final nut set was expressed as a percentage of initial set per raceme per treatment replicate to overcome variability in nut set (% Final set = ((Initial set – Nut drop)/ Initial set)) x 100).

3.5.2 Nut yield and quality

Mature orchard trees were sprayed with ethylene seven days before harvest, upon nut maturation, whereafter trees were harvested by hand and nut in husk (NIH) yield was determined separately for each treatment replicate. A representative 5 kg NIH sample was taken from the middle replicate tree of each treatment, dehusked and husk- to wet nut in shell (WIS) mass was determined. A representative 2 kg WIS sample was taken and dried to a 1.5% kernel moisture content. Representative samples were cracked at 1.5% kernel moisture content in order to ultimately determine the percentage total kernel recovery and nut quality parameters for each of the respective treatments. Nut quality parameters include, the percentage total kernel recovery (TKR), percentage sound kernel recovery (SKR), percentage unsound kernel recovery (USK), percentage sinkers, percentage first grade kernel, percentage immature kernel and the percentage discolouration/onion ring of the kernel. Yield and quality data were not determined for the 2019/2020 season due to the Covid-19 national lockdown from 27 March 2020 to

1 May 2020 interfering with the harvesting process as researchers could not travel to the orchard at this time.

3.6 Data analysis

In the mature orchard, a randomized complete block design with seven treatments and three replicate blocks consisting of three trees per treatment was used. Yield and quality data were statistically compared between all seven treatments for the 2018/19 harvest. Yield and quality data for the 2017/18 and 2018/19 seasons were, however, only compared for T1, T2 and T3 treatments since treatments T4 - T7 were only implemented from July 2018. Analysis of variance (ANOVA) in the Variance Estimation, Precision & Comparison methodology (VEPAC) of Statistica (TIBCO Software Inc. Version 13.3) were used to assess the effect of treatments on yield and quality parameters for each year of harvest. Treatment means were compared using the least significance difference (LSD) procedure with a significance level $p = 0.05$. A two-way ANOVA was used for comparisons between different yield and quality parameters between the 2017/18 and 2018/19 harvests, whilst a one-way ANOVA was used to statistically compare yield and quality parameters between different treatments for the 2018/19 season. A one-way ANOVA was similarly used to detect significant differences ($p \leq 0.05$) between water potential measurements of well-watered and water deficit treatments.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Weather variables

Understanding the weather conditions under which the trial was conducted is important when considering if any weather conditions may have been limiting to yield and quality of the macadamia trees. When assessing weather variables over a season (July to June), mean seasonal temperature was fairly similar between two of three production seasons, with the average daily temperature for the 2017/18 season being 18.27 °C, whilst it was 18.63 °C and 19.32 °C for the 2018/19 and 2019/20 seasons respectively. The 1.05 – 0.69°C difference in average daily temperature between the 2019/20 season and 2017/18, 2018/19 seasons can be attributed to a much warmer July to November period for the 2019/20 season, with mean daily temperatures being 1.83 °C higher than what was recorded for the same period over the two previous seasons (**Figure 4.1A**). Average daily temperatures were particularly high for July, October and November in the 2019/20 season, with mean daily temperatures being 3 - 4 °C higher over these months, compared to the previous two seasons. The highest average daily maximum temperatures were recorded over the four month period from December to March in all three seasons.

Average daily air vapour pressure deficit (VPD_{air}) was fairly similar between the three seasons, with the average daily VPD_{air} being 0.97 kPa, 0.98 kPa and 1.04 kPa over the 2017/18, 2018/19 and 2019/20 seasons respectively. The highest daily average VPD_{air} values were observed from August to October over all three seasons (**Figure 4.1B**). Daily average VPD_{air} values recorded for July of the 2019/20 season were substantially higher (1.55 kPa) than those recorded during the same time for the 2017/18 (1.05 kPa) and 2018/19 (0.85 kPa) seasons. Total reference evapotranspiration (ET_o) was similar for the 2017/18 (1190.4 mm), 2018/19 (1164.4 mm) and 2019/20 (1154* mm estimated) seasons. Average daily ET_o was also similar between the 2017/18 (3.26 mm/day), 2018/19 (3.19 mm/day) and 2019/20 (3.18 mm/day) seasons. Highest daily ET_o values were recorded during September and October periods over all three seasons (**Figure 4.1C**). Mean daily solar radiation was fairly similar for the 2017/18 (17.08 MJ m^{-2} day^{-1}), 2018/19 (16.17 MJ m^{-2} day^{-1}) and 2019/20 (16.83 MJ m^{-2} day^{-1}) seasons (**Figure 4.1D**). The total annual rainfall was, however, substantially higher during the 2018/19 (894 mm) season compared to that of the 2017/18 (759.4 mm) and 2019/20 (759.2 mm) seasons. Rainfall followed a typical summer rainfall pattern over all three seasons, but was well below the long term mean rainfall (854 mm) for the Nelspruit growing area for both the 2017/18 and 2019/20 seasons.

The potential impact of weather conditions on trial results can be further evaluated by assessing average weather conditions experienced during each phenological stage for the duration of the water deficit trial (**Table 4.1**). Air temperature from the nut sizing stage through to oil accumulation was significantly higher than during flowering and fruit set, except for the 2018/19 season during nut sizing. Despite the lower temperatures during flowering and nut set VPD_{air} was higher, possibly reflecting low rainfall (<40 mm for both seasons) at this time, resulting in low relative humidity. The hottest conditions occurred during shell hardening, which is not surprising as this stage occurred over December and January and was associated with high solar radiation and fairly high ET_o. However, this stage was also associated with significant rainfall, especially in the 2018/19 season (490 mm; **Table 4.2**) and as a result, VPD_{air} was not as high as during flowering and nut set. Whilst, temperatures were still quite high during oil accumulation, VPD_{air} was moderately low and so was ET_o. This could be attributed to higher rainfall during this period, which would have resulted in higher relative humidity and more overcast conditions. Whilst there were clear differences in weather conditions between phenological stages, this is to be expected due to seasonal weather patterns. These differences were mostly consistent between seasons and any differences could largely be attributed to differences in rainfall between seasons.

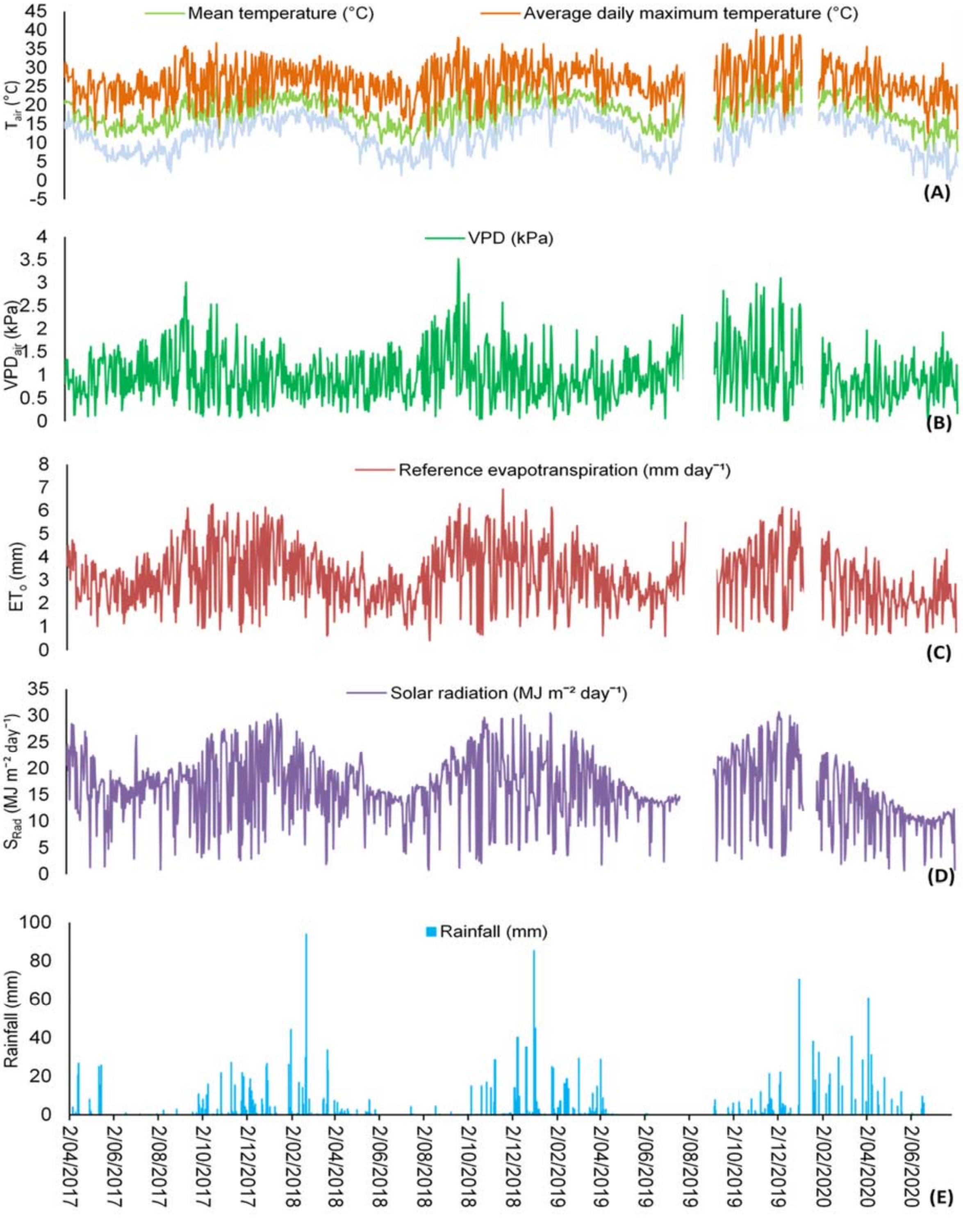


Figure 4.1 (A) Maximum, minimum and mean air temperature (T_{air}) (°C), (B) air vapour pressure deficit (VPD_{air}) (kPa), (C) reference evapotranspiration (ET_o) (mm day^{-1}), (D) solar radiation (S_{Rad}) (MJ m^{-2} day^{-1}) and (E) total daily rainfall (mm) obtained from an automatic weather station located close to the orchard over a three-season period (01 April 2017 to 27 July 2020). Missing data was due to battery failure of the automatic weather station

Table 4.1 Weather data summary over the three-season trial period and during the different phenological stages of the mature orchard trial. Daily average values were used for air temperature (T_{air}), total incident solar radiation (S_{Rad}), air vapour pressure deficit (VPD_{air}) and total reference evapotranspiration (ET_o)

Phenological stage (Treatment)	Year	Time (days)	T_{air} (°C) (Av ± Std)	S_{Rad} (MJ m^{-2} day^{-1}) (Av ± Std)	VPD_{air} (kPa) (Av ± Std)	ET_o (mm) (Av ± Std)	Rain - ET_o (mm)
Season data	2017/18	365	18.27 ± 3.33	17.08 ± 5.87	0.97 ± 0.50	3.26 ± 1.24	-431.04
	2018/19	365	18.63 ± 3.90	16.17 ± 6.15	0.98 ± 0.56	3.19 ± 1.28	-270.48
	2019/20	365	19.32 ± 4.00	16.83 ± 6.68	1.04 ± 0.65	3,18 ± 1.23	-394.8*
Flowering and nut set	2018/19	82	17.84 ± 3.90	16.52 ± 5.12	1.31 ± 0.74	3.44 ± 1.33	-267.16
	2019/20	80	18.06 ± 3.39	16.52 ± 6.38	1.35 ± 0.75	3.15 ± 1.08	-197.87*
Nut sizing and premature nut drop	2018/19	41	17.35 ± 2.76	18.55 ± 9.72	0.90 ± 0.53	3.53 ± 1.70	-19.29
	2019/20	67	22.07 ± 3.20	18.69 ± 8.31	1.23 ± 0.83	3.57 ± 1.51	-125,66
Shell hardening	2018/19	59	22.20 ± 2.35	16.55 ± 7.05	0.84 ± 0.51	3.48 ± 1.34	283.31
	2019/20	39	23.11 ± 2.58	18.60 ± 6.99	1.10 ± 0.65	3.76 ± 1.32	-37.79*
Oil accumulation	2018/19	81	21.30 ± 1.70	16.13 ± 5.79	0.75 ± 0.38	3.06 ± 1.03	13.75
	2019/20	71	20.37 ± 2.32	13.20 ± 5.63	0.70 ± 0.44	2.63 ± 1.08	96.43

Missing ETo data for August 2019 and January 2020 was estimated by multiplying the mean value for the season with the total missing days. Estimated data is indicated by values with an asterisk (*)

It is therefore unlikely that any extreme weather events throughout the trial would have caused a reduction in yield, which would have masked any differences between treatments. However, the higher rainfall in the 2018/19 season influenced the ease with which deficit treatments were implemented from December through to early April (shell hardening and oil accumulation). Although plastic was placed under the trees to exclude rainfall from treatment trees, the likelihood that rain was excluded completely from the treatments is highly unlikely, especially during prolonged periods of rainfall or during heavy showers. This would have resulted in the wetting of soil in water deficit treatments, thereby interrupting the stress period. Alternatively, hotter temperatures in the 2019/20 season may have increased atmospheric evaporative demand at the start of the season, thereby possibly increasing the rate of soil water depletion and hence the quicker implementation of stress due to water deficit conditions. However, other than these differences weather conditions in the three seasons were fairly similar and typical of the region.

4.2 Soil matric potential and water application parameters

Control trees were well irrigated, with relative soil water content (RWC) being kept close to field capacity according to readings from the capacitance probe installed within the trial block. Measurements of RWC beneath the control trees showed that, within the top 60 cm of the soil profile, RWC was maintained between field capacity (FC) and permanent wilting point (PWP) over all three seasons (2017/18 – 2019/20) (**Figure 4.2**). According to these measurements (**Figure 4.2**), macadamias extracted water from within the top 60 cm of the soil profile. The withdrawal of water from this depth is expected seeing that findings of a whole tree excavation study by Firth et al. (2003b), showed that the taproot of a 12-year-old grafted macadamia tree was 60 cm in length and the highest density of fibrous roots were found within the top 40 cm of the soil profile. Similarly, an in-field lysimeter study by Stephenson et al. (2003) showed that unstressed trees extracted water primarily from the top 70 cm of the soil profile. Since trees used in this study were grafted and of similar age (14-16 years old), it can be assumed that the root distribution, root density and water extraction patterns of unstressed trees were similar to those described by Firth et al. (2003b) and Stephenson et al. (2003). Based on the results from this study it is therefore reasonable to assume that if soil water content is maintained close to FC within the top 60 cm of the soil, macadamias should not be water stressed.

Irrigation frequency increased from June (pre-flowering) to April (harvest) over each of the three seasons, as observed by the spikes in RWC in the top 20 cm of the soil profile (**Figure 4.2**). The time between irrigation events decreased during the flowering and nut set, and nut sizing and premature nut drop phenological stages (July to November), overlapping with periods of high atmospheric evaporative demand (ET_o) and low rainfall (**Figure 4.1**). Irrigation water was intentionally restricted over the dormant post-harvest (27 April) to pre-flowering (1 June) period of the 2019/20 season, whereafter soil water content was replenished to FC by the grower (as observed from the large spike in RWC in August 2019 presented in **Figure 4.2**). This pre-meditated water restriction is a common water saving practice implemented by macadamia producers in the South African macadamia industry, based on findings made in other crops such as peaches (Marsal et al., 2003) and on findings made by Stephenson et al. (2003). Stephenson et al. (2003) found that mild water stress after crop maturity is unlikely to be detrimental to yield and quality in the following season and that it may be beneficial to crop yield as one could potentially manipulate flushing patterns during critical stages.

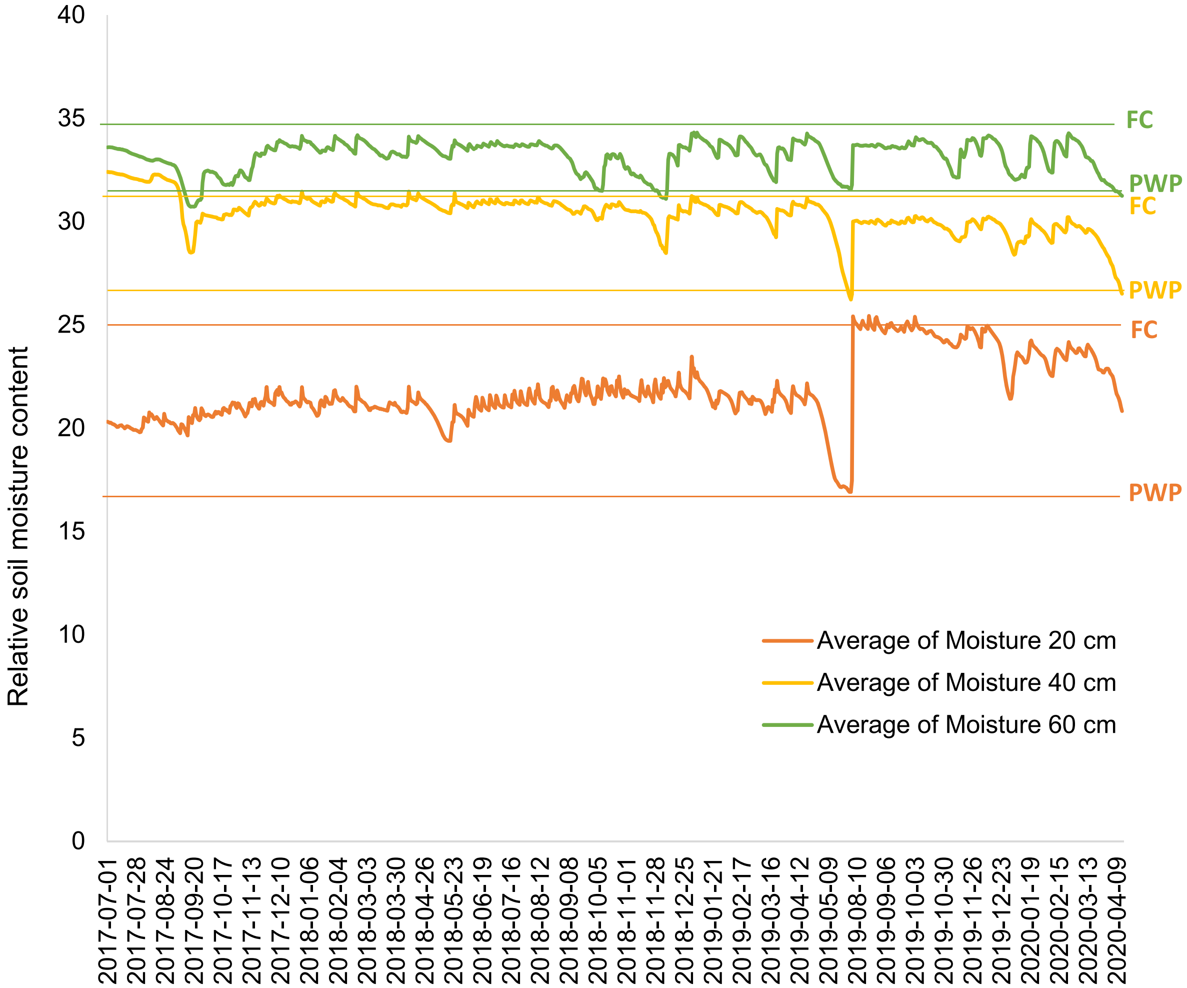


Figure 4.2 Relative soil moisture content at three different soil depths (cm) of the fully irrigated treatment control. Values were recorded in the mature orchard over a three-season period (01 July 2017 to 04 April 2020). Field capacity (FC) and permanent wilting point (PWP)

Total ET_o exceeded total rainfall during most phenological stages over both the 2018/19 and 2019/20 seasons (**Table 4.2**). Rainfall was particularly low from May to October, meaning that supplementary irrigation was necessary to meet the full estimated ET requirement of the crop. The highest estimated irrigation requirements were observed during the flowering and nut set phenological period, where total crop evapotranspiration ($ET_c = ET_o \times K_c$) (based on a constant $K_c = 0.65$ used by Carr (2013)) exceeded total rainfall by 169.4 mm and 113.5 mm during the 2018/19 and 2019/20 seasons respectively (**Table 4.2**). However, total seasonal rainfall and irrigation were sufficient to meet the seasonal ET_c requirement of the control trees over all three seasons. In this study, the total seasonal rainfall and irrigation were also sufficient to meet the seasonal ET_c requirement of the half irrigation treatment trees, over all three seasons. Similarly, total rainfall met the seasonal ET_c requirement of rainfed trees over both the 2018/19 and 2019/20 seasons, but rainfed trees were exposed to an estimated 14 mm water deficit during the 2017/18 season (**Table 4.2**).

Despite rainfall and irrigation being sufficient to meet the seasonal ET_c requirement of both half irrigation and rainfed treatment trees, trees were subjected to substantial water deficits during the flowering and nut set phenological stage over both the 2018/19 and 2019/20 seasons. Water deficits of 140.5 and 83.8 mm were experienced by trees in the half irrigation treatment during the flowering and nut set phenological stage in the 2018/19 and 2019/20 respectively, whilst rainfed trees were exposed to a 169.4 and 113.5 mm water deficit during the same time. Rainfed trees were also exposed to a 26.5 mm water deficit during the nut sizing and premature nut drop phenological stage of the 2019/20 season. Furthermore, through the exclusion of rainfall and restriction of irrigation water, treatment trees on which a mild water deficit was implemented, were exposed to a calculated water deficit during all of the intended phenological stages, over both the 2018/19 and 2019/20 seasons (**Table 4.2**). Therefore, even though annual rainfall may be sufficient, it is important to evaluate rainfall distribution throughout the seasons when assessing the need for irrigation, as a seasonal rainfall pattern may result in tree water deficit periods, if no supplementary irrigation is applied.

Nonetheless, soil matric potential readings over the 2017/18 and 2018/19 seasons correlated with intended water deficits periods, as well as periods of high ET_o and low rainfall for all treatments in the mature orchard block (**Figure 4.3**). Measured soil matric potential readings, using Chameleon probes, of the control treatment ranged mainly (63% of readings) between 0 – -20 kPa (blue) throughout the course of both the 2017/18 and 2018/19 seasons, with some fluctuations in soil water content noted over both seasons (**Figure 4.3A**). Soil matric potential readings were substantially lower for the rainfed (**Figure 4.3C**) and half irrigation treatments (**Figure 4.3B**) over both the 2017/18 and 2018/19 seasons, with 74% and 58% of the 48 chameleon readings being in the -50 kPa range (red) for these treatments.

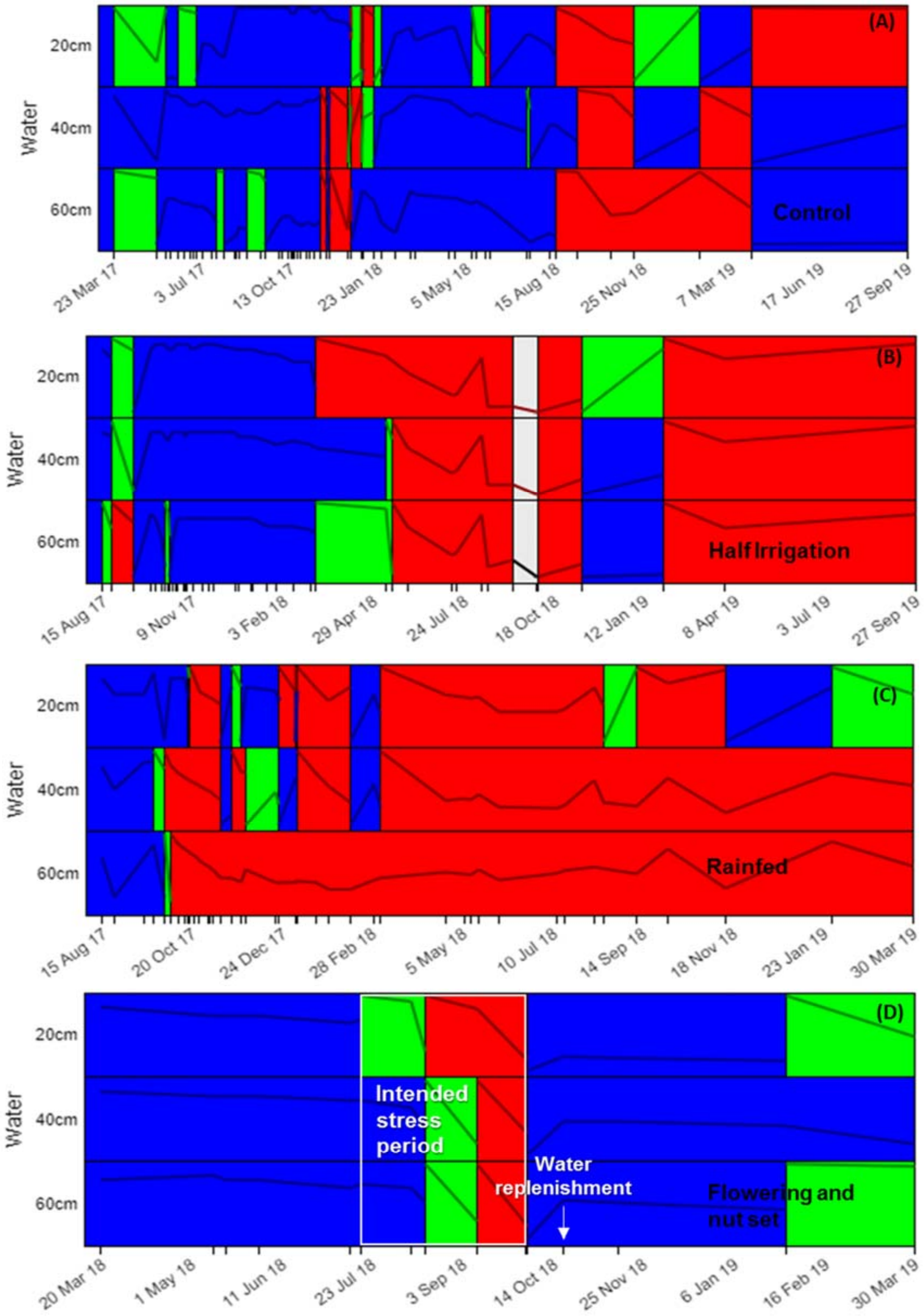
Table 4.2 Irrigation, rainfall, reference evapotranspiration (ET_o) and crop reference evapotranspiration ($ET_c = ET_o \times K_c$) ($K_c = 0.65$) summary for the mature bearing orchard over a three-season period (July 2017 – July 2020) and during different treatment stages. Values in brackets represent the quantity of rain and irrigation water received by the control treatment during the respective phenological stage

Phenology	Year	ET_o (mm)	Rain (mm)	Irrigation (mm)	Rain + Irrigation – ET_o (mm)	ET_o x K_c (mm)	Rain + Irrigation – ET_c (mm)
Control	2017/18	1190.4	759.4	160.6	-270.4	773.5	146.5
	2018/19	1164.4	894.0	156.0	-114.4	756.8	293.2
	2019/20	1154.0*	759.2*	177.8	-217.0	750.1	186.9
Half irrigation	2017/18	1190.4	759.4	80.3	-350.7	773.5	66.2
	2018/19	1164.4	894.0	78.0	-192.4	756.8	215.2
	2019/20	1154.0*	759.2*	88.9	-305.9	750.1	98.0
Rainfed	2017/18	1190.4	759.4	0	-431.0	773.5	-14.1
	2018/19	1164.4	894.0	0	-270.4	756.8	137.2
	2019/20	1154.0*	759.2*	0	-394.8	750.1	9.1
Flowering and nut set	2018/19	278.9	0 (11.8)	0 (57.8)	-278.9	181.2	-181.2
	2019/20	225.5*	0 (33.1)	0 (59.3)	-225.5	146.6	-146.6
Nut sizing and premature nut drop	2018/19	145.0	0 (125.8)	0 (15.6)	-145.0	94.5	-94.5
	2019/20	283.0	0 (157.4)	0 (60.9)	-283.0	183.9	-183.9
Shell hardening	2018/19	205.8	0 (489.2)	0 (14.0)	-205.8	133.7	-133.7
	2019/20	218.6*	0 (241.3)	0 (10.9)	-218.6	142.1	-142.1
Oil accumulation	2018/19	248.0	0 (261.8)	0 (40.6)	-248.0	161.2	-161.2
	2019/20	184.5	0 (281.0)	0 (43.7)	-184.5	119.9	-119.9

Missing ETo data for August 2019 and January 2020 was estimated by multiplying the mean value for the season with the total missing days. Estimated is data indicated by values with an asterisk (*)

Furthermore, soil water content was successfully depleted during the flowering and nut set phenological stage (August – October 2018) (**Figure 4.3D**), with matric potential readings exceeding -50 kPa (red) from 10 September 2018 to the end of October 2018. The replenishment of soil water upon the completion of nut set resulted in the rapid increase in measured soil matric potential, as subsequently observed on 2 October 2018 (**Figure 4.3D**). Soil water content was similarly depleted during the remaining phenological stages which included nut sizing and premature nut drop, shell hardening and oil accumulation stages (**Figure 4.3E, F, G**). Water replenishment following these respective phenological stages was not measured, due to a limited number of measurement points obtained after the completion of the different treatment stages. Following the completion of each treatment stage, all treatment replicates were irrigated according to the same irrigation cycle determined for the control treatment and similar water replenishment patterns would most likely have been observed. Furthermore combined rainfall and irrigation also exceeded total ET_c following the nut sizing and premature nut drop, shell hardening and oil accumulation treatment stages

(**Table 4.1**), which would also have contributed to ensuring adequate soil water replenishment following the water deficittreatment.



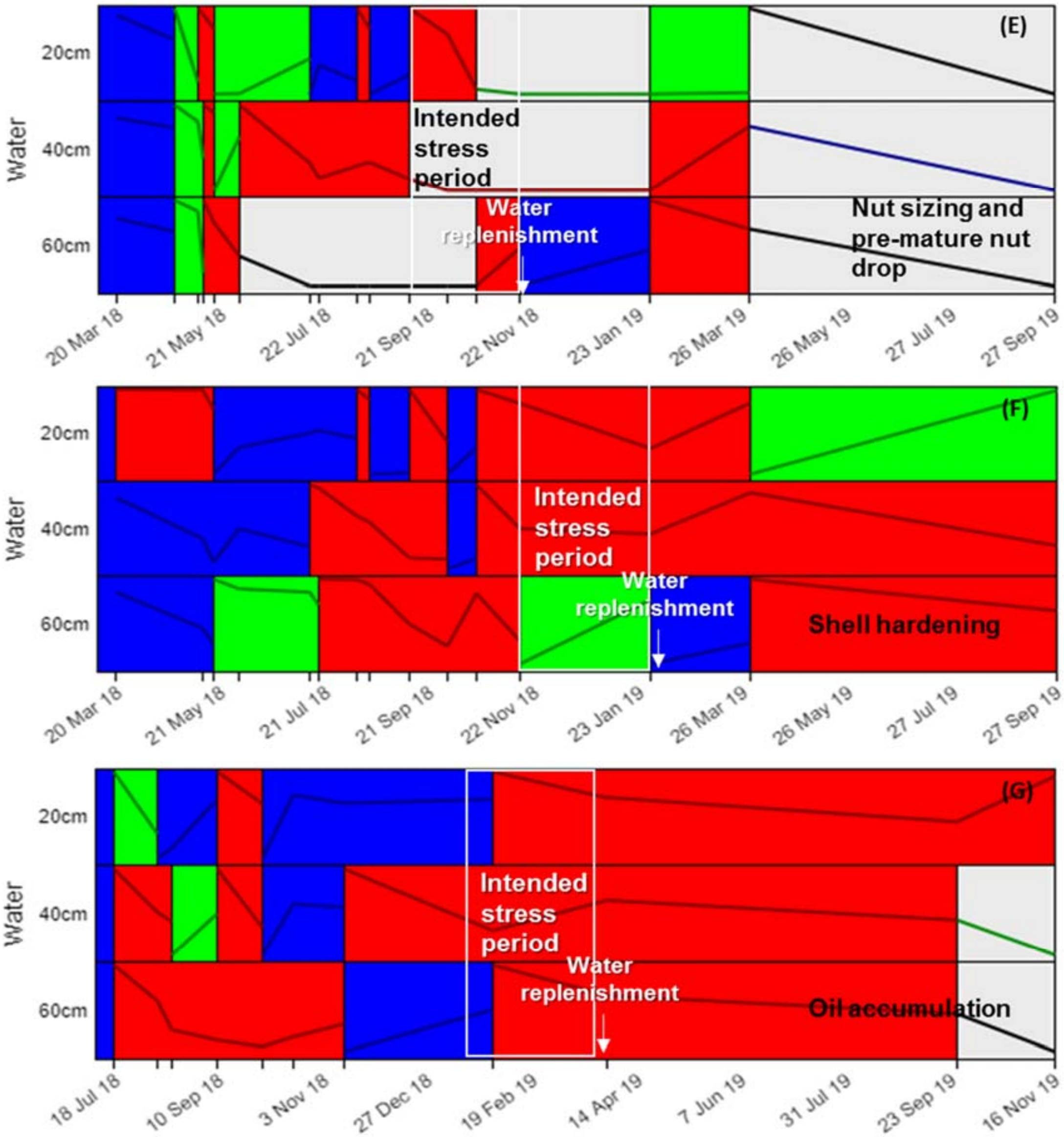


Figure 4.3 Soil matric potential estimates of seven different water deficit treatments, which included (A) normal irrigation (control); (B) half irrigation; (C) no irrigation/rainfed; (D) water stress during flowering and nut set; (E) water stress during nut sizing and pre-mature drop; (F) water stress during shell hardening; and (G) water stress during oil accumulation measured at three different depths over the course of the 2017/18 and 2018/19 season (July 2017 – April 2019). The measurement range included: Blue (0-20 kPa); Green (-20 - -50 kPa); Red (<-50 kPa); Grey (Unreadable). Solid lines represent relative trends in water extraction and replenishment

Soil matric potential measurements were primarily used to demonstrate that soil water was limiting during each of the four different phenological stages at which a water deficit was implemented. Recommendations made by Stephenson and Searle (2014) suggests that soil water tension readings between -20 and -40 kPa at a 60 cm depth should be used as critical trigger points for deficit irrigation studies. Based on these recommendations, data presented in **Figure 4.3**, suggest that all treatments were exposed to mild water deficits during each of the intended phenological stages, as soil matric potential readings at all of the measured depths exceeded -40 kPa for a prolonged period (>4 weeks), during each of the treatment stages. Stephenson and Searle (2014), however, also noted that soil water replenishment trigger points should be fine-tuned for different soil types and tree rooting patterns and that these values should be validated through monitoring different physiological responses to developing water deficits.

4.3 The effect of water deficits on macadamia pre-dawn leaf water potential

One of the most common methods of evaluating the impact of water deficits on plant water relations is the use of pre-dawn leaf water potential (Ψ_{pd}) (Jones, 2004). Using Ψ_{pd} as an indicator of the level of crop water stress has been shown to be an accurate indicator of water deficit conditions in almond (Nortes et al., 2005), lime (Silva et al., 2005), plum (Intrigliolo and Castel, 2006) and apple and walnut trees (Valancogne et al., 1996). At night, when transpiration is negligible, the plant continuously refills xylem and other tissues with water. Tissue refill occurs as a result of the water potential difference between the leaves and the soil and refill will continue until an equilibrium is reached (Jones, 2004). This equilibrium between the leaves and soil usually occurs just before dawn, before water is again lost from leaf tissue as a result of transpiration. Hence, at zero plant water flux (no transpiration), Ψ_{pd} is considered to be in equilibrium with the soil water status in the plant root zone, making Ψ_{pd} a good indicator of the plant water status (Chone et al., 2001). Therefore, when Ψ_{pd} is more negative, it is reasonable to assume that soil water potential is more negative and vice versa. Under conditions of more negative soil matric potential, Ψ_{pd} tends to be more negative than under conditions of higher (less negative) soil matric potential, mainly as a result of incomplete water replenishment of stem and leaf tissues.

In this study soil water deficits during the different phenological stages (**Figure 4.3)**, were confirmed by the reduction in Ψ_{pd} during each of the respective phenological stages (**Figure 4.4**). Subsequent to a four-month (July – October) period without any soil water replenishment, average Ψ_{pd} values in the flowering and nut set treatment were -0.78 MPa and -0.73 MPa for stressed trees and -0.18 MPa and -0.06 MPa for control trees during 2018/19 and 2019/20 seasons respectively (**Figure 4.4**). Similar trends in the reduction of the average Ψ_{pd} were observed for trees exposed to water deficits during nut sizing and premature nut drop, shell hardening and oil accumulation over both seasons (**Figure 4.4**). Average minimum Ψ_{pd} levels reached during the nut sizing and premature nut drop, shell hardening and oil accumulation phenological stages were, however, noticeably higher than the average Ψ_{pd} that was recorded for trees stressed during flowering and nut set, over both seasons. The higher Ψ_{pd} levels observed during these stages could possibly be attributed to water extraction from deeper soil layers during the rainy season, as opposed to the drier flowering period, with total ET_c greatly exceeding rainfall during the flowering and nut set phenological stage over both seasons (**Table 4.2**). Searle and Lu (2002) demonstrated that soil water was depleted more gradually at depth than near the surface, which presumably provides a buffer against the onset of water deficit stress in deep rooted trees.

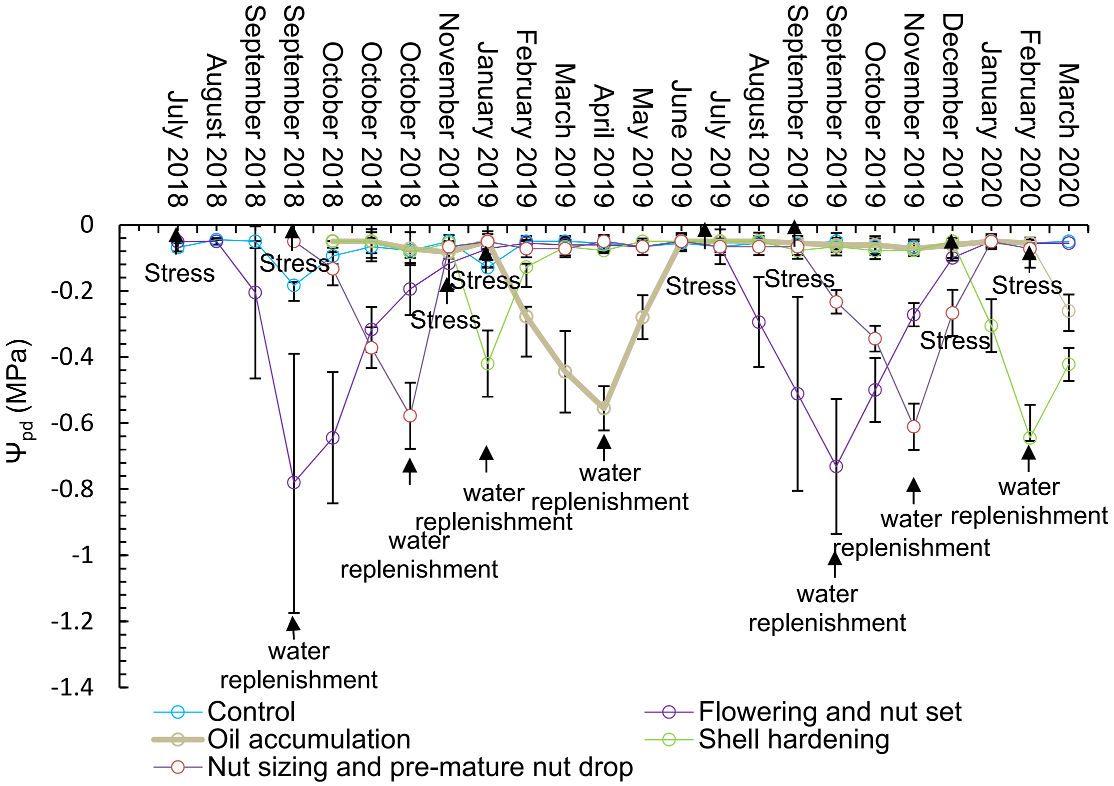


Figure 4.4 Average pre-dawn leaf water potential (Ψ_{pd}) (MPa) over nine measurement replicates per treatment over both the 2018/19 and 2019/20 seasons. Water deficit periods are indicated at the base of the figure.

The lower rainfall and higher ET_o during flowering and nut set, combined with the fact that irrigation only aims at replenishing soil water within the top 60 cm of the soil profile (limited water replenishment >60 cm), could provide a possible explanation for the more negative Ψ_{pd} values observed for control, half irrigation and rainfed treatments during the September-October period (**Figure 4.5**). As discussed in section 4.2, ET_c exceeded total rainfall and irrigation volumes in the control and half irrigation treatments during the flowering and nut set phenological stage, leading to a water deficit period. Therefore, limited water availability at depth (>60 cm) may have inhibited deep water extraction during these water deficits periods, leading to the more negative Ψ_{pd} levels observed at this time. Differences between 2018/19 (-0.18 MPa) and 2019/20 (-0.06 MPa) Ψ_{pd} levels recorded for the control treatment during the flowering and nut set phenological stage, can possibly be related to the grower replenishing the soil water content to FC, prior to flowering (1 June 2019), leading to some soil water reserves being available at depth (>60 cm) during the water deficit period. In addition, rainfall was higher and more evenly distributed during the flowering and nut set period in the 2019/20 season with a total rainfall of 33.1 mm, as opposed to 11.8 mm during the 2018/19 flowering and nut set period. This led to more regular soil water replenishment within the top 20 cm of the soil profile (**Figure 4.2**).

These findings are supported by Stephenson et al. (2003), who demonstrated that soil water was rapidly depleted within the top 40 cm of the soil surface by the surface mat of roots of stressed trees after which water extraction occurred at greater depth (>130 cm) during a prolonged dry spell. A study by Lloyd et al. (1991) complemented the findings of Stephenson et al. (2003) and this study by reporting that macadamia roots have the ability to extract sufficient water at depth, even at low water content. These abilities, combined with possible water table replenishment by rain, could have led to the observed higher Ψ_{pd} levels for trees stressed during nut sizing and premature nut drop, shell hardening and oil accumulation.

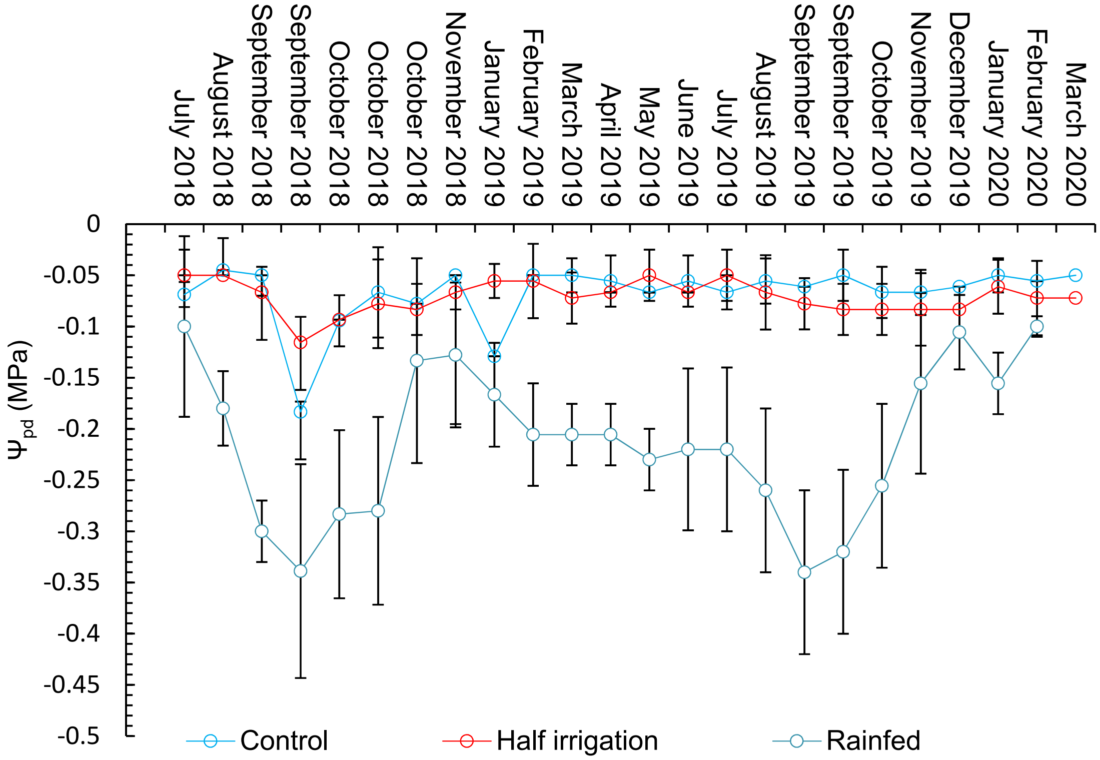


Figure 4.5 Average pre-dawn leaf water potential (Ψ_{pd}) (MPa) over nine measurement replicates for control, half irrigation and rainfed treatments, over both the 2018/19 and 2019/20 seasons

The study by Lloyd et al. (1991), further confirmed results from this study, with similar Ψ_{pd} levels (-0.05 to -0.1 MPa) reported for macadamia trees under well irrigated conditions. Despite occasional soil water depletion, Ψ_{pd} levels recorded over the 2018/19 and 2019/20 seasons seldom surpassed -0.1 MPa in both the control and half irrigation treatments (**Figure 4.5**). Minimum Ψ_{pd} levels recorded during this study was, however, substantially lower (-1.2 MPa) (**Figure 4.**4) than what is reported by Lloyd et al. (1991). These authors reported a minimum Ψ_{pd} of approximately -0.22 MPa (-0.12 MPa more negative than the control) after a four-month dry period in well drained red krasnozem (Hutton) soil. Average minimum Ψ_{pd} levels recorded over all treatments in this study (-0.61 MPa) was similar to the minimum Ψ_{pd} levels (-0.58 MPa) observed by Firth et al. (2003a) in an unirrigated one-year-old macadamia orchard.

There was, however, substantial standard deviation between the minimum Ψ_{pd} reached over the various treatment replicates which can be attributed to the variation in soil characteristics within the experimental block. Treatment replicates situated in the southern parts of the experimental block generally had higher (more positive) minimum Ψ_{pd} levels in comparison to treatment replicates in the northern sections. Due to a shallow soil profile and poor orchard drainage, treatment replicates in the southern parts of the experimental block were likely subjected to fluctuations in the soil water table, which would have led to occasional tree water access and therefore the substantial standard deviation observed between measured replicates.

4.4 The effect of mild water stress on macadamia yield and quality

By using the universal accepted Ψ_{pd} approach as an indicator of the level of crop water stress, a decrease in Ψ_{pd} (increase in water stress) at various phenological stages appeared to have little effect on macadamia yield. For the 2018/19 season yield per tree, in terms of both nut in husk (NIH) and wet in shell (WIS), was not significantly different between trees subjected to water deficit at different phenological stages. The treatments, however, differed relative to the control, with all treatments yielding significantly ($p \leq 0.05$) more nuts than the control (**Figure 4.6**). Similar findings have previously been reported in a lysimeter study by Stephenson et al. (2003), where trees exposed to water deficits during floral initiation and floral development yielded more than the well-irrigated controls over a single season. Increased yield in the water deficit treatments relative to the control could be related to possible improvements in tree health (root longevity) (Stephenson, 2004) and improved nutrient use efficiency (Kiggundu et al., 2012) for trees subjected to some degree of water deficit. It is possible that by reducing irrigation to the various treatments, waterlogged conditions were avoided in these treatments, resulting in improved root growth and reduced disease incidence, which could both have led to improved yields. Furthermore, conditions with raincovers may have been less stressful due to reduced evaporation, effectively buffering soil moisture. Although not measured, the influences of raincovers on the soil microclimate may have stimulated root growth but the imposition of stress may have countered this. A study by Stephenson et al. (2003), however, showed that the yields of well watered trees with raincovers were lower compared to those without which may indicate some adverse effects of raincovers on tree performance, although these appeared to be minor and inconsistent. The effect of raincover on the tree microclimate, especially the level of diffused radiation should, however, be measured in future studies. Nonetheless, the possibility of the control being over-irrigated is supported by comparing the yield in the half irrigation treatment to treatments where trees were exposed to water deficit conditions at different phenological stages. Trees receiving half the normal irrigation yielded significantly more ($p \leq 0.05$) than all water deficit treatments. It is, therefore, possible that trees in the control were stressed due to excess water.

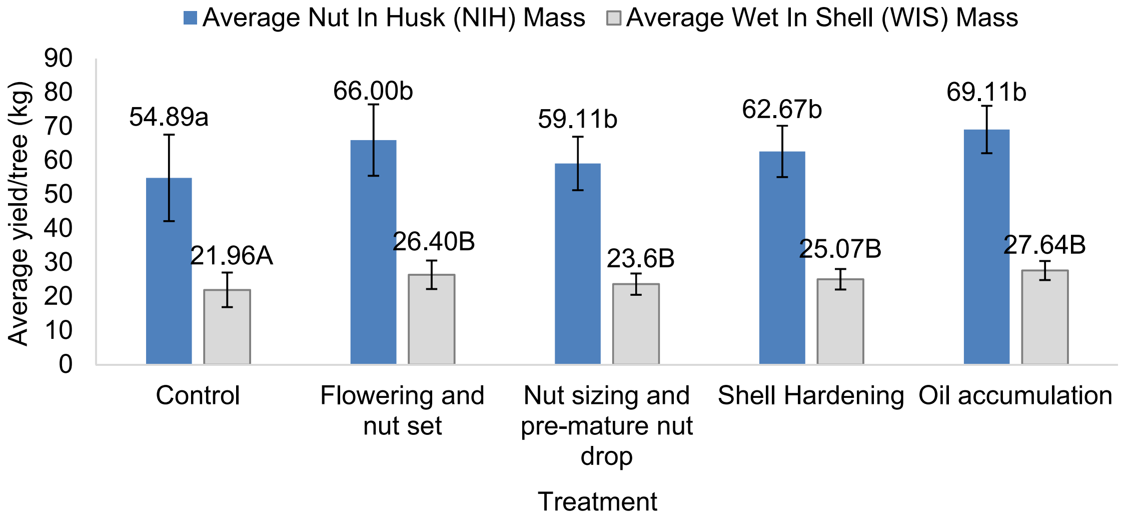


Figure 4.6 Averaged harvested nut mass (kg) per tree over nine measurement replicates per treatment for the 2018/19 season. Different letters indicate a significant difference ($p \leq 0.05$) between treatment means. NIH:WIS = 60:40

Yield increases in water deficit treatments could also be linked to a reduction in vegetative growth (although not measured in this study) during the respective phenological stages, as carbohydrate reserves previously partitioned to vegetative growth could potentially be assigned to the developing crop, which is the dominant sink (Stephenson et al., 1989a). For example, Stephenson et al. (1986) showed that yields may be decreased by active vegetative growth in late spring. This, however, is unlikely as current photosynthesis would have also been affected by water deficit conditions, likely having an adverse effect on yield during the current or next phenological stage of the respective treatment. In fact, Stephenson et al. (1989a) concluded that managerial practices affecting the availability of carbohydrate reserves are more likely to affect reproductive than vegetative growth. Stephenson et al. (2003) further showed that vegetative growth in macadamias was suppressed (not observed in this study) by a mild water deficit, where after trees responded to re-watering with substantial vegetative growth. If this was indeed the case, reduced vegetative growth during a specific phenological stage was more likely to affect nut development at a later stage. For example, a delay in early spring flush may have improved initial set, but re-watering would have resulted in vegetative growth during the next phenological stage being the nut sizing and premature nut drop stage, thereby negatively affecting the yield of this treatment. This is, however, not supported by the findings of this study with all treatments showing improved yields relative to the control. Importantly, the level of stress achieved in this study needs to be considered in relation to the draining lysimeter study by Stephenson et al. (2003). Silva et al. (2005) reported the Ψ_{pd} of lime trees to be substantially higher (-0.89 MPa) for field-grown trees than for lysimeter grown trees (-2.70 MPa), after the same waterless period. Silva et al. (2005) attributed the observed difference to the probability that the root systems of field-grown trees were larger than that of the lysimeter grown trees, allowing better water uptake by the increased, exploited soil volume. It is, therefore, possible that only a mild water deficit was achieved in this current study and Stephenson et al. (2003) concluded that, since improved yields can be achieved by a change in macadamia phenology, the cautious application of mild water stress may be useful to manipulate macadamia trees to achieve higher yields.

Water deficits during nut sizing and premature nut drop is often associated with increased premature nut drop, potentially contributing to the natural shedding process of the tree (Stephenson and Gallagher, 1987). Nut sizing and premature nut drop during the 2018/19 season also coincided with high VPD_{air} levels during late October and early November (**Table 4.1**), which, in combination with low soil water availability has been shown to significantly increase premature nut abscission (Stephenson et al., 2003). In an earlier study, Stephenson and Gallagher (1987) reported that high daytime temperatures (>30 °C) can induce greater premature nut drop and that young nuts were sensitive to increases in temperature during endosperm development. Despite evidence in literature supporting the possibility of increased nut abscission if external stresses coincide with the nut sizing and premature nut drop phenological stage, similar findings of increased nut abscission (reduced final set) were not made over the course of this study (**Figures 4.7 and 4.8**). Findings could be linked to the fact that all treatments were exposed to the same environmental extremes, leading to a similar increase in nut abscission over all treatments, which overridden the impact of a soil water deficit.

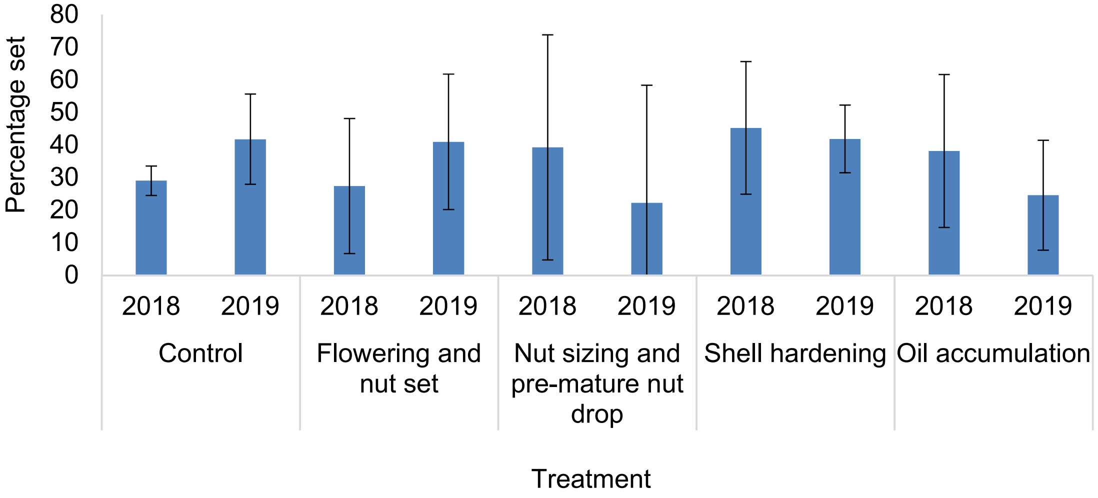


Figure 4.7 Average percentage final nut set over nine measurement replicates per treatment for the 2018/19 and 2019/20 seasons

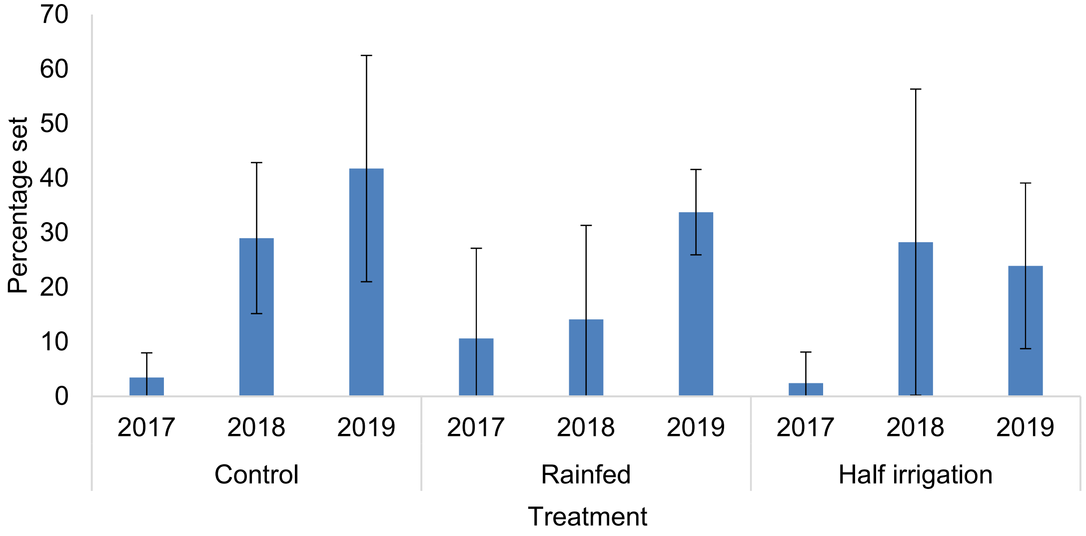


Figure 4.8 Average percentage final nut set over nine measurement replicates per treatment for the 2017/18, 2018/19 and 2019/20 seasons

In addition, the lack of differences in final nut set between the various treatments can potentially be attributed to limitations that arose in the methodology used during the current trial. A single macadamia tree produces >10000 inflorescence, each containing approximately 200 – 300 flowers, which are born on hardened wood in all parts of the canopy (Carr, 2013). Although between 5 -10% of these flowers set fruit (Carr, 2013), only 0.3% may reach maturity (Nagao, 2011). Hence, by selecting a single branch or multiple racemes within the canopy, it may still not provide a representative data set of the percentage final nut set (% Final set = ((Initial set – Nut drop)/ Initial set)) x 100) as a crop load adjustment could have been made in a different part of the canopy. Initial nut set per raceme is further affected by possible pest and disease incidence and the potential lack of cross pollination (Carr, 2013). In some instances, entire racemes were lost due to strong winds or mechanical damage by farm equipment. It is therefore recommended that whole tree nut abscission for each of the respective treatments should be measured, as this will provide the best possible indication of the effect of water deficits on nut abscission and reduce the substantial standard deviation observed in **Figures 4.7 and 4.8**.

Both rainfed and half irrigation treatments resulted in significantly ($p \leq 0.05$) higher yields (NIH and WIS) than the fully irrigated control when considering cumulative yield over both the 2017/18 and 2018/19 seasons, and yield for each season individually (**Figure 4.9**). Yields were also significantly higher ($p \leq 0.05$) during the 2018/19 season for all treatments (**Figure 4.9**). Trochoulias and Johns (1992) similarly reported an inconsistent response of macadamia to irrigation in high rainfall areas, with improved yields in irrigated trees in only 5 years of the 8-season study period. Trees used in the study by Trochoulias and Johns (1992) were, however, widely spaced (11m x 11m) which could have resulted in trees having a much larger root volume, as well as a large soil water reservoir to buffer against the development of water stress. Yield differences between half irrigation and control trees could be possibly linked to the limitation of nutrient losses as a result of leaching in control trees due to higher irrigation volumes. Control trees were, however, irrigated according to a capacitance probe, the industry norm, which raises concerns with the current industry irrigation recommendations. Similar to trees exposed to water deficit conditions during different phenological stages, trees receiving half the normal irrigation may have experienced improved root growing conditions and/or lower disease incidence which may have improved the tree yield response. Nevertheless, rainfed trees further showed a 7 kg WIS increase and a 2% increase in total kernel recovered (TKR) (**Figure 4.10**) in the 2018/19 season when compared to the 2017/18, suggesting no knock-on effect of water deficits in the 2017/18 season on macadamia yield and quality in the second season of measurement. **Figure 4.10** further shows no significant difference ($p > 0.05$) in the TKR percentage between the rainfed, half irrigation and control treatments over a two-season period, while the percentage discolouration was significantly ($p \leq 0.05$) higher during the 2017/18 season. Consequently, the percentage first grade kernel was significantly ($p \leq 0.05$) higher during the 2018/19 season than during the 2017/18 season.

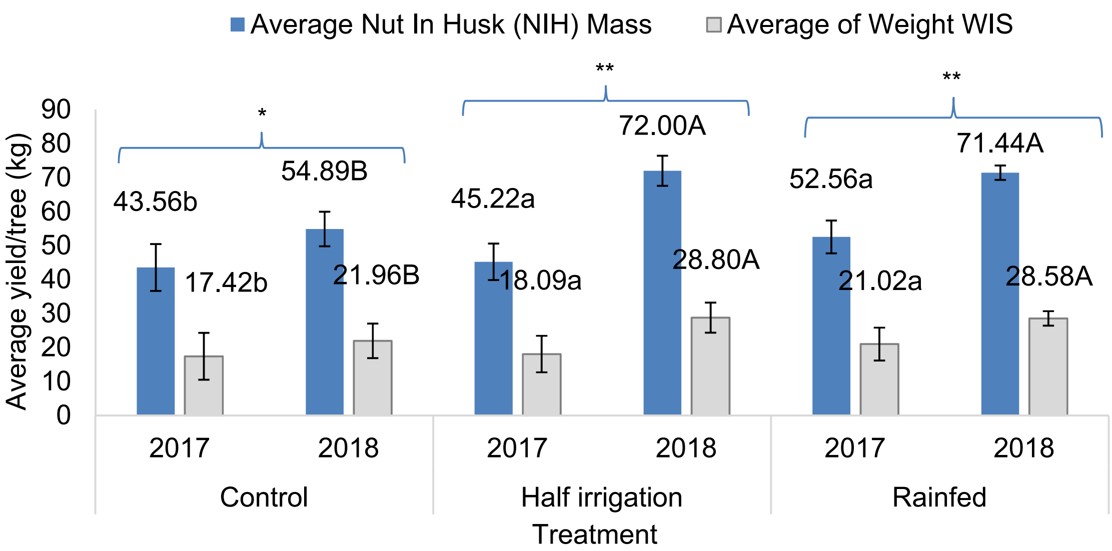


Figure 4.9 Average harvested mass (kg) of nut in husk per tree over nine measurement replicates per treatment for the 2017/18 and 2018/19 seasons. Sample means across both seasons with a different number of * indicate significant differences ($p \leq 0.05$) between treatments. Sample means with a different letter indicate significant differences ($p \leq 0.05$) between seasons. Capitalised letters (AB) represent significant differences between 2018/19 season treatment means while lower case letters (ab) represent significant differences between 2017/18 season treatment means. NIH:WIS = 60 :40

Two way ANOVA, however, showed no significant difference ($p > 0.05$) between both treatment and year, showing no seasonal effect on different treatment outcomes. Although annual environmental conditions were not significantly different, seasonal conditions such as temperature and VPD_{air} during more sensitive phenological stages, such as anthesis, could possibly have had an over-riding effect on the increase in yield over the two seasons (Stephenson et al., 2003, Smit et al., 2020, Stephenson et al., 1989b)

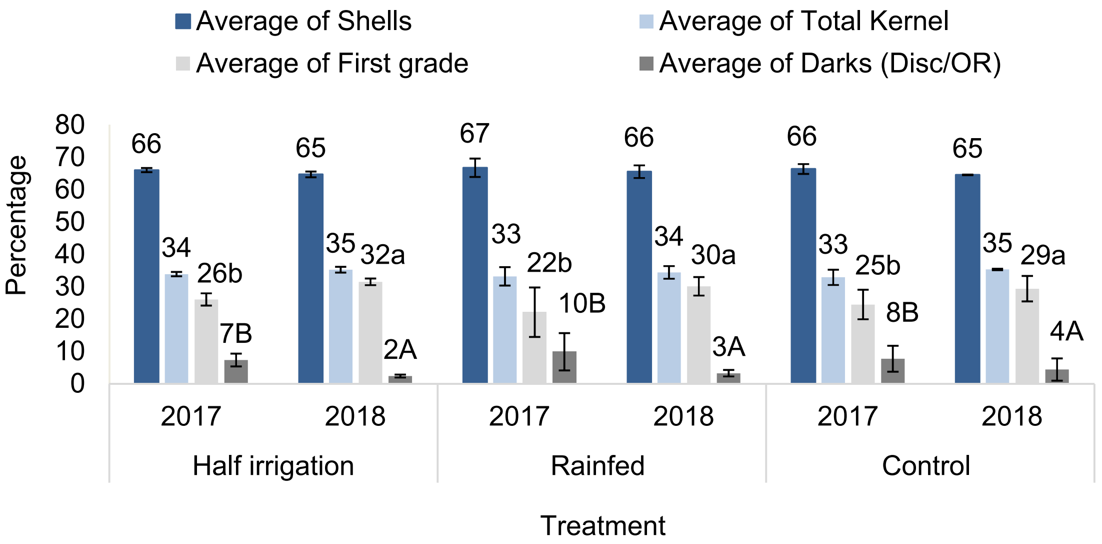


Figure 4.10 Average quality parameters over nine measurement replicates per treatment for the 2017/18 and 2018/19 seasons. A 2000g sample was used for each treatment. First grade sample means with different lower-case letters (ab) indicate significant differences ($p \leq 0.05$) between seasons. Darks sample means with different capitalised letters (AB) indicate significant differences ($p \leq 0.05$) between seasons

In contrast to the effect of water deficits during different phenological stages on yield, a significant difference ($p \leq 0.05$) in quality was observed between the seven experimental treatments in the 2018/2019 season (**Figure 4.11**). Trees stressed during flowering and nut set, as well as trees stressed during shell hardening had a significantly ($p \leq 0.05$) higher shell percentage and therefore significantly ($p \leq 0.05$) lower total kernel recovery percentage (TKR), when compared to the treatment control. Water deficits during flowering and nut set and shell hardening stages had 3.35 % and 1.42 % lower TKR than the treatment control. This was unexpected as water deficits during flowering is not expected to have an influence on fruit quality. The reduction in TKR observed could possibly be attributed to a lag in recovery in physiological functioning after rewatering, leading to a possible deficit in carbohydrate availability during the nut sizing and premature nut drop phenological stage. Stephenson et al. (1989a) showed that the macadamia flowering period coincides with the spring vegetative flush, utilizing large amounts of stored carbohydrate reserves. A reduction in cumulative diurnal photosynthesis at this time could lead to a deficit in carbohydrate reserves during the nut sizing stage as a result of the onset of water stress in flowering and nut set. This is supported by findings from Stephenson et al. (2003) who demonstrated that floral development and premature nut drop are the most sensitive phenological stages to water deficits in terms of yield. Yield improvements through irrigation in areas where relative dry periods coincides with the flowering and early nut sizing stages have also been reported (Carr, 2013). Stephenson et al. (2003), however, noted an increase in TKR for trees subjected to water deficits during the premature nut drop stage, attributing the increase in TKR to a fruit 'thinning' effect which allows for the greater partitioning of carbohydrate the remaining nuts.

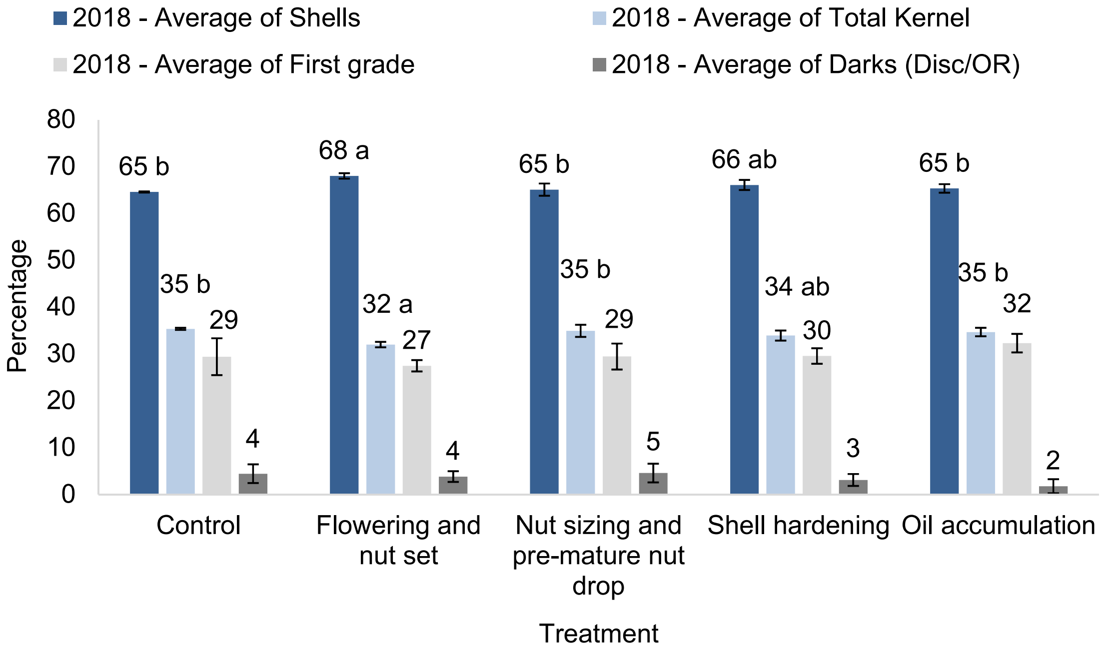


Figure 4.11 Average quality parameters over nine measurement replicates per treatment for the 2018/19 season. A 2000g sample was used for each treatment. Sample means with different letters indicate significant differences (*p* ≤ 0.05) between treatments

Even though Stephenson et al. (2003) did not distinguish between the shell hardening and oil accumulation phenological stages, the authors similarly reported that water deficit conditions during nut maturation/oil accumulation stage (December - January) was highly detrimental to yield and quality. A study by Awada et al. (1967) also reported sufficient soil water to be essential for nut maturation. Hence, although similar phenological stages were identified with this study, the contrast in the degree of sensitivity during the two different phenological stages can possibly be attributed to the higher level of stress achieved in the lysimeter study by Stephenson et al. (2003). Furthermore, rainfall and other relevant environmental factors, such as VPD_{air} (**Table 4.2**), were most favourable during the oil accumulation stage in this study (VPD_{air} <0.8

kPa and rainfall >260 mm), significantly reducing the probability of inducing sufficient stress during this stage to cause an impact on yield and quality.

The study by Stephenson et al. (2003) further reported that water stress during floral initiation had no effect on yield or nut number, and also had no consistent impact on the flowering intensity of stressed trees. Similar findings were made in this study for trees exposed to water deficits during the floral initiation period (rainfed treatments), with no observed difference in flowering intensity, yield or quality.

4.5 The effect of plant and soil water deficits on macadamia physiological functioning

There is limited literature available on the physiological responses of macadamia to water deficits, with most available literature assessing light saturated net photosynthetic assimilation rate (A_{max}), stomatal conductance (g_s), leaf water potential (Ψ_L), pre-dawn leaf water potential (Ψ_{pd}) and soil water deficits independently (Carr, 2013, Huett, 2004). This information is critical for understanding thresholds for stress in macadamia, as this could have an impact on yield, irrigation and ultimately water use efficiency.

In order to try and understand the response of macadamias to water deficit conditions, various levels of water deficits were imposed in an intermediate macadamia orchard. These deficits were used to try and identify a Ψ_{pd} threshold at which g_s and A_{max} are significantly reduced. Trees undergoing a 30 day period without irrigation were exposed to a 40.52 mm water deficit, where a water deficit was calculated as the difference between ET_c (ET_o x (K_c = 0.65)) and rainfall. In addition, trees undergoing a 25, 20 and 15 day period without water replenishment were subjected to a 32.93, 25.16 and 11.74 mm water deficit over each respective period (**Table 4.3**). Throughout the trial weather conditions remained fairly cool, with moderate VPD (<1 kPa). The combination of well-drained soil and the withholding of irrigation in the intermediate orchard resulted in a reduction in the average minimum Ψ_{pd} from -0.1 MPa for control trees to an average minimum of -0.48 MPa for trees receiving, no water over a 30 day period (**Figure 4.12**). A reduction in Ψ_{pd} was correlated with the duration of the different water deficit treatments, with trees exposed to a 25, 20 and 15-day water deficit having an average minimum Ψ_{pd} of -0.26 MPa, -0.13 MPa and -0.12 MPa respectively.

Table 4.3 Weather data summary over the 30 day trial period in the intermediate orchard. Daily average values were used for air temperature (T_{air}), total incident solar radiation (S_{Rad}), air vapour pressure deficit (VPD_{air}) and total reference evapotranspiration (ET_o)

Treatment	Year	Date	Time (days)	T_{air} (°C) (Av ± Std)	S_{Rad} (MJ m^{-2} day^{-1}) (Av ± Std)	VPD_{air} (kPa) (Av ± Std)	ET_o (mm) (Av ± Std)	ET_o (mm) (Total)
Control	2019/20	25 May - 24 Jun	31	12.99 ± 2.17	9.65 ± 2.12	0.83 ± 0.32	2.01 ± 0.56	62.35
15 day water deficit	2019/20	9 Jun - 24 Jun	16	11.90 ± 2.06	9.10 ± 2.73	0.75 ± 0.33	1.88 ± 0.58	18,16
20 day water deficit	2019/20	4 Jun - 24 Jun	21	12.21 ± 1.88	9.27 ± 2.48	0.74 ± 0.31	1.84 ± 0.53	38,72
25 day water deficit	2019/20	30 May - 24 Jun	26	12.77 ± 2.06	9.53 ± 2.28	0.76 ± 0.28	1.88 ± 0.48	50,67
30 day water deficit	2019/20	25 May - 24 Jun	31	12.99 ± 2.17	9.65 ± 2.12	0.83 ± 0.32	2.01 ± 0.56	62.35

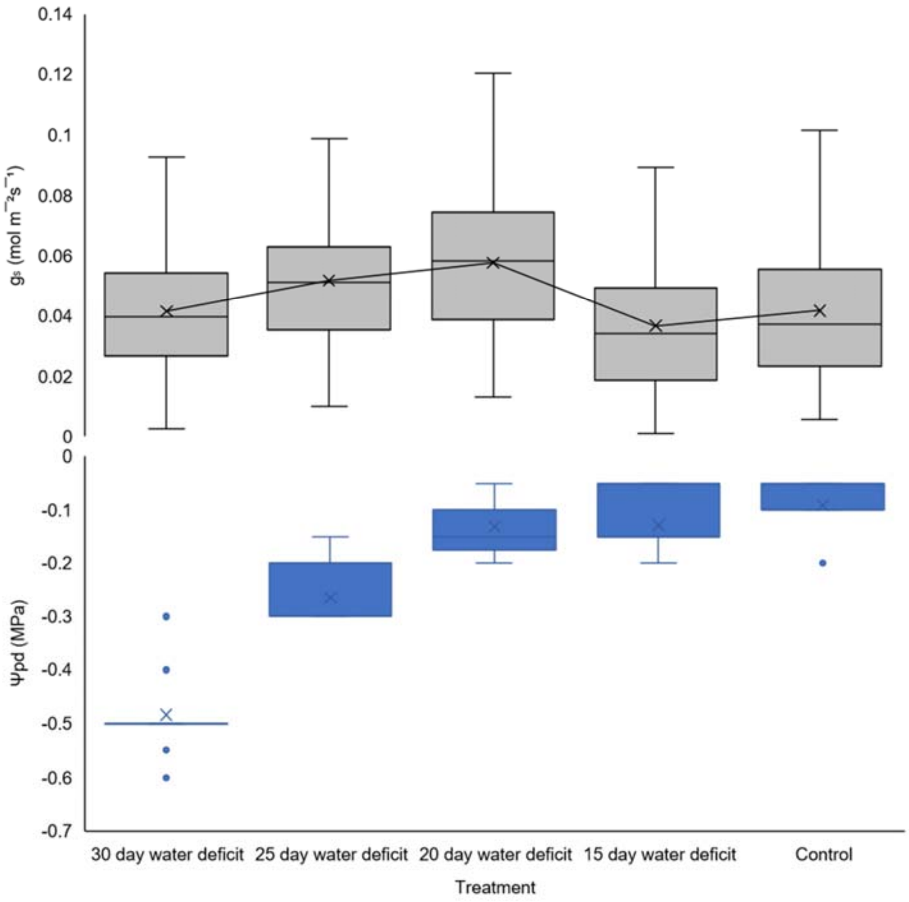


Figure 4.12 Stomatal conductance (g_s) (mol $m^{-2}s^{-1}$) for four water deficit treatments and a well-watered control for corresponding pre-dawn water potentials (Ψ_{pd}) (-MPa). Stomatal conductance data points were obtained over an eight-hour measurement period during three measuring campaigns (22 June – 24 June 2020). Pre-dawn water potentials were recorded prior to sun-rise during each of the measuring campaigns

A linear reduction in g_s with more negative Ψ_{pd} levels was, however, not observed in the intermediate orchard, with g_s remaining relatively constant for Ψ_{pd} readings between -0.05 and -0.5 MPa. Stomatal conductance was the same for trees subjected to a 30 day water deficit (0.41 mol $m^{-2}s^{-1}$) and for control trees (0.41 mol $m^{-2}s^{-1}$) (**Figure 4.12**). Stomatal conductance values reported in this trial were substantially lower than the average diurnal g_s values recorded in a lysimeter study by Stephenson et al. (2003). Stephenson et al. (2003) reported mean maximum g_s values of approximately 0.28 mol m^{-2} s^{-1} and 0.25 mol m^{-2} s^{-1} for stressed and unstressed trees during a five-hour measurement campaign. Maximum g_s values recorded in the intermediate orchard was approximately 0.13 mol m^{-2} s^{-1}. Maximum g_s was, however, similar to the levels reported by Lloyd et al. (1991) (g_s ±0.15 mol m^{-2} s^{-1}) for five-year-old, unirrigated macadamia trees after a 30 day rainless period (Ψ_{pd} ± -0.15 MPa). These authors first noted a reduction in g_s, relative to irrigated trees (g_s ±0.20 mol m^{-2} s^{-1}), after approximately 30 days without water. This was unexpected, as Lloyd et al. (1991) noted that at the stage when a reduction in g_s was first observed, soil water would have already been depleted by more than 125 mm (K_c = 0.6) which is substantially more than what was previously reported to affect the physiological functioning of peach (75 mm) (Chalmers et al., 2012) and citrus (60 mm) (Buss, 1988). The low g_s values in this study were most likely a result of prevailing weather conditions, such as air temperature, VPD_{air} and solar radiation, which were below the optimum required for optimal physiological functioning (Stephenson et al., 1986). In both, the study by Lloyd et al. (1991) and by Stephenson et al. (2003) g_s was measured during spring (September-October), which coincided with much warmer temperatures, higher VPD_{air}, solar radiation and ET_o values than what was experienced during the respective measurement campaigns in this study (**Table 4.4**). Seasonal effects on leaf gas exchange have been previously noted by Machado et al. (2002) who recorded lower leaf gas exchange rates in citrus trees during winter than in summer, due to lower VPD_{air} levels and lower air temperatures.

The lack of response of g_s to water deficit treatments is also reflected in the response of A_{max} to these treatments (**Figure 4.13**). Although g_s and A_{max} were highly correlated (r^2 0.87-0.92) over all treatments, there was no substantial difference recorded in the response of A_{max} to g_s between the different water deficit treatments and well irrigated trees (**Figure 4.14**). This suggests that both A_{max} and g_s were unaffected by the level of stress achieved in the orchard over a 30 day period and that during this trial macadamia gas exchange parameters are more affected by changes in atmospheric conditions, such as VPD_{air}, than soil conditions. It can thus be tentatively proposed that Ψ_{pd} above -0.6 MPa do not result in stressful conditions that lead to changes in g_s or A_{max}.

Table 4.4 Mean weather variables, including air temperature (T_{air}), maximum temperature (T_{max}), vapour pressure deficit (VPD_{air}), solar radiation (SRad) and daily total reference evapotranspiration (ET_o) during each of the 10 leaf gas exchange measurement campaigns. Measurements performed during the 2018/19 season were conducted in the mature orchard while measurements in 2020 were performed in the intermediate orchard.

Date	Year	T_{air} (°C)	T_{max} (°C)	SRad (MJ m^{-2} day^{-1})	VPD_{air} (kPa)	ET_o (mm)
1 June	2018/19	17.7	27.7	15.8	1.45	3.11
2 June	2018/19	14.1	23.5	15.8	1.05	2.68
1 October	2018/19	18.5	31.6	24.8	1.91	5.14
2 October	2018/19	23.3	35.1	25.3	2.75	6.11
30 January	2018/19	21.8	28.0	18.5	0.62	3.46
31 January	2018/19	21.7	28.8	20.4	0.66	3.82
22 June	2019/20	12.4	20.1	10.2	0.63	1.76
23 June	2019/20	12.7	23.2	9.4	1.01	2.14
24 June	2019/20	11.6	20.6	10.1	0.62	1.69
9 July	2019/20	13.9	22.4	10.3	0.54	1.71

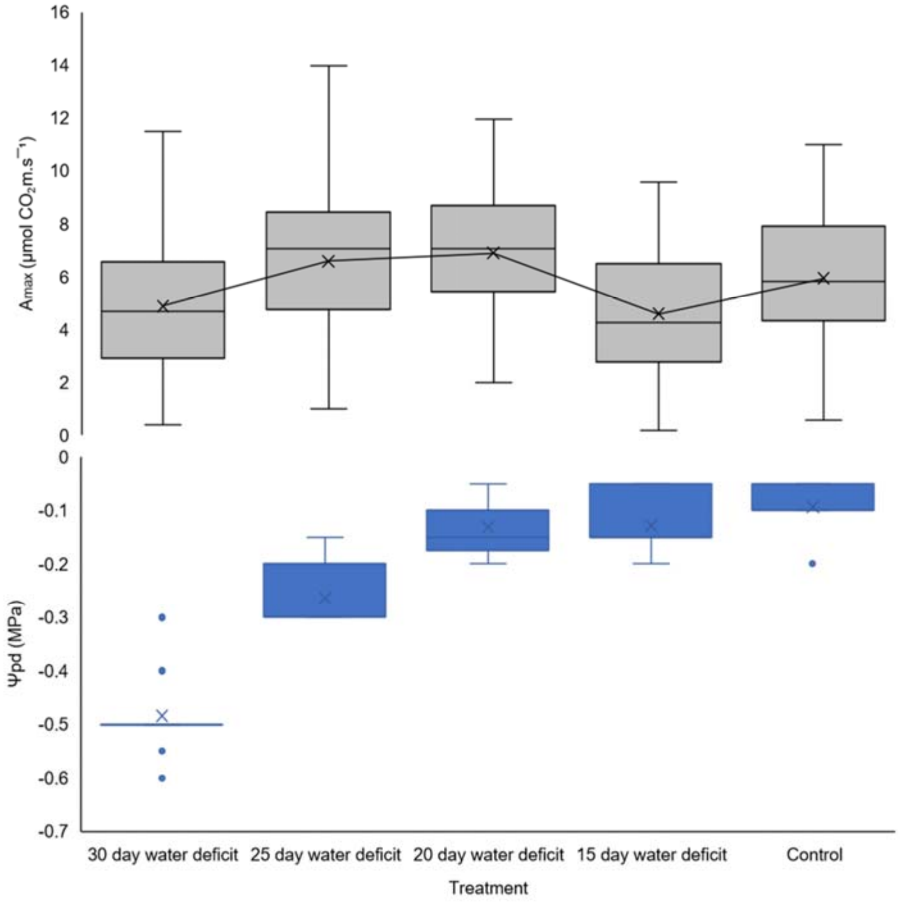


Figure 4.13 Maximum net photosynthetic assimilation rate (A_{max}) at different pre-dawn water potentials (Ψ_{pd}) (MPa) for four water deficit treatments and a well-watered control. A_{max} data points were obtained over an eight-hour measurement period during three measuring campaigns (22 June – 24 June 2020). Pre-dawn water potentials were recorded prior to sunrise during each of the measuring campaigns

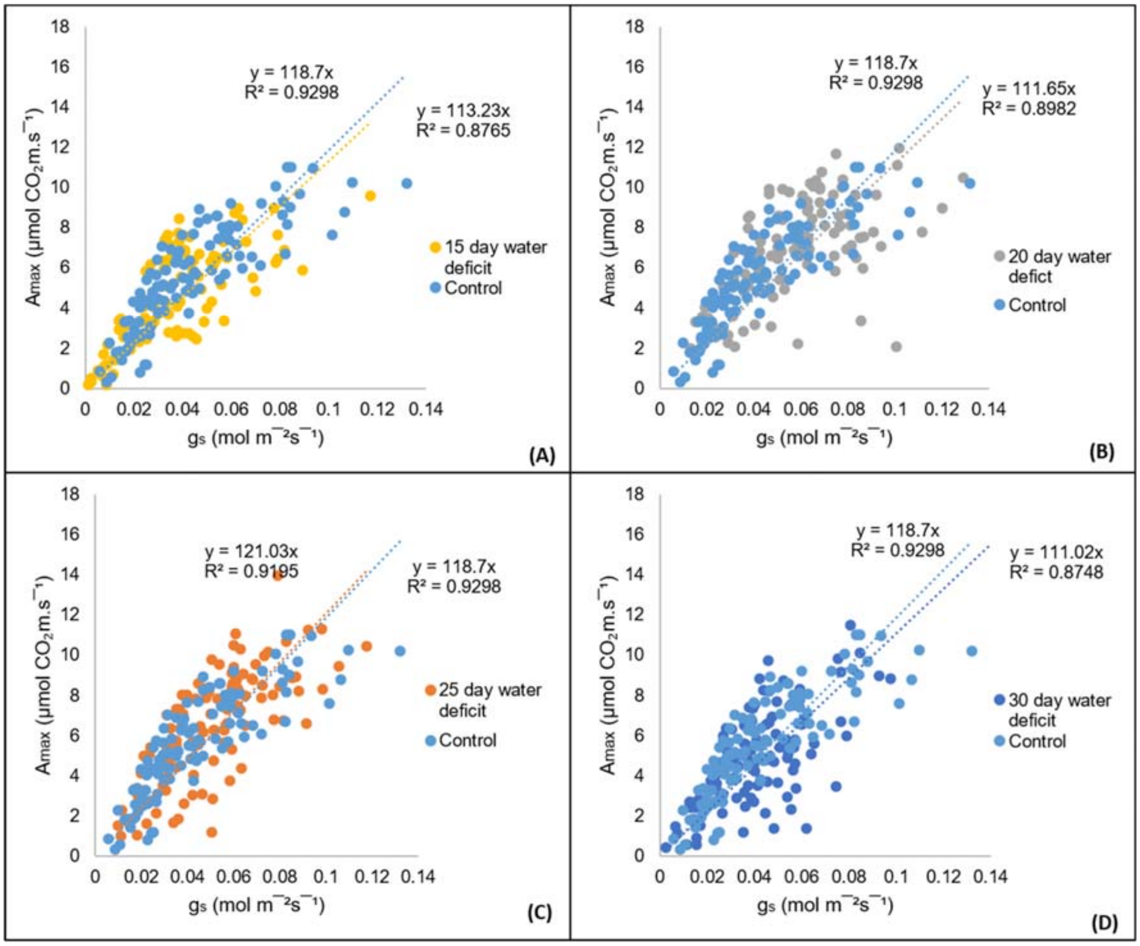


Figure 4.14 The relationship between the maximum net photosynthetic assimilation rate (A_{max}) and stomatal conductance (g_s) (mol $m^{-2}s^{-1}$) of four water deficit treatments (A: 30 day, B: 25 day, C: 20 day and D:15 day) and a well-watered control. Data points were obtained over an eight-hour measurement period during three measuring campaigns (22 June – 24 June 2020)

Stephenson et al. (2003) showed the g_s of stressed trees to be similar to that of unstressed trees, until peak VPD_{air} levels (1.6 kPa) were reached. The authors found that g_s of stress trees declined and remained low after the midday depression (caused by peak VPD_{air}), whereas unstressed trees recovered. Stephenson et al. (2003) attributed the lower gas exchange values after midday to a root signal induced reduction in g_s, caused by low root zone water potentials. During the current study, g_s of stressed and control trees showed a similar response to changing levels (<3 kPa) of VPD_{leaf} (assuming that $T_{leaf} = T_{air}$) (**Figure 4.15**). Stomatal conductance of stressed trees was, however, substantially reduced, relative to the control, at VPD_{leaf} levels >3 kPa. Hence, suggesting a similar repose to what was observed by Stephenson et al.

(2003), with a substantial reduction in g_s of stressed trees, once a threshold VPD level is reached. The findings by Stephenson et al. (2003) combined with those observed over the course of this study may improve our understanding of why water deficit conditions imposed during the flower initiation had a limited effect on the yield and quality of macadamias. Flower initiation typically coincides during winter periods (May – July) in the southern hemisphere, which is characterised by lower atmospheric temperatures, low solar radiation and low VPD_{air}. Under these conditions, threshold VPD levels will seldom be exceeded and as a result gas exchange of trees experiencing a mild water deficit will not be impacted.

Stephenson et al. (2003) further showed that photosynthesis and g_s are highly but negatively correlated with VPD. Similar observations were, however, not made in the intermediate orchard, as observed from **Figure 4.15** and **Figure 4.16**. Since A_{max} is a function of g_s, the correlation between A_{max}, water deficits and VPD are most likely related to the sensitivity of g_s to these factors. Hence, the effect of VPD on macadamia gas exchange may have influenced the interpretation of tree physiological responses to water deficits under field conditions.

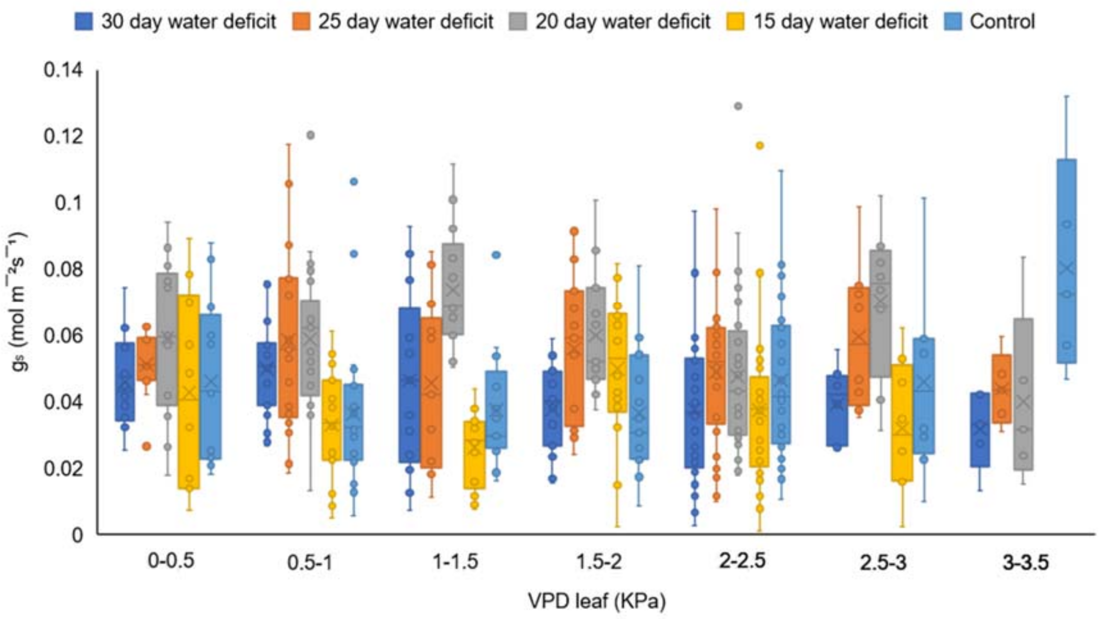


Figure 4.15 Stomatal conductance (g_s) (mol $m^{-2}s^{-1}$) at different leaf vapour pressure deficits (VPD_{leaf}) (kPa) for four water deficit treatments and a well-watered control. Data points were obtained over an eight-hour measurement period during three measuring campaigns (22 June – 24 June 2020)

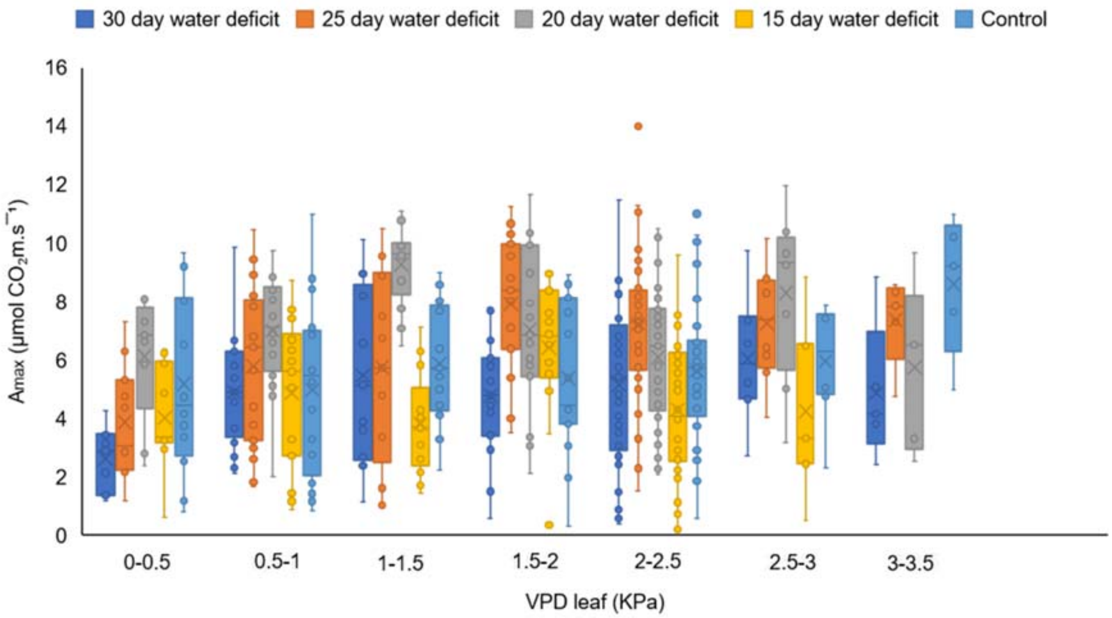


Figure 4.16 Maximum net photosynthetic assimilation rate (A_{max}) at different leaf vapour pressure deficits (VPD_{leaf}) (kPa) for four water deficit treatments and a well-watered control. Data points were obtained over an eight-hour measurement period during three measuring campaigns (22 June – 24 June 2020)

When assessing the response of macadamia A and g_s to different Ψ_{pd} levels for a range of atmospheric variables, including VPD (**Table 4.4**), g_s and A_{max} were shown to be highly but negatively correlated with different water deficit levels (**Figure 4.17** and **Figure 4.18**). In this study, measurements conducted in both mature and intermediate orchards showed a substantial reduction in average g_s (r^2 0.76) and A_{max} (r^2 0.79) with every unit change in Ψ_{pd}. A considerable difference in average g_s was observed between well-watered trees (Ψ_{pd} -0.05 MPa) and trees subjected to water deficits with a 26%, 30% and 74% reduction in average g_s observed for trees at a Ψ_{pd} of -0.4 MPa, -0.7 MPa and -1.2 MPa respectively (**Figure 4.17**). The response of g_s to water deficits was reflected in A_{max} (**Figure 4.18**). Lloyd et al. (1991) found g_s of unirrigated macadamia trees to be 30% lower than that of the irrigated trees after a two-month rainless period, when measured in spring. Although much lower Ψ_{pd} (<-0.6 MPa) were recorded in the mature orchard than what was reported by Lloyd et al. (1991) (± -0.22 MPa), lowest recorded g_s and Ψ_{pd}, in both studies, were recorded during spring (October) which coincided with substantially high ET_o and VPD_{air} levels (**Table 4.4**). Hence, significant stomatal down regulation due to high atmospheric evaporative demands and soil water deficits was observed, as these conditions were more likely to lead to an imbalance between atmospheric evaporative demand and the ability of the plant to supply the leaves with water (Campbell and Turner, 1990).

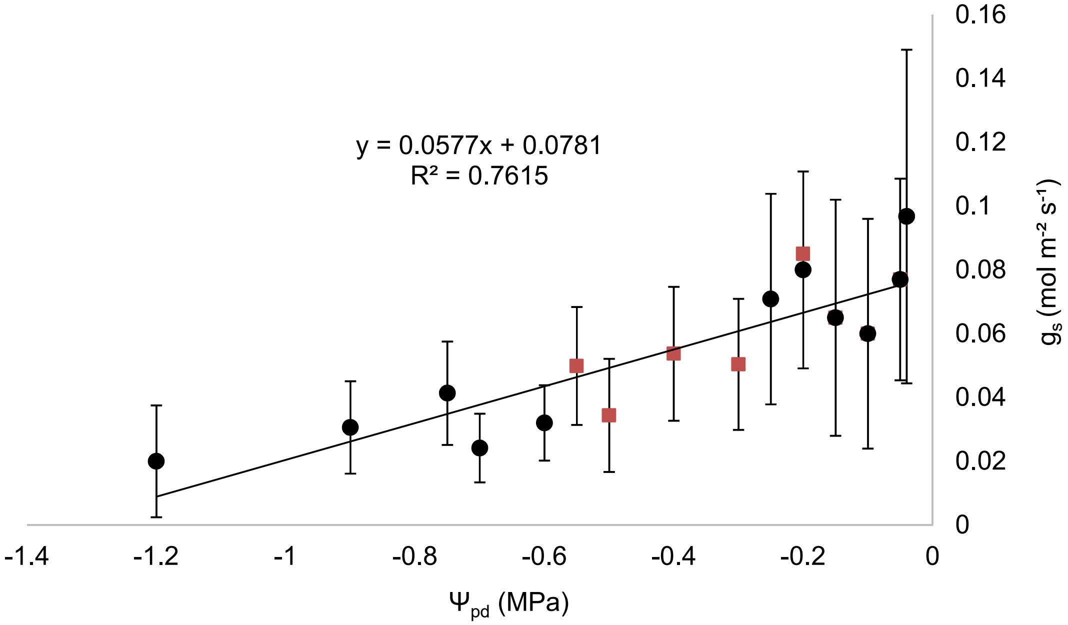


Figure 4.17 Mean stomatal conductance (g_s) (mol $m^{-2}s^{-1}$) following different pre-dawn water potential levels (Ψ_{pd}) (-MPa). Gas exchange data presented was collected over an eight-hour measurement period during 10 different measurement campaigns. Orange data points were obtained during the intermediate orchard measuring campaign while black data points were obtained during the mature orchard measurement campaigns

The down regulation in A_{max} in response to water deficits (**Figure 4.17**) can be related to the reduction in g_s for trees subjected to water deficits. A reduction in g_s leads to a decrease in transpiration and therefore a decrease in CO_2 entering the leaf mesophyll (site of assimilation), ultimately resulting in a lower CO_2 assimilation rate and an increase in leaf temperature (Ribeiro and Machado, 2007). It seems that from the data collected in this trial, macadamia trees start experiencing water deficit stress when Ψ_{pd} <-0.6 MPa. In most instances in this trial these values were only achieved after water was withheld for a period of a month and under drier conditions, reflected in higher ET_o and VPD.

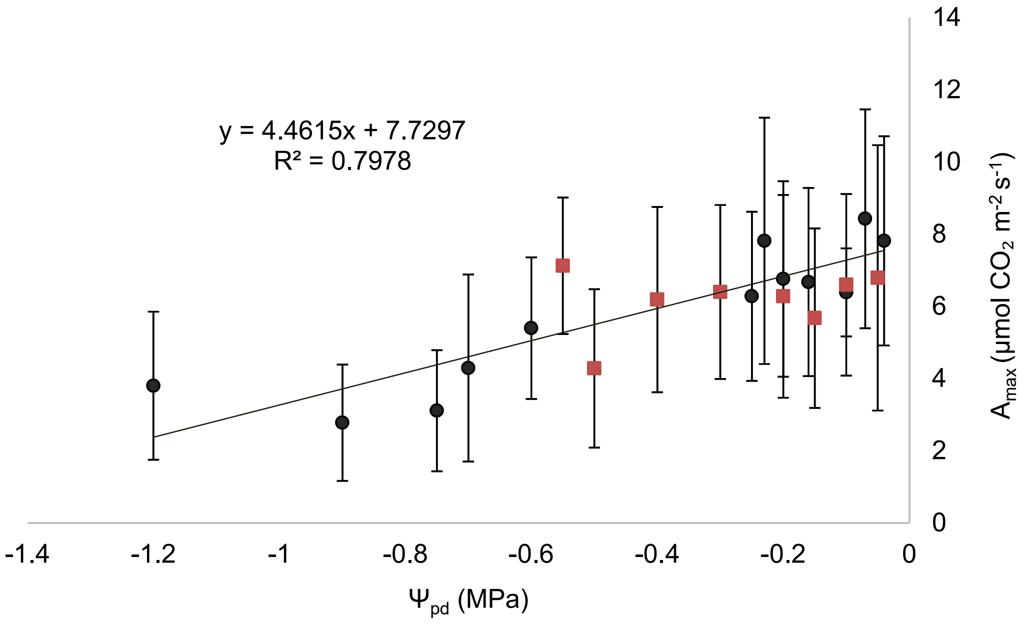


Figure 4.18 Mean maximum net photosynthetic assimilation rate (A_{max}) at different pre-dawn water potential levels (Ψ_{pd}) (-MPa). Gas exchange data presented was collected over an eight-hour measurement period during 10 different measurement campaigns. Orange data points represent data obtained during a measurement campaign in the intermediate orchard, while black data points represent data obtained during measurement campaigns in the mature orchard

4.6 Midday stem and leaf water potentials as indicators of macadamia water deficits

Plant tissue water potential at any time depends on both the bulk soil water content and the hydraulic conductivity of the plant, as well as the resistance to water flow between the bulk soil and plant tissue (Jones, 2004). The scheduling of irrigation, based on plant water status, requires some reference or threshold value for when irrigation is required, which is related to the development of stress which impacts plant performance. Whilst, Ψ_{pd} is widely regarded as one of the best measures of developing water stress, measurements of Ψ_x and Ψ_L have been investigated as they may be more practical for commercial use (Jones, 2004). Diurnal $\Psi_{L,}$ however, imitates the effect of multiple, often fluctuating variables at a single leaf level such as local leaf water demand (VPD, leaf intercepted radiation), soil water availability, internal plant hydraulic conductivity and stomatal regulation (Chone et al., 2001, Naor and Cohen, 2003). Hence, potentially making Ψ_L an unsuitable indicator of water deficit conditions, especially in isohydric plants (Jones, 2004). Since, bagged Ψ_x are not as subjected to environmental fluctuations, these measurements are preferred as they have shown to be more stable and more closely related to soil water status than Ψ_L (Correia et al., 1995, McCutchan and Shackel, 1992). Results from this study suggest Ψ_{pd} measurements in macadamias are strongly correlated (r^2 0.79) to midday Ψ_x measurements (**Figure 4.19**). This relationship should, however, be determined in a range of growing regions which differ substantially in climate, as it may differ between humid and more arid regions. Based on the strong correlation between Ψ_{pd} and macadamia gas exchange, it is therefore reasonable to assume that a reduction in midday Ψ_x will result in a similar reduction in gas exchange. Similar observations, in terms of a strong correlation between Ψ_{pd} and $\Psi_{x,}$ over a range of water deficits have previously been made by Nortes et al. (2005) in almonds. Study findings, therefore, indicate both Ψ_{pd} and bagged midday Ψ_x to be accurate indicators of macadamia water deficits with Ψ_{pd} = -0.6 MPa and midday Ψ_x = -0.9 MPa indicating a mild macadamia water deficit. Extrapolating **Figure 4.19**, trees displaying midday Ψ_x within the range -0.3 to -0.5 MPa can be considered as well irrigated and non- water stressed.

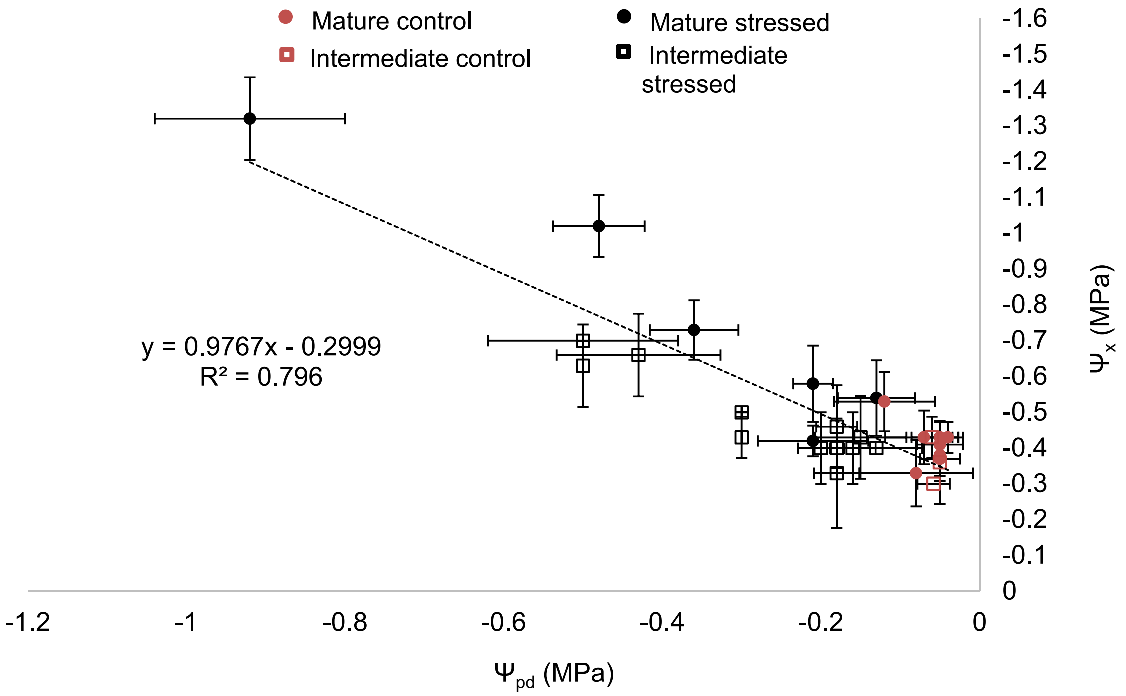


Figure 4.19 The relationship between predawn (Ψ_{pd}) (MPa) and midday stem water potential (Ψ_x) (MPa) measurements (Mean ± std). Data points represent data obtained during both the mature and intermediate orchard measurement campaigns. Non-filled data points represent measurements made under stressed conditions while filled data points represent measurements made under well-watered conditions

In terms of Ψ_L, the large variability between measurements and measurement campaigns failed to produce clear differences between different treatments (**Figure 4.20**). Similar observations in terms of no significant differences between the day-time Ψ_L of irrigated and non-irrigated macadamia trees were made by Lloyd et al. (1991), after a two-month rainless period. A study by Smit et al. (2020) has similarly reported that macadamias maintain a relatively constant midday Ψ_L, complementing the theory by Sperry et al. (1993) that stomata act as internal pressure regulators, which attempt to maintain the hydraulic continuum. This theory is also complemented by a strong linear relationship between g_s and Ψ_L amongst all treatments observed in this study (**Figure 4.21**). As expected, the response of g_s to Ψ_L is reflected in the strong linear relationship between A_{max} and Ψ_L over all treatments (**Figure 4.22**). Based on the findings of this study the use of Ψ_L as an indicator of macadamia water stress wound not be recommended due to the lack of response of Ψ_L to different water deficit levels. A review by Jones (2004) highlighting the potential pitfalls of plant-based irrigation scheduling tools has highlighted that Ψ_L is a function of underlying physiological responses and is, therefore, an unreliable indicator of water stress.

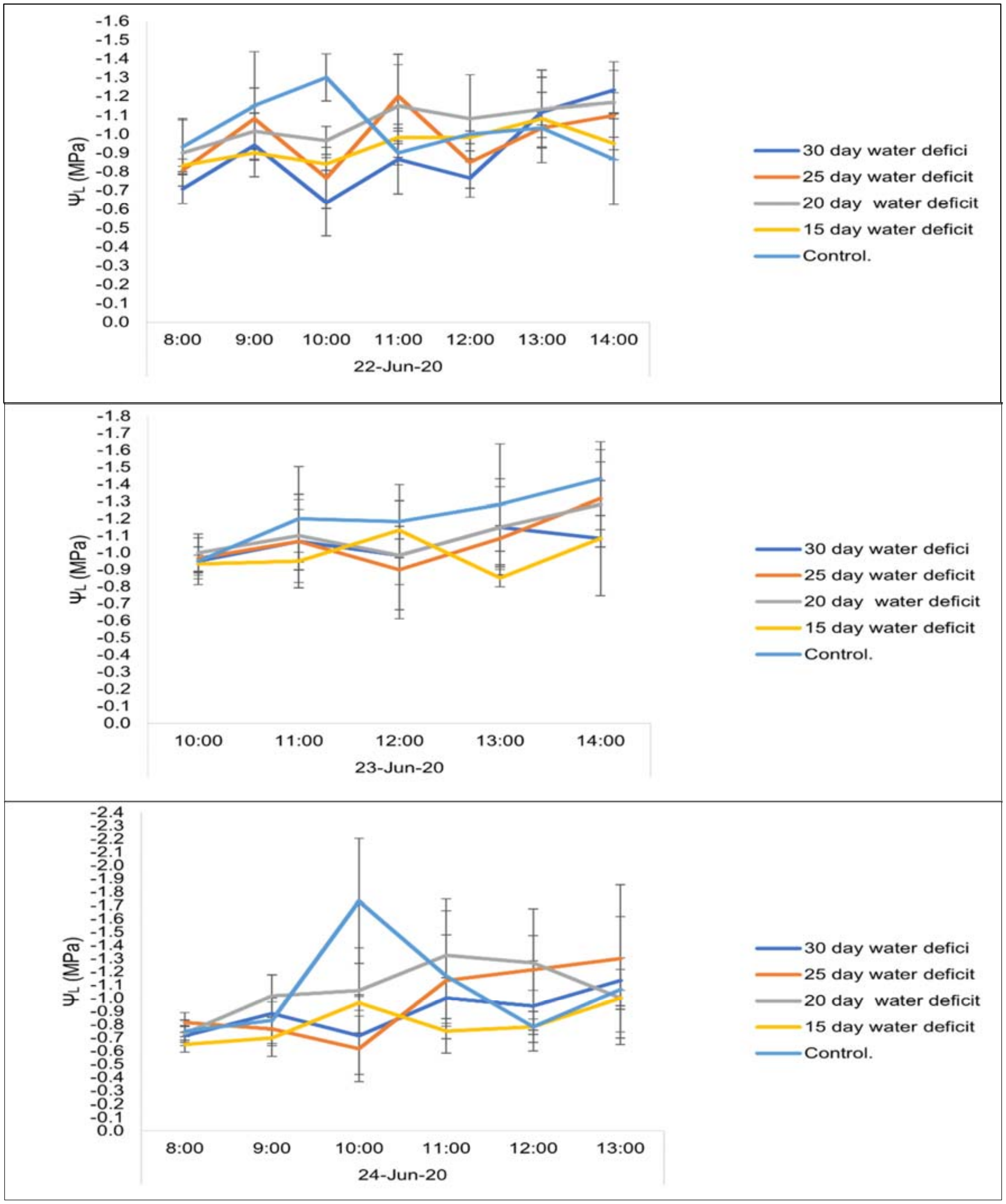


Figure 4.20 Diurnal leaf water potential Ψ_L (-MPa) of four water deficit treatments and a well-watered control. Data points were obtained during three measuring campaigns in the intermediate orchard (A) 22 June 2020, (B) 23 June 2020 (C) 24 June 2020

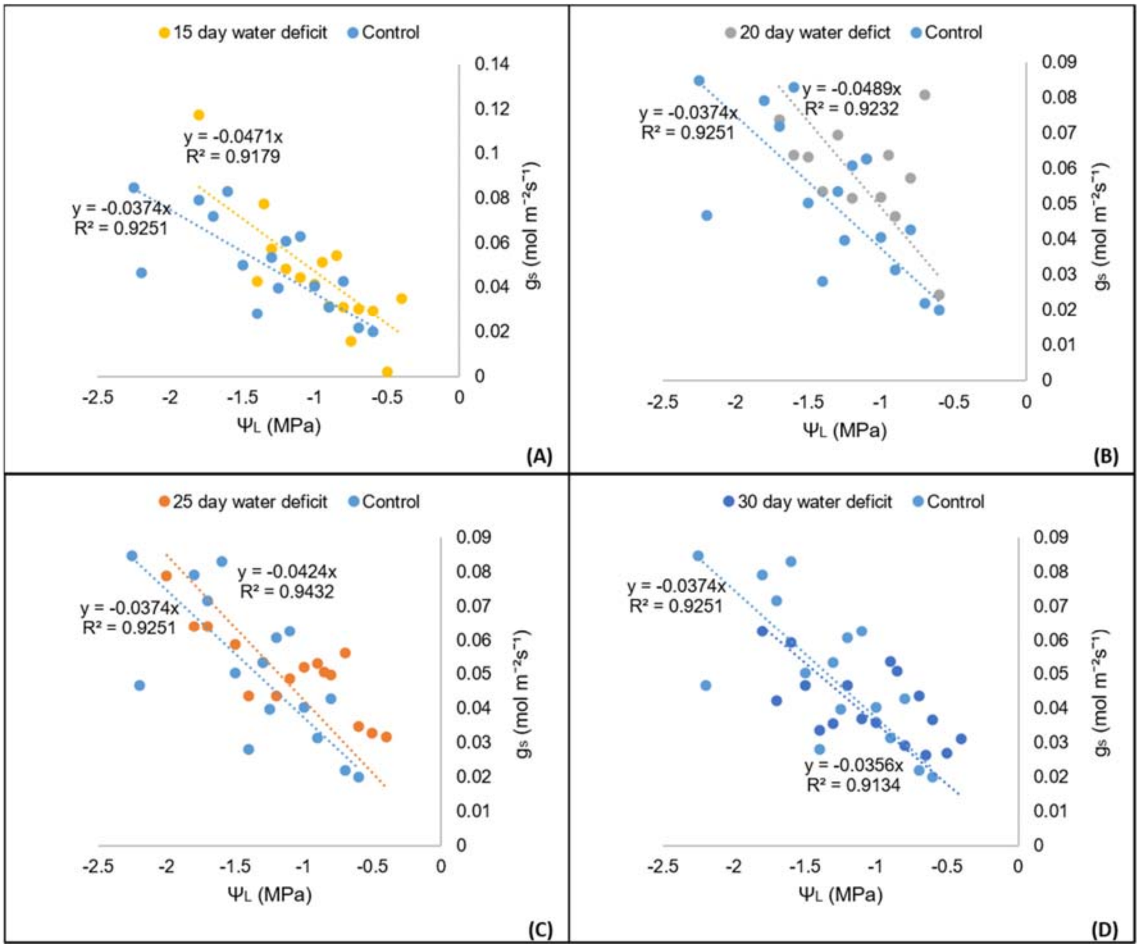


Figure 4.21 The relationship between the stomatal conductance (g_s) (mol m^{-2}s^{-1}) and leaf water potential Ψ_L (MPa) of four water deficit treatments (A: 15 day, B: 20 day, C: 25 day and D:30 day)and a well-watered control. Data points were obtained over an eight-hour measurement period during three measuring campaigns (22 June – 24 June 2020)

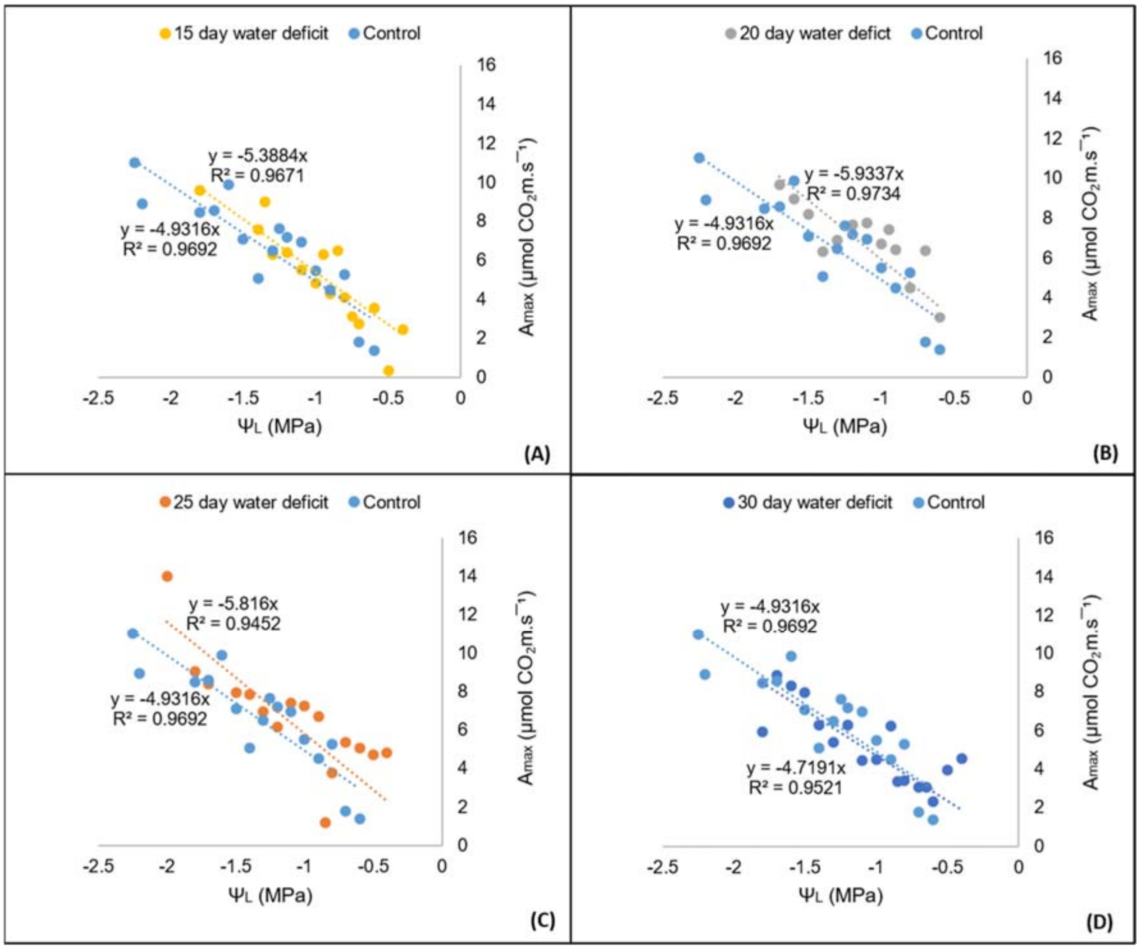


Figure 4.22 The relationship between the maximum net photosynthetic assimilation rate (A_{max}) and leaf water potential Ψ_L (MPa) of four water deficit treatments (A: 15 day, B: 20 day, C: 25 day and D:30 day) and a well-watered control. Data points were obtained over an eight-hour measurement period during three measuring campaigns (22 June – 24 June 2020)

CHAPTER 5: CONCLUDING REMARKS AND RECOMMENDATIONS

Total seasonal rainfall and irrigation were sufficient to meet the seasonal ET_c requirement of the control trees over all three seasons. Although a constant K_c of 0.65 was used over the season, it can be suggested that during any of the non-treatment stages trees were well-irrigated and not water stressed, meaning that comparisons between irrigated and stressed trees can be assumed to be fair in terms of a water deficit. Well-irrigated conditions were reflected in the capacitance probe readings over the 2017/18, 2018/19 and 2019/20 seasons and through the above industry standard yields (>4 ton/ha DIS) recorded for this orchard over a number of years.

Through the exclusion of rainfall and restriction of irrigation water, treatment trees on which a mild water deficit was implemented, were exposed to a calculated water deficit during all the intended phenological stages, over both the 2018/19 and 2019/20 seasons. Soil matric potential readings correlated with intended water deficits periods, as well as periods of high ET_o and low rainfall for all treatments in the mature orchard block. Plant water deficits during the different phenological stages were further confirmed by a reduction in pre-dawn water potential (Ψ_{pd}) during each of the intended water deficit periods relative to the control trees.

As proposed by the study hypotheses, mild water deficits during flower initiation had no impact on yield and quality, whilst a mild water deficit during flowering and nut set, nut sizing and premature nut abscission, oil accumulation and shell hardening similarly did not reduce yield relative to the control. Even though there were no significant differences between the yield of control trees and trees exposed to a mild water deficits during different phenological stages, trees exposed to prolonged deficit irrigation (irrigating half the volume of the grower scheduled control) yielded significantly more than all treatments (including the control). In addition, relying solely on rainfall also had no impact on yield over two seasons. This suggested that control trees were potentially over-irrigated, which may have led to the observed yield penalties. Therefore, when using the half irrigation treatment as the “control”, it can be concluded that mild water deficits during flowering and nut set, premature nut abscission, oil accumulation and shell hardening can potentially impact yield negatively, which supports the hypotheses proposed for the study.

The flowering and nut set and shell hardening phenological stages were, however, identified as the most sensitive phenological stages to water deficits. Total kernel recovery of trees stressed during flowering and nut set and shell hardening were significantly impacted relative to the control, thereby negatively affecting macadamia quality. A second season of yield measurements quantifying the impact of water deficits at different phenological stages was unfortunately lost due to the COVID-19 lockdown and these results could have proven invaluable in reaching a more definite conclusion regarding how water stress impacts yield and quality of macadamia orchards.

Results from this study further suggest that macadamias are less sensitive to water deficit conditions than most other horticultural crops. This was illustrated by the fact that, despite withholding irrigation for fairly long periods of time, key indicators of plant stress, did not suggest that the trees were often stressed, when compared to the well-watered control. This highlights macadamia's ability to extract and use water efficiently, with Ψ_{pd} seldom surpassing -0.6 MPa in water stressed trees. Capacitance probe and soil matric potential readings further support these findings, showing that macadamias have the ability to extract sufficient water from depth. This ability appears to buffer macadamias against the onset of water stress during short water deficit periods, which was reflected in the gradual reduction in measurements of plant water status, even after a one-month waterless period. It can therefore be assumed that severe water deficitsat any phenological stage will likely have a negative impact on both yield and quality but in deep soils and under normal rainfall conditions, reaching such a level of stress would be unlikely in most macadamia producing regions of South Africa. In the current study, this was reflected by the fact that there was a large variation between replicates, which was probably indicative of variations in spatial water availability and soil depth, which could have resulted in some trees having access to more water than other trees. Depending on the slope and the orchard drainage patterns, trees in deeper soils generally had higher Ψ_{pd} which declined slowly under water deficit conditions whilst trees in shallow, well drained soils showed more abrupt Ψ_{pd} fluctuations.

The ability of macadamia trees to extract sufficient water from depth and at low soil water content also influence the interpretation of measurements of soil water content in macadamia orchards. Even though soil matric potential readings within the bulk rootzone (up to 60 cm in depth) reflect water deficit conditions, this did not correspond with physiological water stress in macadamia orchards. This is of fundamental importance, as this soil-based measurement, is commonly accepted by macadamia growers as an indicator of macadamia water stress. Measurements of soil matric potential can, however, be used as an indication of conditions conducive to the development of water stress in macadamia trees, but the rate and severity of stress development will greatly vary with atmospheric evaporative demand, soil depth and tree rooting patterns. Soil matric potential readings, at a 60 cm depth, of -50 kPa can be used as critical irrigation trigger points in macadamia orchards, but caution should be exercised when constantly maintaining matric potential readings above -20 kPa, as this may lead to deep drainage, waterlogging and/or nutrient losses.

Nevertheless, tentative results from this study suggest that both Ψ_{pd} and midday stem water potentials (Ψ_x) can be used as indicators of water stress in macadamia orchards, with Ψ_{pd} <-0.6 MPa and Ψ_x <-0.9 MPa probably indicating the onset of stress that could impact yield and quality. Measurement sensitivity (rate of change), however, varied with atmospheric evaporative demand and soil characteristics, which could lead to difficulty in interpreting measurement data in commercial orchards.

Although it was not the intended purpose of the trial, this trial has also demonstrated that the “traditional” ways of scheduling irrigation with a single point measurement of soil water content may not be appropriate for macadamia orchards, as a slight yield penalty was noted in the grower control when compared to the half irrigation and rainfed treatments. This reiterates the importance of choosing the right control in these types of experiments and ensuring that this treatment is irrigated optimally. It also challenges the perception of what is the best way to manage irrigation in macadamia orchards. Since mild stress was achieved in some trees during the flowering and fruit set stage, when conditions were fairly dry, due to increasing temperatures in spring but as yet no rainfall, this may be a critical time for irrigation in macadamia orchards. It is therefore recommended to fill the orchard soil profile to field capacity (FC) prior to forecasted rainless periods, such as the spring flowering and nut set period in the southern hemisphere. This can potentially buffer macadamias against the onset of water stress during times when rainfall and irrigation cannot meet the full ET requirement of the trees. Caution should, however, be exercised when maintaining soil water content at FC during high rainfall periods as this may lead to excessive run-off, waterlogging and nutrient losses. Furthermore, the impact of VPD on stomatal conductance needs to be considered during these times as relying solely on ET_o to schedule irrigation may result in over-irrigation. Due to the isohydric nature of macadamias, stomatal conductance (g_s) will be down-regulated in response to high VPD which in-turn may limit canopy transpiration. This means that tree water use may decline under conditions of high atmospheric evaporative demand which could lead to waterlogging if more water is applied. At times, transpiration may therefore be limited by high VPD and not soil water content.

This study has provided a good foundation for future research, but there are still some unresolved issues. Due to the limited amount of stress measured in the current trial, it will be very important to continue this work in a location where it is easier to implement water deficit conditions. For this, a more uniform orchard needs to be chosen with a deep, well-drained soil. This should hopefully allow the determination of more accurate pre-dawn and midday stem potential thresholds for stress in macadamia and allow for improvements in irrigation scheduling that ensure optimal utilisation of a scarce and finite resource. Furthermore, field studies evaluating the effect of a specific parameter on the yield and quality of perennial crops should be conducted over a prolonged period (minimum 5 years) as seasonal characteristics may lead to variable results. It will also allow a more in depth analysis of which are the most sensitive phenological stages to water deficit conditions in terms of yield and quality.

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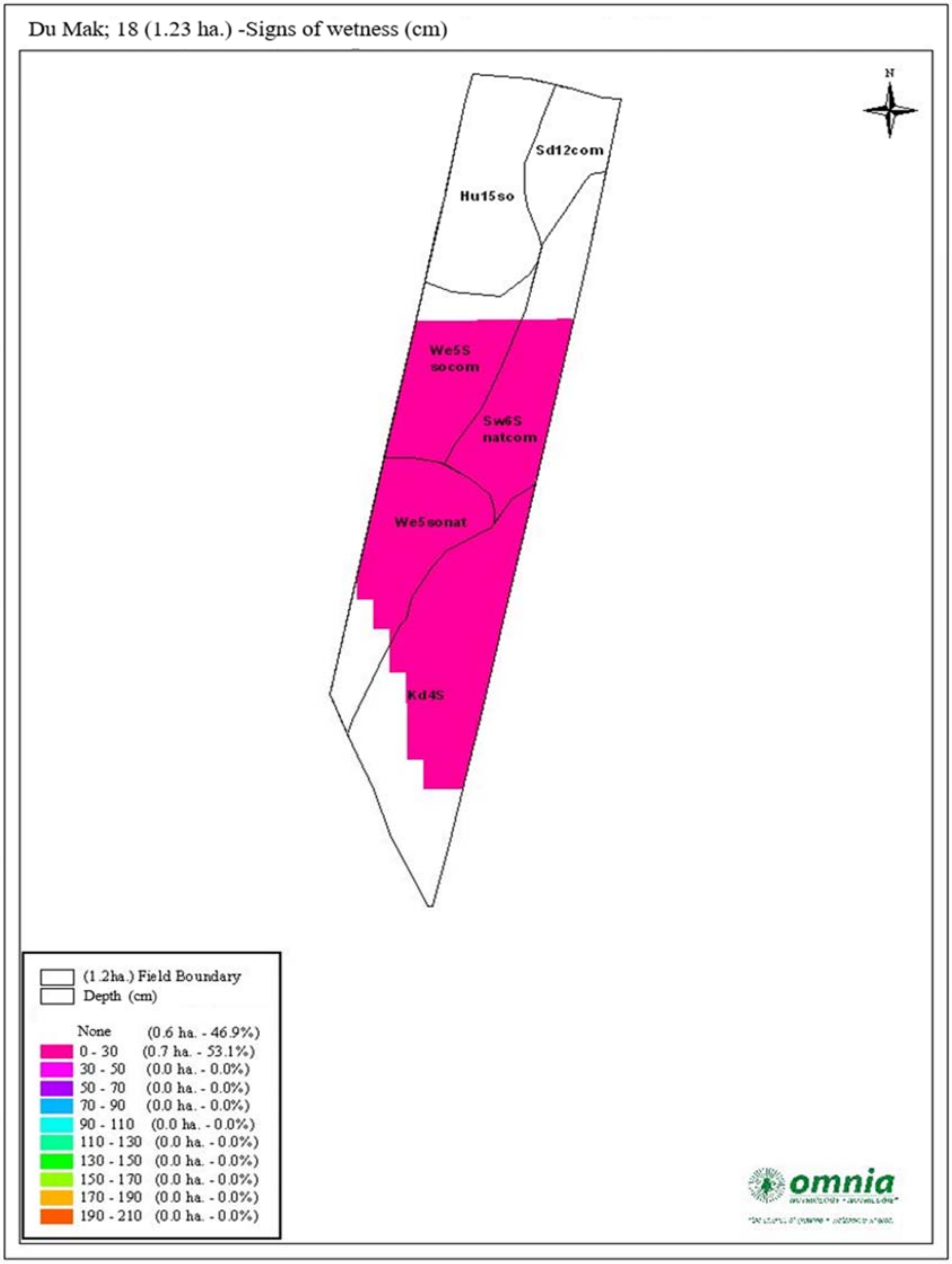
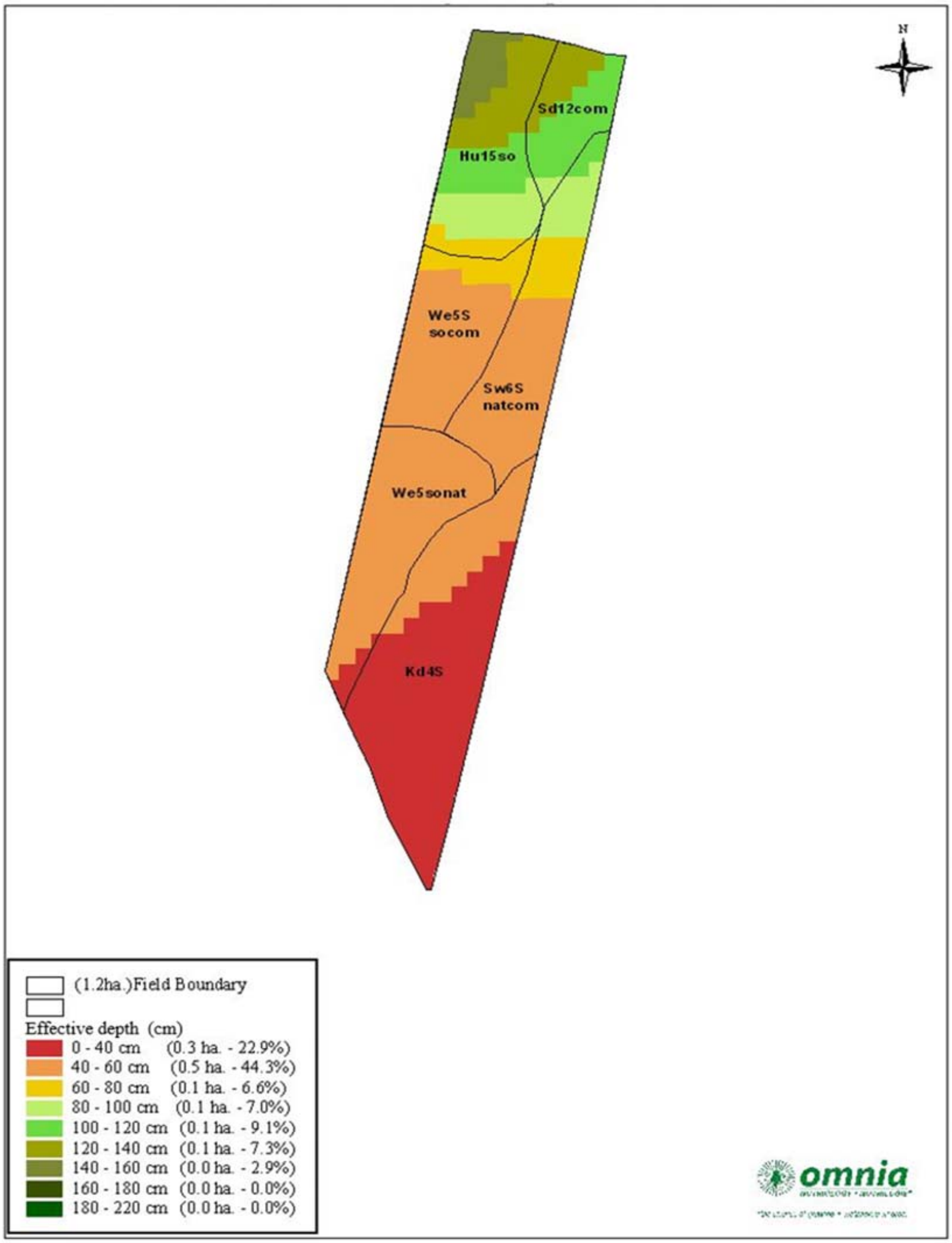
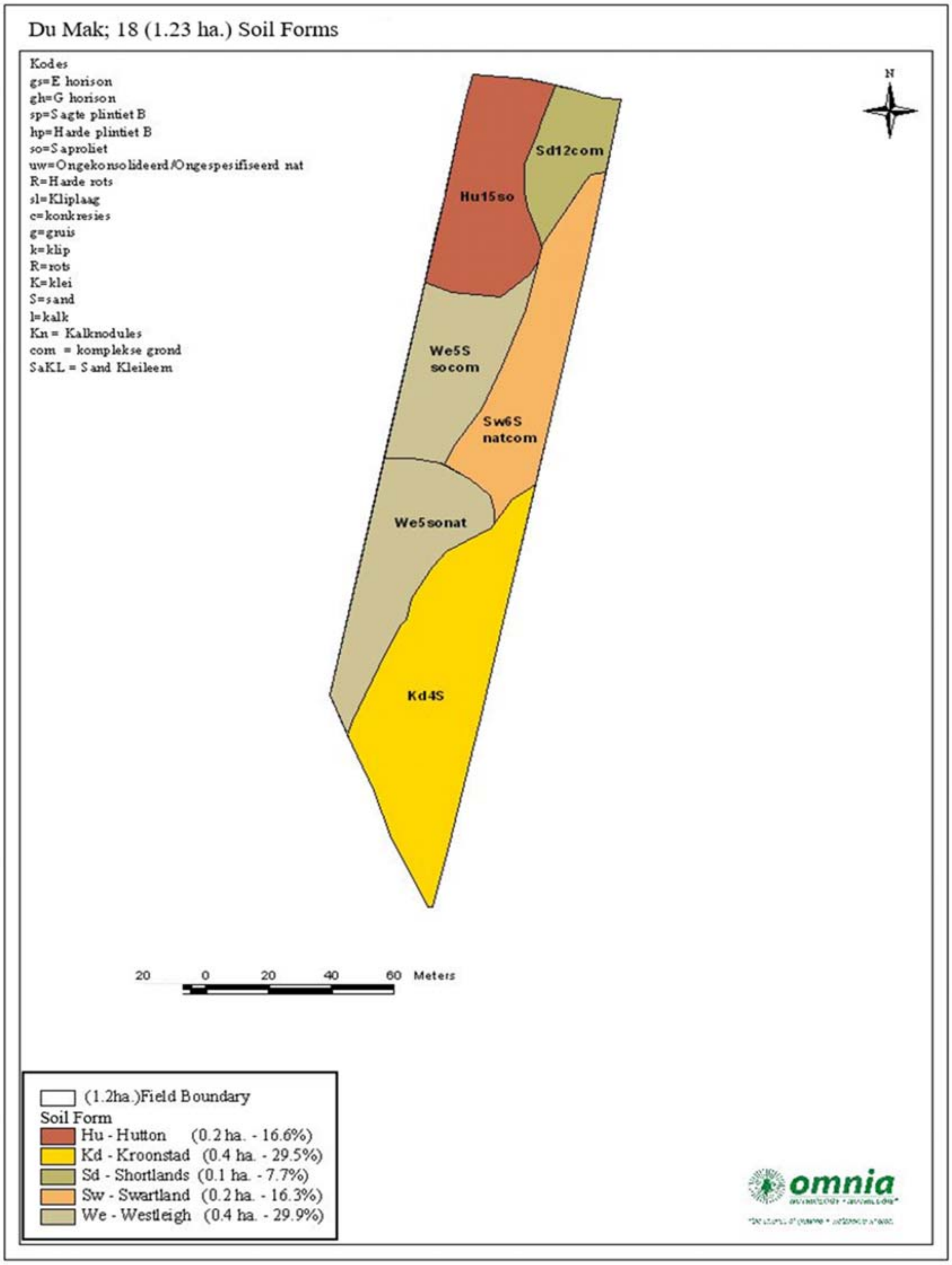
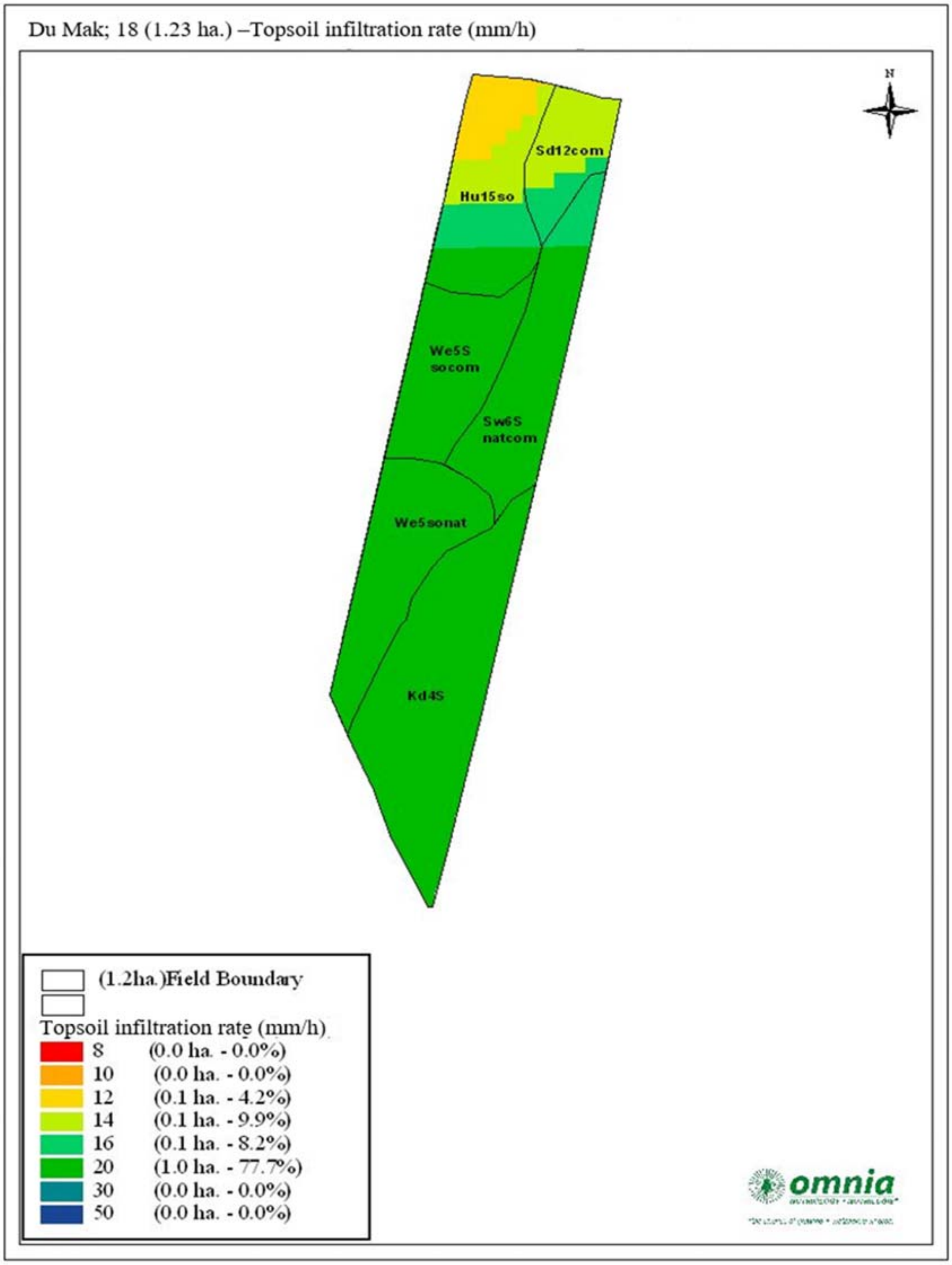
ANNEXTURE 1


Du Mak; 18 (1.23 ha.) -Signs of wetness (cm)
N
Sd12com
Hu15so
We5S socom
Sw6S natcom
We5sonat
Kd4S
(1.2ha.) Field Boundary
Depth (cm)
None (0.6 ha. - 46.9%)
0 - 30 (0.7 ha. - 53.1%)
30 - 50 (0.0 ha. - 0.0%)
50 - 70 (0.0 ha. - 0.0%)
70 - 90 (0.0 ha. - 0.0%)
90 - 110 (0.0 ha. - 0.0%)
110 - 130 (0.0 ha. - 0.0%)
130 - 150 (0.0 ha. - 0.0%)
150 - 170 (0.0 ha. - 0.0%)
170 - 190 (0.0 ha. - 0.0%)
190 - 210 (0.0 ha. - 0.0%)
omnia

Du Mak; 18 (1.23 ha.) – Effective soil depth (cm)




Du Mak; 18 (1.23 ha.) Soil Forms
Kodes
gs=E horison
gh=G horison
sp=Sagte plintiet B
hp=Harde plintiet B
so=Saproliet
uw=Ongekonsolideerd/Ongespesifiseerd nat
R=Harde rots
sl=Kliplaag
c=konkresies
g=gruis
k=klip
R=rots
K=klei
S=sand
l=kalk
Kn = Kalknodules
com = komplekse grond
SaKL = Sand Kleileem
N
Sd12com
Hu15so
We5S socom
Sw6S natcom
We5sonat
Kd4S
20 0 20 40 60 Meters
(1.2ha.)Field Boundary
Soil Form
Hu - Hutton (0.2 ha. - 16.6%)
Kd - Kroonstad (0.4 ha. - 29.5%)
Sd - Shortlands (0.1 ha. - 7.7%)
Sw - Swartland (0.2 ha. - 16.3%)
We - Westleigh (0.4 ha. - 29.9%)
omnia



Du Mak; 18 (1.23 ha.) –Topsoil infiltration rate (mm/h)

Du Mak; 18 (1.23 ha.) – Plant available water between PWP and FC (mm)