The impact of water stress at key phenological stages on yield and quality of pecans (*Carya illinoinensis* Wangenh. K.Koch).

by

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DECLARATION

I, Seluleko Celumusa Kunene, declare this as my own work, except where secondary material is used, and it has however been duly acknowledged. I also certify that no plagiarism was done in compiling this thesis.

SIGNATURE: 

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ABSTRACT

An important part of developing sound water management strategies for crops is to identify phenological stages which are sensitive and insensitive to water stress in terms of yield and quality. This is particularly important for regions which are prone to droughts and where water resources are limited. As pecans are largely produced in semi-arid regions in South Africa and are reported to have very high annual water requirements, this information is particular important for this valuable crop. This study therefore attempted to evaluate how water stress at different phenological stages (flowering and nut set, nut sizing, nut filling and shuck dehiscence) impacts pecan (*Carya illinoinensis* Wangenh. K.Koch) yield and quality. A trial was conducted in a 12-year-old pecan orchard at Innovation Africa @UP over three consecutive seasons. Measurements included several plant physiological responses to quantify the level of plant stress throughout the growing season and yield and quality measurements at the end of each season. Water stress (midday stem water potential <-0.9 MPa) led to a decline in stomatal conductance, photosynthesis, transpiration, and growth (especially during bud break). At the flowering and nut set stage, water stress led to significant flower abortion and nut fall, which reduced the final number of nuts by 60%, which ultimately reduced the final yield compared to well-water control. Water stress at nut growth stage, did not necessarily reduce the final yield at this stage, but rather reduced the nut size (many smaller nuts), thereby compromising the nut quality and income. Water stress at the nut filling stage significantly reduced both the yield and nut quality, reducing the nut mass, due to a high percentage of wafers/air pockets as a result of poor nut filling. Stress during the final phenological stage, shuck dehiscence, did not have a major impact on final yield or quality, but there was an increase in the number of stick-tights. However, over the three seasons the increase in the number of stick tights did not always translate into a significant reduction in yield when compared to the control. Therefore, in seasons where water allocations are reduced, it may be possible to make some water savings during nut sizing and shuck dehiscence without compromising yield. However, some reduction in quality and income may result if the trees are stressed during these stages.

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LIST OF ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS

ABA – Abscicic acid

O_2 – Oxygen

CO_2 – Carbon dioxide

H_2O – Water

RH – Relative Humidity

R_n – Net Radiation

ROS – Reactive oxygen species

Rubisco – Ribulose-1,5-bisphosphate carboxylase/oxygenase

NAD – Nicotinamide adenine dinucleotide

NADP – Nicotinamide adenine dinucleotide phosphate

ET - Evapotranspiration

ET_c – Crop Evapotranspiration

ET_o – Reference Evapotranspiration

DI – Deficit Irrigation

NIS – Nut in shell

Ψ_{smd} – Stem water potential

Ψ_{leaf} – Leaf water potential

Ψ_{pd} – Predawn leaf water potential

(T_{leaf}) – leaf temperature

VPD – Vapour Pressure Deficit

VPD_{leaf} – Leaf-to-air Vapour Pressure Deficit

g_s – Stomatal Conductance

A_n – Net assimilation CO_2 rate

C_i – Intercellular CO_2 concentration

PAR – Photosynthetically Active Radiation

IRGA – Infra-red gas analyser

Q – Photo flux

CWSI – Crop Water Stress Index

TDR – Time Domain Reflectomery

HPV – Heat Pulse Velocity

ACT – Automatic Colour Threshold

ʎ - Latent heat of vaporization

SAPPA - South African Pecan Nut Producers' Association

Dissertation outline

This dissertation is comprised of six chapters. **Chapter 1** of this thesis entails a general introduction, presenting arguments on the importance and relevancy of the study to the pecan industry. In this section, the hypotheses, aims, and objectives are outlined. **Chapter 2** reviews the existing literature related to water stress in plants, covering the general physiological responses and the effects of water stress on final yield and quality in fruit trees. This chapter also gives insight into knowledge of the effects of water stress at different phenological stages on plant performance in various fruit trees and pecans in particular. Moreover, the various methods and techniques for the assessment of plant stress, both direct and indirect, are outlined. **Chapter 3** focuses on the methodology and provides a description of the experimental site. The hypotheses and objectives formulated for the study are tested in the next two chapters. **Chapter 4** presents evidence that the trees experienced water stress at each phenological growth stage (flowering and nut set, nut sizing, nut filling and shuck dehiscence) through the assessment of the impact on plant water stress on the physiology of the tree. **Chapter 5** presents the effects of water stress at different phenological stages on the final yield and quality of pecans. Water stress sensitive and insensitive stages were identified and are presented in this chapter. **Chapter 6** summarizes the key results obtained, which contribute to a better understanding of the effects water stress at key phenological stages on yield and quality of pecans. Recommendation and future perspectives are also highlighted in this chapter.

CHAPTER 1: GENERAL INTRODUCTION

Water scarcity and drought threaten agricultural productivity, which is being exacerbated by global climate change (Wujeska et al. 2013). Drought have, however, always existed and their persistence, together with seasonal rainfall, has led to an increase in irrigated agriculture, which in turn increases agricultural water use (FAO 2017). In addition, increased pressure from urbanization and industrialization has resulted in agriculture facing increased competition for this scarce resource (Ashley and Cadilhon 2018). As a result, agriculture is having to rethink how water is used within this sector, with the aim of being more resource use efficient.

In South Africa, the gap between demand and supply of water is estimated to increase in the coming years (Figure 1.1a), which will increase water scarcity further. The average annual rainfall is approximately 450mm in South Africa, which means it is classified as a water stressed country. with irrigated agriculture currently accounting for approximately 60% of freshwater use (Figure 1.1b) (Hedden and Cilliers 2014). Continuous improvements in sustainable water management in the agriculture sector is therefore vital for food production. This is particularly important in crops, such as pecans (*Carya illinoinensis* Wangenh. K.Koch), which consume large volumes of water annually. If the pecan industry in South Africa is to grow sustainability, water needs to be used judiciously and efficiently.

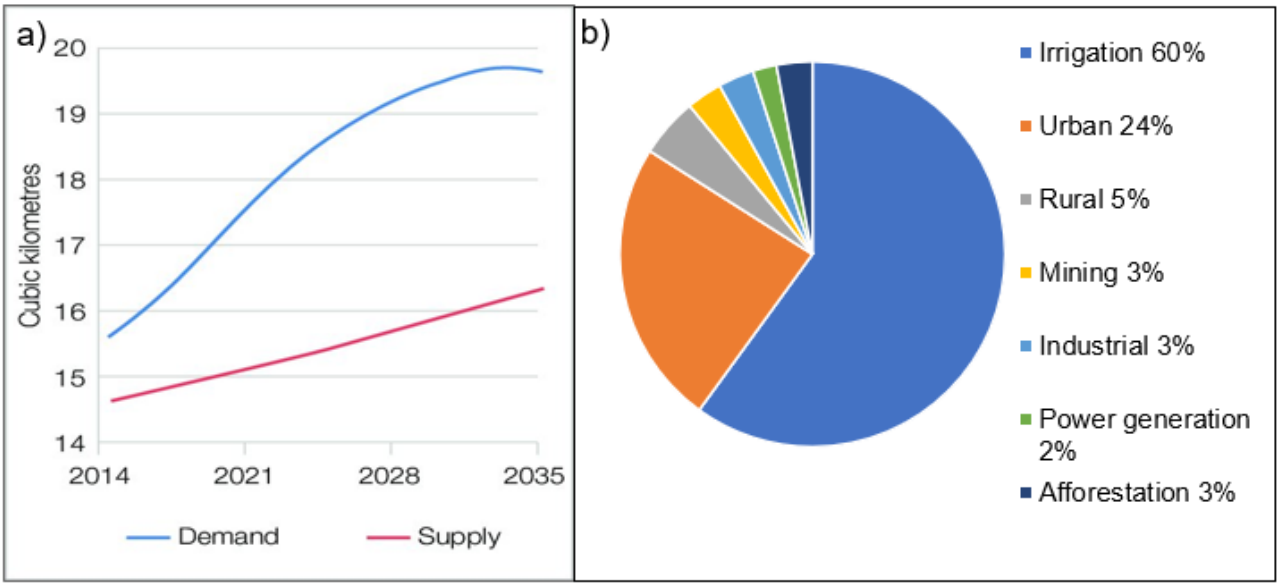


Figure 1.1 a) Gap between demand and supply of water as forecasted to 2035 and b) water use among the different sectors in South Africa (Hedden and Cilliers 2014).

Singh et al. (2018) defined pecans as “a valuable horticultural gift of North America to the world”. It is considered as the “Queen of nuts” in the USA due to its value both as a cultivated and wild nut (Singh et al. 2018). Pecans have gained enormous popularity due to their excellent nutty flavor, highly nutritive energy-giving nuts, and rich source of fat, protein, carbohydrates, and minerals. In South Africa, pecans are considered as an important cash crop, with 80% of the crop exported as nut in shell (NIS). Statistics by South African Pecan Nut Producers’ Association (SAPPA) indicates a continual expansion of the pecan industry since 2010 (Figure 1.2). Importantly, a lot of this expansion has taken place in the drier areas of the country, where irrigation is critical for optimal production. According to the information provided by SAPPA, in South Africa ‘Wichita’, ‘Choctaw’, ‘Barton’, ‘Elliot’, ‘Ukulinga’ and ‘Shoshoni’ are the most common planted cultivars. Other promising cultivars include ‘Mohawk’, ‘Cherokee’, ‘Caspiana’, ‘Nellis’ and ‘Western Schley’. Cultivars that are currently planted in the western parts are mostly ‘Wichita’ and ‘Navaho’, while ‘Choctaw’ and ‘Western’ are also planted to some extent. In the east, ‘Ukulinga’ and ‘Barton’ are planted as they show some resistance to scab, which is a problem in more humid regions. Pecan production is widespread in South Africa, with plantings in all nine provinces (Figure 1.3). However, the majority of the plantings are found in the Northern Cape.

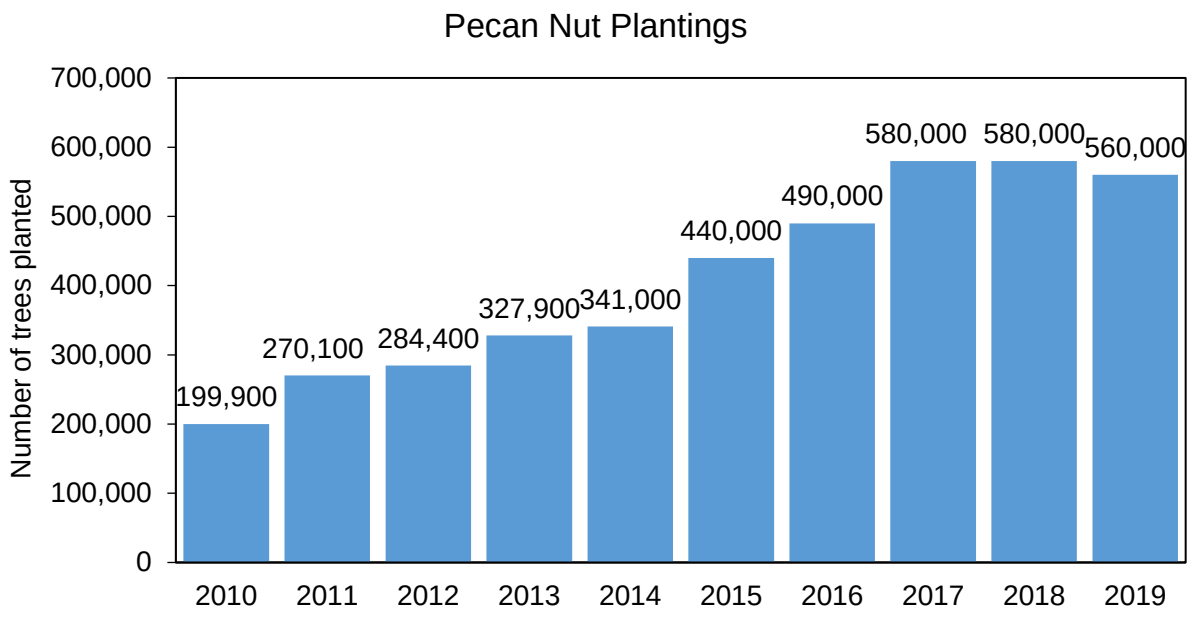


Figure 1.2 Total number of planted pecan nut trees in South Pecan as of the year 2010 to 2019 (Figures obtained from SAPPA).

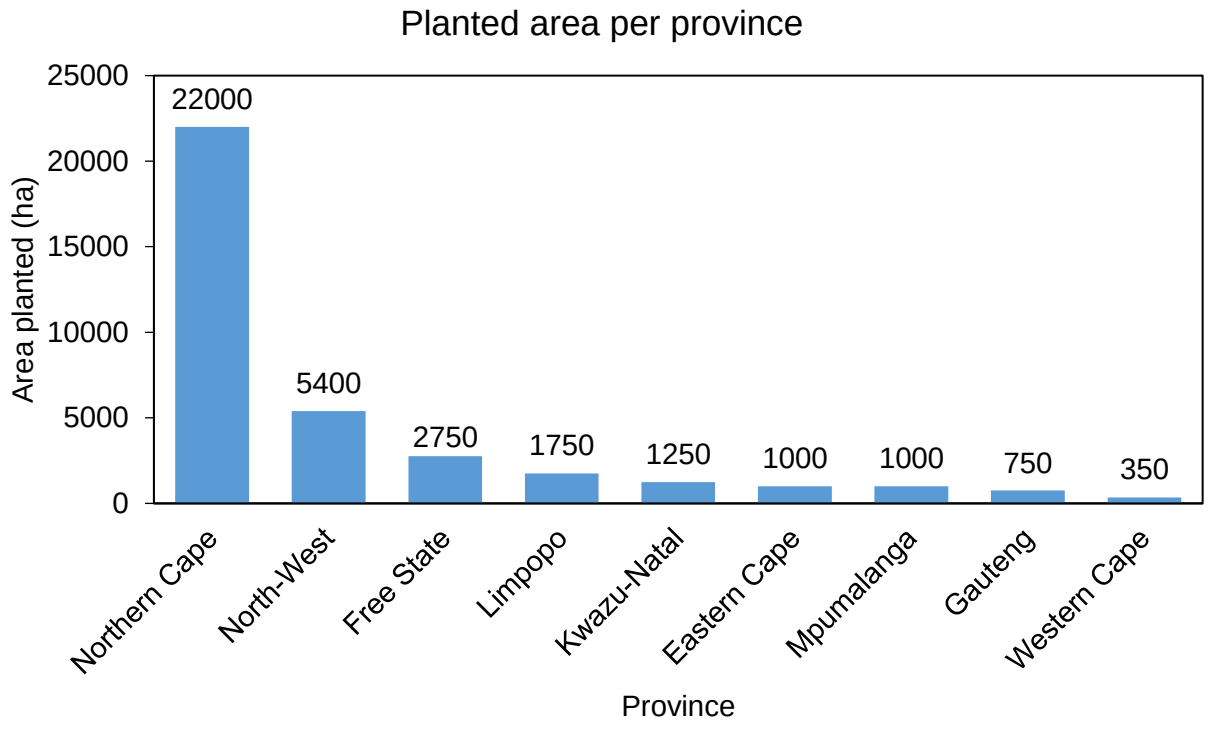


Figure 1.3 Total area planted (ha) in 2019 in each province (Figures obtained from SAPPA).

Pecans are deciduous trees and are characterized by a large canopy, resulting in high water use and high water requirements for optimal growth and production of good quality nuts, relative to other irrigated annual crops (Sammis et al. 2004). The water requirement of the tree, however, depends on the season, tree age and size, soil type and irrigation type. A study conducted in Cullinan, South Africa, revealed that mature pecans consumed 943 to 1035 mm of water per growing season (Ibraimo et al. 2016). At the peak of summer, a mature tree can use approximately 500 L of water per day (Taylor and Gush 2014). Like any other crop, pecans are sensitive to water stress, defined as water deficit for this study, which has a profound effect on the physiology and productivity of the tree, thereby affecting both yield and nut quality (Garrot Jnr et al. 1993). Numerous negative effects of water stress have been recorded in many plants, which include stomatal closure, decrease of photosynthetic activity, decline in transpiration rates and growth inhibition (Cornic and Massacci 1996, Dodd and Ryan 2001, Cifre et al. 2005, Flexas et al. 2012). Several studies on fruit trees revealed that water stress reduces fruit growth (Stein et al. 1989), resulting in small sized fruits e.g. apples (Naor 2006) and pears (Shackel 2007) and reduces kernel size and nut mass in almonds (Goldhamer et al. 2006), walnuts (Fulton et al. 2006) and hazelnuts (Bignami et al.). In pecans, early termination of fruit growth, increase in the number of pops and a decrease in the percent kernel have been recorded(Sparks 2001). Fereres et al. (2012) suggested that water stress should be avoided at all stages of growth and development for fruit tree crops. However, this suggestion may not necessarily hold in many situations. For instance, Moriana et al. (2003) reported that flowering and fruit set are the most water sensitive phenological stages, whereas pit hardening was insensitive to drought stress in Olives. In pecans, Wells (2016a) reported that reduced irrigation early in the season did not impact tree yield, or quality in the southeastern U.S, suggesting that pecan trees can tolerate moderate water stress early in the season. This therefore, can provide an opportunity for water savings with no impact on production (Johnson and Handley 2000).

Identifying water sensitive and non-sensitive phenological stages is therefore critical for managing irrigation in times when water allocations are reduced below the full evapotranspiration requirements of an orchard. It can also allow water savings during a season which can potentially be used to expand the planted area of pecans or for other crops. This study, therefore, aimed to determine the most sensitive growth stages to water stress in pecan, when yield and quality is negatively impacted. This will help farmers manage their irrigation water, by allocating sufficient water to the most sensitive phenological stages and making savings during less sensitive stages

under conditions where their annual water allocation may be reduced due to water scarce conditions. Furthermore, it aimed to determine the point at which pecan trees start experiencing water stress by assessing photosynthesis in stressed and control trees relative to both predawn and midday stem water potentials. This will help guide judicial irrigation scheduling in pecan orchards in future, which will allow increased water use efficiency in the industry.

1.1 Hypotheses

1 Water stress during flowering and fruit set will result in lower nut set and more nut drop relative to a well-watered control. As a result, there will be fewer nuts on the tree, but the nuts are likely to be bigger at harvest than those from the control trees receiving full irrigation.
2 A lack of sufficient water during nut sizing will result in small unmarketable nuts and excessive nut drop.
3 Water stress at the nut filling stage will result in poor filling, since nut filling is mainly influenced by the availability of assimilates from nearby leaves and/or the rate of carbon assimilation (photosynthesis) and may result in significant fruit drop or stick tights since the nuts fail to fill, thus reducing yield and quality.
4 Water stress after nut filling will result in delayed shuck opening thereby producing stick-tights and making harvesting difficult.
5 The photosynthetic rate of sunlit leaves will decline under water stress, mainly as a result of stomatal limitations, as a result of a decline in predawn and midday stem leaf water potentials indicative of a drying soil.

1.2 Aim

To determine how water stress at different phenological stages impacts pecan tree physiology, as well as yield and quality.

1.3 Objectives

1 To successfully implement water stress during the following phenological stages; flowering and nut set, nut sizing, nut filling and shuck dehiscence, whilst maintaining a well-watered control.

2 To assess the growth and development, as well as the final yield and quality, of the nuts in relation to the phenological stage at which the stress was implemented.

3 To measure midday stem and predawn water potentials, in order to determine whether the plants are stressed and to correlate these values with changes in photosynthesis and stomatal conductance.

4 To determine the impact of water stress at different phenological stages on canopy development at the beginning of the season, final canopy size during a season and leaf drop at the end of the season.

CHAPTER 2: LITERATURE REVIEW

2.1 Plant Stress

Plant stress can simply be defined as external conditions that adversely affect growth, development, or productivity. Larcher (1987) described plant stress as a "state in which increasing demands made upon a plant lead to an initial destabilization of functions, followed by normalization and improved resistance," and also, "If the limits of tolerance are exceeded and the adaptive capacity is overworked, the result may be permanent damage or even death." Depending on the crop and stage of development, biotic and abiotic factors can reduce average plant productivity by 65 to 87% (Gursoy et al. 2012). The effects are dependent on the duration of exposure, violence/density, age and developmental stage, with the stress resulting in either temporary or permanent damage (Gursoy et al. 2012).

Environmental or plant stress factors may be classified into two categories; namely biotic and abiotic factors (Schulze et al. 2005). Biotic stresses, are caused by biological agents (Gursoy et al. 2012), therefore they occur as a result of interactions with other organisms including viruses, bacteria, fungi, nematodes, parasites, weeds, beneficial and harmful insects and herbivores. Diseases caused by these pathogens account for major yield losses worldwide (Verma et al. 2013). Abiotic stresses on the other hand are caused by the physical environment and being sessile, plants cannot escape from the prevailing environmental stressors (Verma et al. 2013). These stresses include temperature, humidity, light intensity, carbon dioxide (CO_2) and water and nutrient availability, which all impact plant growth (Duque et al. 2013).

Under normal scenarios, water stress occurs as a result of an excess of water or water deficit. Excess water is as a result of flooding, which primarily reduces oxygen (O_2) supply to the roots (Mahajan and Tuteja 2005). Insufficient O_2 negatively affects critical root functions, resulting in limited nutrient uptake and inadequate respiration. In contrast, water deficits occur when the actual root absorption is lower than the rate of plant water loss through transpiration (Bray 1997) or when the plant water status is reduced to affect regular plant functions (Gimenez et al. 2005). Drought, high soil salinity and low soil temperature are listed as causes of water deficit (Bray 1997, Lisar et al. 2012). During periods of high soil salinity, water exists in the soil solution but cannot be absorbed by the roots due to equal or higher concentration of the solutes in the soil solution as opposed to the root (Lisar et al. 2012). Water deficit is, however, the more common

water stress condition, which is also referred to as drought stress (Mahajan and Tuteja 2005). According to Wilson et al. (2001), during a plant's life cycle, periods of soil or atmospheric water deficit often occur, even outside arid or semi-arid regions. Limited water availability (water stress) has proven to be a critical constraint to primary productivity in many crops (Chaves et al. 2002), nonetheless the impact of water stress on plant growth and yield depend on the timing of the stress, the duration of the stress and the severity of the stress (Gursoy et al. 2012).

2.2 Plants response to water stress

Physiological responses of plants to water deficits vary with the severity and duration of the stress. Furthermore, plants differ in their sensitivity to water stress. In most cases, only the most sensitive processes are altered by mild stress (Lisar et al. 2012). As water deficit increases, these changes intensify, and additional processes become affected in accordance to their relative sensitivities to the stress (Burke 2007). If the stress is prolonged, there is more time for the initial effects to lead to secondary and tertiary responses (Lange 2012). These changes over time often result in the modulation of the system in order to meet the demands of the altered environment and allow the plant to survive (Figure 2.1) (Chaves et al. 2003).

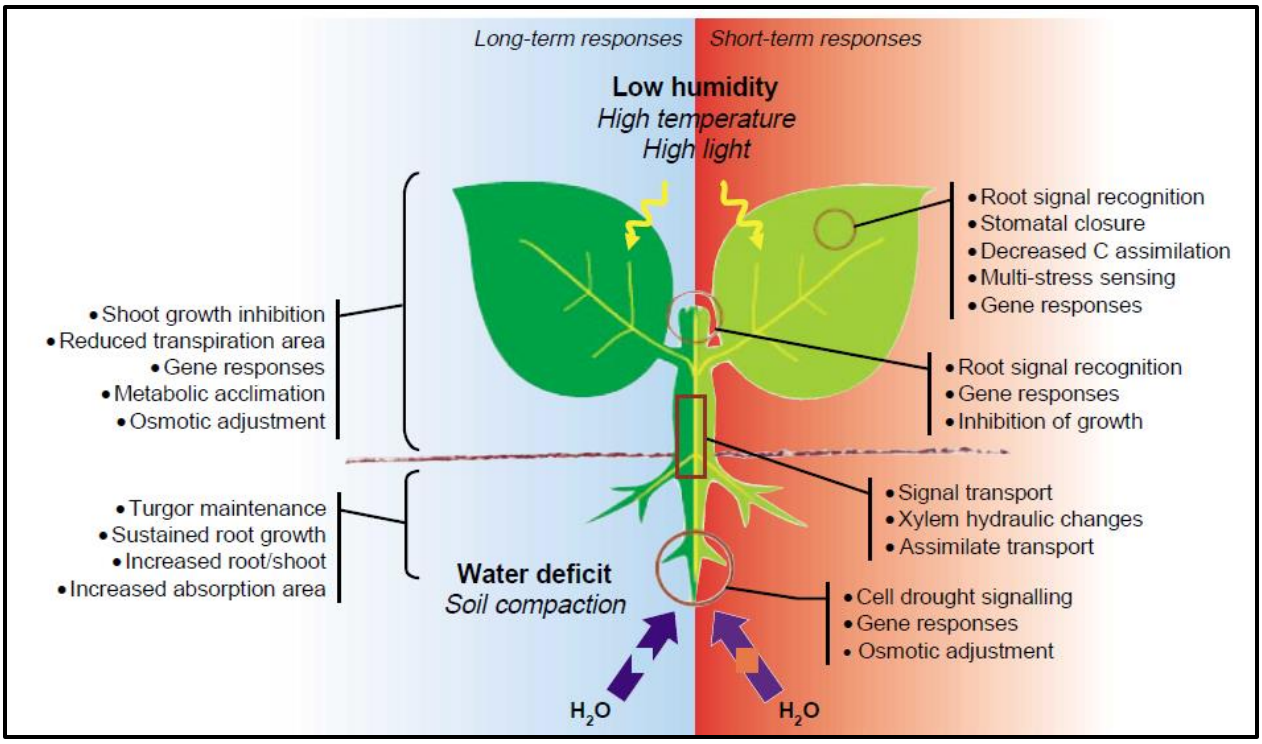


Figure 2.1 Whole-plant responses to drought stress. Left, long-term or acclimation responses; Right, short-term responses (Chaves et al. 2003).

2.2.1 Transpiration and stomata

Transpiration is a vital plant physiological process and is essential as a defense mechanism against overheating (Belozerov and Imanbayeva 2018). It creates a continuous flow of water and nutrients from the root system to the upper part of the plant or other organs. As the plant loses water to the environment, it creates pressure for the root to absorb more water from the soil (Sammis et al. 2004). There are several factors affecting transpiration which include CO_2 concentration, wind, atmospheric humidity, available soil water and internal plant water conditions, to name a few (Krishnan 2012). Stomatal closure, together with leaf growth inhibition, are among the first leaf responses to water deficits (Cifre et al. 2005) and tends to protect the plant from excess water loss, which ultimately leads to cell dehydration and death (Chaves et al. 2003, Lisar et al. 2012). Therefore, under drought conditions transpiration is reduced following stomatal closure, as the plant strives to strike a water balance (Berry et al. 2010, Pirasteh-Anosheh et al. 2016). According to Pirasteh-Anosheh et al. (2016) stomatal closure follows direct evaporation of water from the guard cells when soil-available water content declines. A decline in

transpiration under drought condition, is also associated with the reduction of total nutrient uptake (Garg 2003, Farooq et al. 2009).

As stomata close due to developing water deficits, photosynthetic rates are negatively affected thereby influencing carbon assimilation and plant growth (Pirasteh-Anosheh et al. 2016). Mutava et al. (2015) reported stomatal closure and a decline in photosynthesis in crops even under mild water deficit soils. Chaves et al. (2003) indicated that the mechanism of stomatal responses to water deficit is not easy to justify, since at any given instant, stomata may be responding to an intricate set of factors, sometimes ranging from light intensity to CO_2 concentration, in addition to leaf water status. Nevertheless, Schurr et al. (1992), Farooq et al. (2009) and (Cuevas et al. 2013) explained that early studies indicated that stomata may close in response more to a drying soil than to leaf water status, even when shoot water status was maintained at high turgor either by experimental manipulation or by growing the plant with part of the root system in drying soil and the other half in a wet soil (Gowing et al. 1990). Some fruit trees, for instance olives (Angelopoulos et al. 1996) and grapevines (Socias et al. 1997), conform to this hypothesis. This indicates that stomatal closure is possibly mediated by chemical signals, such as abscisic acid (ABA), travelling from the dehydrating roots to the shoots (Davies and Zhang 1991, Bray 1997, Chaves et al. 2003, Farooq et al. 2009). Stoll et al. (2000) concluded that partial root-zone drying in grapevines resulted in an increase in xylem ABA, which brought about a reduction in stomatal conductance. The accumulation of ABA also signals the reduction in the rate of leaf initiation, final leaf size and leaf transpiration, which ultimately inhibits shoot growth and promotes root growth instead. Another effect of drought in plants is the decrease in Plasma Membrane-ATPase (PM-ATPase) activity. Low PM-ATPase increases the cell wall pH and leads to the formation of ABA^- form of abscisic acid, which cannot penetrate the plasma membrane and is translocated toward the guard cells by the water stream in the leaf apoplasm. High ABA concentration around guard cell results in stomata closure, which helps to conserve water (Lisar et al. 2012).

2.2.2 CO_2 assimilation

A reduction in the leaf water potential and relative water content of the plant results in a decline in photosynthesis of higher plants (Lisar et al. 2012). Stomatal closure is proposed to be the major cause of limitations to photosynthesis under water deficit conditions, as a result of the decline in CO_2 uptake (Chaves 1991, Chaves et al. 2002, Lawlor and Cornic 2002, Pirasteh-Anosheh et al. 2016). Carbon dioxide diffusion restriction can also increase the possibility of disruptions to

photosynthetic pigments, which ultimately leads to reduced plant growth and productivity (Cornic and Massacci 1996, Anjum et al. 2011). Under these conditions, a decrease of photosynthetic activity, which was sometimes coupled with early leaf fall, was reported in hazelnuts (Bignami and Natali 1996, Dias et al. 2004, Bignami et al. 2010), pistachio (Ranjbarfordoei et al. 2000), almonds (Goldhamer et al. 2006) and olives (Angelopoulos et al. 1996).

Water deficits also interrupt photosynthetic components and functions of different Calvin cycle enzymes (Anjum et al. 2003), which ultimately leads to a massive decline in crop yield (Monakhova and Chernyad'ev 2002). Changes in the photosynthetic apparatus lead to excess production of reactive oxygen species (ROS), which exceeds the capacity of the antioxidant defense system, thus inducing oxidative stress in membrane lipids and proteins (Figure 2.2) (Reddy et al. 2004). Among other enzymes, Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is the main enzyme for carbon synthesis in leaves, which acts as an oxygenase during photorespiration and a carboxylase in the Calvin cycle (Lisar et al. 2012). Limited CO_2 concentration within the photosynthetic apparatus, due to stomatal closure, increases photorespiration (binds O_2 instead of CO_2, thus reducing sugars), which ensures partial substrate replenishment and maintains the carboxylation function of Rubisco, thus helping to prevent the chloroplast from oxidative damage, as it reduces the oxygen-free radical production (Lisar et al. 2012, Pirasteh-Anosheh et al. 2016).

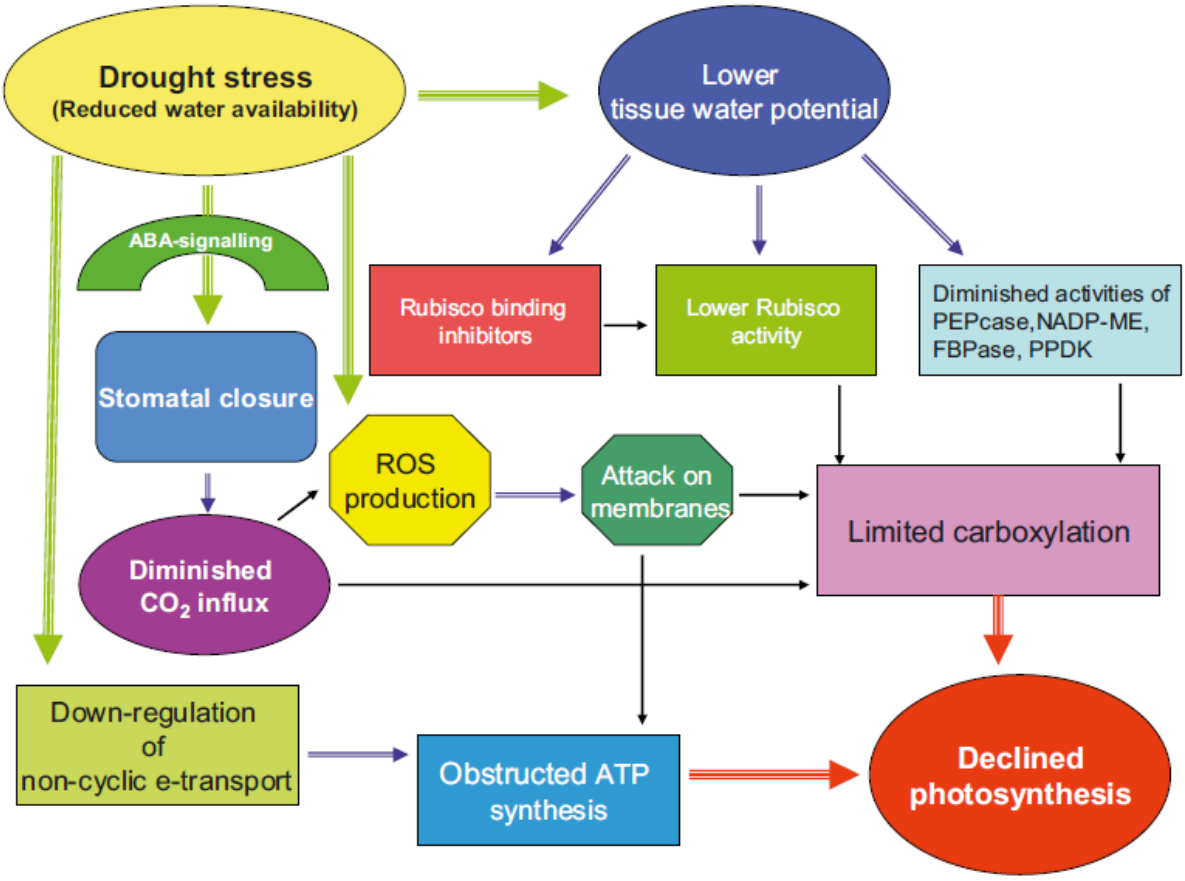


Figure 2.2 Photosynthesis under drought stress. Proposed mechanisms for why photosynthesis is reduced under stress (Farooq et al. 2009).

In severe drought conditions, the amount of Rubisco decreases rapidly within the chloroplast leading to conformational changes. Furthermore, water stress conditions acidify the chloroplast stroma thus lowering its activity (Farooq et al. 2009). Other than Rubisco, drought stress can reduce activity of other photosynthetic enzymes to different extents which include; Nicotinamide adenine dinucleotide phosphate (NADP)-dependent glyceraldehyde phosphate dehydrogenase, phosphoenolpyruvate carboxylase, Nicotinamide adenine dinucleotide (NAD)-dependent malate dehydrogenase, phosphoribulose kinase, fructose-1,6-bisphosphatase and sucrose phosphate synthase (Farooq et al. 2009).

2.2.3 Plant growth: Cell division and cell growth

According to Jaleel et al. (2009), plant growth depends on cell enlargement, cell division and differentiation, which are severely affected by water deficits. Water stress reduces the water potential and turgor of plant cells, compromising cell enlargement which leads to growth inhibition (reduced plant height, leaf area etc.) and reproductive failure (Figure 2.3) (Jaleel et al. 2009, Lisar et al. 2012). Shoot initiation, shoot extension, leaf and fruit growth, and trunk growth are all affected by water deficit. Water stress reduced vegetative growth in hazelnuts (Bignami et al.) and walnuts (Fulton et al. 2006), resulting in a decreased number of leaves, plant height and plant quality. Fereres et al. (2012) mentioned that plants have differential sensitivity to water deficit, with some trees shedding their leaves when only moderately stressed, e.g. almonds and hazelnuts (Bignami et al. 2010). However, for some species like olives (Orgaz et al. 2006), effects require severe water stress to be evident.

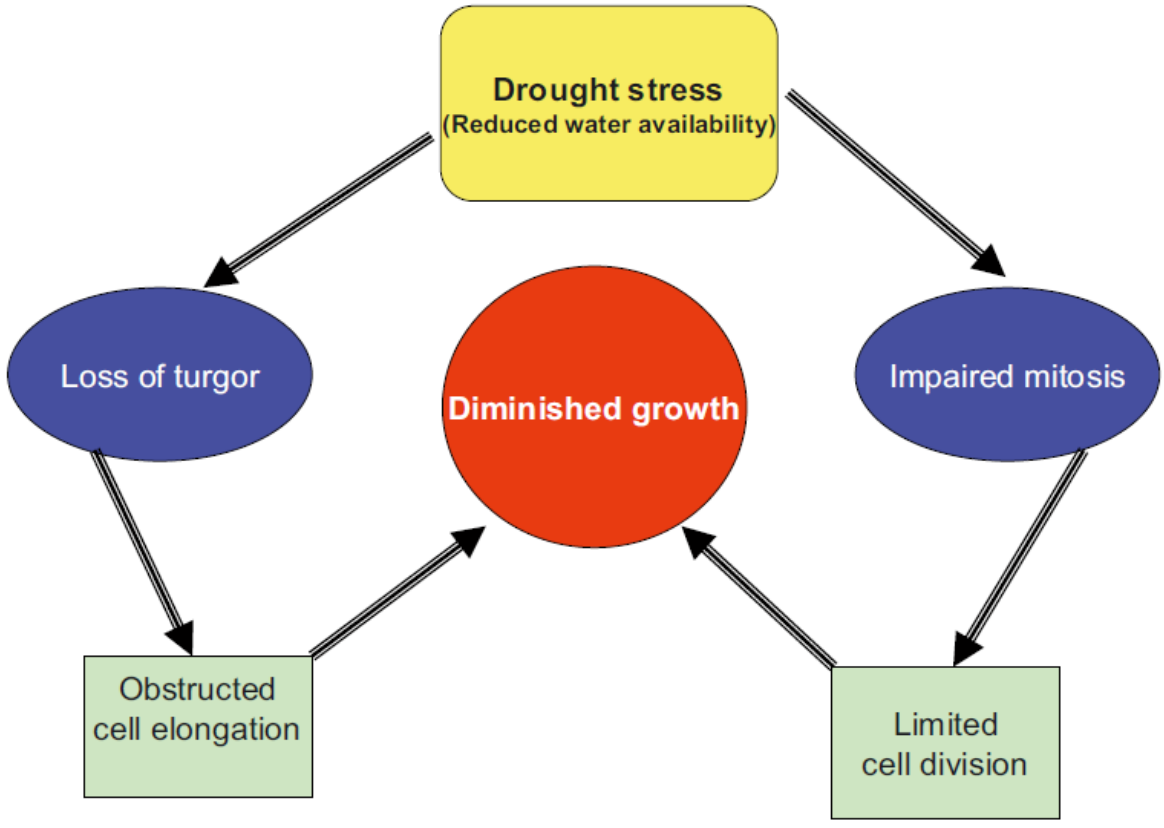


Figure 2.3 Schematic presentation of the impact of drought stress on plant growth (Jaleel et al. 2009).

2.2.4 Yield and quality

Water stress affects many yield-determining physiological processes (Farooq et al. (2009), and as a result, both yield and quality is compromised in many crops due to water deficits. Fereres et al. (2012) stated that the knowledge of the effects of water stress on yield and quality is critical to predict the orchard response to reduced water supply. Several studies on fruit trees revealed that water stress reduces fruit growth, resulting in small sized fruits e.g. apples (Naor 2006) and pears (Shackel 2007) and reduces kernel size and nut mass in almonds (Goldhamer et al. 2006), walnuts (Fulton et al. 2006) and hazelnuts (Bignami et al.). Although Bignami et al. (demonstrated an improvement in yield and quality of hazelnuts by irrigating at 75% crop evapotranspiration (ETc) (Figure 2.4), the percentage of blanks (pops) was significantly higher in water stressed trees (Bignami et al.). Importantly, the effects of water stress on yield and quality are often dependent on the developmental stage at which the stress occurs (Fereres et al. 2012). For instance in almonds (Goldhamer et al. 2006), the crop load was reduced as a result of water stress at flowering and bud differentiation and in olives (Fereres et al. 2012) fruit growth was lowered when water stress occurred during the fruit growth stage.

However, Lopez et al. (2012) stated that in several cases, the response to water deficits can be exploited to improve fruit quality. These improvements were illustrated in almonds (Tejero et al. 2018), pome fruits (Fereres et al. 2012), plum (Crisosto and Crisosto 2005), peach (Jackson 2003), pear (Crisosto et al. 2004), and wine grapes (Bravdo et al. 2003), where sugar/acid ratios or sugar content, together with other chemical compounds responsible for flavor and aroma, were increased under mild water stress. This highlights the importance of managing water as a primary tool for improving fruit quality even in water scarce areas. For instance, practices of deficit irrigation (DI) have been widely adopted in water scarce areas (Fereres and Soriano 2007) and have been implemented in various fruit trees and vines, where it was reported that its effects are not limited to improvements in fruit quality, but also increases water use efficiency in grapes (Bravdo et al. 2003). Fereres and Soriano (2007) defined deficit irrigation as “the application of water below the full ETc requirements”. This concept is often used by farmers receiving water provisions below the full ETc needs of the crop.

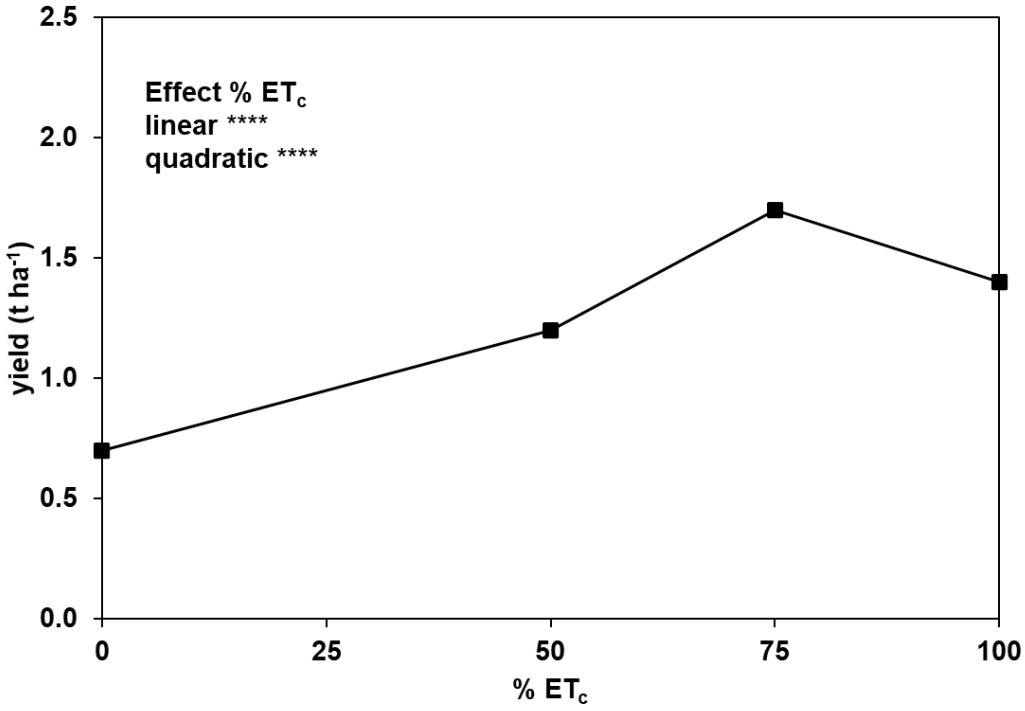


Figure 2.4 Yield as affected by volume of irrigation relative to crop evapotranspiration (ETc) adopted from Bignami et al. (2008).

2.3 How do plants cope with water stress?

2.3.1 Stress tolerance/avoidance

Plants are sessile organisms and are exposed to various unfavorable environmental conditions (Gursoy et al. 2012). For survival, species diversity allows adaptation to different environments, including dry environments (Bray 1997), with genotype determining the ability of the plant to survive and thrive under such environments (water limited). Survival under water deficit conditions may arise from either tolerance of the water deficit or from mechanisms that allow avoidance of the water deficit (Figure 2.5) (Dodd and Ryan 2001). These mechanisms involve the ability of species to maintain a high water potential by closing stomates in water-short environments, for example, pomegranate (Rodríguez et al. 2018), plums (Blanco-Cipollone et al. 2017) and pears (Arndt et al. 2000). These are typically known as isohydric plants (Sade et al. 2012, Skelton et al. 2015). Skelton et al. (2015) further explained that these drought avoiding species tend to minimize

water loss, in order to prevent cavitation from occurring in xylem streams, even though this comes at the expense of carbon assimilation during drought periods. This strategy of saving water was well documented by Dodd and Ryan (2001) in succulents plants e.g. cactus. Some plants may also have improved water use efficiency, found in crassulacean acid metabolism plants by only opening stomata at night and an alternative form of carbon assimilation promotes the use of less water (Ceusters et al. 2009). In most common cases, plants avoid drought through rapid growth and development before soil water depletes or shedding leaves, especially in deciduous species, or reducing branching, and leaf size and morphology (Basu et al. 2016, Polle et al. 2019). This is true for hazelnuts, as specific leaf area of non-watered trees was generally lower than in the well-watered treatment (Awada and Josiah 2007). Basu et al. (2016) suggested sclerophylly as another adaptative technique, where some plants produce hard leaves that will not suffer permanent injury as a result of dehydration.

In contrary, drought tolerant species tend to maintain high stomatal conductance, maintaining CO_2 uptake and risking the possible formation of embolism within the xylem stream. These are often referred to as anisohydric species (Skelton et al. 2015, Anderegg et al. 2016). Basu et al. (2016) described these species as water spenders and tend to maintain higher plant water status through increased hydraulic conductance, and a large rooting system, to maintain water absorption. This was confirmed in a study on *Ziziphus mauritiana*, where Arndt et al. (2000) found that leaf water potential did not vary during the drought period, even with the development of water deficits in the shallow layers, suggesting that this species might have access to ground water.

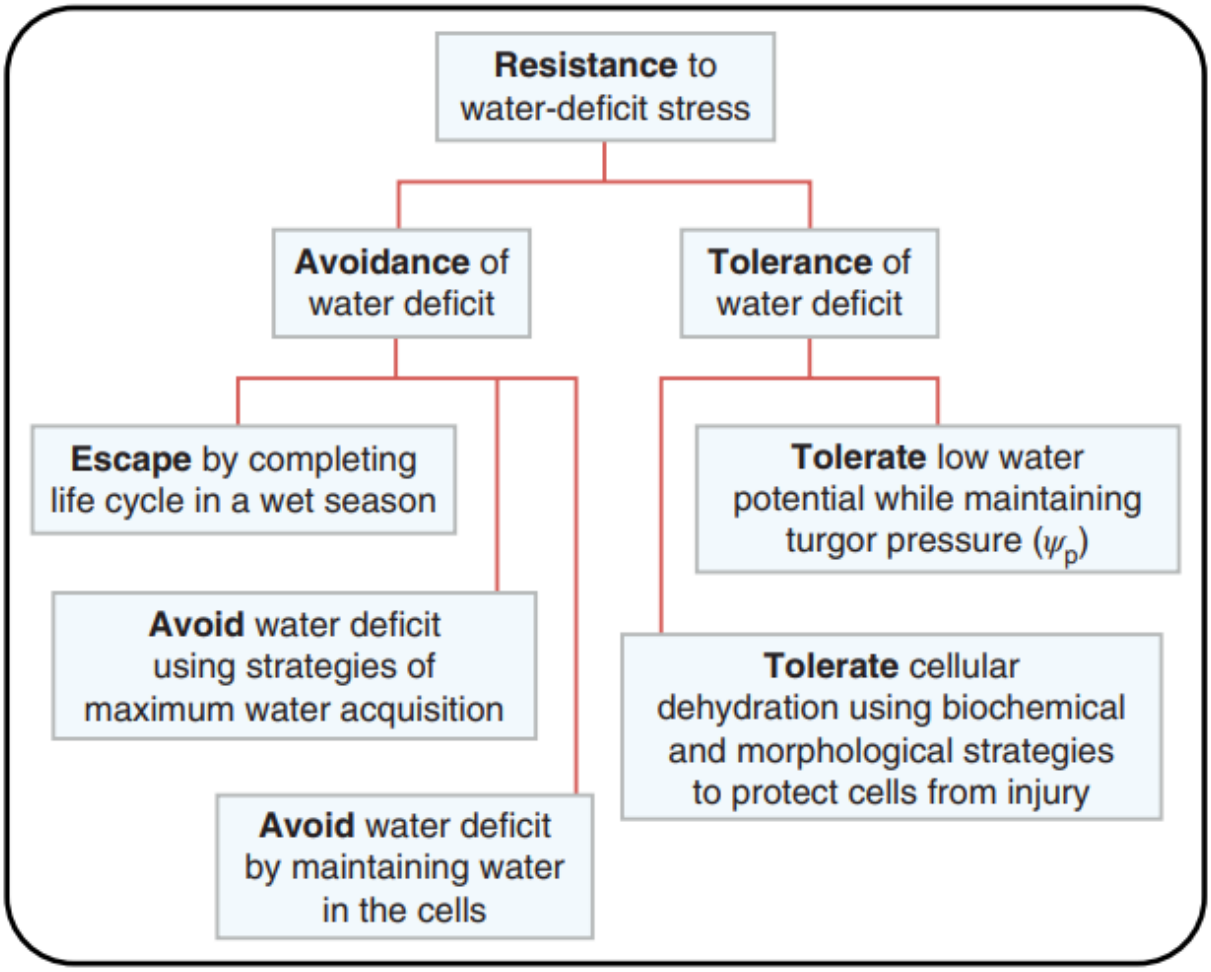


Figure 2.5 Pathways for plants as they try to escape or tolerate water deficits (Dodd and Ryan 2001).

2.3.2 Plant genotype

According to Chaves et al. (2002), plant responses to water scarcity are complex, involving adaptive changes or deleterious effects. Plant strategies to cope with drought normally involve a mixture of stress avoidance and tolerance strategies that vary with genotype. Early responses to water deficit aid immediate survival, whereas acclimation, calling on new metabolic and structural capacities mediated by altered gene expression, helps to improve plant functioning under water stress in the long term, through adjustment of the cellular environment and plant tolerance (Bohnert and Sheveleva 1998, Mahajan and Tuteja 2005). For all plants, a sensing mechanism

initiates the responses to a water deficit, which occurs at molecular, metabolic, physiological and developmental levels (Dodd and Ryan 2001) and many of these responses are driven by changes in gene expression for tolerance. During these periods, Rubisco is down-regulated in the light, due to tight-binding inhibitors and can possibly be critical for stress tolerance and recovery (Griffiths and Parry 2002). Chaves et al. (2002) further mentioned that these cellular adjustments differ with plant genotype and some of these responses occur at the leaf level in response to stimuli generated in the leaf itself or elsewhere in the plant (Figure 2.6).

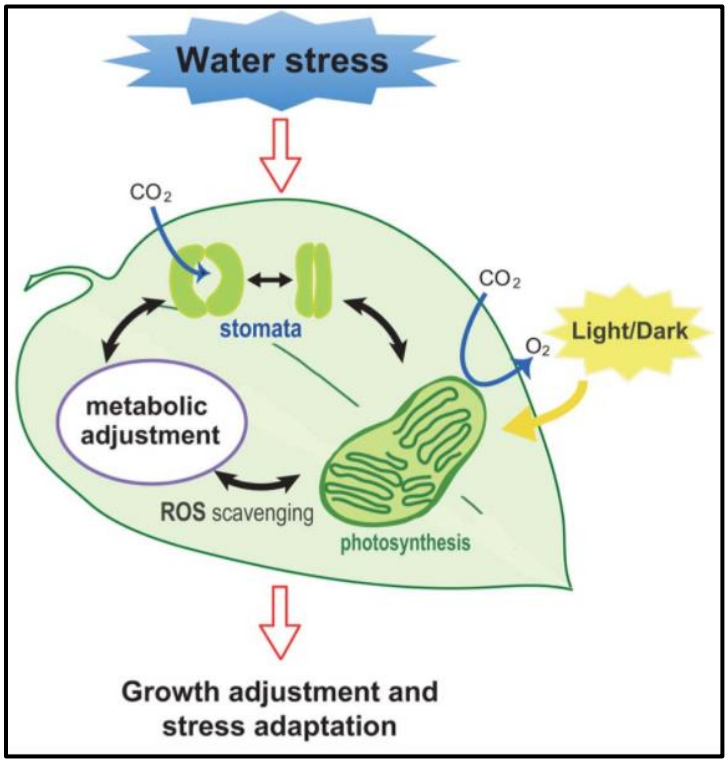


Figure 2.6 The response in plant leaves to water stress. Stomatal responses, metabolic changes, photosynthesis, and ROS scavenging all are affected when plants are subjected to water stress and the combined response leads to changes in the growth rate of plants as an adaptive response for survival (Osakabe et al. 2014).

2.4 Pecan production

Pecan nuts are produced by numerous countries in the world and the top 5 producers are shown in Figure 2.7. The USA is the leading producer (63 887 t), followed by Mexico (58 241 t) and South Africa (5 151 t) on a kernel basis (INC 2017). For over 10 years, the South African pecan industry

has shown considerable expansion (Figure 2.8), with a total production over 18 213 t nut in shell (NIS) in 2018 (statistics from the South African Pecan Nut Producers' Association (SAPPA)). It is worth noting that figures from 2006 to 2011 are not official. Over 80% of this produce is being exported, which has contributed approximately R1 300 million in more than a five-year period (NAMC and DAFF 2013).

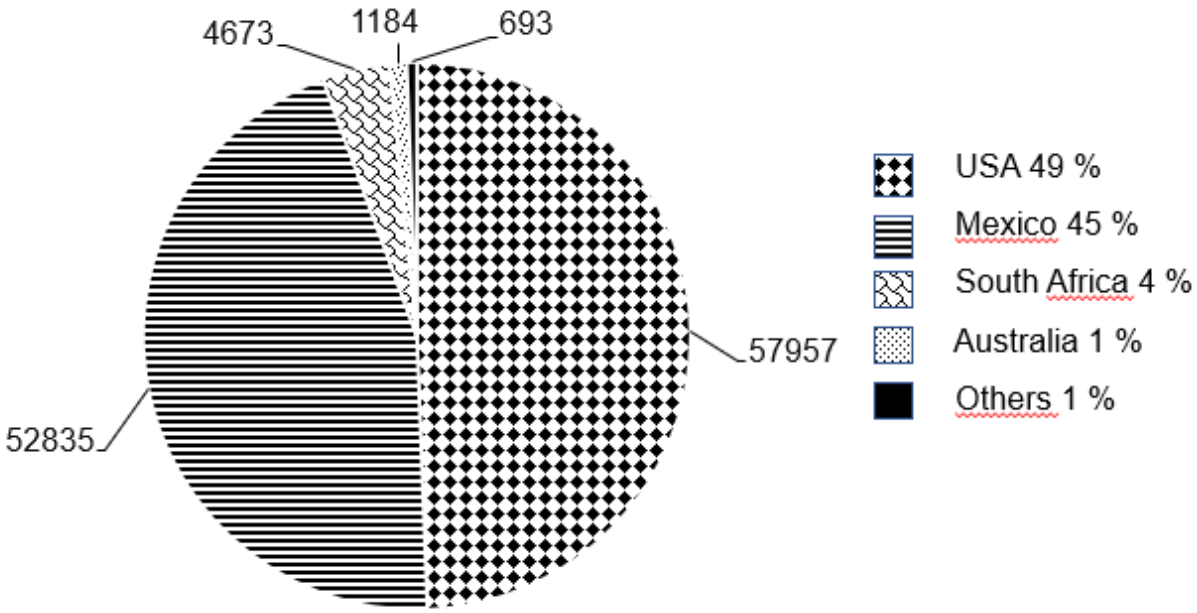


Figure 2.7 Top five pecan nut producing countries globally (INC 2017).

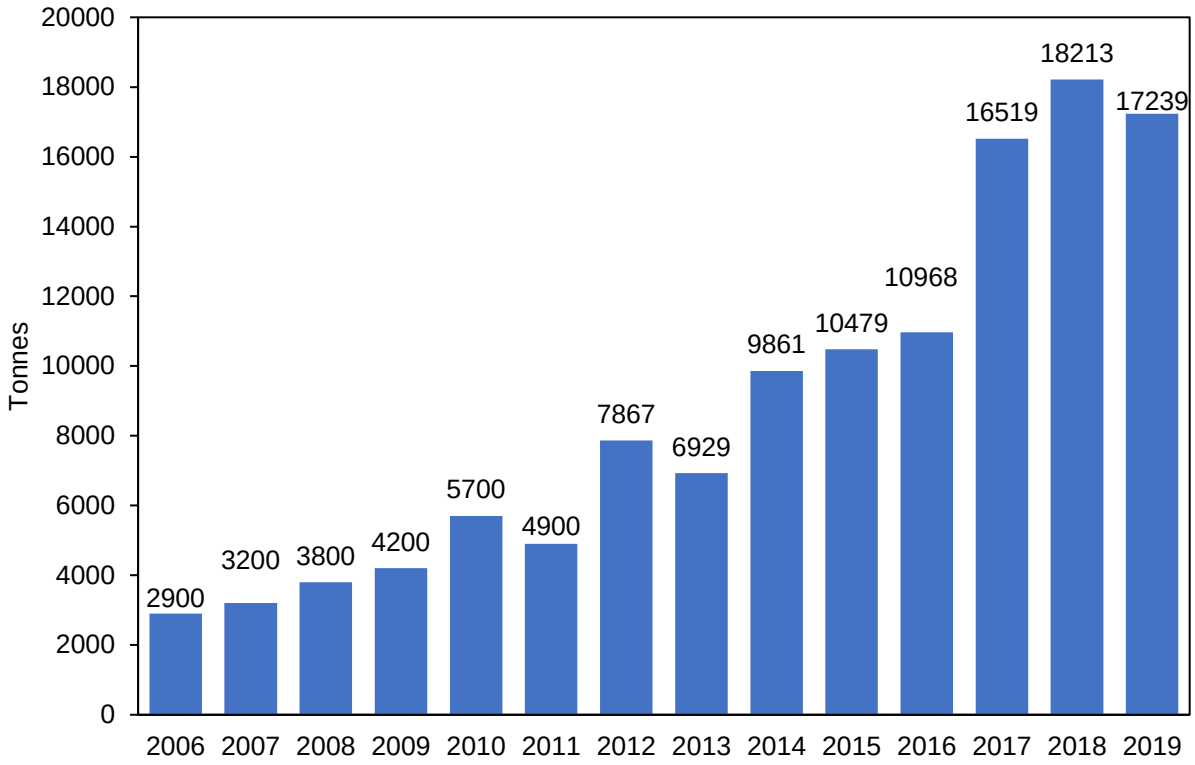


Figure 2.8 Total pecan nut production (nut in shell) figures for South Africa (www.sappa.za.org/industrystatistics/).

The pecan [*Carya illinoinensis* (Wangenh.) C. Koch] tree is a large deciduous tree native to the Mississippi River drainage system of the United States and extends to the north, south and north eastern parts of Mexico (Sparks 2005). Sparks (2005) further stated that in the indigenous pecan area, the climatic conditions range from mild to extremely harsh winters and from very humid to semi-arid conditions. The pecan tree consumes a great amount of water (Miyamoto 1983), thus Blum (1982) commented that pecan growth and production is compromised in soils with a low available water capacity, which includes shallow soils or extremely well drained sandy soils. The trees perform best on well-drained, fertile soils, with adequate oxygen and quality and quantity of water (Nesbitt et al. 2013) and a good availability of nitrogen, zinc, potassium and phosphorus (Sauls et al. 1991).

In South Africa, pecan trees were first imported to KwaZulu-Natal in the late 19th century. However, they are now commonly grown in the arid central and north-western regions of the Northern Cape Province, which have short, cold winters and long, very hot summers. This area is referred to as the “heartland of South Africa’s pecan production” which developed around the

Vaalharts Irrigation Scheme in the Northern Cape (De Villiers and Joubert 2008). Nevertheless, pecan production is recorded across the country, except the humid subtropical regions due to susceptibility to scab disease in humid regions (DAFF 2006). Although pecan nut production suites regions in the Northern Cape Province, these areas are classified as arid to semi-arid since the rainfall is poorly and unevenly distributed with extended dry periods, which can negatively impact crop productivity. Therefore, according to Ibraimo (2018), irrigation becomes vital to meet the crop water needs, especially when the atmospheric evaporative demand exceeds the available rainfall, which is most often the case for all these regions in which pecans are produced.

2.4.1 Growth and development

Vegetative growth

According to Sparks (2005), pecan juvenility may last for up to15 years or even more with sparse branching during this period. Most of the time, noticeable branches are formed during the third year of growth and are more plentiful in the fourth to seventh year of growth, with top growth increasing exponentially (Sparks 2005), as the central shoot maintains strong apical control. Pecan growth and development is dependent on climate, growing best in a mainland climate, with long hot summers and short cold winters and in moist to semi-arid areas, with sufficient water, which is most often provided by irrigation. Basically, areas with long, very hot summers and short, cold winters are ideal, with monthly average summer temperatures ranging from 23^{o}C to 28^{o}C and minimum average temperatures above 16^{o}C in summer and below 8^{o}C in winter. In South African conditions, a base temperature range of 10 to 15.5^{o}C for accumulation of 2700 to 3000 Heat units from October to April is recommended, with average monthly temperatures of <13°C from June to August for adequate chill accumulation (DAFF 2006). Low annually rainfall of <700mm and humidity (<55%) is required during the growing season, to minimize the susceptibility to scab disease, which greatly reduces crop productivity (DAFF 2006).

Reproductive growth and development

Nut production starts with the onset of flowering, followed by pollination, which is facilitated by wind, where pollen is shed from the male flowers (catkins) to pollinate female flowers (Sparks 2005). The number of female flowers determines the potential yield and is affected by the size of the previous crop, often resulting in alternate bearing (Sparks 1992, Sparks 2005). Flowers may

shed prematurely early in the season. These are typically flowers located near the shoot tip, flowers that were not pollinated or pollinated flowers in which nutlets did not develop as a result of unfavourable moisture conditions (Sparks 2005). In December there is typically a second nut drop period, which is associated with incomplete fertilization or in other words poor fruit set (Byford and Herrera 2005a). Nuts are shed because either the egg is not fertilized, or the endosperm fails to develop. Approximately 25% of the total nut crop is shed during the first and second nut drop periods and is dependent on environmental stresses and pollination. Cross pollination is key for reducing fruit drop, as fruit drop has been shown to be correlated with self-pollination. It is at this stage, the end of Stage I, that the third nut drop (late February to March) takes place. The percentage drop can be between 8-10% of large sized nuts and is thought to occur due to embryo abortion. If the embryo aborts after the shell hardens, the nut usually matures on the tree, but will be hollow (often leading to stick-tights). Although the exact causal factors for embryo abortion are unknown, the following situations seem to contribute to embryo abortion: 1) A severe drought or water stress. 2) A prolonged period of excess moisture or waterlogging. 3) Hot, dry winds which increase water loss by increasing the pecan tree moisture requirements due to high transpiration rates. 4) Insects which puncture the ovary wall and cause nuts to fall in 3 or 4 days. 5) Physical damage (e.g. hail) that results in a disturbance of the ovary wall or shell of the nut (Byford and Herrera 2005a).

According to Sparks (2001), nut growth occurs in four stages; flowering and fruit set, nut sizing, nut filling and shuck dehiscence (Figure 2.9). The timing of the stages vary between cultivars, which are either early or late maturing, but generalizations can be made (Sparks 1989, Sparks et al. 1995) and applied to South African conditions. Flowering and fruit set occur in late October to early November. During this stage, buds break, stimulating growth for the new season and flowering, followed by pollination and fruit set occurs shortly afterwards (Byford and Herrera 2005b, Wells 2016b). This is followed by the nut sizing stage, which occurs from mid-November through early February. At this stage, the nuts grow to a maximum size (Sparks 2001, Byford and Herrera 2005b). This is also referred to as the “water stage”, as the endosperm is entirely noncellular (Byford and Herrera 2005b). The third stage is the nut filling stage, which occurs from mid-February to mid-March. Byford and Herrera (2005b) declared this stage as the energy demanding, exhaustive and or draining stage, since storage materials are translocated into the nuts from leaves and shoots in close proximity to the nuts to ensure that nuts are well-filled with oil, protein, water and minerals. Shuck dehiscence marks the final stage of nut development and

occurs in late March to early May. Since this is the maturation stage, the hull splits along four sutures, exposing the nut to dry out and the moisture content of the nut drops from about 30% to 12% at harvest, or 3.5-5% when trees reach dormancy (Byford and Herrera 2005b, Wells 2015).

Sparks (2001) stated that nut growth is affected by various factors which includes pests (insects and diseases), nut volume, soil moisture, temperature, and crop load. Popular pecan nut insects include pecan weevil, aphids, ants, thrips and stink bug, along with pecan scab as a major disease affecting pecan nut growth (Sparks 2001). Temperature affects nut growth and development, with kernel development and nut volume increasing with temperature (Sparks 2001). Sparks (1995) further stated that nut growth is also affected by nutrient availability such as nitrogen, zinc, potassium, and other minor nutrients, which occur under severe deficiency. Nevertheless, soil moisture has major effects on nut growth and affects all stages of development (Sparks 2005).

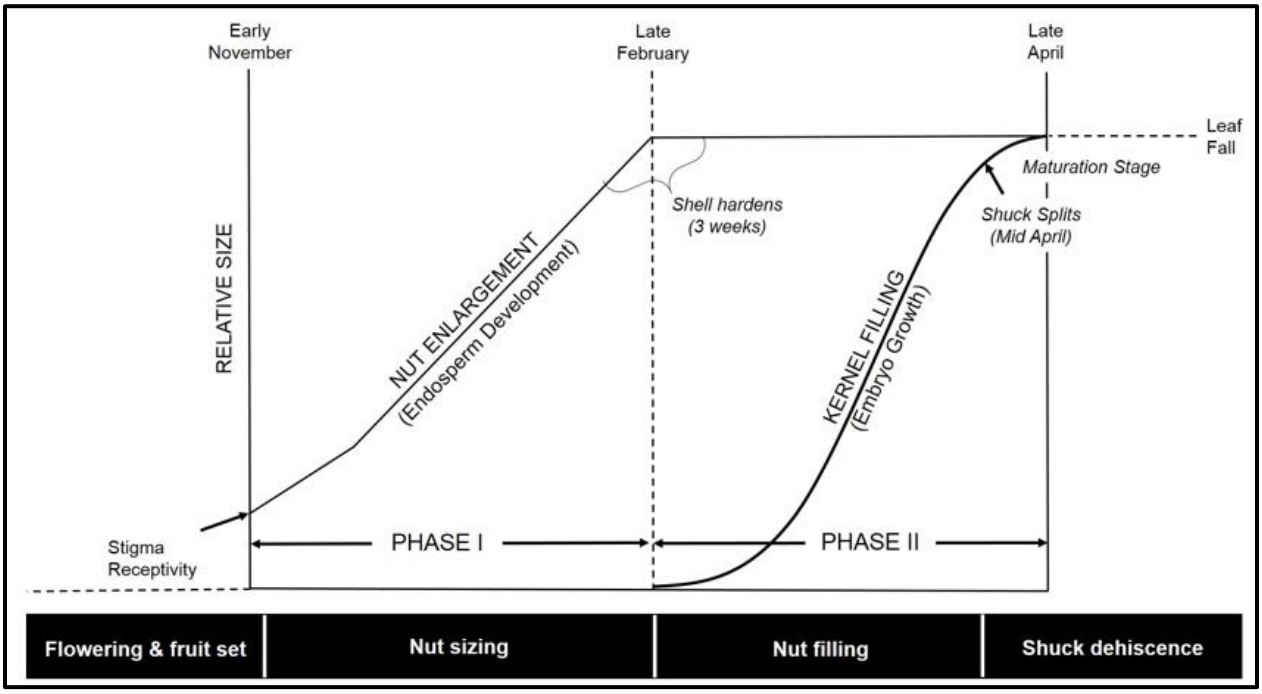


Figure 2.9 Different stages of pecan nut growth and development (Byford and Herrera 2005).

2.4.2 Water requirements

Pecan trees are great water consumers compared to other irrigated orchard trees (Sammis et al. 2004, Samani et al. 2011). According to Sparks (2005), this stems from adaptations to their natural habitat as they originated in river bottoms (Sparks 2005). The quantity of water required by a

pecan tree mainly depends on the season, the tree's age, canopy size, planting density and prevailing weather conditions (Samani et al. 2011), and hence during a hot and dry period, the tree tends to require more water than an extended wet period. Samani et al. (2011) further mentioned that pecan water requirement ranges from 368 mm to 1310 mm per season in USA for optimal growth and development. A study conducted in Cullinan, South Africa, reported that mature pecan tree ET_c was between 943 mm and 1035 mm per growing season (Ibraimo et al. 2016). In New Mexico the amount of water extracted by a pecan tree can vary from approximately 4.0 L day^{-1} for a young tree, to between 567 – 946 L day^{-1} for a mature tree (Sammis and Herrera 1999). According to Sammis and Herrera (1999), water use throughout a season differs with the tree's growth and stage of nut growth and development. Adequate soil moisture is necessary to stimulate strong, vigorous growth from budbreak through shell hardening for nut size, and during the nut filling stage for optimal kernel percentage (Wells and Harrison 2010, Wells 2016b).

Wells et al. (2007) declared two critical stages of pecan development that require adequate water. The first stage is early in the season (around October to February) when the nuts grow to full size, with adequate water at this stage resulting in large nut size and as size is king in the market this is important for profitability. The second stage is late in the season when pecan kernels develop (February to March), and it is during this stage that water demand is at its peak (Wells 2016b) and adequate water at this stage will ensure that kernels are well filled. Water needs continue to stay fairly high through the shuck split stage to promote shuck opening and water stress needs to be avoided. Wells et al. (2007) suggested that the high-water requirement for the trees continues until a week before shuck split. Although, Sammis and Herrera (1999); Call et al. (2006) suggested that the tree's water use decrease in spring (early in the season) and late summer when the nuts begin to fill, stress should still be avoided.

2.4.1.1 Pecan response to water stress: General physiological response

The first response of pecan trees to water stress is a decline in stomatal conductance and a consequent reduction in carbon assimilation (Othman et al. 2014). This indicates that the trees are isohydric species, maintaining high water potential but at a cost of carbon assimilation as a result, leaf and shoot growth is inhibited (Wells 2016b). Othman et al. (2014), established water deficit thresholds for midday stem leaf water potential in New Mexico, with values between -0.40 and -0.85 MPa indicating well-watered conditions, -0.90 to -1.45 MPa for moderately stressed

trees and -1.5 and -2.0 MPa for severely stressed trees. There was a significant decline in photosynthesis when Ψ_{smd} decreased below -0.9 MPa in New Mexico (Othman et al. 2014) and ≈-0.78 MPa in Southern Georgia (Wells 2015). This decline was mostly attributed to stomatal limitations. Furthermore, this decline in both gas exchange and photosynthesis exceeded 50% when Ψ_{smd} ranged from -1.5 to -2.0 MPa. Therefore, it is recommended that pecan orchards be maintained at Ψ_{smd} lower than -0.90 MPa to prevent significant reductions in carbon assimilation and gas exchange (Othman et al. (2014). Importantly Ψ_{smd} thresholds may be related to local weather conditions and may differ under dry and humid conditions. Additionally, the tree shed leaves, drop nuts, or only moderately fill the nuts under excessive drought stress conditions (Miyamoto 1989, Wells and Harrison 2010, Othman et al. 2014, Wells 2016b).

2.4.1.2 Impact of water stress during different key phenological stages on pecan yield and quality

Bud break, flowering, and fruit set

Miyamoto (1989) found that drought during bud break and flowering resulted in non-uniform bud break. At this stage, water stress also reduced the growth vigor, which in turn resulted in misshaped nuts after pollination. Water stress at fruit or nut set resulted in poor fertility, which ultimately led to fruit abortion (Miyamoto 1989). However, according to Wells (2015) pecan trees can tolerate mild water stress early in the season with no significant effect on yield and quality. This may be true for most deciduous species as atmospheric evaporative demand is low at this time due to cooler spring conditions and the canopy is still developing resulting in a fairly small leaf area capable of transpiration.

Nut sizing stage

The general rule for fruit trees is to avoid water stress during the fruit growth period, until fruits have reached their full size (Goldhamer 2003). However, according to Wells (2015), in pecans, the nut sizing stage, also referred to as the “water stage” (Byford and Herrera 2005b) is less sensitive to water stress. Even so, severe drought conditions can impact yield during this period. Wells (2015) further marked prolonged drought to cause excessive nut drop (reducing the final yield), production of small sized, fat and misshapen nuts (Figure 2.10), thereby reducing the yield of marketable nuts (Sparks 2001, Byford and Herrera 2005b, Wells 2016b), thus Sparks (2001)

revealed that soil moisture availability during the fruit elongation and expansion phases influences the final size and shape of pecan nuts. Since this is the water stage, water stage fruit split (causing nuts to abort) may occur upon re-watering following a period of water stress, as a result of a sudden influx of water and increased in turgor pressure within the nuts (Byford and Herrera 2005b).

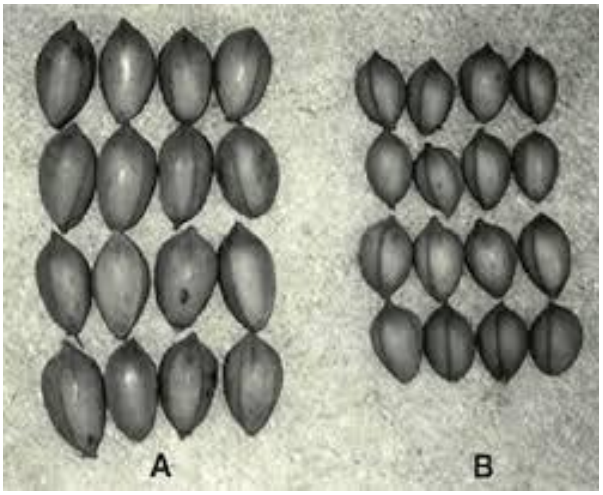


Figure 2.10 The effect of inadequate water during the nut sizing stage. (A) Pecan nuts from irrigated trees, (B) from non-irrigated trees (Wells 2016b).

Nut filling stage

Sparks (1995) reported that the impact of soil water on kernel percentage is dramatic and that kernel quality is a direct function of available soil water. Subject to the level of soil water, kernel quality may range from poor to excellent. Lack of sufficient irrigation water or rainfall during this period leads to poorly filled nuts and shriveled kernels (Sparks 2001, Valentini et al. 2015, Wells 2016b), which reduces both the final nut mass and nut quality. Sparks (1992) established that the kernel percentage was influenced by the availability of water, with a good relationship between kernel percentage and rainfall in orchards without irrigation (Figure 2.11). Results from an irrigation and kernel filling trial for ‘Stuart’ pecans, conducted by Sparks (1995) in Georgia, demonstrated a significantly higher kernel percentage (46.5 %) in irrigated orchards compared to non-irrigated orchards (40.2 %). Nevertheless, a study by Wells (2015) pointed out that at nut filling stage, soil water is not the only factor influencing nut fill, but poor nut fill can also be associated with moderate to heavy crop loads. When there are too many nuts, the tree cannot provide the photosynthates to fill all the nuts and thus a greater percentage of poorly filled nuts

are often associated with heavy crop loads. The degree of poorly filled nuts is thus a function of both water stress and crop load.

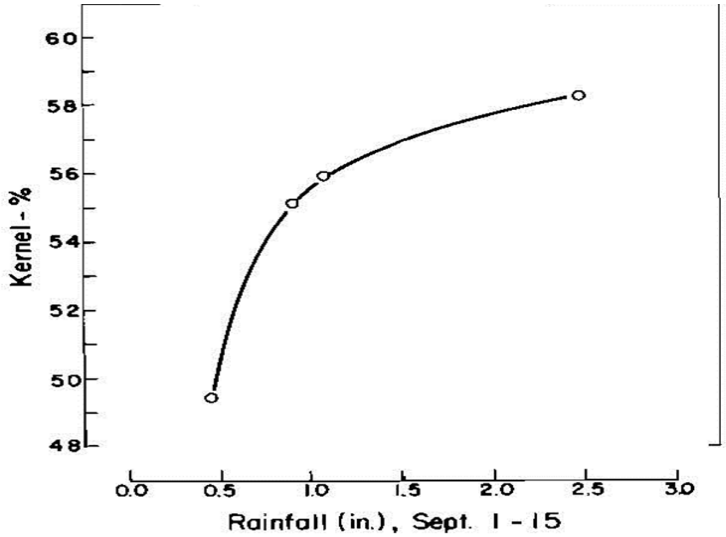


Figure 2.11 Relationship between percentage kernel in pecan and rainfall during the first 15 days in September in Georgia (Sparks 1992)**.**

Shuck dehiscence stage

Water stress during this late stage inhibits and delays shuck split, thereby increasing the percentage of stick-tights (nuts with unopened shucks) (Carroll et al. 2015) and affects the energy reserves of the tree (Sparks 1992, Byford and Herrera 2005b). Most often, stick-tights have poorly filled kernels and the abscission layers at the shuck sutures do not develop, thus the shuck does not open and sticks tightly to the nut (Heerema et al. 2010) (Figure 2.12). These nuts are hard to shake off the tree and the meat percentage is often low, usually between 10 and 30%, thereby reducing the total yield and quality (Call et al. 2006). According to Byford and Herrera (2005c), the effect of drought stress at this stage can be cultivar dependent, with ‘Wichita’ trees being more susceptible to water stress than ‘Western Schley’, causing them to have more stick-tights at the end of the season (Byford and Herrera 2005c).

Figure 2.12 Symptoms and internal quality of pecan nut stick tights with closed shuck (top left) and visible poorly filled nuts (bottom left) (http://northernpecans.blogspot.com/2016/11/stick-tights-when-pecan-kernel-doesnt.html).

2.4.3 Irrigation and scheduling

Water is a limited resource in arid and semi-arid regions due to insufficient and unreliable rains (Heyns 2009). The conditions are usually worsened by high temperatures and dry conditions resulting in low water reserves. This implies that in these regions, the success of pecan production mainly relies on irrigation and indeed requires judicious water management. Pecan irrigation needs for South African conditions according to DAFF (2006) during the production months (Sept to April) with a total of 471 mm presented in Table 2.1. Even though the scientific basis of these figures is questionable as there has not been a concerted effort to quantify water use of pecan trees and water use will differ across South Africa due to the very different climatic conditions in the different growing regions. Nonetheless, this value (471 mm) is generally lower compared to the total seasonal amount of water (1023 mm, three season average) both irrigation plus rainfall Ibraimo et al. (2016), with evapotranspiration (ET) of 1165 mm (three season average) for mature pecans in Cullinan, South Africa. These values may provide an insight of the total seasonal amount of water to be used for sustainable production. However, these values are fairly lower

than the amount used in Las Cruces, New Mexico, Sheng and Liu (2015) recorded the total seasonal amount of 1905 mm and ET of 1215 mm two season average. Commonly adopted irrigation systems in pecan orchards include flood, micro-sprinklers and drip irrigation (Van der Gulik 2006, Wells 2016b), with choice mainly depending on the site.

Table 2.1: Water application rate for mature pecans during the production months (DAFF 2006).

Month	September	October	December	January	February	March	April	Total
Amount (mm/month)	30	47	63	80	94	94	63	471

According to Wells (2016b), knowledge both pecan water requirements and irrigation scheduling are the most important management tools that can be used to ensure the sustainability of pecan production under water scarce conditions. Furthermore, adequate supply of soil water is necessary from budbreak through to shuck split (Wells 2016b). However, water stress sensitivity differs for each phenological growth and development stage, as mentioned in the previous sections. For instance, withholding irrigation early in the season and late in the season at the start of shuck dehiscence, can result in water savings with minimum impact on pecan yield (Call et al. 2006). This was proven in a study conducted in the southeastern U.S. where irrigation was reduced early in the season and provided a 38% decrease in irrigation water use with no significant effect on pecan tree water stress, yield, or quality, signifying that pecan trees can tolerate moderate early season water stress, with no effect on pecan yield or quality (Wells 2016a).

Irrigation scheduling involves applying sufficient water at the right time (Kallestad et al. 2008). The importance of this is emphasized in regions with decreasing supplies of water and forces growers to be water conscious if a profit is to be realized (Garrot et al. 1993). Timely water application especially in water scarce regions, such as the large majority of pecan growing regions in South African, is critical for successful harvest. This might involve the identification of water sensitive and insensitive stages of pecan nut growth and development and the quantity of water to be applied. Also, knowledge of water extraction by pecan trees, soil type and soil water holding capacity is important as this may assist in choosing the most appropriate irrigation system for the farm, for example, either micro-sprinklers or drippers. According to Wells (2016b), mature pecan trees extract water in the upper 80 cm (Wells 2014), and therefore, irrigation should occur until

the water reaches the bottom of the root zone at approximately 80 cm. Watering beyond this depth is considered wasteful. To ensure that irrigation stops once the required depth is reached, soil moisture sensors may be of great use. In this respect, the timing of water application may be determined by observing changes in soil water content or plant water potential (Jones 2007, Othman et al. 2014).

2.5 Determining the degree of water stress in an orchard.

Decreasing water supplies have forced farmers to be more precise with their irrigation scheduling, which typically requires a quantitative measure of soil water content or plant stress. According to Garrot et al. (1993), Marsal Vila (1997), Stephenson et al. (2003), Pérez-Priego et al. (2005), Jones and Schofield (2008), and (Krishnan 2012), water stress assessment relies on indirect methods; soil tensions, soil moisture measurements, crop growth patterns and estimated evapotranspiration; as well as direct measures of plant water stress, which includes measurements of leaf water potential, leaf transpiration rates or stomatal conductance and crop color. Another method, the crop water stress index (CWSI) for assessing crop water stress based on canopy temperature has been tested in pecans (Garrot et al. 1993).

2.5.1 Indirect methods

Indirect methods are typically soil-based methods, which rely on sensors that monitor soil water content or soil matric potential at appropriate locations and depths (Henggeler 2002). As a plant uses water, the root zone soil water reservoir is depleted and the decline in soil water content will be reflected by the sensor. These sensors are used for irrigation scheduling purposes and indicates when the remaining soil water level reaches a critically low value and irrigation must to be applied (Van der Gulik 2006). These sensors, to a lesser extent, can also indicate when irrigation has restored the water to the desired level and the irrigation system is shut off (Henggeler 2002). Jones (2004b) further stated that these sensors are easy to apply and use. Nevertheless, many sensors may be required due to soil heterogeneity and selecting the representative location of the root-zone is tricky (Jones 2004b, Krishnan 2012). There are various soil water sensors. Examples include tensiometers, chameleon sensors, capacitance probes,

gravimetric measurements, electrical resistance blocks, time domain reflectometry (TDR) and the neutron probe.

2.5.2 Direct methods

These methods involve the direct quantification of plant water stress (Garrot et al. 1993, Jones 2004b, 2007, Krishnan 2012). According to Naor (2006), several water-saving irrigation strategies tend to rely on approaches based on sensing the response of the plant to water deficits. Jones (2004) highlighted that the detection of the plant water status is the ideal physiological indicator, because of its dynamic nature and their direct relation with weather and soil conditions, and with crop productivity as well. According to Van der Gulik (2006), there are several plant-based indicators that can be used to determine plant stress. These include direct measurements of some aspect of plant water status and the measurement of a number of plant processes that are known to respond sensitively to water deficits (Jones 2004). Plant-based measurements include stem and leaf water potentials, stomatal conductance (g_s) and transpiration (sap flow) (Peretz et al. 1984, Jones 2004b, Krishnan 2012).

2.5.2.1 Plant water status

According to Jones (2004), the first approach to detect water deficits is the use of the plant itself, such as visible wilting and is still widely adopted to date (Engelbrecht et al. 2007). However, Slatyer (1969), Jones (2004) argued that a significant amount of potential yield might have been lost by the time wilting is visible. This therefore implies that more sensitive measures of plant water status are required. Jones (1990b), Krishnan (2012) presented leaf water potential to be the most applicable determination of developing plant water deficits and can also be used to formulate good decisions for proficient use of irrigation water. However, Jones (2004) argued that a straight leaf water potential is not a suitable measure for determining plant water status, especially in isohydric species since plant water status, and importantly leaf water status, to some extent, is mostly controlled by stomatal closure (Peretz et al. 1984). Jones (1990b) also mentioned rapid temporal variations are often detected as a function of changing environmental conditions for example, passing clouds, which marks leaf water potential not an appropriate indicator of developing plant water deficits or irrigation need.

However, despite the outlined concerns with the use of leaf water potential, Peretz et al. (1984) reported that leaf water potential can provide a sensitive indicator for plant stress and guide for irrigation when corrected for daily and environmental variation. The proposed measure of plant water status involves stem water potential (Ψ_{stem}) or midday (Ψ_{stem}), which is also referred to as xylem water potential (Jones 2004). Stem water potential, defined as "a reading of what is going on within the xylem of the plant", is one of the best indicators of plant water stress, since it measures the integrated effect of the plant, atmospheric conditions and soil water availability on the plant water status (Peretz et al. 1984). It has been used in a wide range of studies to assess plant water stress (Garnier and Berger 1985, Ramos et al. 1993, Berman and DeJong 1996, Naor 1999, Jones et al. 2002). McCutchan and Shackel (1992) presented that stem water potential is measured using a Scholander pressure chamber on leaves enclosed in a plastic bag covered with aluminum foil for some time (about an hour) before measurement while still attached to allow leaf water status to equilibrate with the xylem water potential. This therefore eliminates the short-term environmental variations to represent true water status in both isohydric and anisohydric species (McCutchan and Shackel 1992). However, Jones (2004) presented predawn leaf water potential (Ψ_{pd}) to better estimate the soil water status as (Ψ_{leaf}) is considered to be in equilibrium with soil water potential (Ψ_{soil}) by dawn since it is done at no plant water flux (Chone et al. 2001). Nonetheless, this measure is carried out early in the morning therefore, it is labour intensive and expensive, requires replication, and can only be done once a day (Jones 2004). Furthermore, none of these plant-based measures are automated.

2.5.2.2 Stomatal conductance and thermal sensing

Stomatal closure remains a useful representative measure of water stress in plants, as it is often a reflection of a change in soil water status (Jones 2007). Casson and Gray (2008), Roelfsema and Hedrich (2005) added light, CO_2, and vapor pressure deficit (VPD) as other factors influencing stomatal conductance. However, a decline in stomatal conductance (gs) is a largely attributed to increasing water deficits in plants, as they try to strike the balance the photosynthetic performance of the plant with the available water (Chaerle et al. 2005) and it has been widely adopted (Jones 2004, Naor 2006). This measure, however, is not automated and it is not suitable for commercial use (Jones 2004). Various instruments are available for the measurement of stomatal conductance, which include diffusion porometers and photosynthesis systems (Jones 2004). However, for the success of these methods, determination of leaf water potential threshold values

at which stomatal closure occurs is crucial (Gimenez et al. 2005) and these threshold values vary with species, leaf age, previous exposure to radiation, the stress history of the plant, and environmental conditions (Gimenez et al. 2005).

Since measurements of stomatal conductance are labour intensive and not automated, it has led researchers to develop thermal sensing methods which are based on infrared thermometers to detect plant stress (Jones and Leinonen 2003, Jones 2004, Jones and Schofield 2008). This method however, is an indirect measure of plant stress (Jones 2004b). According to Jones (2004b), this was after the recognition by Raschke (1960) that leaf temperature tends to rise as a result of stomatal closure in water stressed plants, as the main determining factor of leaf temperature is the rate of transpiration or evaporation from the leaf (Jones and Schofield 2008). Jones and Schofield (2008) further highlighted that this method mostly applies in isohydric plants, where stomatal closure is an early response to developing water deficits. Jones and Schofield (2008) explain the concept: “The cooling effect of transpiration arises because a substantial amount of energy (the latent heat of vaporization, λ) is required to convert liquid water to water vapour, and this energy is then taken away from the leaf in the evaporating water and therefore cools it”. To a lesser extent, Jones and Schofield (2008) mentioned that some other plant physiological processes may affect leaf temperature, such as high respiratory rates found in the Arum spadix (Seymour 1999). Nonetheless, leaf temperature is not influenced by respiration since the heat generated is said to be too small to be detectable (Jones and Schofield 2008).

Since a change in leaf temperature is largely attributed to water stress, this allows a detection of water stress by thermal sensors or using high-resolution thermal imagery which can also be used to generate a Crop Water Stress Index (CWSI), which allows the mapping of spatial variability of water stress and detecting potential problems with the irrigation system (Berni et al. 2009). This has successfully been implemented in peach orchards and olives trees to track water stress (Sepulcre-Cantó et al. 2006, Sepulcre-Cantó et al. 2007, Berni et al. 2009).

However, Jones and Schofield (2008) indicated that it is difficult to estimate stomatal conductance or transpiration from thermal sensing techniques, as leaf temperature (TI) at any time depends on wind speed (u), air temperature (Ta), air humidity (e) and absorbed net radiation (Rn) as presented by equation 2.1 (Guilioni et al. 2008).

$$T_I - T_a = [r_{HR}(r_{aW} + r_s)\gamma R_{ni} - \rho c_P r_{HR} D]/[\rho c_P[\gamma(r_{aW} + r_s) + s r_{HR}]] \quad \text{Equation 2.1}$$

Where, r_s is the leaf resistance to water vapour (equal to the reciprocal of the stomatal conductance) and assumed to be dominated by the stomatal resistance component (s m^{-1}), r_{aW} the boundary layer resistance to water vapour (s m^{-1}), R_{ni} the net isothermal radiation (the net radiation that would be absorbed by a leaf if it were at air temperature, W m^{-2},), ρ the density of air (kg m^{-3}), c_p the specific heat capacity of air (J kg^{-1} K^{-1}), s the slope of the curve relating saturating water vapour pressure to temperature (Pa ˚C^{-1}), γ the psychrometric constant (Pa K^{-1}) and r_{HR} the parallel resistance to heat and radiative transfer (s m^{-1}) and D the air vapour pressure deficit (Pa). It is possible to measure all the variables in order to determine conductance, however, it is much easier to make use of reference model leaves, which includes the use of 'dry' and 'wet' reference surfaces. This allows the comparison of leaves that are wet and transpiring at a maximum rate with those that are dry and are not transpiring (Jones 1990b). In this scenario, stomatal resistance can be estimated from equation 2.2 and where the first term depends primarily on wind speed (Jones and Schofield 2008).

$$r_s = (r_{aW} + \left(\frac{s}{\gamma}\right) r_{HR} \cdot (T_I - T_{wet})/(T_{dry} - T_I) \quad \text{Equation 2.2}$$

The common measure for the detection of water stress involves the definition of (CWSI) according to Idso et al. (1981) as

$$CWSI = (T_{canopy} - T_{nws})/(T_{max} - T_{nws}) \quad \text{Equation 2.3}$$

where T_{max} is the temperature of a dry surface and T_{nws} is the empirical "non-water-stressed baseline" temperature (that of a well-watered crop transpiring at the potential rate in the same environment). However, as Jones and Schofield (2008) suggest the CWSI is more an indication of stomatal opening than of the water stress itself. However, Jones (2004) presented this method to be less useful in cloudy or more humid climates as it was developed in Arizona (clear arid climate).

2.5.2.3 Transpiration (Sap flow)

According to Jones (2004b), the development of energy balance and heat pulse thermal sensors for measurements of sap-flow in the stems of plants (Green et al. 2003, Allen et al. 2011) has opened up an alternative approach to assess plant water stress based on the measurement of sap-flow rates. This is because sap-flow rates are anticipated to be sensitive to water deficits and particularly to stomatal closure. The use of sap-flow measurements for detecting increasing water deficits and to schedule irrigation has been practiced in various crops, including fruit trees e.g. peaches, (Remorini and Massai 2003), apples and pears (Fernández et al. 2008) olive trees (Giorio and Giorio 2003) and grapevine (Ginestar et al. 1998, Fernández et al. 2008). The heat pulse velocity (HPV) method measures sap flow rates by determining the velocity of a short pulse of heat carried by the moving sap stream (Smith and Allen 1996). This method is only appropriate for use on woody stems and orchards since it requires woody stems larger 40mm diameter (Allen et al. 2011). Green et al. (2003) classified HPV systems as cheap, easy to install and suited to automated data collection. However, for the success of this technique for irrigation scheduling, sap flow ‘control thresholds’ need to be derived through calibration for appropriate applications, particularly for larger trees. Irrigation decisions are based on the analysis of the diurnal patterns of sap flow, with midday reductions being indicative of developing water deficits. However, Wronski et al. (1985) highlighted problems with sap flow, firstly, diurnal fluctuations due to environmental conditions can mimic the diurnal patterns of sap flow due to water stress and also very humid conditions mimic typical water stress patterns for diurnal sap flow, thus presenting problems with specific reference to accurate irrigation scheduling . Secondly, sap flow tends to lag-behind changes in transpiration rate owing to the hydraulic capacitance of the stem and other tissues of the plant.

2.6 Conclusions

Water scarcity is a concern in agriculture, and this is particularly so in the pecan sector in South Africa. The high-water demand by these trees requires careful water management strategies to ensure profitability of these orchards. The constant expansion of the industry combined with a decline in this limited resource and increased competition requires detailed knowledge to ensure that water is managed judiciously. Knowledge gaps include the impact of water stress at each phenological stage on growth, physiology and the final yield and quality. This information is required in order to identify phenological stages that are either sensitive or insensitive to water stress.

This knowledge will assist in developing accurate irrigation scheduling frameworks, without compromising the growth, yield and quality of the trees and nuts. This framework will be based on estimating the threshold at which the trees will be stressed and evaluating the effects of water stress on yield and quality. Various measurements for the determination of plant water stress responses are available and include plant gas exchange (CO_2 and H_2O), stomatal conductance, photosynthesis, leaf water potential and soil water content. However, according to Jones (2007) a single measure will not necessarily determine if the plant is stressed or not and therefore a combination of measurements is required. Most of the information currently available is from the USA in the Northern Hemisphere, which in some cases differs quite substantially from South African conditions. As a result, it is necessary to develop guidelines for sensitivity of different pecan phenological stages to water stress under local conditions.

CHAPTER 3: MATERIALS AND METHODS

3.1 Experimental site description

The experiment was conducted at the University of Pretoria's Experimental Farm (Innovation Africa) South Africa (25°4'55.85" S, 28°15'3.88'' E, 1372 altitude) from April 2018 to May 2020. The Experimental Farm is located in the country's summer rainfall region, characterized by high intensity and short duration rainfall events, with sunny periods in between rains. Weather data (rainfall, relative humidity, solar radiation, vapor pressure deficit, and air temperature) was collected by an automatic weather station situated 230 m from the pecan orchard. The pecan research orchard was 3 ha in size (Figure 3.1) with mixed varieties on 'Ukulinga' rootstocks, however, the focus in this study was on the cultivar 'Wichita' for measurements. The majority of the orchard was planted with alternate rows of 'Wichita' and 'Western Schley' trees. The trees were planted in a north–south orientation at two planting densities i.e., 10 x 10 m and 5 x 10 m. For the experimental purposes, the 10 x 10m planting density (marked in Figure 3.1) was used since it is the recommended planting density for commercial production (DAFF 2006). The soil type in the orchard was a clay loam (45% sand, 36% clay and 19% silt). The orchard was irrigated using pressure compensated drippers. Emitters were spaced 0.6 m apart and each emitter delivered 1.6 $L.h^{-1}$. Three dripper lines were laid parallel to each other, with the middle dripper under the trees and the other two on either side of the trees, 1 m away from the tree trunk. Each tree was fertilized with LAN, superphosphate, potassium chloride, and zinc at the beginning of each season. The trees were pruned before the start of each season to a modified central leader.

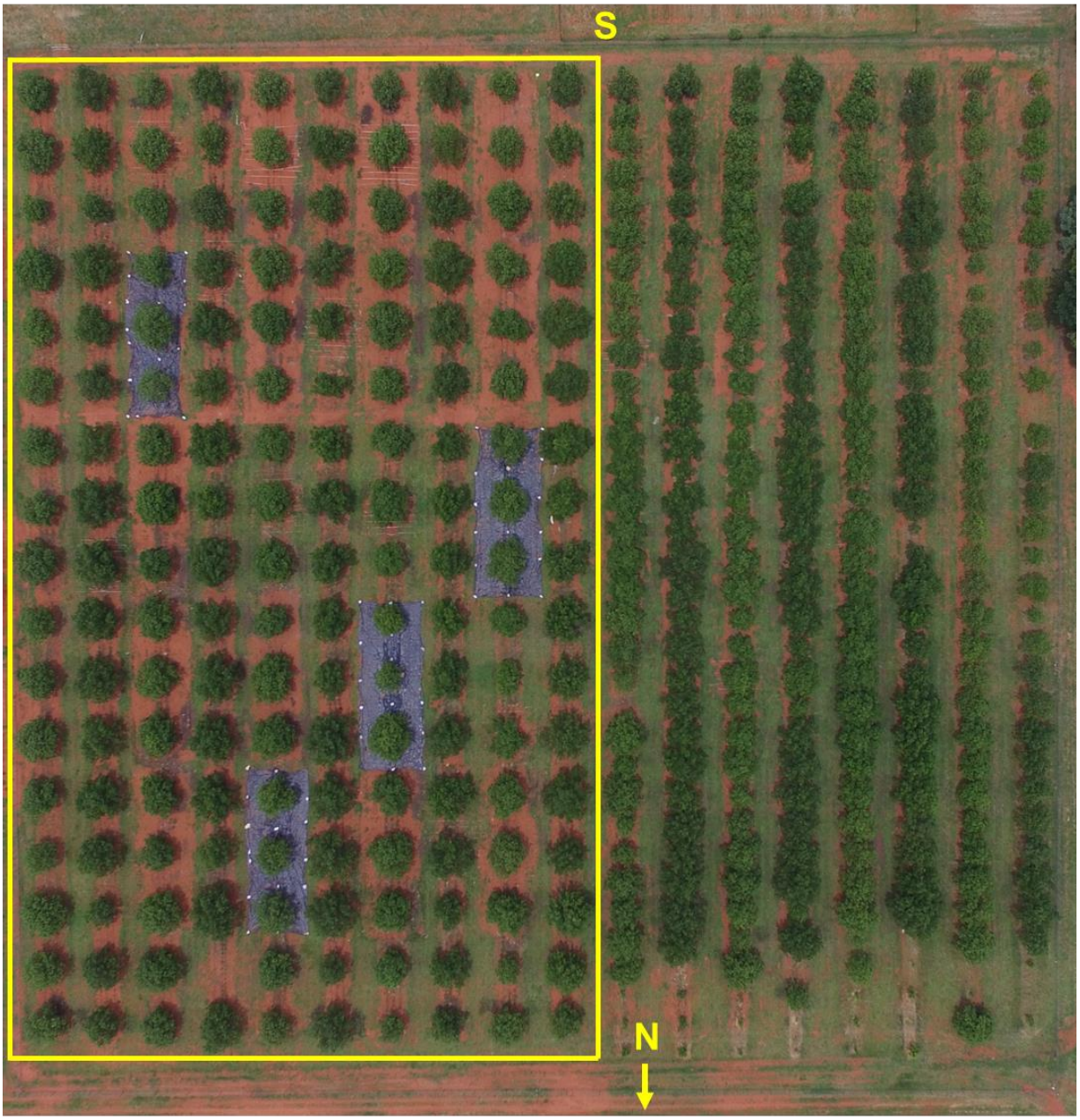


Figure 3.1 Overview of the pecan research orchard on the University of Pretoria's Experimental Farm (Captured 11 November, 2019)

3.2 Water stress treatments

The experiment was laid in a random complete block design (RCBD), with each treatment (of three trees) replicated four times (Figure 3.2). Data was collected from the middle tree of all treatments (Figure 3.2). There were five treatments; 1) the well-watered control, 2) water stress imposed at flowering and nut set, 3) water stress imposed during nut sizing, 4) water stress imposed during nut filling and 5) water stress imposed during shuck dehiscence (maturity). Stress was implemented by withdrawing irrigation for a specific treatment and covering the soil allocated to the trees with black plastic to eliminate rainfall (Figure 3.3). There was a temperature probe attached to the Chameleon sensor at 50 c m below the soil surface and the data was used to assess temperatures under the black plastic which was compared to the control treatment. The temperature for the control ranged between 18 and 21 °C, which was slightly lower than the stressed treatments (covered by the black plastic), which ranged between 20 and 25 °C. This indicates that the black plastic mulch could have impacted soil temperature, but the probe was a bit too deep to determine the impact on shallow roots. Woodroof and Woodroof (1934) reported optimal root growth temperatures to range between 15 – 30 °C measured at 30 cm. The probe was a bit too deep to determine the impact of temperature on roots within the top 10 – 15 cm, therefore, the recovery of predawn and midday water potentials following the removal of the plastic was assessed and, in most cases, they were at levels similar to the control within a week, which suggests it was unlikely that the high temperatures caused by the plastic would have killed feeder roots. The stress levels were kept within moderate stress limits, as are outlined by Othman et al. (2014) where a midday stem water potential (Ψ_{stem}) between –0.40 and –0.85 MPa indicated well-watered trees, moderately stressed values lay between –0.90 to –1.45 MPa and severely stressed between –1.5 and –2.0 MPa. The control was irrigated optimally and was scheduled according to Chameleon soil moisture sensors (https://via.farm) where a colour change from green to red indicated a developing soil water deficit, with predawn and midday leaf water potentials kept within –0.40 and –0.85 MPa, respectively. During stress periods, midday stem water potential was maintained between –0.90 to –1.45 MPa for moderate stress, thereby avoiding severe stress. Predawn stem water potentials were also used as an indication of stress, with the aim of determining threshold values during the study. When Ψ_{stem} dropped below the acceptable limit, the trees were irrigated to slightly refill the profile and alleviate some of the stress.

All measurements were taken in comparison to the well-watered control at all phenological stages. After the completion of each phenological stage, stressed trees were irrigated back to field

capacity, which differed slightly from the irrigation for the well-watered control. The period of stress recovery was monitored through the assessment of pre-dawn leaf water potentials and midday stem water potentials and/or stomatal conductance measurements.

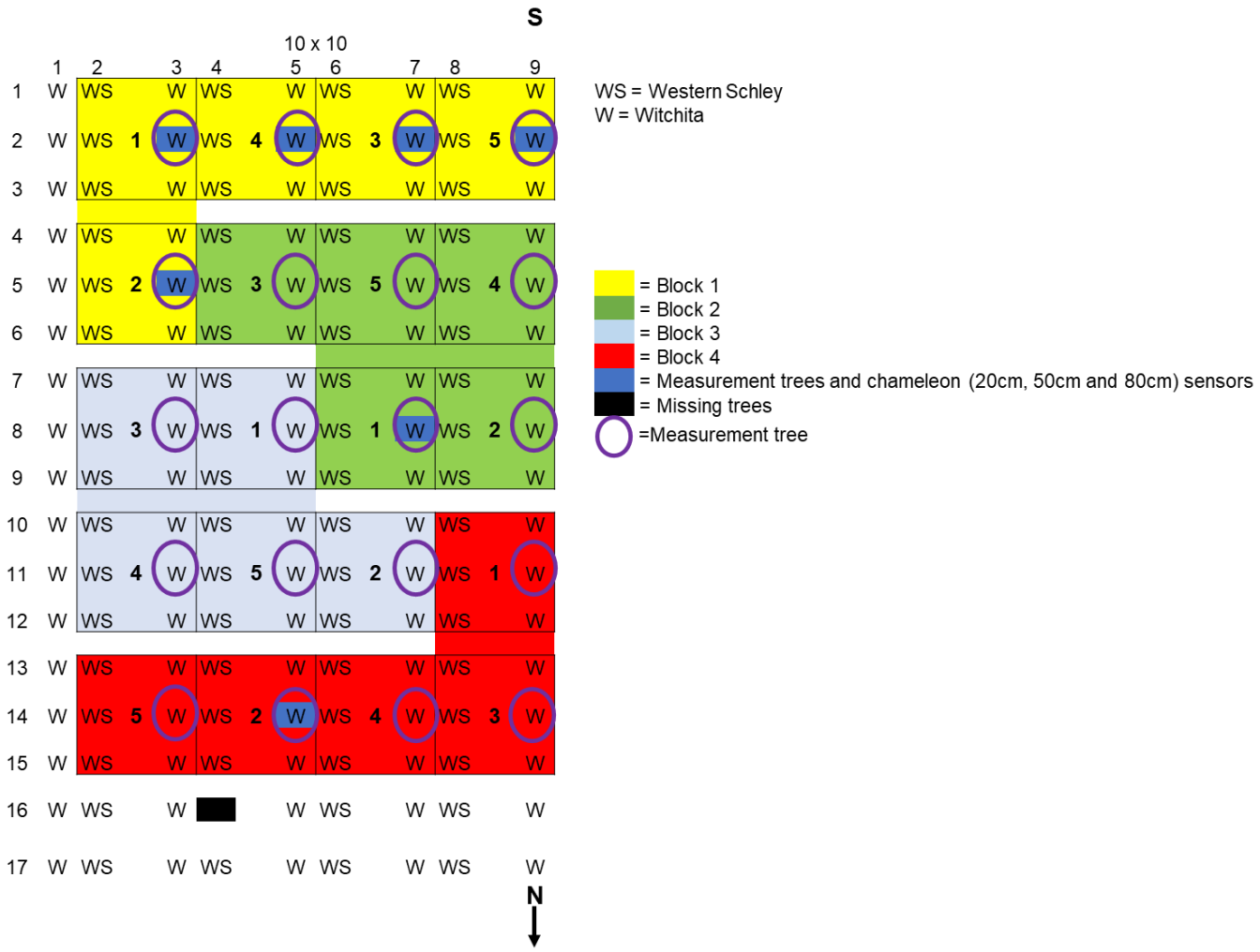


Figure 3.2 Orchard layout showing all treatments (Numbers within each block: 1=Well-watered control, 2=Flowering and nut set, 3=Nut sizing, 4=Nut filling and 5=Shuck dehiscence) and replicates or blocks, measurement trees and location of soil water monitoring equipment (Chameleons).

Figure 3.3 Rainfall was eliminated from the stressed treatments by covering the area allocated to the trees with black plastic.

3.3 Ecophysiology measurements

3.3.1 Leaf water potential

Water stress was assessed through the measurements of pre-dawn and midday stem water potential on the middle tree in each replicate, for all treatments every 5–6 days. Leaf water potential was determined using a Scholander type pressure chamber (Model 3005, Soil Moisture Equipment Co., Santa Barbara, CA) immediately after leaf removal. Pre-dawn leaf water potential was determined on eight leaves from each measurement tree, with measurements taking place prior to sunrise. For midday stem water potentials eight leaf samples were selected from the inside of the canopy only and were enclosed in a plastic bag covered with aluminium foil for a period of 30 – 60 minutes prior to measurement. This was done to stop the leaves from transpiring and allowing them to equilibrate to the water potential of the stem (Scholander et al. 1965).

3.3.2 Transpiration

Transpiration was determined using the heat ratio method of the heat pulse velocity technique as described by Burgess et al. (2001) and Ibraimo et al. (2016) on four trees (selected trees with more or less same size canopy through the measurement of canopy size (using techniques discussed in the next few sections) in block one representing 1) the well-watered control (Tree 1), 2) water stress at flowering and nut set (Tree 2), 3) water stress at nut sizing (Tree 3) and 4) water stress at nut filling stage (Tree 4). Four custom made heat pulse probe sets were inserted at four different depths (Figure 3.4a) in each tree trunk to account for the radial variation in sap flux within the conducting sapwood. Each probe set consisted of two Type T (copper/constantan) thermocouples (embedded in 2.0 mm outside diameter polytetrafluoroethylene (PFTE) tubing) placed equidistantly (0.35 cm) upstream and downstream of the heater probe inserted into a brass collar (2.5 mm) (Figure 3.4b). These probe sets were inserted above the rootstock in the scion and below the lowest branch, with probes being equally spaced around the trunk and randomly arranged, taking care to avoid any abnormalities in the trunk. The heat pulse velocity (V_h) in cm h^{-1} for each probe set was calculated following Marshall (1958) as:

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

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

$$V_c=bV_h+cV_h^2+dV_h^3 \qquad \text{Equation 3.2}$$

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

$$b=6.6155w^2+3.332w+0.9236 \qquad \text{Equation 3.3}$$

$$c=-0.149w^2+0.0381w-0.0036 \qquad \text{Equation 3.4}$$

$$d=0.0335w^2-0.0095w+0.0008 \qquad \text{Equation 3.5}$$

The wound width was assessed through visual inspection and subsequent measurement of the outer diameter of the wound.

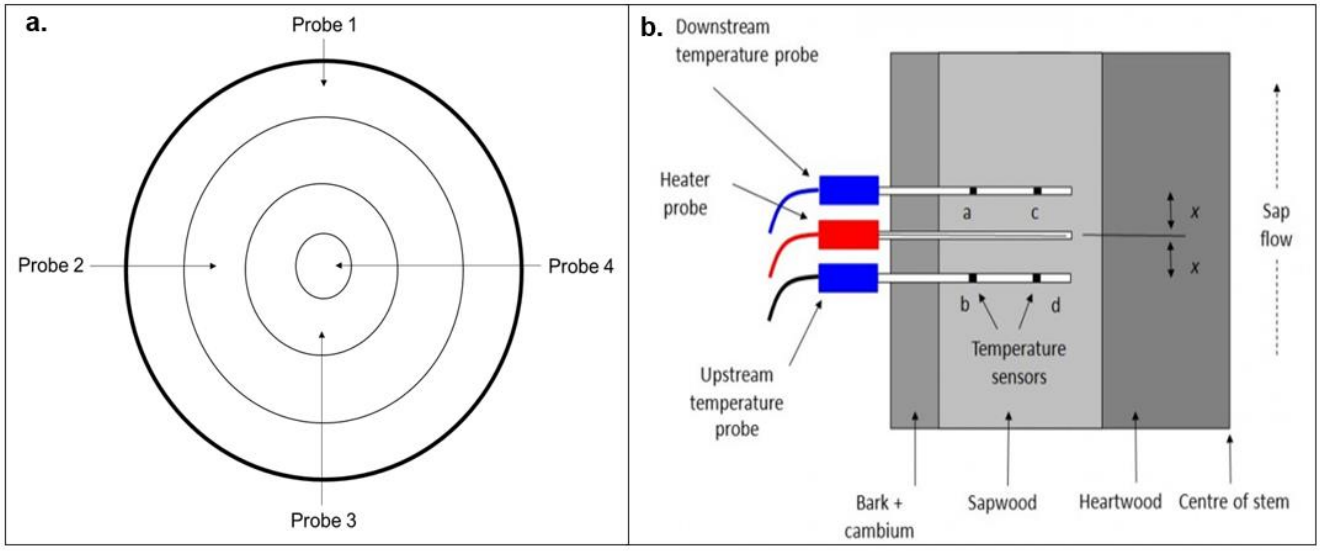


Figure 3.4 (a) Typical installation of the heater and sensor probes into the stem for the heat ration method (Bleby et al. 2004), (b). Demonstration of the insertion of four probes into the stem to account for the radial variation in sap flow within conducting sapwood.

The presence of heartwood was determined by taking wood cores with an incremental borer. These core samples were stained using safranin, with unstained areas being marked as non-conducting wood. Other wood characteristics, including sapwood moisture content (m_c) and density (ρ_b) were determined from additional core samples taken during the measurement period. Following the determination of m_c and ρ_b, sap velocity (V_s) was calculated from the corrected heat pulse velocity using the equation suggested by Marshall (1958) that was later modified by Barrett *et al*. (1995):

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

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

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

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

3.3.3 Gas exchange and photosynthesis

Measurements of leaf gas exchange included, but were not limited to, net assimilation CO_2 rate (A_n), stomatal conductance (g_s), and intercellular CO_2 concentration (Ci) and were measured using an infra-red gas analyser (IRGA) (Model: LI-6400 XT, LI-COR, Lincoln, Nebraska, USA).

Sensors inside the cuvette monitored leaf surface temperature (T_{leaf}) and leaf-to-air vapour pressure deficit (VPD_{leaf}). Measurements of A_n and g_s were performed on the third fully expanded leaf (third leaflet) from the apex of each shoot for each water stressed treatment and the well-watered control every 5–6 days to assess the impact of water stress on gas exchange. For the diurnal changes, spot measurements of leaf gas exchange measurements were made on four leaves on the outside of the canopy at the four cardinal points on one tree per treatment and per block. Chamber CO_2 concentration was maintained at 400 µmol mol^{-1}, the flow rate was 400 µmol s^{-1}, photosynthetically active radiation (PAR) inside the chamber was maintained between 1500 – 2000 µmol m^{-2} s^{-1} (LI-6400 XT LED light source), and RH was maintained at more than 50% (to prevent stomatal oscillations).

CO_2 response curves were determined by varying partial pressure of CO_2 surrounding the leaf (400, 300, 200, 100, 50, 0, 200, 300, 400, 600, 700, 1000, 2000 µmol CO_2 mol^{-1}) and calculating C_i from stomatal conductance measurements (Long and Bernacchi 2003). The response of A to photo flux (Q) was determined by varying the PAR within the chamber and determining the response of A (2000, 1500, 1000, 600, 400, 200, 100, 50, 0 µmol m^{-2} s^{-1}). For light response curves the CO_2 concentration was controlled at 400 µmol mol^{-1}, whilst PAR was set at 1500 µmol m^{-2} s^{-1} for A/C_i curves. Measurements were taken throughout the day and were used to determine the impact of water stress on photosynthesis of pecan trees at each phenological stage in relation to the control treatment.

3.3.4 Stomatal conductance

Additional measurements of g_s were performed using an AP4 porometer (Delta-T Devices Ltd, Cambridge, United Kingdom). Six leaves were selected from one experimental tree per block, with four sunlit leaves and two shaded leaves under the canopy measured at midday on a weekly basis. Diurnal measurements were conducted when there was a difference in predawn leaf water potentials between the well-watered control and water stressed treatments.

3.3.5 Canopy cover

Canopy cover was determined by measuring fractional interception of (PAR) using a Decagon AccuPAR LP-80 ceptometer (Decagon Devices, Pullman, WA, USA). Sampling of PAR below the canopy was conducted across and within the row (covering the total area allocated to one tree) at pre-determined 1 m intervals, whilst a full sun reading was taken in an open area next to the

orchard. Measurements were taken weekly on one experimental tree in all blocks, in the 2018/19 and 2019/20 seasons (middle tree of all treatments) during canopy growth at the beginning of the season and during the end of the season as the leaves senesced (Mid-April to May). In the middle of the season, measurements were taken at two to three-week intervals. A Phantom 3 drone, fitted with a RGB camera, was also flown 15m above the canopy to take photographs of the experimental trees at the same height (the middle tree of all treatments in all blocks) at midday on a weekly basis during canopy development and end of the season as leaf senescence began. In the middle of the season, it was flown every two to three weeks.

These measurements were then used to determine canopy cover by analysing the images using the Canopeo application in MATLAB R2017a software (Patrignani and Ochsner 2015). As defined by Liang et al. (2012), canopeo is an automatic colour threshold (ACT) image analysis tool developed in the Matlab programming language (Mathworks, Inc., Natick, MA) using colour values in the red–green–blue (RGB) system. Canopeo analyses and classifies all pixels in the image (Liang et al. 2012).

3.4 Nut growth and development

Nut growth and development was assessed from flowering and nut set to shuck dehiscence or the maturity stage. Nut set was determined by labelling three clusters per tree in all the treatments. The number of flowers per cluster were counted soon after the female flowers were visible, with the number of nuts set per cluster recorded following pollination and fertilization. The number of nuts remaining on the tree were recorded on a weekly basis and was used to determine nut drop for the first three stages (flowering and nut set, nut sizing and nut filling) during the trial. The increase in nut size (length and diameter) was measured from nut set to final nut size (when no further increases in nut size were recorded) on weekly basis using Vernier calipers (GripsWorks, Arnold, Missouri, USA) on the marked clusters per experimental tree whilst attached to the tree.

For nut development, three nuts on each treatment were harvested every two weeks. A cross section cut at the mid-point of the nut was made on two nuts and a perpendicular cut was made on one nut (Figure 3.5). The nuts were dissected with a sharp knife in the first half of the growing season. Later in the season, as the shell hardened, nuts were sectioned with a saw. Stages that were analysed included kernel deposition (dough), ovary wall (shell) lignification, cotyledon thickening (nut filling) and fruit maturity, as judged by involucre (hull) dehiscence. This was used

to classify nut development, that is, how the nuts develop in response to water stress treatments as compared to the well-watered control and to determine the different phenological stages.

Figure 3.5 Cross-section cut at the mid-point of the nut and perpendicular cut along the longitudinal dimensions of the nut.

3.5 Yield and Quality

At the end of the season, nuts were harvested from each experimental tree in each of the four replications using a mechanical shaker (for the 2018/19 and 2019/20 seasons). However, during the first season (2017/2018), the nuts were harvested by hand. All nuts were collected on the floor after shaking or beating the trees to determine sound kernel % and unsound kernel % (including pops). Sub-samples of 1 kg were randomly taken from each crate of the different treatments (Figure 3.11a). The yield was adjusted to 4% moisture. Nut quality parameters that were assessed included wet in shell mass, dry in shell mass, average size distribution and shape. The nuts were then cracked for the analysis of total kernel %, kernel colour, moisture content and storage stability at a local processor in Cullinan (Elandsdraai Pecan Growers). The nut size was determined as the number of nuts per kg and using Vernier calipers (GripsWorks, Arnold, Missouri, USA). Kernel

shell-out percentage was calculated from the total nut and kernel mass after drying. Kernel moisture content was determined by drying a 5 g ground kernel sample per treatment in a moisture content analyser (MB23 Food Moisture Analyser Ohaus).

3.6 Irrigation and soil water management

The volume of applied irrigation was recorded using water meters (Multi Jet Water Meter w/o EV ARAD) plumbed into the irrigation at the at the start of the drip line for each treatment replicate in block 1. These meters were read following each irrigation event. Soil water matric potential measurements were made using Chameleon soil water sensors (Virtual Irrigation Academy, www.via.farm) (Figure 3.6a). The Chameleon sensor estimates soil water matric potential and displays colours which correspond to a certain range of soil water tension (Figure 3.6b). These colours are displayed by a hand-held portable reader, which is connected to the sensor array. The chameleon has three sensors in one sensor array which are installed at different depths in the soil profile. In this study the depths were 20 cm, 50 cm and 80, determined with multiple sensor arrays. The colours displayed can be blue (wet), green (moist) or red (dry). When the light is blue it indicates that the soil matric potential is between approximately 0 and -20 kPa (wet). Typically, under these conditions water is moving in the soil and leaching is possible. At approximately -20 kPa to -40 kPa the light colour changes to green (intermediate), which means the water is still readily available to the plant, but unlikely to be moving in the soil, hence leaching cannot occur. Red indicates a decline in soil water potential, below -40 kPa (dry) and this means the plant struggles to extract water and water stress is likely to occur in plants and could result in a yield penalty. However, when the soil becomes too dry the Chameleon lights are not turned on and they are considered grey. This indicates a severe soil water deficit. The sensors were installed in all treatments, and all replication under the drippers.

In this trial Chameleon sensor readings were taken three times a week. The reason for this was to schedule irrigation in the control, to manage the level of stress in the other treatments and to ensure that soil water was being depleted too much in the water stressed treatments. The non-stressed treatments were irrigated when one of the top layers (20 and 50cm) turned red in the control treatments, whilst in the stressed treatments, the two top layers were kept red and irrigation was only performed if one of the layers turned grey. However, this data was used in conjunction with midday stem leaf water potentials, that is, maintaining a value between –0.40 and –0.85 MPa in the control and –0.90 to –1.45 MPa in the stressed treatments, when the values dropped beyond the minimum threshold (-1.45 MPa), the trees were irrigated.

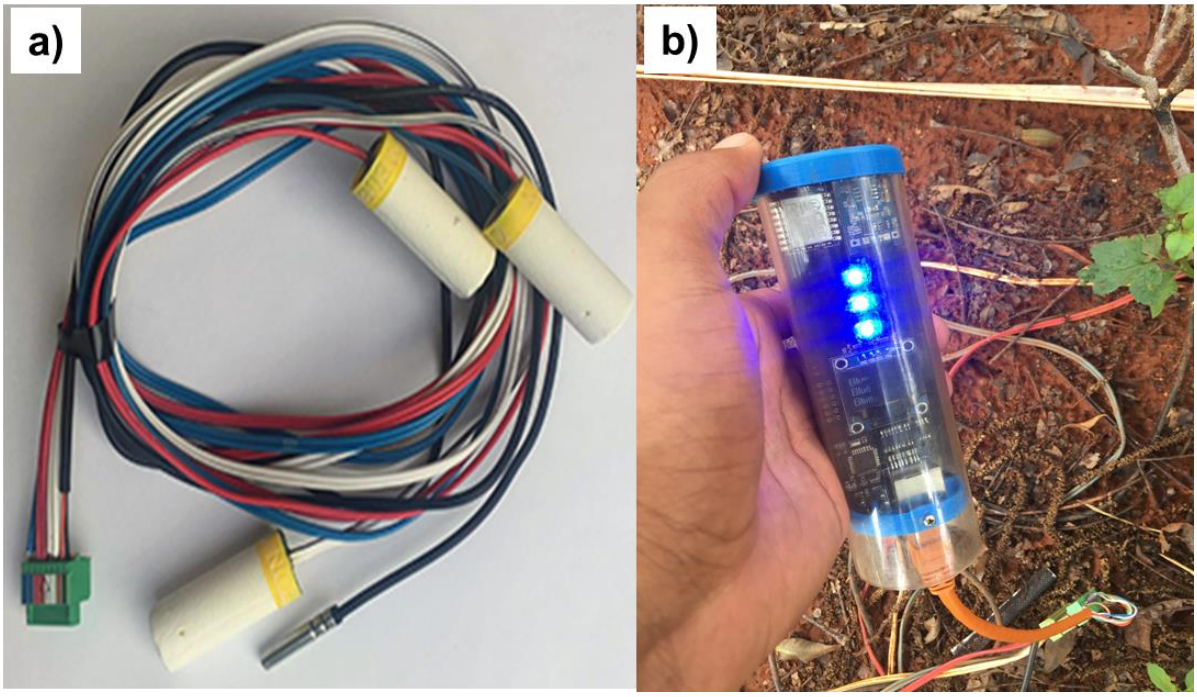


Figure 3.6 (a) Chameleon soil water sensors buried in the ground (b) Chameleon reader connected to the sensor array with colour display in the well-watered control during the course of the trial.

3.7 Statistical analysis

Excel statistical analysis were used to determine statistical differences between stomatal conductance, photosynthesis and yield (flower set, nut drop) and quality (average nut size, number of nuts kg $^{-1}$, kernel %, moisture %, unsoaund kernel %, waifer/air pockets % and stick tights) of the water stressed treatments relative to the well-watered treatment. The differences between treatment means were determined using the F test and T test mean separation. The significance levels were $p<0.05$ (or 95% confidence level).

CHAPTER 4: THE PHYSIOLOGICAL RESPONSE OF PECAN TREES TO WATER STRESS DURING DIFFERENT PHENOLOGICAL STAGES

4.1 INTRODUCTION

Plants have differential sensitivity to water deficit, with some trees shedding their leaves when only moderately stressed (Fereres et al. 2012). This has been shown in trees like almonds and hazelnuts (Bignami et al. 2010). However, for some species like olives (Orgaz et al. 2006), stress needs to be severe in order for effects to be evident. Lisar et al. (2012) highlighted that water stress reduces the water potential and turgor of plant cells required for cell enlargement, which leads to growth inhibition (for example reduced plant height and leaf area) and reproductive failure. Shoot initiation, shoot extension, leaf and fruit growth, and trunk growth are all affected by water deficits. Reduced vegetative growth (decreasing number of leaves, plant height and plant quality) in apples (Kuroda et al. 1992), hazelnuts (Bignami et al.) and walnuts (Fulton et al. 2006), were observed as a result of drought stress. Garrot et al. (1993) reported that pecans are sensitive to water stress, with profound effects on the physiology and productivity of the tree, thereby affecting both yield and nut quality. Physiological effects include stomatal closure, decreased photosynthetic activity and a decline in transpiration rates (Cornic and Massacci 1996, Dodd and Ryan 2001, Cifre et al. 2005, Flexas et al. 2012). Water stress is commonly detected in a range of tree organs, such as leaves, stem, roots and Bréda et al. (1993) proposed that the sensitivity of these organs may differ. Therefore, Leuschner et al. (2001) suggested that a whole-tree approach for stress detection is preferred to one restricted to the leaf level. Furthermore, Gieger and Thomas (2002) stated that water deficit responses are species dependent and may sometimes differ in closely related species. Therefore, for assessing a plant's physiological response to water stress, various plant stress measurements have been developed (Jones 2007, Krishnan 2012). This includes the measurement of gas exchange (photosynthesis, stomatal conductance), transpiration and leaf water potentials (midday stem leaf and predawn stem leaf water potentials) (Jones 2004, Othman et al. 2014, Pirasteh-Anosheh et al. 2016). Such measurements are said to be a good integrator of the soil, water and weather parameters and are considered as the ideal criteria for stress detection (Jones 2007).

Understanding the physiological responses of plants to drought stress during different phenological stages is a crucial aspect in many water management strategies. This concept has

gained much popularity to date due to rising water scarcity concerns around the globe (Pereira et al. 2002, Pegram 2010, Mekonnen and Hoekstra 2016). Much attention has been given to water stress responses across a wide variety of fruit trees, which includes a range of deciduous fruits (Shackel et al. 1997) e.g. peaches (Rahmati et al. 2018), apples (Jackson 2003), pears (Poni et al. 1992, Crisosto et al. 2004), almonds (Tejero et al. 2018) and hazelnuts (Marsal Vila 1997). This is due to water stress posing a significant constraint on plant growth and productivity in many crop plants (Churkina and Running 1998, Leuzinger et al. 2005). Although, Fereres et al. (2012) suggested that water stress should be avoided at all stages of growth and development, this suggestion may not necessarily hold in many situations. For instance, Moriana et al. (2003) reported flowering and fruit set as the most water sensitive phenological stages, whereas pit hardening was insensitive to drought stress in olives (Goldhamer 1997, Moriana et al. 2013, Girón et al. 2015). This suggests that water stress also differs with the different phenological stages. In pecans, Miyamoto (1989) found that drought during early developmental stages (bud break and flowering) resulted in non-uniform bud break. In some cases of excessive drought stress, the tree shed leaves and moderately filled the nuts at the nut filling stage (Miyamoto 1989, Wells and Harrison 2010, Othman et al. 2014, Wells 2016b). In addition, early termination of nut growth resulted in an increased number of pops, as well as a decrease in the percent kernel (Sparks 2001). In contrast, Call et al. (2006) stated that withholding irrigation early in the season and late in the season at the start of shuck dehiscence, though there is a lack of evidence, can provide the opportunity for water savings with no impact on production for the current season. However, Wood et al. (2002) demonstrated that water stress late in the season reduces carbohydrates reserves which result in poor flowering in the following season, reducing yield, also referred to as alternate bearing.

Through the identification of sensitive and insensitive phenological stages to water stress, in terms of yield and quality, an opportunity is created to develop sound water management strategies. Water savings can be made during insensitive stages, whilst optimal irrigation should be practiced during sensitive stages. This is particularly important for regions which are prone to droughts and where water resources are limited. Since pecans are largely produced in semi-arid regions (low and unreliable rainfall) in South Africa and are reported to have very high annual water requirements, this information is particularly important for this valuable crop. However, this requires a proper assessment of plant stress, which involves the establishment of threshold values. For example, for pecans in New Mexico, Othman et al. (2014), established water deficit thresholds for midday stem leaf water potential. Values between -0.40 and -0.85 MPa were

determined to represent well-watered conditions, -0.90 to -1.45 MPa represented moderate stress and -1.5 to -2.0 MPa severe stress. Importantly, these values appear to be region specific and may depend on the prevailing weather conditions. For instance, in southern Georgia, which is more humid, this threshold was reported to be ≈-0.78 MPa (Wells 2015). It is therefore important that these thresholds are determined for local conditions to aid with local water management.

It was hypothesized that the photosynthetic rate of sunlit pecan leaves would decline under water stress, mainly due to stomatal limitations, as a result of the decline in predawn and midday stem leaf water potentials indicative of a drying soil. In addition, it was hypothesized that withholding irrigation early in the season (bud break and flowering) and late in the season at the start of shuck dehiscence will result in delayed canopy development and hastened leaf fall respectively. To test these hypotheses, the following objectives were formulated: (1) To successfully implement water stress during the following phenological stages; flowering and nut set, nut sizing, nut filling and shuck dehiscence, whilst maintaining a well-watered control. (2) To measure midday stem and predawn water potentials, in order to determine whether the plants are stressed and to correlate these values with changes in photosynthesis and stomatal conductance. (3) To determine the impact of water stress at different phenological stages on canopy growth at the beginning of the season, final canopy size during the season and leaf drop at the end of the season.

4.2 Materials and methods

Detailed materials and methods are presented in Chapter 3: General materials and methods, section 3.3.

4.3 Results

4.3.1 Weather variables

Table 4.1 presents the seasonal weather variables for the three respective seasons. The first season was the warmest season recorded, with the highest seasonal average daily maximum temperature (32.0°C) and average daily minimum temperature (15.2°C) presented by the control across all seasons. However, the second season recorded the highest seasonal maximum vapor pressure deficit (VPD) (1.11 kPa). Average daily solar radiation was 21.34 MJ m^{-2} day^{-1} for the

2017/18 season and 20.66 MJ m^{-2} day^{-1} for the 2018/19 season, which was slightly higher than the third season. The third season presented the lowest average daily maximum temperature (25.8°C), VPD (0.93 kPa) and average daily solar radiation (19.79 MJ m^{-2} day^{-1}). Table 4.1 also presents the averages of the weather variables for the four different phenological stages when water stress was implemented. In the last two seasons, higher temperatures together with higher VPD were observed during the flowering and nut set stage. However, lower temperatures, VPD and daily solar radiation were recorded during the last stage (shuck dehiscence) in the last two seasons, as compared to the first season. In the first season, the highest VPD was recorded in the nut sizing stage, during which higher average daily maximum and minimum temperatures were recorded compared to the other two seasons.

Table 4.1: Seasonal weather variables including average maximum temperature (°C), average minimum temperature (°C), maximum vapour pressure deficit (VPD, kPa) and average daily solar radiation (MJ m^2 day^{-1}) for three seasons. Season 1 (2017/2018), season 2 (2018/2019) and season 3 (2019/2020).

Season 1				
Treatment	Avg Max (°C)	Avg Min (°C)	VPD (kPa)	Avg daily solar radiation (MJ m^2 day^{-1})
Control	32.0	15.2	1.04	21.34
Flowering and nut set	27.3	13.6	0.98	24.89
Nut sizing	31.0	15.9	1.40	25.23
Nut filling	38.8	17.2	1.16	20.04
Shuck dehiscence	30.4	14.1	0.73	16.88

Season 2				
Treatment	Avg Max (°C)	Avg Min (°C)	VPD (kPa)	Avg daily solar radiation (MJ m^2 day^{-1})
Control	28.4	11.4	1.11	20.66
Flowering and nut set	33.7	9.1	1.48	23.67
Nut sizing	29.7	16.0	1.22	24.93
Nut filling	28.6	15.7	0.95	22.43
Shuck dehiscence	24.6	9.2	0.89	16.26

Season 3				
Treatment	Avg Max (°C)	Avg Min (°C)	VPD (kPa)	Avg daily solar radiation (MJ m^2 day^{-1})
Control	25.8	12.3	0.93	19.79
Flowering and nut set	29.7	15.4	1.44	24.68
Nut sizing	27.1	16.0	0.92	21.13
Nut filling	28.2	15.7	0.94	22.39
Shuck dehiscence	23.1	8.6	0.74	16.60

4.3.1 Irrigation management

The volume of water received by each treatment, both rainfall received, and irrigation applied, and the reference evapotranspiration (ET_o) for the three seasons are presented in Table 4.2. As expected, the well-watered treatment (control) received the highest irrigation across all treatments in all three seasons, as it was irrigated optimally. As water stress was implemented at each phenological stage, a reduction in irrigation water at flowering and nut set, nut sizing, nut filling and shuck dehiscence resulted in water savings of 13%, 14%, 16% and 42% in the first season, 24%, 33%, 36% and 22% in the second season, and 33%, 24%, 32% and 22% in the third season, when compared to the well-watered control (Table 4.2). Seasonal total ET_o was 1277 mm for the first season, 1312 mm for the second season and 1246 mm for the third season, which reflected the period when the trees were in leaf (September to May).

Table 4.2: Seasonal water application including irrigation, rainfall water applied and reference evapotranspiration (ET_o) for the different phenological stages when a water stress was implemented for the three seasons. Season 1 (2017/2018), season 2 (2018/2019) and season 3 (2019/2020)

Season 1					
Treatment	**Rainfall (mm)**	**Irrigation (mm)**	**Irrigation water saved**	**Total (mm)**	**ET_o (Sept - May) (mm)**
Control	638	144		**782**	1277
Flowering and nut set	568	125	13%	**693**	225
Nut sizing	378	124	14%	**497**	244
Nut filling	597	123	15%	**720**	191
Shuck dehiscence	375	84	42%	**459**	182

Season 2					
Treatment	**Rainfall (mm)**	**Irrigation (mm)**	**Irrigation water saved**	**Total (mm)**	**ET_o (Sept - May) (mm)**
Control	500	215		**715**	1312
Flowering and nut set	383	163	24%	**546**	503
Nut sizing	354	144	33%	**498**	301
Nut filling	232	137	36%	**369**	234
Shuck dehiscence	419	168	22%	**587**	305

Season 3					
Treatment	**Rainfall (mm)**	**Irrigation (mm)**	**Irrigation water saved**	**Total (mm)**	**ET_o (Sept - May) (mm)**
Control	730	220		**950**	1246
Flowering and nut set	567	147	33%	**715**	440
Nut sizing	354	168	24%	**522**	216
Nut filling	594	149	32%	**743**	199
Shuck dehiscence	675	171	22%	**846**	262

4.3.2 Canopy cover

Canopy cover was assessed throughout the second and third season (2018/19 and 2019/20) to determine the effect of water stress on canopy size (refer to Chapter 3, section 3.3.5 for the materials and methods). Early canopy growth was impacted by water stress implemented during the flowering and nut set stage, with clear differences between the stressed and well-watered treatments. Canopy growth was slightly delayed by water stress from October to November in both seasons, reaching the maximum canopy size a week after the well-watered treatment (Figure 4.1a). As the water stress at the nut sizing and nut filling stages were implemented when the canopy had achieved its final size, no differences in growth were detected in this treatment when compared to the well-watered control, with trees in all treatments achieving a similar final fractional cover. Water stress at the end of the season (final stage, shuck dehiscence) hastened leaf fall or senescence, resulting in a faster decline in canopy size in the treatment where a water stress was applied during shuck dehiscence. This is evident by the clear differences observed from April to June in both seasons, with the stressed treatment reaching 0% canopy cover a week before the well-water treatments (Figure 4.1b).

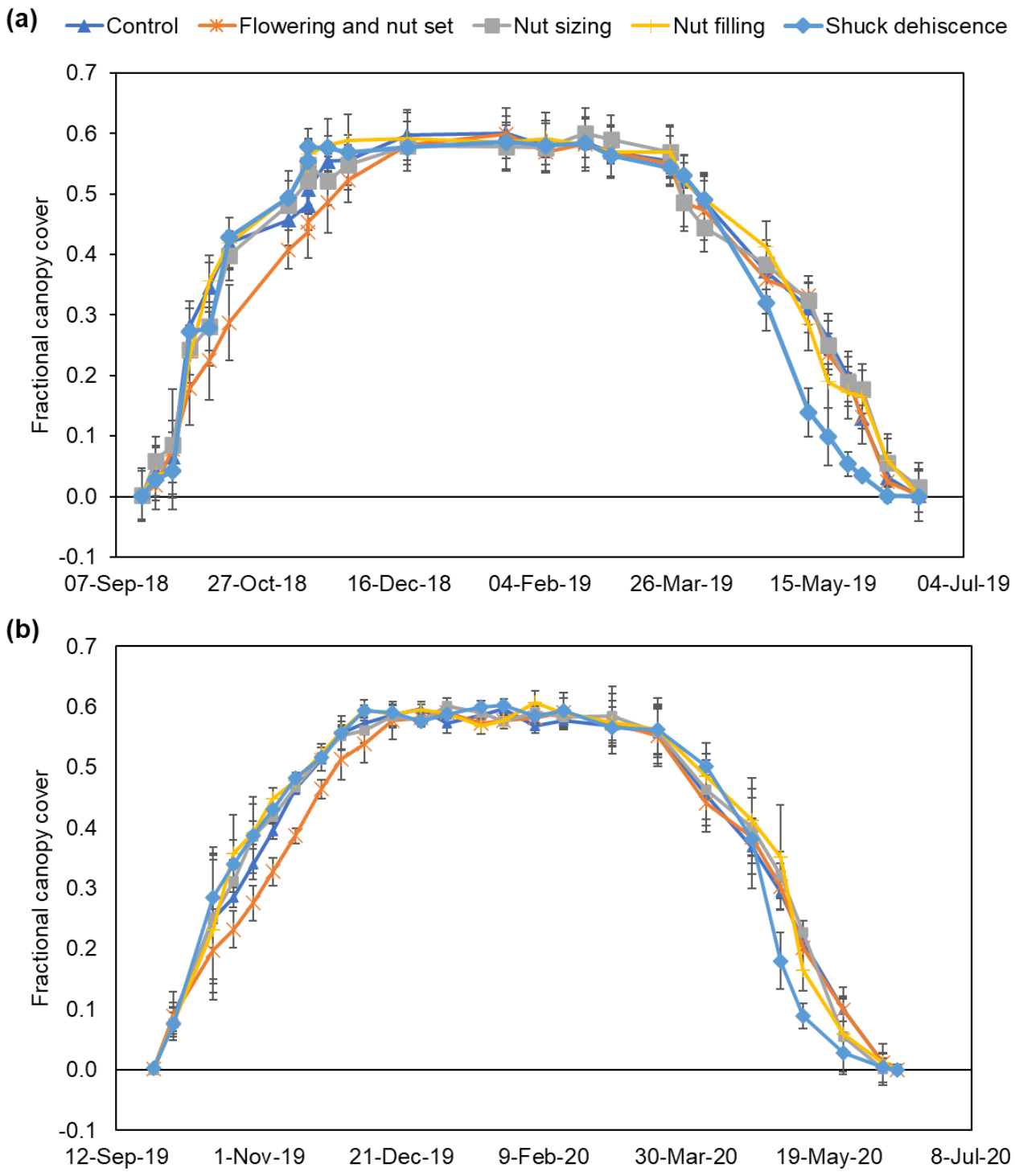
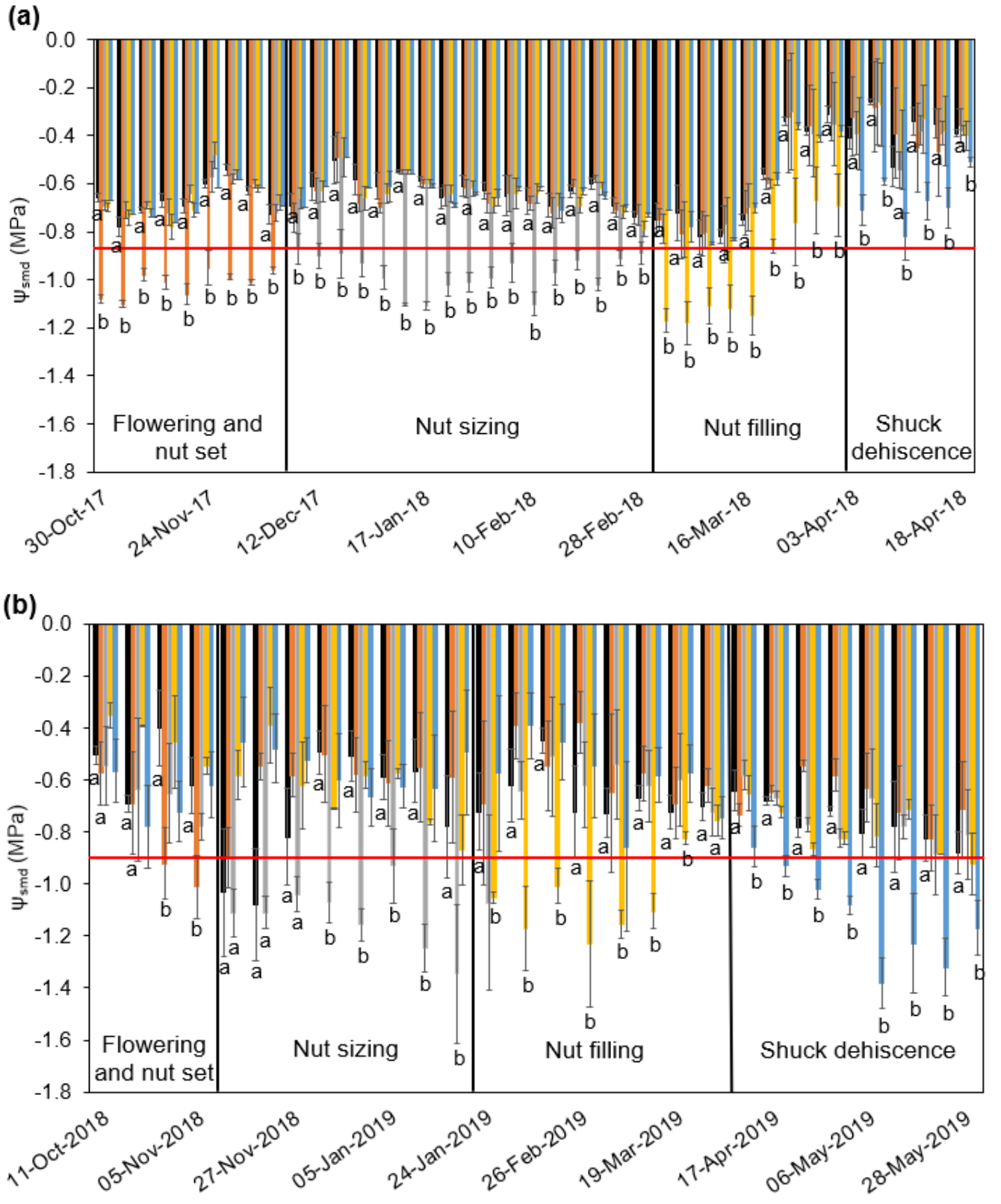


Figure 4.1 Fractional canopy cover in the five treatments (four replicates) throughout the seasons (a) 2018/2019 season and (b) 2019/2020 season in the pecan orchard in Pretoria. Fractional canopy cover was determined by measuring the fraction of photosynthetically active radiation intercepted by the tree canopies.

4.4.3 Predawn leaf water potential and midday stem water potential

Measurements of predawn leaf water potential (ψ_{pd}) and midday stem water potentials (ψ_{smd}) were taken throughout three growing seasons from 2017 to 2020 to monitor plant water stress, with stress at each phenological stage being compared to the well-water control (refer to Chapter 3, section 3.3.1 for the materials and methods). Increasing soil water deficits as a result of the cessation of irrigation at each phenological stage led to decreasing midday stem water potential (ψ_{smd}) values (Figure 4.2). Water stress was evident at all phenological stages in the second and third season as midday stem water potential values fell below the midday stem water potential threshold value of -0.90 MPa, as suggested by Othman et al. (2014). However, for the first season (Figure 4.2a), the trees did not experience significant water stress at the final phenological stage (shuck dehiscence), with minimum ψ_{smd} values of -0.82 MPa. This was due to the high rainfall received at this stage, with 189 mm received in a single week. Nevertheless, in this season as irrigation was withdrawn during the flowering and nut set stage, the ψ_{smd} decreased to -1.10 MPa, -1.11 MPa at nut sizing and fell to -1.18 MPa during nut filling. In the second season, the lowest ψ_{smd} value obtained at flowering and nut stage was -1.01 MPa, -1.35 MPa at nut sizing, -1.23 MPa at nut filling and -1.38 MPa at shuck dehiscence (Figure 4.2b). Importantly, during this season there was a decrease in ψ_{smd} in the control treatment during the flowering and nut set stage, which was caused by a temporary breakdown in the irrigation system to this treatment, which then had to be irrigated by hand using a water tanker. Water stress was also achieved in the third season, with lowest ψ_{smd} values of -1.12 MPa, -1.15 MPa, -1.17 MPa and -1.32 MPa at flowering and nut set, nut sizing, nut filling and shuck dehiscence (Figure 4.2c). During this season, a sudden increase of water potentials was observed in all treatments during the nut sizing stage due to the very high rainfall during December 2019 (260 mm in 8 days). In all three seasons, after the completion of each phenological stage, the trees that were subjected to stress were re-watered and as a result the water potentials increased, reaching the well-watered control midday stem water potential values after a week.



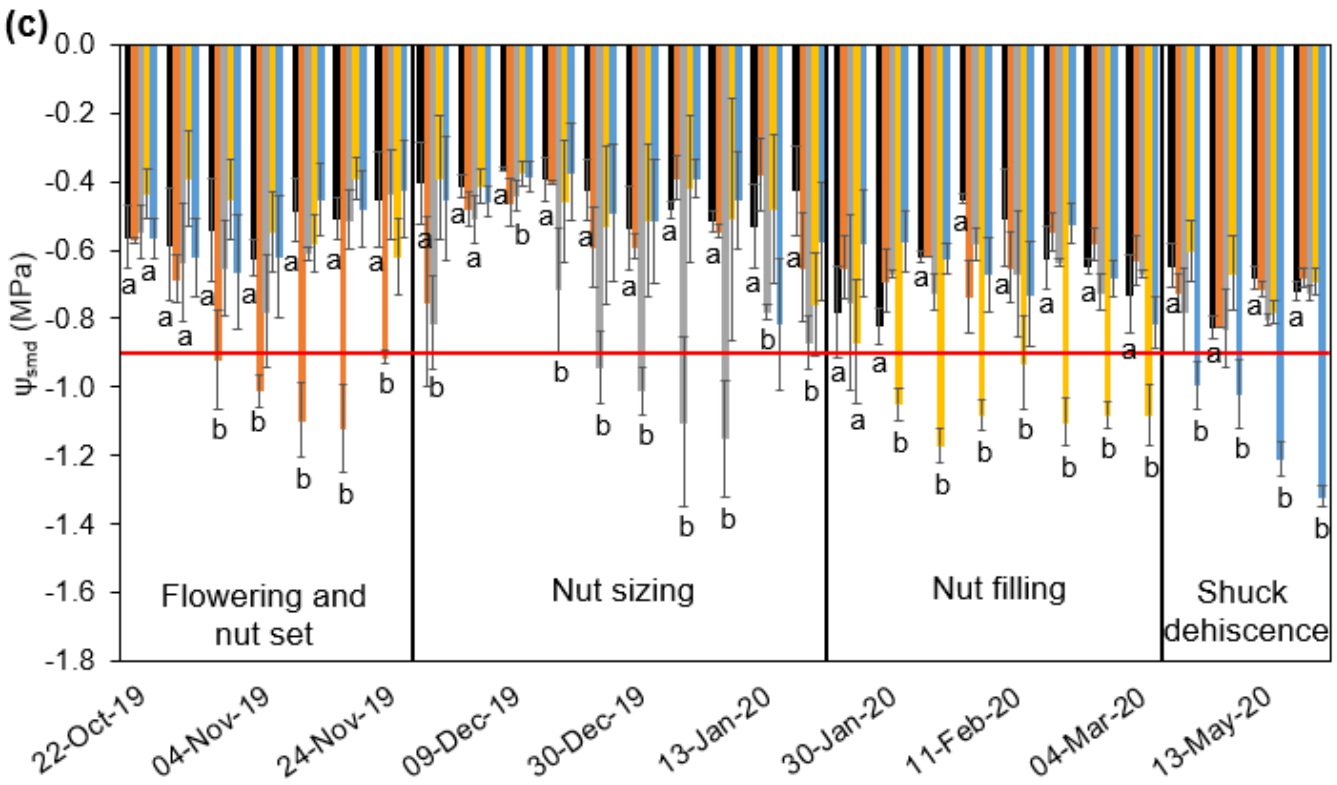
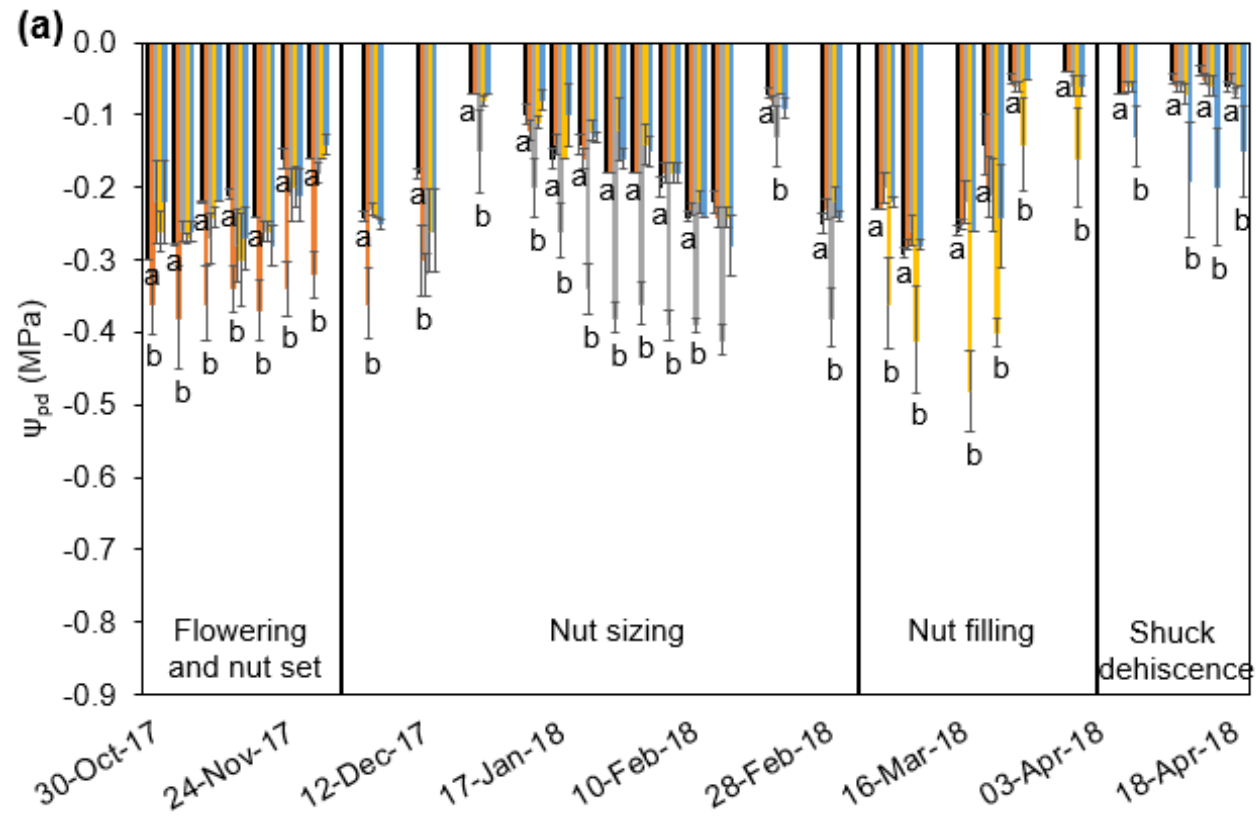
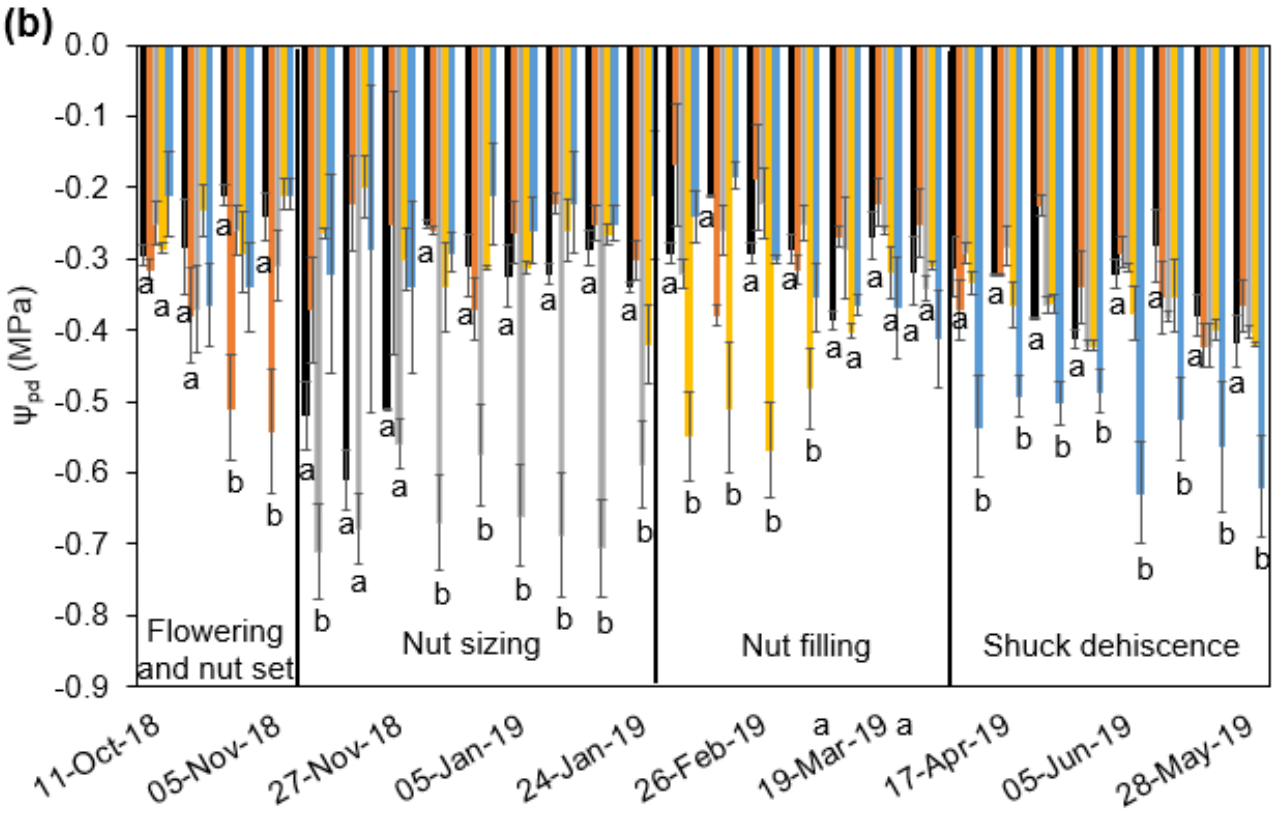


Figure 4.2 Midday stem water potential (ψ_{smd}) measurements for each phenological stage and for the three seasons. (a) Season one (2017/18), (b) season two (2018/19) and (c) season three (2019/20). The vertical lines on each figure indicates the different phenological stages (flowering and nut set, nut sizing, nut filling and shuck dehiscence) from left to right. The black color lines within the graph presents the well-watered control. The horizontal red line demonstrates the stem water potential threshold value (-0.90 MPa) for mild stress as outlined by Othman et al. (2014). Statistical analyses were done at each phenological stage between the control and the water stressed treatment. Each value was an average of 4-6 leaves from 4 trees ± standard error. Mean values with the same letters are not significantly different from each other ($p>0.05$).

Predawn leaf water potential measurements were also performed for the different seasons (Figure 4.3) and these values exhibited the same pattern as the ψ_{smd}. For the first season, minimum ψ_{pd} values of -0.38 MPa, -0.41 MPa, -0.48 MPa and -0.20 MPa were recorded during stress at flowering and nut set, nut sizing, nut filling and shuck dehiscence (Figure 4.3a). Figure 4.3b, presents ψ_{pd} values during water stress for the second season and minimum values of -0.54 MPa at flowering and nut set stage, -0.71 MPa at nut sizing, -0.57 MPa at nut filling and -0.63 MPa at

the final stage of nut development (shuck dehiscence) were recorded. Likewise, a similar response to water stress imposed at different phenological stages was observed during the third season, with minimum ψ_{pd} values of -0.66 MPa during stress at flowering and nut set, -0.76 MPa at nut sizing, -0.58 MPa at nut filling and -0.68 MPa at the shuck dehiscence stage (Figure 4.3c).





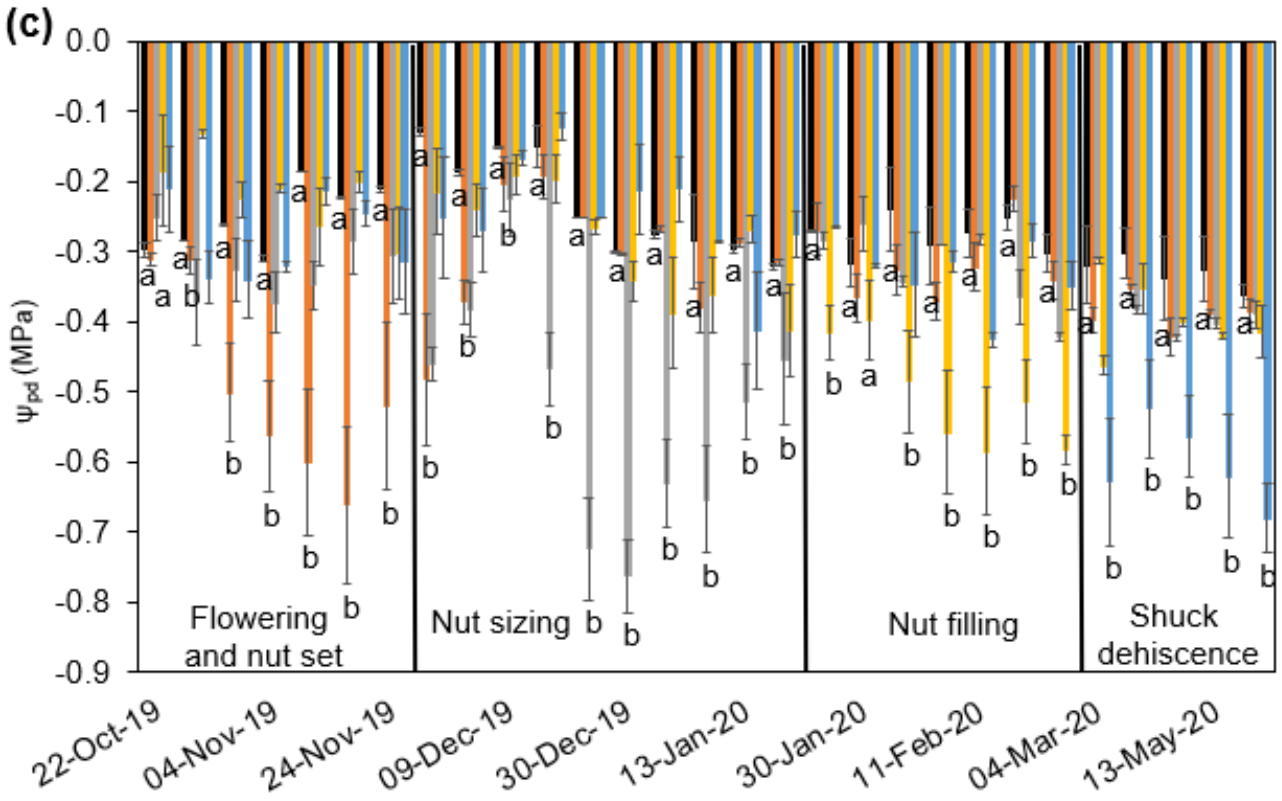


Figure 4.3 Predawn leaf water potential (ψ_{pd}) measurements for each phenological stage and for the three seasons. (a) Season one (2017/18), (b) season two (2018/19) and (c) season three (2019/20). The vertical lines on each figure indicates the different phenological stages (flowering and nut set, nut sizing, nut filling and shuck dehiscence) from left to right. Statistical analyses were done at each phenological stage between the control and the water stressed treatment. Each value was an average of 4-6 leaves from 4 trees ± standard error. Mean values with the same letters are not significantly different from each other ($p>0.05$).

Since similarities in ψ_{smd} and ψ_{pd} were observed, the data was correlated to establish the relationship between the two parameters (Figure 4.4). A close correlation existed between ψ_{smd} and ψ_{pd}, with both values tending to decrease as available soil water was depleted. R^2 values of 0.90, 0.89 and 0.81 were obtained for season one, two and three respectively (Figure 4.4a, b & c). Lastly, regardless of the low R^2 value of 0.61, there was still a reasonable relationship between ψ_{smd} and ψ_{pd} when the data for all three seasons was combined (Figure 4.4d). Considering a ψ_{smd} threshold of -0.90 MPa for stress, the corresponding predawn values for the three seasons were

-0.25 MPa, -0.48 MPa and -0.49 MPa for the first, second and third season respectively. For the three seasons combined, the predawn value was -0.42 MPa.

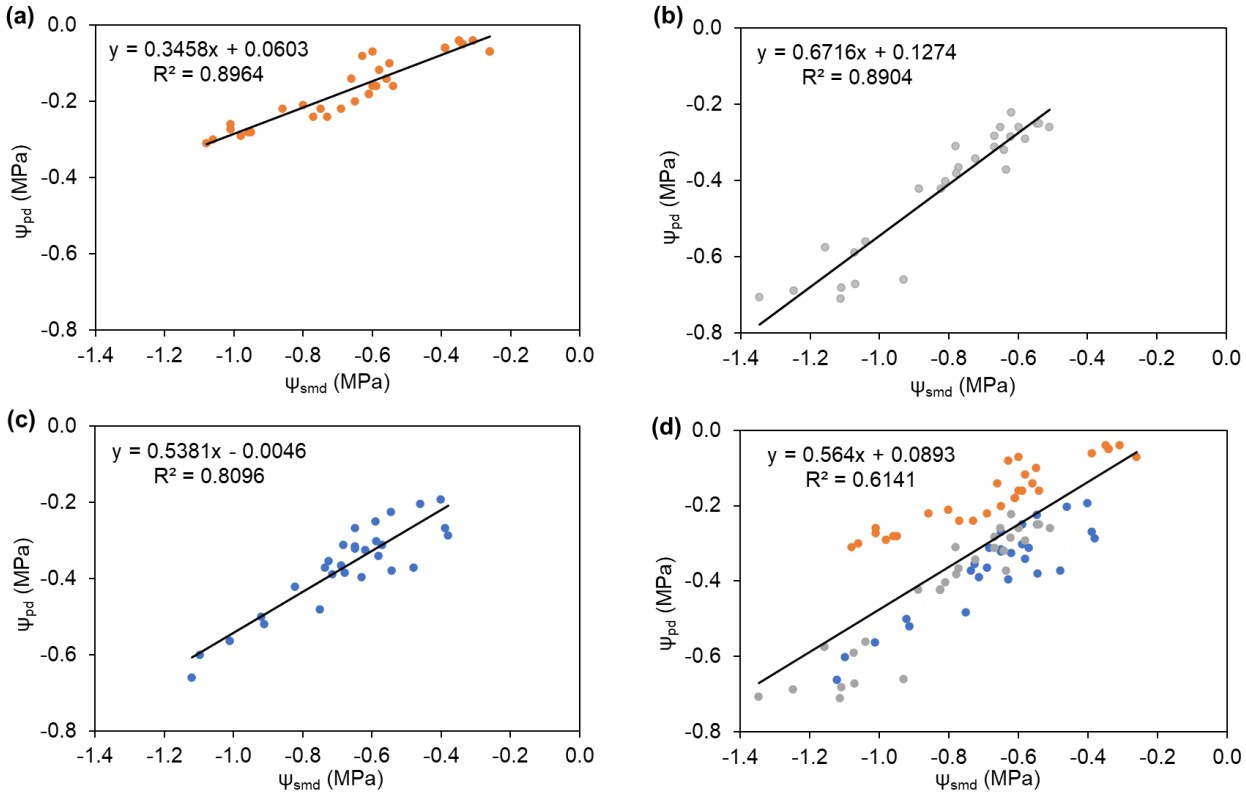


Figure 4.4 Relationship between midday stem water potential (ψ_{smd}) and predawn leaf water potential (ψ_{pd}) for the three seasons. (a) season one (2017/18), (b) season two (2018/19), (c) season three (2019/20) and (d) combined data for the three seasons.

4.4.4 Gas exchange

4.4.4.1 Photosynthetic light response curves

Photosynthetic light response curves (A_n/PPFD) for the well-watered and water stressed treatments were evaluated for the second season (2018/19) to assess the impact of water stress on the ability of pecan leaves to utilize available PAR (refer to Chapter 3, section 3.3.3 to 3.3.4 for materials and methods). Photosynthetic light response curves for all treatments did not differ before the implementation of water stress across all phenological stages and maximum photosynthetic rate (A_{max}), light compensation point (LCP) (when net photosynthesis is 0), and quantum use efficiency (Φ) were not significantly different between treatments (Figure 4.5, Table 4.3). However, after four weeks of no irrigation, midday stem water potentials dropped within the mild stress threshold defined by Othman et al. (2014) and the P_n/PPFD curves differed significantly from the well-watered control across all the different phenological stages (Figure 4.5, Table 4.3). The net A_{max} was significantly higher in the well-watered control as compared to the water the stressed treatments. Maximum photosynthetic rate dropped by 23%, 33%, 32% and 26% relative to the control during stress at flowering and nut set, nut sizing, nut filling and shuck dehiscence respectively (Figure 4.5, Table 4.3). However, at the last stage (shuck dehiscence), there were no significant differences between the two treatments. This was most probably as a result of the start of leaf senescence during this stage, as the trees were entering dormancy. A significant decline in the LCP of the water stressed treatments from the control was observed at each stage as a result of water stress. Water stress led to a 35%, 41% and 46% decline of LCP from the control in the at flowering and nut set, nut sizing, nut filling stages (Figure 4.5, Table 4.3). However, there was no significant difference in the LCP between the control and when the stress was applied during the shuck dehiscence stage. A significant decline in Φ was only observed when the trees were stressed at the nut filling stage.

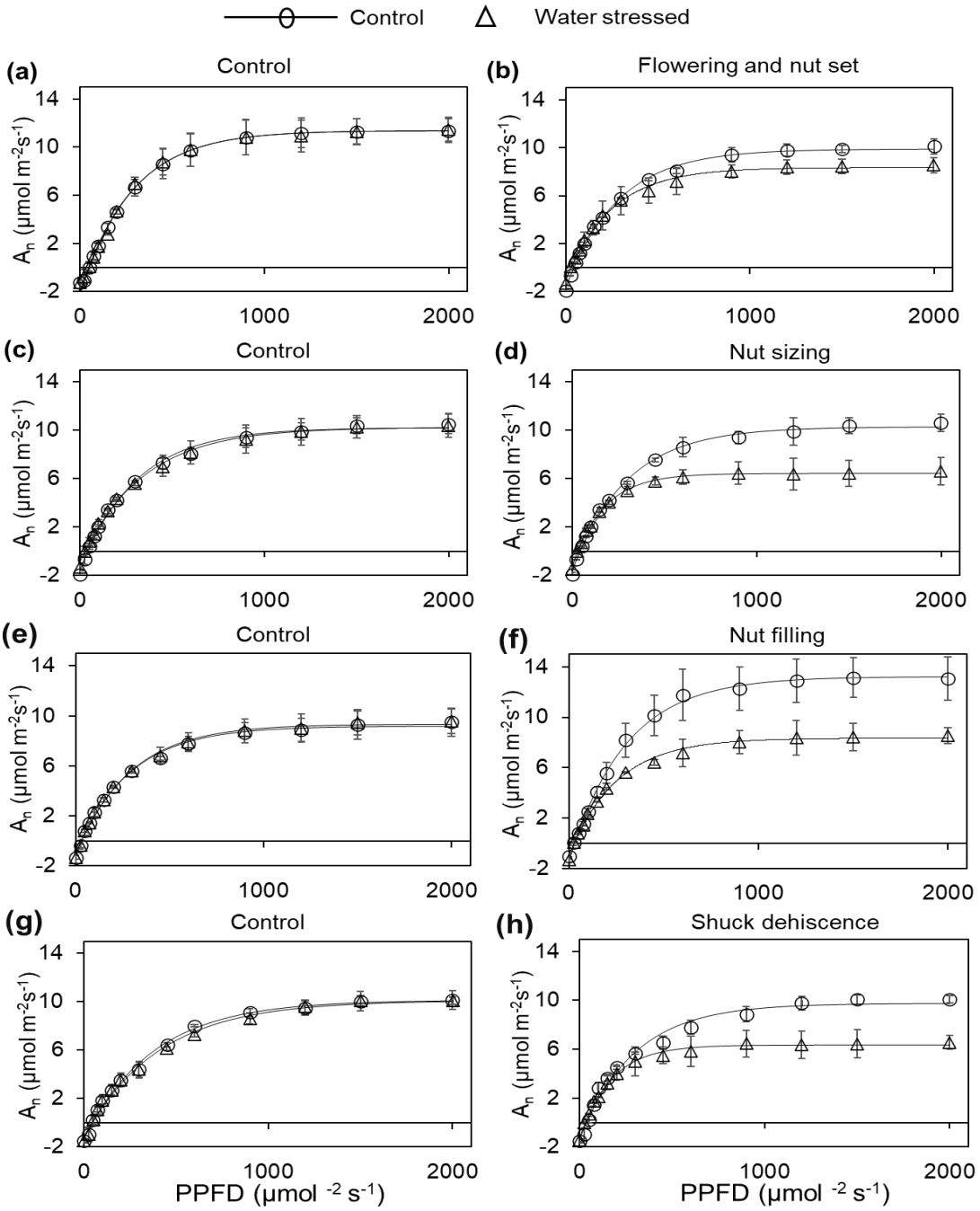


Figure 4.5 Net photosynthetic rate (A_n) versus photosynthetic photon flux density (PPFD) curves for pecan trees exposed to water stress (triangles) or well-watered (circles), measured before the stress implementation: (a), (c), (e) and (g), and during water stress: (b), (d), (f) and (h) at flowering (a, b), nut sizing (c, d), nut filling (e, f) and shuck dehiscence (g, h), for the second season (2018/19). Lines were fitted to these data using the rectangular-hyperbolic equation of Lobo et al. (2013). n = 4 plants per treatment combination.

Table 4.3 Mean (n = 4 plants per treatment) maximum rate of net photosynthesis (A_{max}), the light compensation point (LCP) and apparent quantum yield [AQE (Φ)], derived from A_n/PPFD curves measured before and during water stress implemented at flowering and nut set, nut sizing, nut filling and shuck dehiscence. The results of a 2-way ANOVA (F-statistics with 1 effect and 5 error degrees of freedom) (*p≤0.05, **p≤0.01). Mean values in the same row with different letters are significantly different (p<0.05).

Stages		Before stress		During stress	
Flowering and nut set	Parameters	Control	Flowering and nut set	Control	Flowering and nut set
	A_{max} [μmol(CO_2) m^{-2} s^{-1}]	10.89 ± 0.50a	11.01 ± 0.58a	10.10 ± 0.28a	7.82 ± 1.35b
	LCP [μmol(photon) m^{-2} s^{-1}]	47.95 ± 5.49a	46.27 ± 3.81a	42.37 ± 0.47a	27.48 ± 2.49b
	AQE(Φ) [μmol(CO_2) μmol(PPFD)$^{-1}$]	0.041 ± 0.01a	0.05 ± 0.004a	0.04 ± 0.002a	0.07 ± 0.006a
Nut sizing		Control	Nut sizing	Control	Nut sizing
	A_{max} [μmol(CO_2) m^{-2} s^{-1}]	10.12 ± 0.05a	10.13 ± 0.02a	11.09 ± 0.14a	7.48 ± 1.29b
	LCP [μmol(photon) m^{-2} s^{-1}]	43.62 ± 2.46a	43.10 ± 1.07a	45.03 ± 0.39a	26.51 ± 5.05b
	AQE(Φ) [μmol(CO_2) μmol(PPFD)$^{-1}$]	0.04 ± 0.003a	0.04 ± 0.003a	0.04 ± 0.004a	0.04 ± 0.006a
Nut filling		Control	Nut filling	Control	Nut filling
	A_{max} [μmol(CO_2) m^{-2} s^{-1}]	11.34 ± 0.76a	10.81 ± 0.44a	11.19 ± 0.09a	7.60 ± 0.93b
	LCP [μmol(photon) m^{-2} s^{-1}]	50.05 ± 5.81a	47.51 ± 3.90a	48.92 ± 2.98a	26.55 ± 1.23b
	AQE(Φ) [μmol(CO_2) μmol(PPFD)$^{-1}$]	0.04 ± 0.005a	0.04 ± 0.006a	0.05 ± 0.01a	0.03 ± 0.002b
Shuck dehiscence		Control	Shuck dehiscence	Control	Nut dehiscence
	A_{max} [μmol(CO_2) m^{-2} s^{-1}]	9.65 ± 0.53a	9.51 ± 0.62a	7.94 ± 0.46a	5.84 ± 0.39a
	LCP [μmol(photon) m^{-2} s^{-1}]	28.56 ± 1.34a	29.95 ± 0.60a	28.40 ± 0.81a	32.30 ± 2.25a
	AQE(Φ) [μmol(CO_2) μmol(PPFD)$^{-1}$]	0.04 ± 0.002a	0.04 ± 0.001a	0.04 ± 0.003a	0.03 ± 0.008a

4.4.4.2 A/Ci response curves

Assessing net photosynthetic response (Assimilation, A_n) to variations of intercellular CO_2 concentration (Ci) was done to evaluate the effect of water stress on photosynthesis of pecan trees during the second season (2018/19). Parameters derived from *A/Ci* curves for all treatments did not differ before stress implementation at all phenological stages and included rubisco carboxylation efficiency (V_{cmax}), RuBP (Ribulose-1,5-bisphosphate)-regeneration (J_{max}) (expressed as the maximum rate of electron transport), triose phosphate use (TPU) and dark respiration (R_d) (Figure 4.6, Table 4.4). As observed for the light response curves, after four weeks without irrigation, and when the midday stem water potentials had dropped within the mild stress threshold as outlined by Othman et al. (2014), parameters determined from *A/Ci* curves differed significantly between the water stressed treatments and the well-watered control at all the different phenological stages (Figure 4.6, Table 4.4). Water stress led to a significant decline in the maximum photosynthetic rate, V_{cmax} and J_{max} in all water stressed treatments, relative to the control (Figure 4.6, Table 4.4). Relatively high average A_n values of approximately 26 µmol m^{-2} s^{-1} were determined for the well-watered control throughout the measurement period indicating that it was unlikely that trees in the control treatment were experiencing stress in the main part of the season that would have limited photosynthesis. However, maximum A_n declined to 19 µmol m^{-2} s^{-1} for the well-watered control towards the end of the season during the shuck dehiscence stage, when leaf senescence had started (Figure 4.6g & h). Water stress resulted in a decline in V_{cmax} by 28%, 42%, 46% and 30% from the control at flowering and nut set, nut sizing, nut filling and shuck dehiscence stages, respectively (Table 4.4). This also impacted the rate of RuBP-regeneration, significantly, by lowering the rate of electron transport at all phenological stages relative to the control. At flowering and nut set, water stress lowered electron transport by 43%, by 41% at nut sizing, 55% at nut filling and 32% at shuck dehiscence when compared to the well-watered control. As with the other parameters, the effects of water stress were particularly noticeable during the nut filling stage. The use of triose phosphate declined significantly by 43%, 29%, 52% and 46% as a result of water stress at flowering and nut set, nut sizing, nut filling and shuck dehiscence, respectively in comparison to the control (Table 4.4). Dark respiration values for the stressed treatments were reduced relative to the control treatment (Table 4.4).

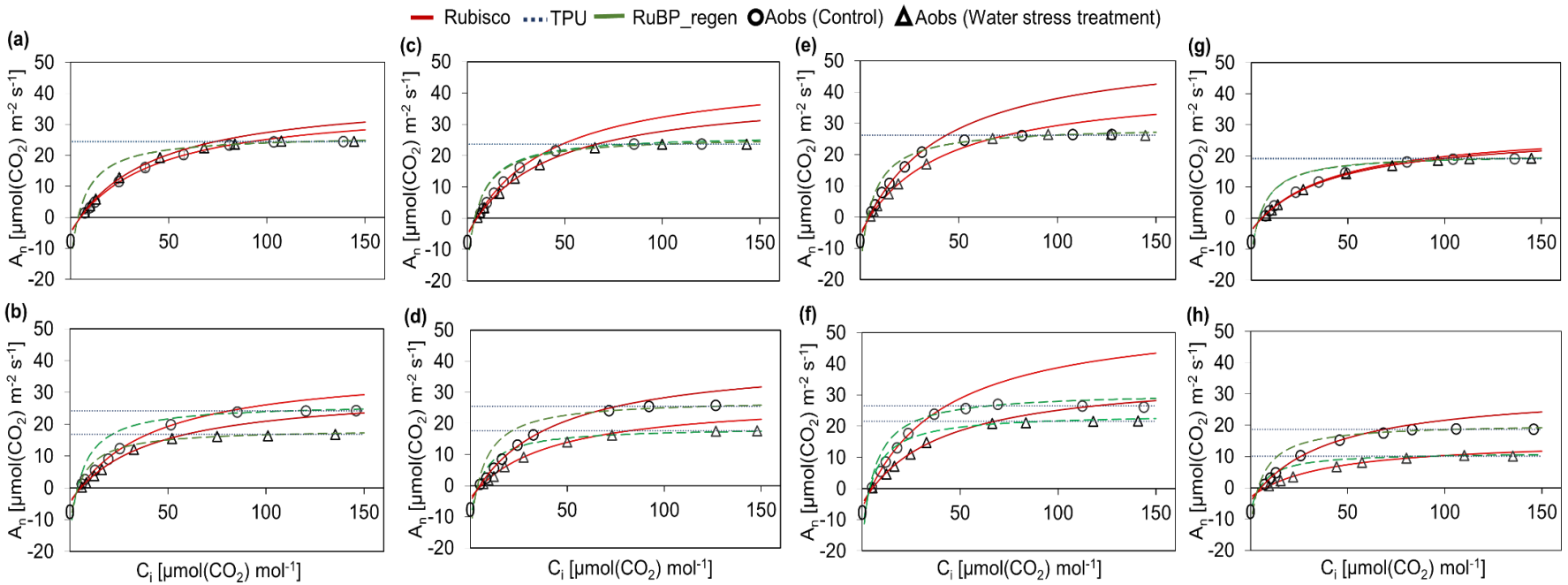


Figure 4.6 Net photosynthetic rate (A_n) vs. intercellular CO_2 concentration (*C*i) curves for pecan trees exposed to water stress (triangles) and the well-watered control (circles), measured before stress implementation: (a), (c), (e) and (g) and during water stress: (b), (d), (f) and (h) at flowering and nut set (a & b), nut sizing (c & d), nut filling (e & f) and shuck dehiscence (g & h), respectively for the second season (2018/19). *Solid lines* indicate the limitation imposed by the photosynthetic rate of Rubisco carboxylation (V_{cmax}), *dotted lines* indicate the limitation imposed by photosynthetic electron transport (J_{max}), and the *dot-dashed lines* indicates the rate of photosynthesis limitation imposed by the use of triose phosphate (*TPU*) calculated using the photosynthesis model of (Sharkey et al. 2007). Mean (*n* = 4 plants per treatment combination).

Table 4.4: Mean (n = 4 plants per treatment) Rubisco carboxylation efficiency (V_{cmax}), RuBP (Ribulose-1,5-bisphosphate)-regeneration (J_{max}) (expressed as the maximum rate of electron transport), triose phosphate use (TPU) and dark respiration (R_d) derived from A/Ci curves measured before and during water stress implemented at flowering and nut set, nut sizing, nut filling and shuck dehiscence. The results of a 2-way ANOVA (F-statistics with 1 effect and 5 error degrees of freedom) (*p≤0.05, **p≤0.01). Mean values in the same row with different letters are significantly different (p<0.05).

Before water stress			During water stress		
Parameters	Control	Flowering and nut set	Parameters	Control	Flowering and nut set
V_{cmax} [μmol(CO_2) m^{-2} s^{-1}]	60.66 ± 0.81a	58.94 ± 1.53a	V_{cmax} [μmol(CO_2) m^{-2} s^{-1}]	61.21 ± 0.97a	43.90 ± 5.72b
J_{max} [μmol(CO_2) m^{-2} s^{-1}]	97.90 ± 11.90a	109.30 ± 15.34a	J_{max} [μmol(CO_2) m^{-2} s^{-1}]	153.00 ± 4.08a	82.15 ± 20.81b
TPU [μmol(CO_2) m^{-2} s^{-1}]	7.07 ± 0.60a	9.71 ± 1.74a	TPU [μmol(CO_2) m^{-2} s^{-1}]	12.18 ± 0.40a	6.90 ± 1.44b
R_d [μmol(CO_2) m^{-2} s^{-1}]	2.06 ± 0.40a	2.072 ± 0.31a	R_d [μmol(CO_2) m^{-2} s^{-1}]	2.23 ± 0.19a	1.91 ± 0.30a
Parameters	Control	Nut sizing	Parameters	Control	Nut sizing
V_{cmax} [μmol(CO_2) m^{-2} s^{-1}]	66.60 ± 6.04a	62.47 ± 5.49a	V_{cmax} [μmol(CO_2) m^{-2} s^{-1}]	71.56 ± 5.49a	41.48 ± 1.34b
J_{max} [μmol(CO_2) m^{-2} s^{-1}]	135.11 ± 11.53a	140.43 ± 17.23a	J_{max} [μmol(CO_2) m^{-2} s^{-1}]	148.16 ± 6.28a	86.80 ± 1.93b
TPU [μmol(CO_2) m^{-2} s^{-1}]	9.90 ± 1.46a	10.79 ± 1.91a	TPU [μmol(CO_2) m^{-2} s^{-1}]	10.46 ± 1.54a	7.41 ± 0.35b
R_d [μmol(CO_2) m^{-2} s^{-1}]	3.27 ± 1.49a	2.90 ± 1.14a	R_d [μmol(CO_2) m^{-2} s^{-1}]	3.43 ± 1.28a	1.76 ± 0.13a
Parameters	Control	Nut filling	Parameters	Control	Nut filling
V_{cmax} [μmol(CO_2) m^{-2} s^{-1}]	75.68 ± 12.34a	73.25 ± 10.82a	V_{cmax} [μmol(CO_2) m^{-2} s^{-1}]	67.79 ± 1.334a	36.96 ± 4.41b
J_{max} [μmol(CO_2) m^{-2} s^{-1}]	157.34 ± 20.49a	156.78 ± 10.71a	J_{max} [μmol(CO_2) m^{-2} s^{-1}]	152.39 ± 1.43a	68.39 ± 25.07b
TPU [μmol(CO_2) m^{-2} s^{-1}]	11.54 ± 1.71a	10.79 ± 2.56a	TPU [μmol(CO_2) m^{-2} s^{-1}]	11.67 ± 0.27a	5.61 ± 1.57b
R_d [μmol(CO_2) m^{-2} s^{-1}]	2.01 ± 0.37a	2.17 ± 0.32a	R_d [μmol(CO_2) m^{-2} s^{-1}]	2.17 ± 0.32a	1.67 ± 0.05a
Parameters	Control	Shuck dehiscence	Parameters	Control	Shuck dehiscence
V_{cmax} [μmol(CO_2) m^{-2} s^{-1}]	47.07 ± 3.66a	45.01 ± 3.27a	V_{cmax} [μmol(CO_2) m^{-2} s^{-1}]	40.90 ± 0.62a	28.73 ± 2.98b
J_{max} [μmol(CO_2) m^{-2} s^{-1}]	112.51 ± 5.80a	119.24 ± 5.16a	J_{max} [μmol(CO_2) m^{-2} s^{-1}]	106.02 ± 3.31a	71.90 ± 12.13b
TPU [μmol(CO_2) m^{-2} s^{-1}]	9.06 ± 0.33a	8.89 ± 0.96a	TPU [μmol(CO_2) m^{-2} s^{-1}]	8.27 ± 0.89a	4.46 ± 3.13b
R_d [μmol(CO_2) m^{-2} s^{-1}]	1.85 ± 0.32a	2.01 ± 0.37a	R_d [μmol(CO_2) m^{-2} s^{-1}]	1.76 ± 0.13a	1.86 ± 0.31a

4.4.4.3 Photosynthesis and stomatal conductance

The impact of water stress on gas exchange and photosynthesis at the different phenological stages was evaluated, since stomatal closure is amongst the first plant responses to rising soil water deficits (Cifre et al. 2005). Diurnal measurements of photosynthesis and stomatal conductance were taken before and during the implementation of water stress at each phenological stage (flowering and nut set, nut sizing, nut filling and shuck dehiscence) and

compared with the well-watered control. Measurements were made during the second season (2018/2019) on specific dates.

Flowering and nut set

During the flowering and nut set stage in season two (2018/19), a mild water stress (ψ_{smd} <-0.9 MPa) was only successfully implemented towards the end of this phenological stage. Before stress implementation, there were no differences in g_s and A_n between the well-watered control and stress treatment (Figure 4.7a & c). In the morning, both g_s and A_n were low and started to increase as the day progressed, with the highest values obtained close to midday, following which values began to decline. During the water stress period, g_s of the water stressed treatment was not significantly different to the control at the start of the day, but there were clear differences between the control and stressed treatment as g_s declined during the afternoon, although these differences were not significant (Figure 4.7b & d). The highest g_s values were g_s = 0.314 mol m^{-2} s^{-1} for the well-watered treatment and 0.215 mol m^{-2} s^{-1} for the water stressed treatment (Figure 4.7b). Photosynthesis values for the well-watered control were also not significantly different from the water stressed treatment at the start of the day, however, the value obtained for the control at 11:00 am was significantly higher than the water stressed treatment. The highest A_n values were A_n = 15.03 µmol m^{-2} s^{-1} and 11.98 µmol m^{-2} s^{-1} (Figure 4.7d) for the well-watered and water stressed treatments, respectively.

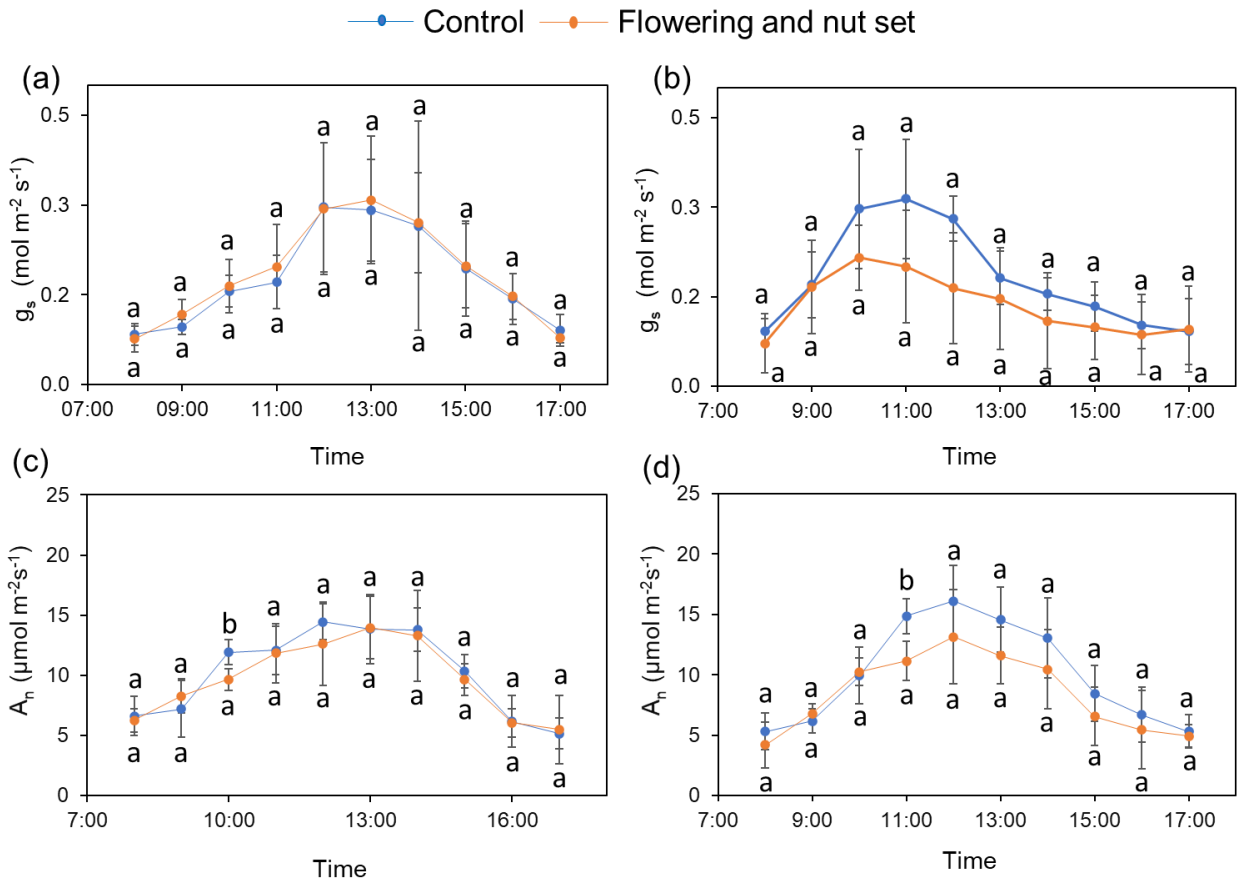


Figure 4.7 Diurnal stomatal conductance (g_s) (a) before and (b) during the water stress and photosynthesis (A_n) (c) before and (d) during the water stress at the flowering and nut set stage. Data was taken 24/10/2218 before stress and 06/11/2018 when the trees were moderately stressed (midday stem water potential was -1.01 MPa). Each value was an average of 4-6 leaves from 4 trees. Mean values with the same letters are not significantly different from each other ($p > 0.05$).

Nut sizing

Before the trees were exposed to water stress, there were no statistical differences between g_s and A_n for trees from the well-watered control and those trees stressed during the nut sizing stage. The effects of water stress during this stage were, however, noticeable by a decline in g_s and A_n at midday in the water stressed treatment as compared to the well-watered control. During the stress period, a significant decline in g_s at this stage was evident during the late hours of the day (16h00 and 17h00) between the well-watered control and the stressed treatment (Figure 4.8b).

Midday stomatal conductance was 0.280 mol m^{-2} s^{-1} for the well-watered control and 0.201 mol m^{-2} s^{-1} for the water stressed trees (Figure 4.8b). Photosynthesis declined significantly in the water stressed treatment at midday and from 14:00 to 15:00, as compared to the well-watered control (Figure 4.8d). Midday photosynthesis values of 15.03 µmol m^{-2} s^{-1} and 11.98 µmol m^{-2} s^{-1} were recorded for the control and the water stressed treatment, respectively (Figure 4.8d)

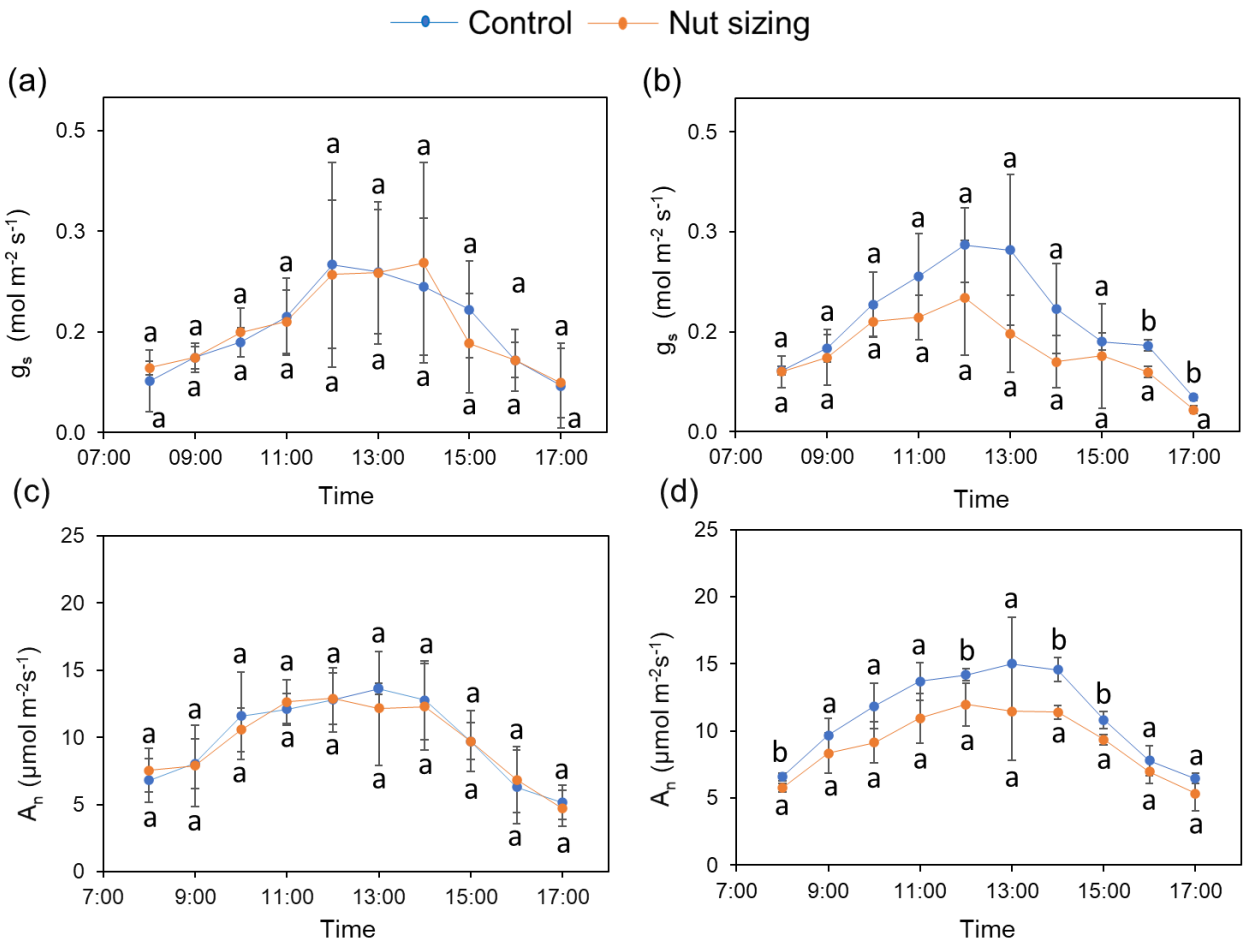


Figure 4.8 Diurnal stomatal conductance (g_s) (a) before and (b) during the water stress and photosynthesis A_n (c) before and (d) during the water stress at the nut sizing stage. Data was taken 11/12/2018 before stress and 19/01/2019 when the trees were moderately stressed (midday stem water potential was -1.35 MPa). Each value was an average of 4-6 leaves from 4 trees. Mean values with the same letters are not significantly different from each other ($p>0.05$).

Nut filling

Before stress implementation at the nut filling stage, there were no significant differences between the two treatments, with the highest g_s values of 0.236 mol m^{-2} s^{-1} and 0.253 mol m^{-2} s^{-1} recorded for the control and the water stressed treatment respectively (Figure 4.9a). The highest values for A_n were 17.12 µmol $m^{-2}s^{-1}$ and 17.45 µmol $m^{-2}s^{-1}$ for the well-watered and stress treatment respectively (Figure 4.9c). There were no significant differences between the well-watered control and water stressed treatment during the implementation of the water stress, but g_s and A_n tended to be lower in the water stressed treatment (Figure 4.9b & d). In the well-watered control g_s of 0.280 mol m^{-2} s^{-1} was recorded, which was higher than water stressed treatment with a value of 0.201 mol m^{-2} s^{-1} (Figure 4.9b). However, due to the large amount of variation in the measurements, this difference was not significant. The highest average values for A_n were 14.56 µmol m^{-2} s^{-1} and 11.90 µmol m^{-2} s^{-1} (Figure 4.9d) for the control and stressed treatment, respectively, but this difference was again not significant.

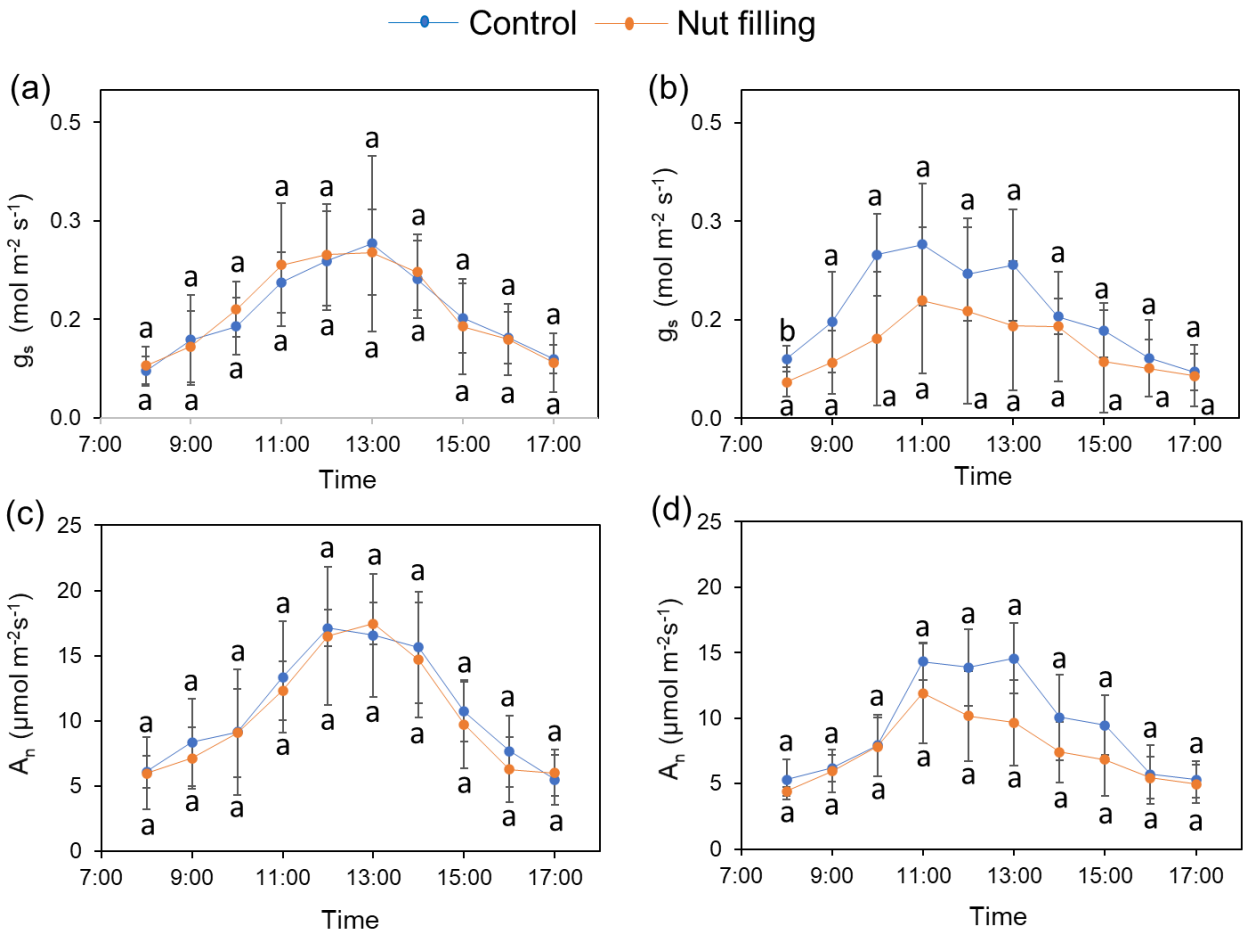


Figure 4.9 Diurnal stomatal conductance g_s (a) before and (b) during the water stress and photosynthesis A_n (c) before and (d) during the water stress at the nut filling stage. Data was taken 24/01/2019 before stress and 14/03/2019 when the trees were moderately stressed (midday stem water potential was -1.11 MPa). Each value was an average of 4-6 leaves from 4 trees. Mean values with the same letters are not significantly different from each other ($p > 0.05$).

Shuck dehiscence

The shuck dehiscence stage is the maturation stage of nut development. During this stage, an overall decrease in g_s and A_n was observed due to the start of leaf senescence before entering the dormant stage. Before the onset of water stress there were no significant differences in g_s or A_n between the well-watered and the water stressed trees. The highest g_s values before stress implementation were 0.207 mol m^{-2} s^{-1} and 0.214 mol m^{-2} s^{-1} for the well-watered and water stressed treatment respectively (Figure 4.10a). Photosynthesis values before stress

implementation were more or less the same, with A_n = 11.31 µmol m^{-2} s^{-1} and 10.22 µmol m^{-2} s^{-1} for the well-watered and water stressed treatment respectively (Figure 4.10c). During the stress period, significantly lower g_s values were recorded for the water stressed treatment when compared to the well-watered control for most of the measurement period (Figure 4.10b). The values were 0.241 mol m^{-2} s^{-1} for the well-watered treatment and 0.128 mol m^{-2} s^{-1} for the water stressed treatment. Similar differences were observed for A_n during the stress period, with the water stressed treatment recording significantly lower values than the well-watered control (Figure 4.10b). These values were A_n = 9.95 µmol m^{-2} s^{-1} and 5.70 µmol m^{-2} s^{-1} for the well-watered and water stressed treatment, respectively.

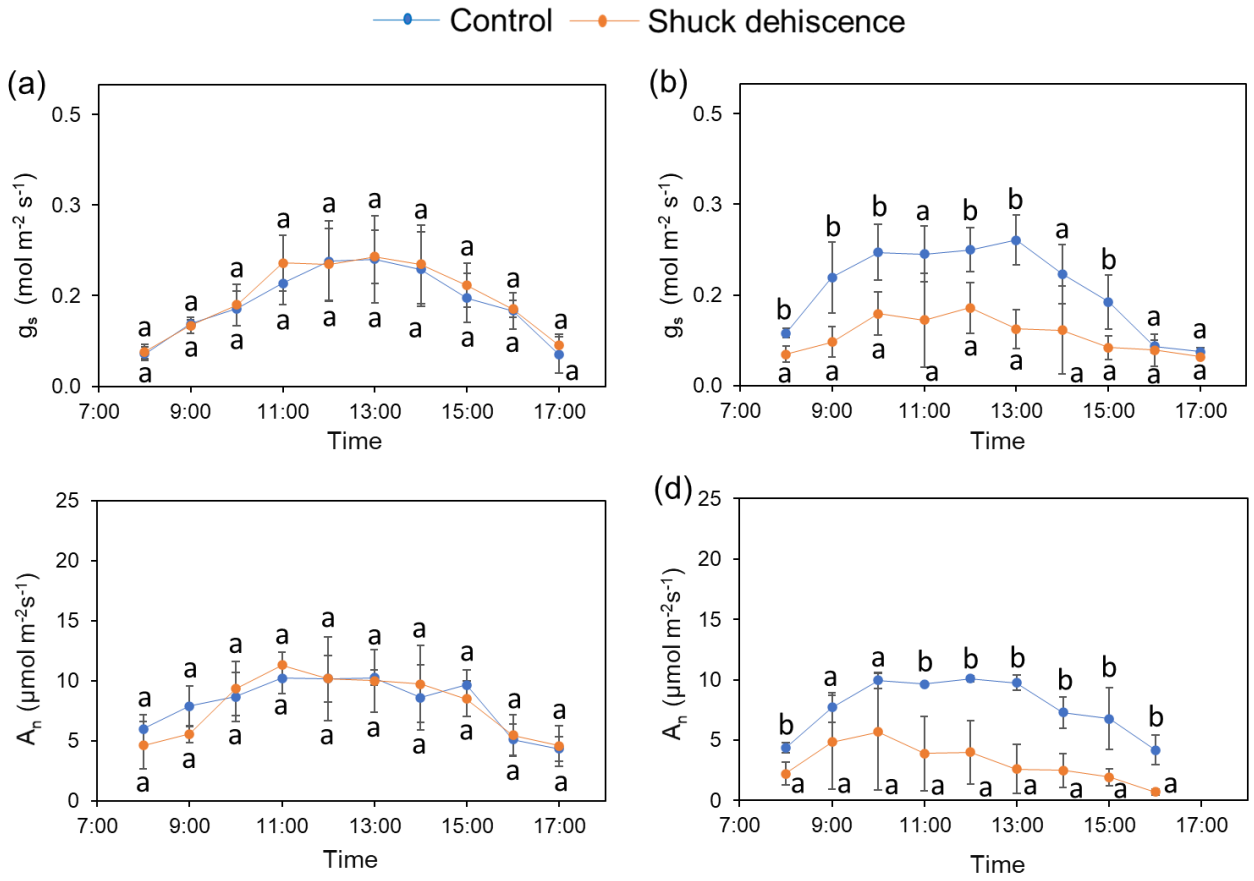


Figure 4.10 Diurnal stomatal conductance (a) before and (b) during the water stress and photosynthesis (c) before and (d) during the water stress at the shuck dehiscence stage. Data was taken 11/03/2019 before stress and 30/04/2019 when the trees were moderately stressed (midday stem water potential was -1.08 MPa). Each value was an average of 4-6 leaves from 4 trees. Mean values with the same letters are not significantly different from each other ($p > 0.05$).

A clear response of g_s and A_n to increasing water stress was evident throughout the trial, with both g_s and A_n declining as ψ_{smd} decreased (Figure 4.11a & b). A decline in leaf water potential, as a result of a drying soil, likely brought about a decline in g_s, which in turn resulted in a reduction in gas exchange and therefore photosynthesis.

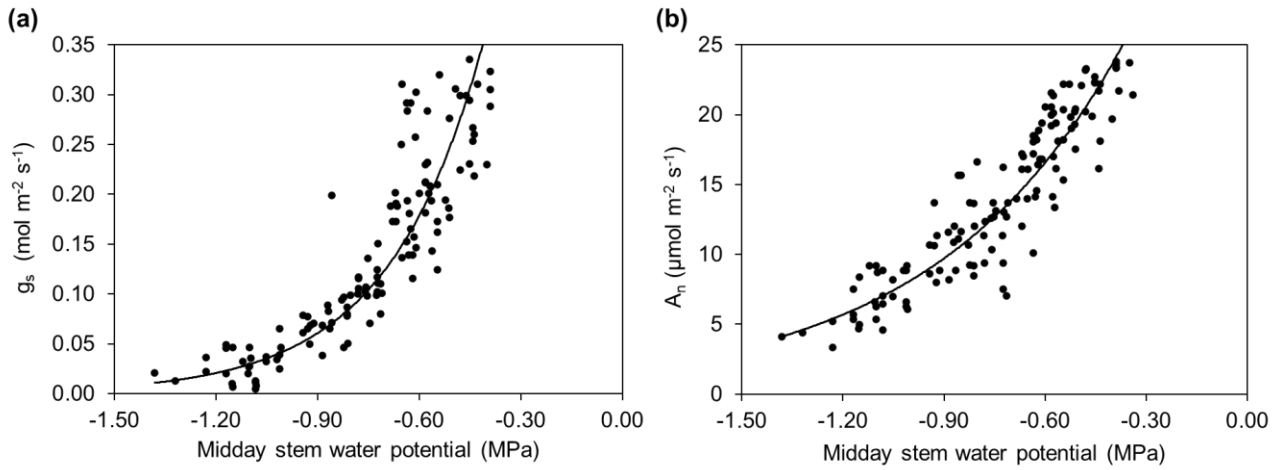


Figure 4.11 The response of stomatal conductance and photosynthesis to increasing water stress as indicated by midday stem water potential in pecan trees: (a) stomatal conductance versus midday stem water potential and (b) photosynthesis versus midday stem water potential. Data was pooled from all treatments and at all measurement dates.

4.3.5 Transpiration

Diurnal measurements of transpiration when the trees were mildly stressed, as indicated by A_n, g_s and ψ_{smd} measurements, at three phenological stage (flowering and nut set, nut sizing and nut filling) showed clear differences between water stress treatments and the well-watered control (Figure 4.12). Though more or less the same canopy sized trees were selected, canopy size differences were observed during the first and the last phenological stage. The control typically had greater transpiration rates throughout the day as compared to the water stress treatments across all phenological stages. This was particularly evident at midday. When integrating the area under the curve for Figure 4.12 to obtain a daily value, it was evident that transpiration was higher in controlled trees than stressed trees. Water stress at flowering and nut set decreased daily transpiration rate from a value of 0.88 mm day^{-1} for the well-watered control to 0.67 mm day^{-1} for the stressed tree, 1.17 to 0.93 mm day^{-1} for the well-watered control relative to the stress

treatments during stress at nut sizing and from 1.24 to 0.81 mm day^{-1} during water stress at nut filling stage.

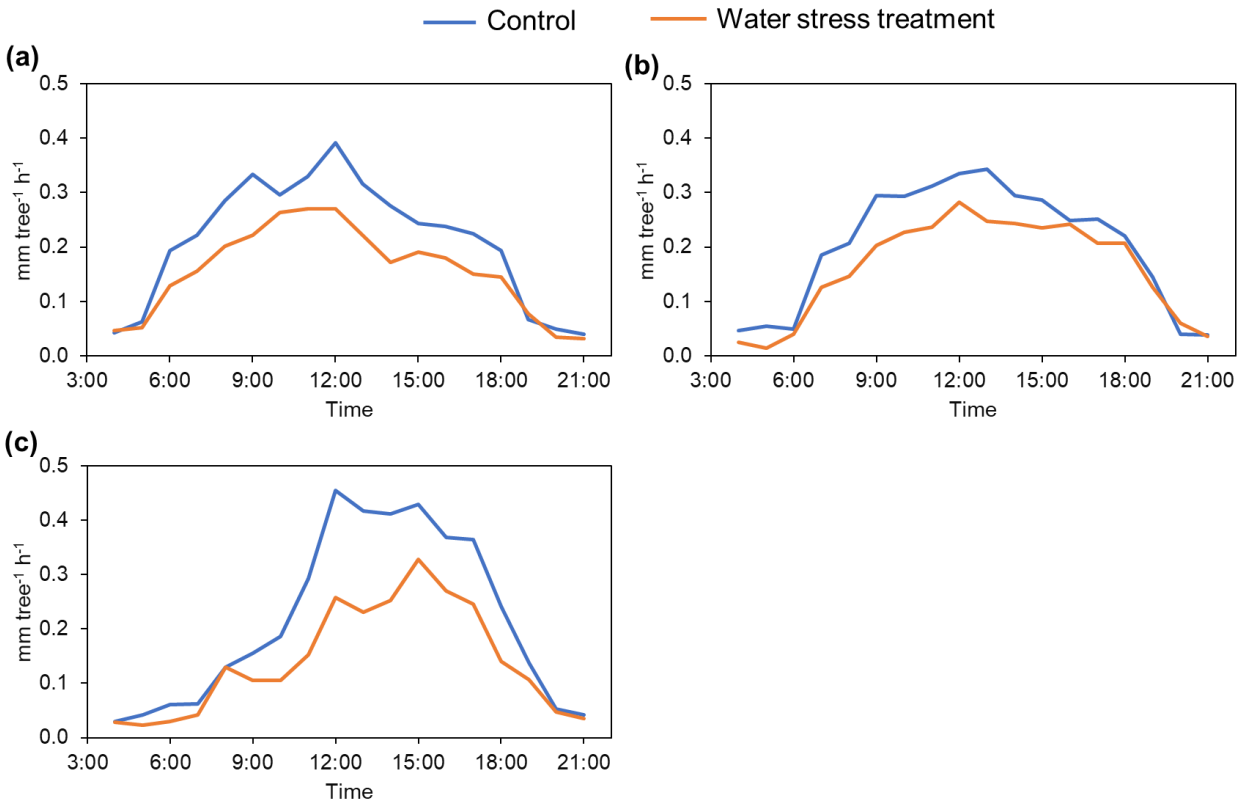


Figure 4.12 Diurnal transpiration at three phenological stages: (a) flowering and nut set (06/11/2018), (b) nut sizing (19/01/2019) and (c) nut filling stage (14/03/2019). Data was for different specific days when the midday stem water potential measurements indicated the trees were mildly stressed and when stomatal conductance and photosynthesis measurements took place.

Daily cumulative transpiration rates were calculated for each phenological stage in the second season (2019/20) and are presented in Figure 4.13a, b & c, to understand the effect of water stress at each phenological stage on transpiration relative to the well-watered control. The daily cumulative transpiration rates over each phenological stage were lower in the water stressed treatment compared to the well-watered control. The highest cumulative transpiration was recorded during the second phenological stage of nut sizing (Figure 4.13b), as this stage occurred over the hottest part of the season, when canopy cover was at a maximum.

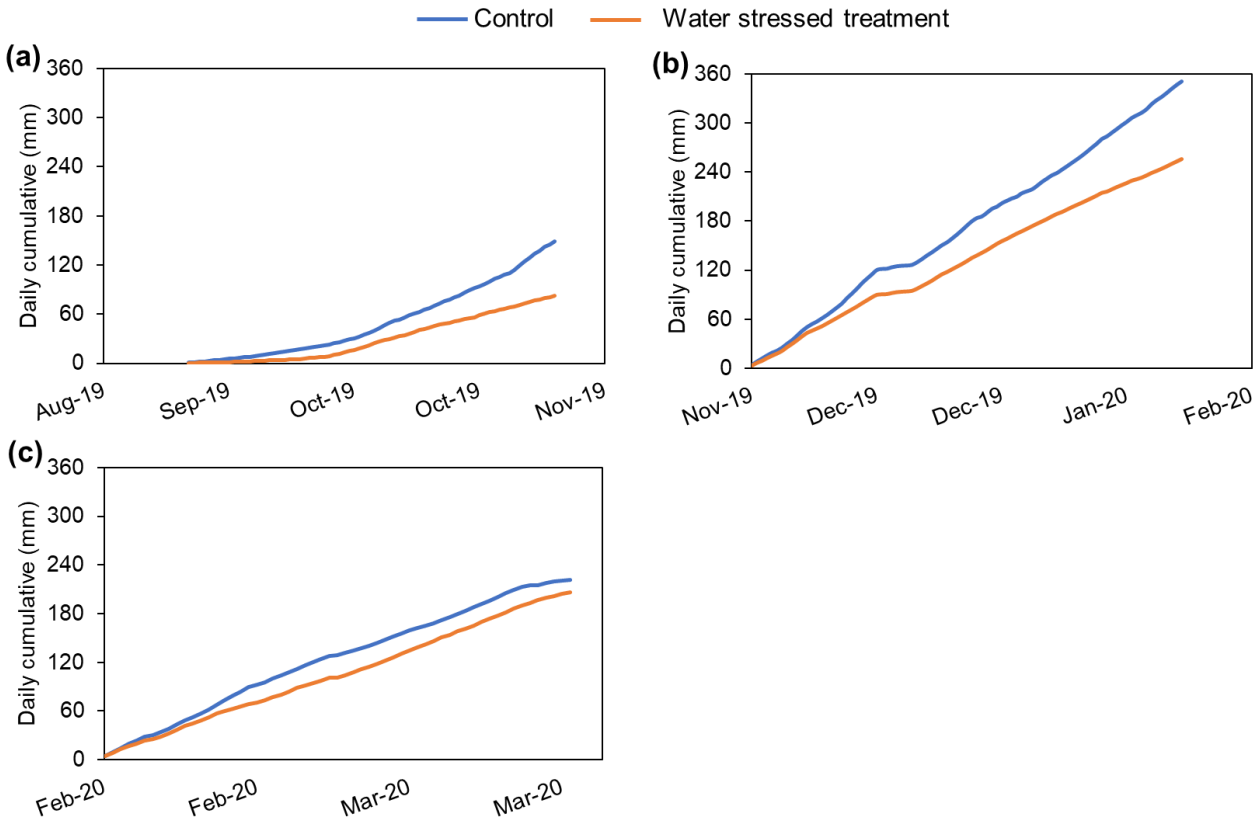


Figure 4.13 Cumulative transpiration rates between the well-watered control and the water stressed treatments: (a) flowering and nut set (September to November, 2019), (b) nut sizing (November, 2019 to February, 2020) and (c) nut filling stage (February to March 2020). Data presented was taken in the 2019/20 season.

4.4 Discussion

The weather conditions during the three seasons of the study were favourable for pecan production and it is unlikely that any weather conditions compromised production, for example, hail events, extreme temperatures or very humid conditions that can cause scab. Differences between treatments were therefore most likely attributable to the imposed water stress and not vastly different conditions during different phenological stages: Nonetheless, Wells (2015) pointed out that at nut filling stage, soil water is not the only factor influencing nut fill, but poor nut fill can also be associated with moderate to heavy crop loads. When there are too many nuts, the tree cannot provide the photosynthates to fill all the nuts and thus a greater percentage of poorly filled nuts or pops are often associated with heavy crop loads. The degree of poorly filled nuts is thus a function of both water stress and crop load. Moreover, various authors reported that high crop loads lead to higher photosynthetic rates (Syvertsen et al. 2003, Silber et al. 2013, Bustan et al. 2016). Even so, this study does not support either of the two scenarios, since the percentage pops in the control treatment was low and crop load was very similar between the control and stress treatment at nut filling at the start of the stress period. This suggests that the differences observed between the control and water stress treatments were due largely to water limitation, with crop load not having a major contribution. However, the significant rainfall event at the end of the nut filling and start of the shuck dehiscence stage (March – April 2018) in the first season resulted in significant fruit split, as a result of the sudden increase in turgor in the stressed trees during these stages. Seasonal weather conditions over the course of each season also impacted irrigation volumes. Periods with higher VPD and ET_o and lower rainfall required higher irrigation volumes, for example, during nut sizing in the first season and during flowering and nut set in the second and third season. In general, the 2018/19 season required higher irrigation volumes relative to the other two seasons, due to hotter and drier conditions during this season, as indicated by higher seasonal ET_o.

Through measurements of ψ_{smd} and ψ_{pd} throughout the three seasons at each phenological stage, it was evident that a water stress was successfully implemented at each phenological stage for the majority of the trial. During each phenological stage ψ_{smd} and ψ_{pd} for the water stressed trees was significantly lower than the control, with ψ_{smd} falling below the threshold value of -0.90 MPa defined by Othman et al. (2014). The only exception was during the shuck dehiscence stage in the first season when ψ_{smd} did not fall beyond the desired threshold value (-0.90 MPa). However, during this period there were significant differences between stressed treatment and the control.

In addition, a short period of water stress was experienced by the well-watered control during the nut sizing stage during the second season due to an irrigation system breakdown for a few weeks. In the third season, heavy rains (260 mm in 8 days) during the nut sizing stage led to a sudden increase in ψ_{smd}, which was also above the threshold value (-0.90 MPa). However, this lasted for a short period of approximately 3 weeks. Based on the ψ_{smd} threshold value of -0.90 MPa identified by Othman et al. (2014) and the relationship between ψ_{smd} and ψ_{pd} determined in this study, it is suggested that a ψ_{pd} value of -0.42 MPa corresponds to the onset of moderate stress in pecans.

The decline in plant water status of the trees, as observed when Ψ_{smd} fell below -0.90 MPa, led to a reduction in g_s in the water stressed treatments relative to the control at most phenological stages, as also observed by Samani et al. (2011) and Othman et al. (2014). Importantly, the reduction in g_s in the water stressed treatments relative to the control was not always significant, as the standard error was quite high. However, g_s was significantly reduced during the nut filling stage. According to Dalmolin et al. (2013), strong stomatal control can be considered to be a survival mechanism for plants in flood and drought conditions, as water loss by transpiration under these conditions cannot be counterbalanced by absorption. Therefore, Cifre et al. (2005) considered stomatal closure to be among the first leaf responses to water deficits and tends to protect the plant from excess water loss, which ultimately leads to cell dehydration and death (Chaves et al. 2003, Lisar et al. 2012). Consequently, the swift decline in stomatal conductance in water stressed trees led to reduction in A_n. This is because stomatal closure is proposed to be the major cause of limitations to photosynthesis under water deficit conditions, as a result of the decline in CO_2 uptake (Chaves 1991, Chaves et al. 2002, Lawlor and Cornic 2002, Pirasteh-Anosheh et al. 2016). Water stress was demonstrated to result in a decrease in photosynthetic activity in a variety of crops, including hazelnut, which was sometimes also coupled with early leaf fall in a number of crops (Bignami and Natali 1996, Dias et al. 2004, Bignami et al. 2010) including, pistachio (Ranjbarfordoei et al. 2000), almonds (Goldhamer et al. 2006), olives (Angelopoulos et al. 1996) and pecan (Smith and Huslig (1990), Othman et al. (2014)).

Davies and Zhang (1991), Chaves et al. (2002) and Farooq et al. (2009) reported that stomatal closure is associated with a drying soil, and that this response is possibly mediated by chemical signals such as abscisic acid (ABA) travelling from the dehydrating roots to the shoots. A decline in soil water, coupled with an increase in ABA, may possibly be responsible for the decrease in

light utilization observed for pecan trees exposed to water stress in the different phenological stages. Light response curves (*A_n/PPFD)* of the stressed treatments at all stages revealed that water stress caused significant reductions in A_n and the light compensation point compared to the well-watered control. These reductions might be due to a low light utilization or the existence of other factors (carbon dioxide, temperature) which are limiting to photosynthesis by the water stressed trees. In this study R_d values for the water stressed treatments were reduced relative to the well-watered control treatment. This is in contrast to the findings of Flexas et al. (2005), where water stress tended to increase respiration. The reasons for reduced respiration in pecans under water stressed conditions therefore requires further investigation. However, the reduced R_d for the water stressed treatments could explain the lower light compensation point determined from the light response curves. Lower respiration means the trees require slightly less photosynthesis to reach the compensation point where photosynthesis balances respiration.

Again, since stomatal conductance declines with a rising soil water deficit, A_n is inhibited due to low intercellular CO_2 *(Ci)* (Tezara et al. 1999, Cifre et al. 2005, Othman et al. 2014). This is an indication of a stomatal limitation to photosynthesis, with A_n decreasing because not enough CO_2 enters the leaf. However, from all the parameters determined from the A/C_i curves, the reduction in photosynthesis in water stressed pecan trees may not solely result from stomatal limitations, but also non-stomatal limitations may also be present. The analysis of A/C_i response curves showed a significant decline in CO_2 assimilation rates in water stressed trees at higher CO_2 concentrations and corresponded with a reduction in RuBP carboxylation capacity (V_{cmax}), RuBP regeneration (expressed as the maximum rate of electron transport, J_{max}) and triose phosphate use (TPU) at all phenological stages relative to the control. Various studies have suggested that a decline in photosynthesis under water stressed conditions is attributed to the impact on the photosynthetic apparatus, which includes a shortage of ATP, RuBP carboxylation capacity and $J_{max,}$ limitation, for example this has been found in olives (Giorio et al. 1999), *Vochysia divergens* (Dalmolin et al. 2013) and apples (Wang et al. 2018).

There was also marked decline in transpiration in water stressed trees relative to the well-watered control, which was most likely attributable to the reduction in stomatal conductance in most of the phenological stages. Berry et al. (2010) and Pirasteh-Anosheh et al. (2016) mentioned stomatal closure as a plant water control mechanism following a rising soil water deficit, which serves to reduce transpiration and ensure that the balance is maintained between water uptake and water

loss. In line with our results, Álvarez et al. (2011) reported a reduction in the rates of transpiration by 63% in water stressed Callistemon plants compared to control plants (well-watered) as a result of the substantial decline in stomatal conductance in the water stressed plants (60 mmol m^{-2} s^{-1}) as compared to control plants (130 mmol m^{-2} s^{-1}) in. These physiological changes also led to a slight delay in canopy growth to full size following water stress at the start of the season, when water stress was implemented during bud break, flowering and nut set. This could have been due to non-uniform bud break as reported by Miyamoto (1989) under water stress conditions during bud break and flowering. Furthermore, Miyamoto (1989) reported that tree growth vigour was reduced under water stress conditions, impacting nut growth and development, resulting in poor fertility, which ultimately led to fruit abortion. Reports by Wells and Harrison (2010) and Wells (2016b), indicated that adequate soil moisture in pecans is necessary to stimulate strong, vigorous growth from budbreak through shell hardening for nut size, and during the nut filling stage for optimal kernel percentage. The reduction in canopy growth as a result of water stress at the start of the season agrees with reports by Rahmati et al. (2018), where a decline in vegetative growth due to a decrease in plant water status was demonstrated in peaches. In addition, our study demonstrated that water stress at the late stage (shuck dehiscence) hastened leaf senescence compared to the well-watered control. Brevedan and Egli (2003) found similar results, highlighting that water stress hastened leaf fall, more especially when the crop approaches maturity. Moreover, Sparks (1992), Byford and Herrera (2005b) and Wells and Conner (2007), also observed water stress at the late stage to negatively impact shuck split and energy reserves for the next season's growth, which also has implications for yield in the following season.

4.5 Conclusion

Water stress was successfully induced in the trees for each phenological stage for most of the trial, however, issues were experienced during periods of heavy rains and due to irrigation breakages at some stages, though to a minor extent. A drop in ψ_{smd} below -0.90 MPa and a corresponding and ψ_{pd} value of -0.42 MPa was observed during the majority of the trial as a result of water stress and was associated with a decline in the well-known physiological measures, that is, g_s, A_n and transpiration, and suggests that these values indicated the onset of stress in this trial. The reduction in stomatal conductance ultimately led to decline in carbon assimilation and transpiration. In addition, water stress tended to delay canopy development and hasten leaf

senescence. Early leaf senescence could compromise reserves for the following season's growth. Early season stress could also possibly impact nut set and development, especially at flowering and nut set stage, which may lead to greater fruit abortion, thereby reducing the final yield. Thus also, the decline in photosynthesis during each stage could possibly impact both yield and quality. As a result, smaller sized nuts, which fetch a lower price, poorly filled nuts (increased number of pops) and increased number of stick-tights could possibly be produced due to a decline in CO_2 assimilation as a result of water stress at nut sizing, nut filling and shuck dehiscence stages.

CHAPTER 5: THE IMPACT OF WATER STRESS AT DIFFERENT PHENOLOGICAL STAGES ON YIELD AND QUALITY OF PECANS

5.1 INTRODUCTION

Fernandes et al. (2018) stated that yield is the final target in agricultural systems and is highly determined by fruit growth and development in fruit trees. Production is greatly determined by soil water availability, among other factors, for instance leaf area and crop load (Naor et al. 2001, Morandi et al. 2014). Fruit growth is sensitive to water deficits, which decreases fruit biomass accumulation, tissue expansion and cell number (Tardieu et al. 2011). Small sized fruits as a result of water stress were reported in apples (Naor 2006) and pears (Shackel 2007), whilst reduced kernel size and nut mass were reported in almonds (Goldhamer et al. 2006), walnuts (Fulton et al. 2006) and hazelnuts (Bignami et al.). Therefore, Johnson and Handley (2000), concluded that water stress reduces yield in cultivated crops, with negative impacts demonstrated in a number of studies (Hanks 1983, Fereres et al. 2012). Fereres et al. (2012) suggested that water stress should be avoided at all stages of growth and development. However, this suggestion may not necessarily hold true in many situations and there may be opportunities for water savings with no impact on production (Johnson and Handley 2000). This is mostly true for several fruit trees and vines, where water deficits have been manipulated through deficit irrigation strategies to improve yield and quality of the fruits (Marsal Vila 1997, Goldhamer et al. 2006, Fereres and Soriano 2007, Tejero et al. 2018). Such improvements were found in almonds (Tejero et al. 2018), plum (Crisosto and Crisosto 2005), peach (Jackson 2003), pear (Crisosto et al. 2004), olives (Moriana et al. 2003) and in vine grapes (Bravdo et al. 2003), where sugar/acid ratios or sugar content, oil accumulation, together with other chemical compounds responsible for flavour and aroma, were increased under mild water stress.

In pecans, yield is a function of the number of nuts per cluster, determined by flowering and nut set, the number of fruiting positions, determined by shoot growth, and nut mass, determined by nut growth (Miyamoto 1989, Sparks 2001, Goldhamer 2003, Byford and Herrera 2005b, Call et al. 2006, Valentini et al. 2015, Wells 2015). Yield of pecan trees is also influenced by the alternate bearing nature of these trees, where a large crop is produced in one year, followed by a smaller crop in the following year (Sparks 2005). In pecans, water stress reduces flowering, nut set and yield during the early stages of development (Miyamoto 1989). Sparks (2001) found that soil water availability during the nut elongation and expansion phases influenced the final size and shape of pecan nuts, with water stress resulting in small sized and misshaped nuts. However, a study by

Wells (2016a) in the southeastern U.S.A., indicated that reduced irrigation early in the season did not impact tree yield, or quality, suggesting that pecan trees can tolerate moderate water stress early in the season. This suggests that in pecan not all phenological stages are equally sensitive to water stress and stress during some stages may not result in a drastic reduction in yield and quality. It was hypothesized that: 1) water stress during flowering and fruit set will result in lower nut set and more nuts drop. As a result, there will be fewer nuts on the tree, but the nuts are likely to be bigger at harvest than on the control trees receiving full irrigation, 2) a lack of sufficient water during nut sizing will result in more small unmarketable nuts and excessive nut drop, 3) stressing trees at the nut filling stage will result in poor nut filling, since nut filling is mainly influenced by the condition of the leaves and may result in nut drop since the nuts fail to fill, thus reducing yield and quality, and 4) stressing the trees after nut filling will result in delayed shuck opening thereby producing more stick-tights, reducing yield and making harvesting difficult. To test these hypotheses, the following objective was formulated: To assess the growth and development, as well as the final yield and quality, of the nuts in relation to the phenological stage at which the stress was implemented.

5.2 Materials and methods

Detailed materials and methods are presented in Chapter 3: General materials and methods, sections 3.4 and 3.5.

5.3 Results

5.3.1 Flowering and nut set

The presence of female flowers on the trees were recorded from 5 October 2018 to 6 November 2018 in the second season (Figure 5.1a) and from 3 October 2019 to 13 November 2019 in the third season (Figure 5.1b). The duration of flowering and nut set was approximately five weeks in both seasons (refer to Chapter 3, section 3.4 for materials and methods). During the first week after female flower appearance (WAFFA), the average number of female flowers per cluster was approximately 8 across all the treatments, with no statistical differences between treatments (Figure 5.1a,b). However, differences between treatments started to become apparent from the second WAFFA in both seasons, with a reduction in flower number in the treatment where water

stress was applied during flowering and fruit set. These differences became significant in the fourth and fifth WAFFA, when the total number of female flowers or nuts set per cluster in the water stressed treatment dropped significantly compared to the other treatments in both seasons. The final average nut set in the last week (week 5) for the water stressed treatment was approximately 3 nuts per cluster (43% nut set) and approximately 5 nuts set per cluster (63% nut set) in the other treatments, which received sufficient water during this stage in both seasons (Figure 5.1a,b). This indicates that water stress at this stage (flowering and nut set) resulted in an increased abortion of flowers, and thus, assuming a similar number of clusters per tree, a reduction in the final number of nuts set compared to the well-watered treatments.

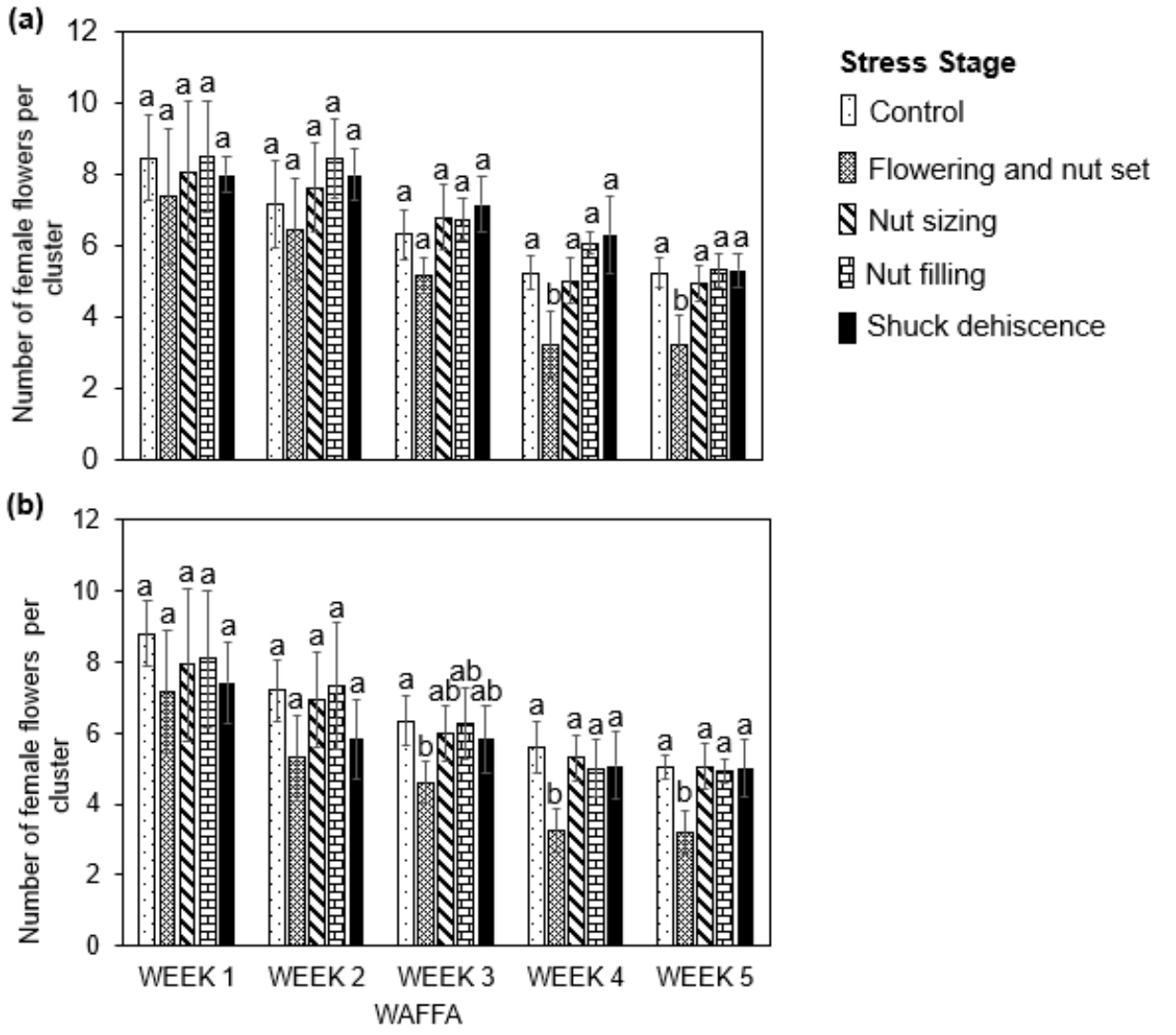


Figure 5.1 The impact of water stress during the flowering and nut set stage on the number of female flowers per cluster for the (a) 2018/2019 season and (b) 2019/2020 season. Only the flowering and nut set treatment was stressed during this period. Treatments with the same letter are not significantly different from each other ($p < 0.05$). WAFFA is week after female flower appearance.

5.3.2 Nut growth

Nut growth, in terms of both length and diameter, was evaluated on a weekly basis after nuts were successfully set until they reached their final size, as indicated by a plateau in growth (Figure 5.2). Nut growth lasted for 18 weeks after nut set (WANS) from the 1 November 2018 to 19

February 2019 in the second season and from 10 November 2019 to 22 February 2020 in the third season. In the 2018/19 season nut length of the control treatment increased from 0.83 cm to 5.01 cm, whilst in the water stressed treatment nut length increased from 0.79 cm to 4.80cm (length) (Figure 5.2a). Nut diameter of both the control and water stressed treatment increased from 0.41 cm to 2.72 cm (Figure 5.2c). Likewise, in the 2019/20 season, there was a similar increase in nut length from 0.79 cm to 5.08 cm in the control and 0.80 cm to 4.79 cm for the water stressed treatment and in terms of nut diameter there was an increase from 0.49 cm to 3.01 cm in the control and 0.46 cm to 2.85 cm in the water stressed treatment (Figure 5.2b,d). Final nut size, both length and diameter, was achieved 15 weeks after the start of measurements in both seasons. However, what is worth noting are the differences from week number 7 (second season) and week 9 (third season) in terms of nut elongation rate, which was slightly decreased in the water stressed treatment. This resulted in slightly reduced final nut size by 0.21 cm in the second season and 0.29 cm in the third season compared to the control (Figure 5.2a,b). Differences in nut expansion (diameter) were also observed from week number 13 (second season) and week number 12 (third season), with the nut expansion rate decreased slightly in the water stressed treatment. Final nut size (diameter) was reduced by 0.07 cm in the second season and 0.16 cm in the third season relative to the nuts in the well-watered control (Figure 5.2c,d).

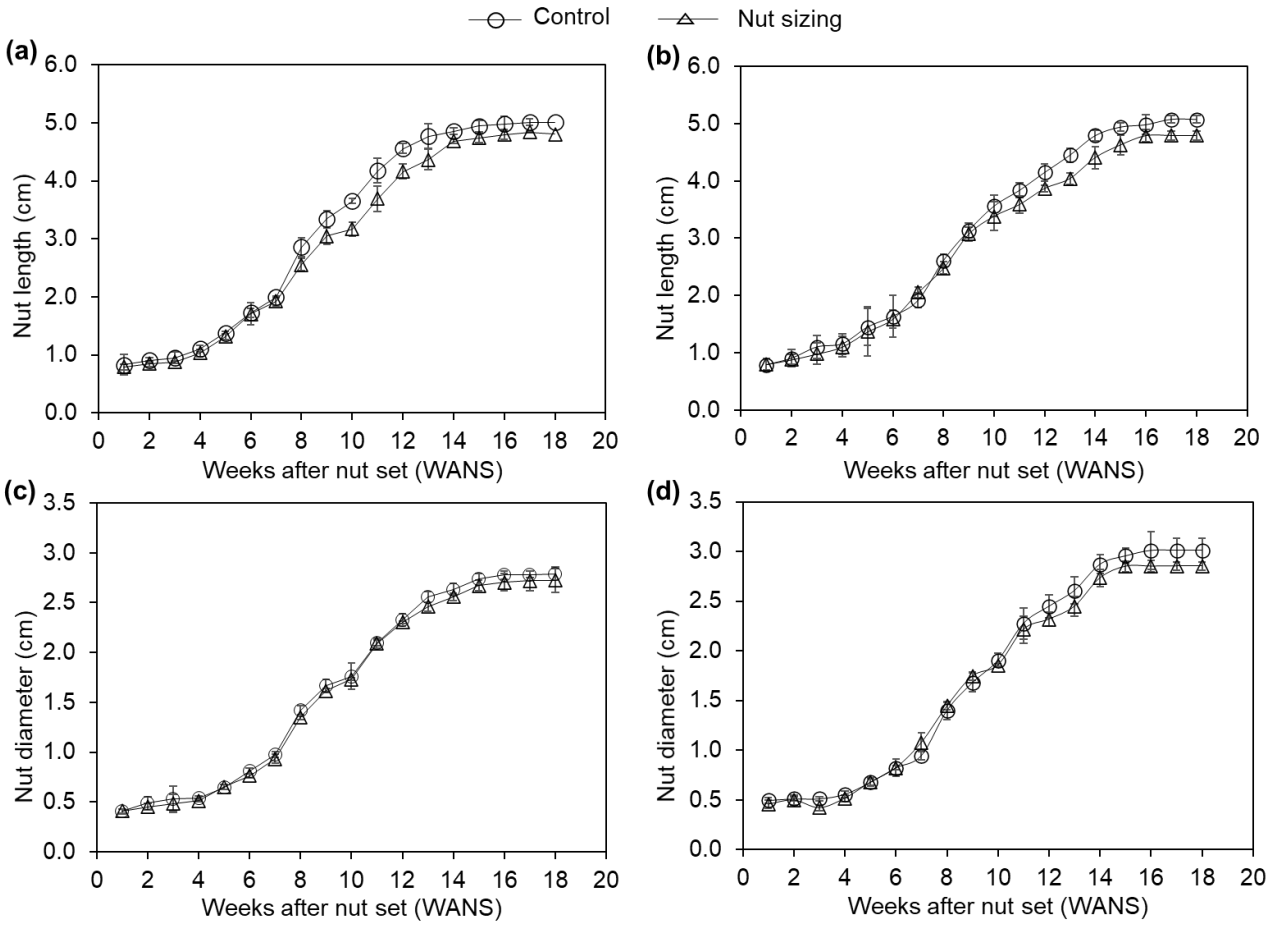


Figure 5.2 Nut elongation (length) during the (a) 2018/19 season (1 November to 19 February) and (b) 2019/20 season (10 November to 22 February) and nut expansion (diameter) during the (c) 2018/19 season, (d) 2019/20 season as influenced by water stress during the nut sizing stage.

5.3.3 Nut drop

The assessment of nut drop was done for three seasons after pollination, when nut set had been completed, to evaluate the effects of water stress at different phenological stages on nut retention for the first three stages (flowering and nut set, nut sizing and nut filling) during the trial, for the three seasons (refer to Chapter 3, section 3.4 for materials and methods). A significantly higher percentage of nut drop was evident when stress was implemented during flowering and nut set across all seasons, where 20% was observed during the first, 22% during the second and 19% during the third season (Figure 5.3a). Nut drop was also noted at nut sizing during the first season

(18 %) and last season (13 %), (Figure 5.3a,c). However, nut drop during nut sizing stage was only significantly different to the control during the first season. In the second season, a significant difference was only observed at flowering and nut set stage. Water stress during nut filling did not result in any nut drop that was significantly different to the well-watered control in all seasons.

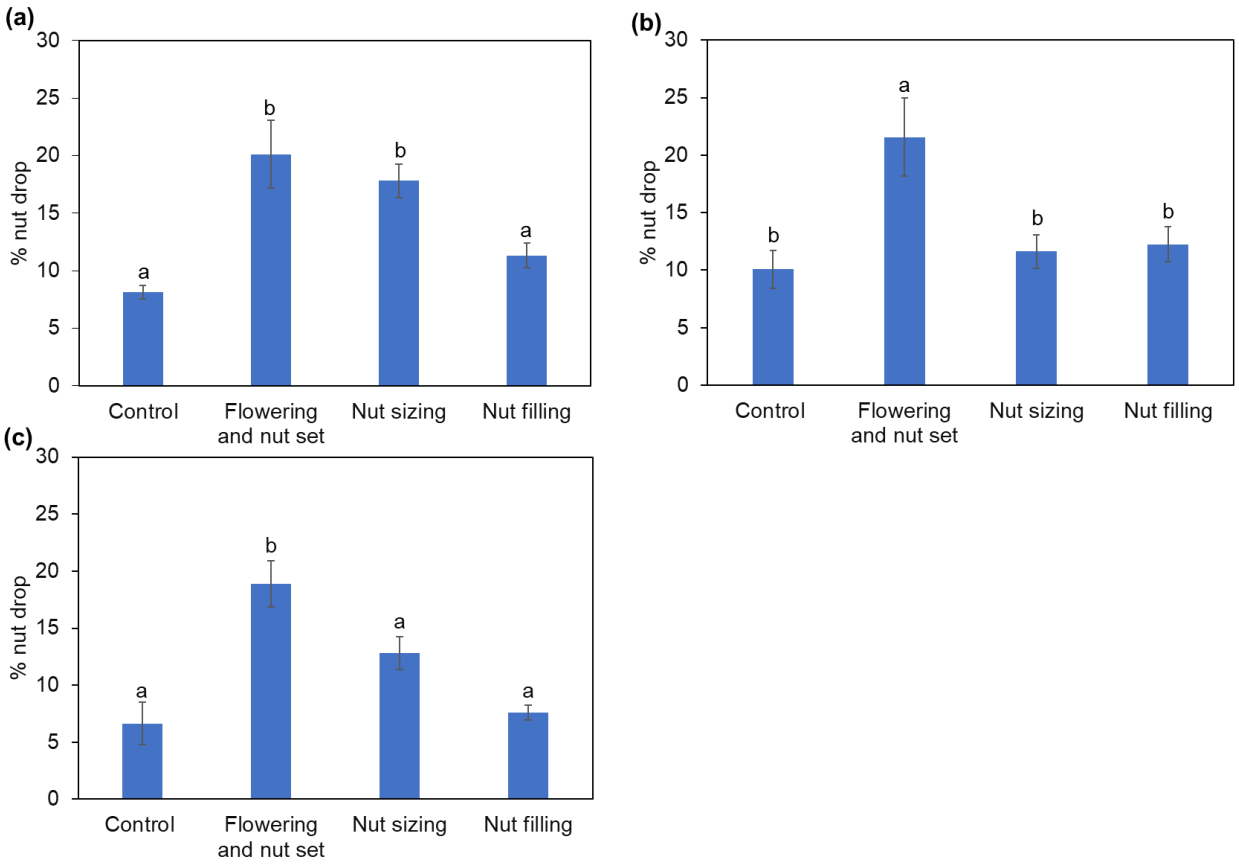


Figure 5.3 Percentage nut drop in the (a) 2017/2018 season, (b) 2018/2019 season and (c) 2019/2020 season for the first three stages (flowering and nut set, nut sizing and nut filling) during the trial. Treatments with the same letter are not significantly different from each other ($p<0.05$).

5.3.4 Yield

Yield per tree was determined immediately after completion of the harvest and the results are shown in Figure 5.4. For the first season in 2017/2018, water stress during flowering and nut set, and nut filling, significantly reduced yield relative to the well-watered control from 1.70 to 0.89 t

ha^{-1} for water stress during flowering and nut set and from 1.70 to 1.05 t ha^{-1} for water stress during nut filling (Figure 5.4a). However, water stress during nut sizing and shuck dehiscence did not result in any differences in yields between these treatments and the control. In the second season in 2018/2019, stress during flowering and nut set yielded 1.03 t ha^{-1} and nut filling was 1.15 t ha^{-1}, which was significantly lower compared to the control with 1.53 t ha^{-1} (Figure 5.4b). However, stress during nut sizing and shuck dehiscence did not show any effect on yield when compared to the well-watered control. In the third season in 2019/2020, stress during flowering and nut set yielded 2.78 t ha^{-1}, which was not significantly different to the control which was 2.68 t ha^{-1}. This was probably due to a breakdown in the irrigation system supplying the control treatments during the flowering and nut set period in this season, which resulted in stress in the control treatment. Nonetheless, water stress at the nut filling stage significantly reduced the final yield to 2.21 t ha^{-1} compared to the 2.68 t ha^{-1} of the control. Again, there were no significant differences in the yields obtained when water stress was implemented during nut sizing and shuck dehiscence and the control. Worth noting in this season is the higher yields obtained compared to the previous two seasons across all the treatments (Figure 5.4c). This could be due to the alternate bearing nature of the trees as described by Sparks (2005). It could also be attributed to the trees reaching maturity and ideal conditions during the growing season.

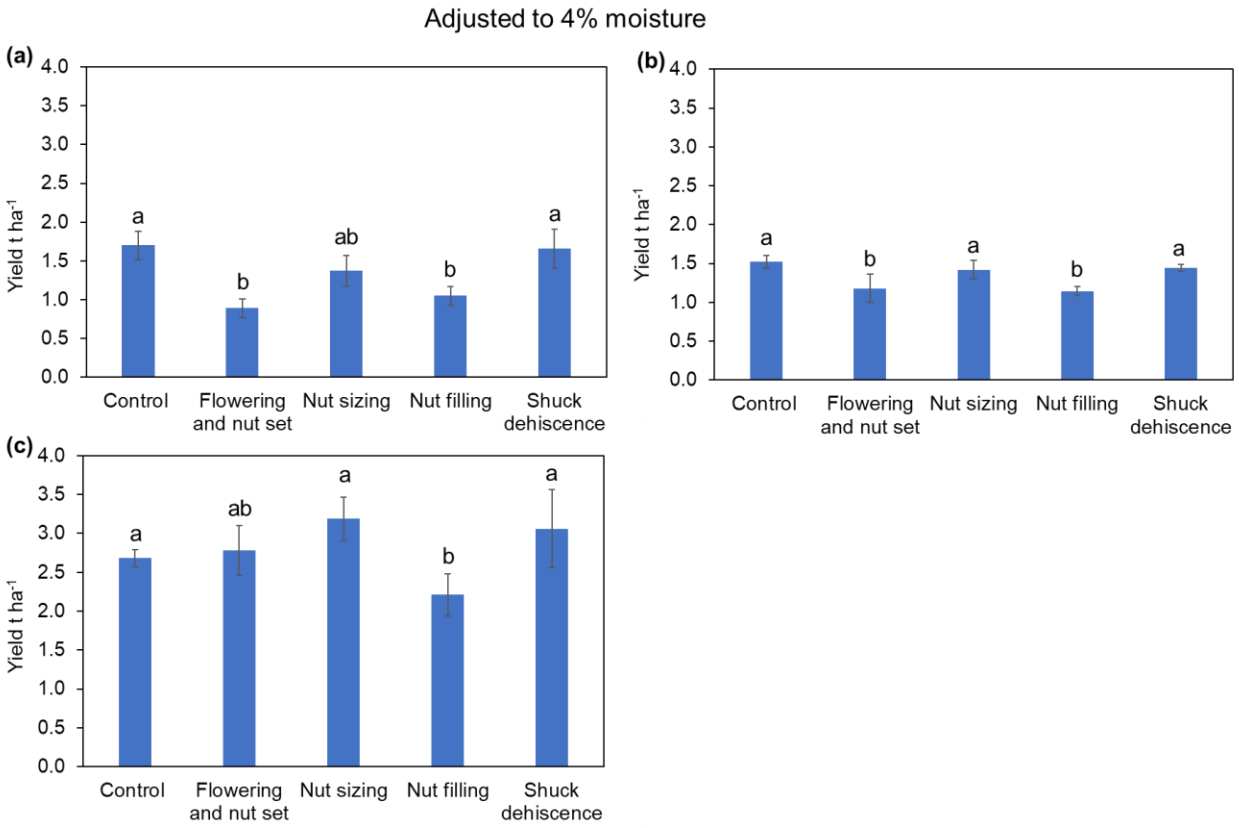


Figure 5.4 Average yield for the different stress treatments (n=4) in the (a) 2017/2018 season, (b) 2018/2019 and (c) 2019/2020 season. Yield was adjusted to 4% moisture content. Treatments with the same letter are not significantly different from each other ($p < 0.05$).

5.2.5 Quality

The quality parameters for the three seasons are presented in Tables 5.1, 5.2 and 5.3. For the first season (2017/2018), stress during the nut filling stage resulted in a significantly great percentage of wafer/air pockets and higher number of nuts per kg when compared to the other treatments (Table 5.1). Water stress had no significant effect on kernel percentage during any of the phenological stages, excluding pops. For the second season 2018/2019, there were no significant differences in the average diameter of nuts, the kernel % and the moisture % between the different treatments (Table 5.2). There was a significantly higher average number of nuts per kg for the treatments where water stress was implemented during the nut sizing and nut filling stages compared to the well-watered control, indicating smaller nuts for these treatments relative to the control. Water stress at nut filling also resulted in an increase in the % of unsound kernel and wafers or air pockets relative to the well-watered control. Lastly, stress during maturity and

the shuck dehiscence stage resulted in an increased percentage of stick tights (14.4%) (both kernel filled and pops), which was significantly higher than any other treatment.

There were no significant differences in the average diameter, kernel % and moisture % between treatments in the 2019/2020 season (Table 5.3). Stress implemented during nut sizing resulted in a greater number of nuts per kg as compared to all the other treatments, once again indicating smaller nuts compared to the other treatments. As in the previous two seasons, water stress at nut filling resulted in poorly filled nuts (greater wafers or air pockets %) and a greater percentage of unsound kernel. Water stress during maturity and shuck dehiscence stage resulted in an increased percentage of stick tights (10%), which was significantly higher than in any other treatment.

Table 5.1: Pecan nut quality parameters including number of nuts per kg, percent kernel, moisture percentage and wafers/air pockets percentage for the well-irrigated control and water stressed treatments in the 2017/2018 season. Letters denotes size distribution (L for large, M for medium).

Treatment	No. of nuts kg^{-1}	Average nut size	Kernel %	Moisture %	Wafer/air pockets %
Control	163a	L	55.2a	2.2a	15.8a
Flowering and nut set	166a	L	54.3a	2.5b	15.4a
Nut sizing	161a	L	55.5a	2.3a	15.4a
Nut filling	177b	M	55.6a	2.1a	24.1b
Shuck dehiscence	164a	L	54.4a	2.3a	15.5a

Treatments with the same letter are not significantly different form each other ($p<0.05$)

Table 5.2: Pecan nut quality parameters including number of nuts per kg, nut size, percent kernel, moisture percentage, unsound kernel (percentage pops), wafers/air pockets percentage and percent of stick tights for the well-irrigated control and water stressed treatments in the 2018/2019 season. Letters denotes size distribution (L for large, M for medium).

Treatment	No. of nuts kg^{-1}	Average nut size	Kernel %	Moisture %	Unsound Kernel (% pops)	Wafer/air pockets %	Stick tights %
Control	153 ± 1.07a	L	58.4 ± 0.57a	3.0 ± 0.59a	2.5 ± 0.47a	13.9 ± 0.34a	6.2 ± 0.76a
Flowering and nut set	152 ± 0.89a	L	62.0 ± 1.09a	2.2 ± 0.09a	2.0 ± 0.43a	14.1 ± 0.44a	6.8 ± 0.73a
Nut sizing	174 ± 1.88b	M	57.0 ± 0.64a	2.0 ± 0.18a	1.6 ± 0.32a	14.7 ± 0.46a	7.2 ± 0.58a
Nut filling	172 ± 2.23b	M	58.0 ± 0.46a	2.0 ± 0.17a	7.9 ± 0.68b	22.3 ± 0.67b	7.1 ± 0.67a
Shuck dehiscence	159 ± 1.61a	L	57.0 ± 0.55a	2.0 ± 0.07a	2.9 ± 0.67a	14.3 ± 0.43a	14.4 ± 1.25b

Treatments with the same letter are not significantly different form each other ($p < 0.05$).

Table 5. 3: Pecan nut quality parameters including number of nuts per kg, nut size, percent kernel, moisture percentage, unsound kernel (percentage pops), wafers/air pockets percentage and percent stick tights for full irrigated control and water stressed treatments in the 2019/2020 season. Letters denotes size distribution (L for large, M for medium and S for small).

Treatment	No. of nuts kg^{-1}	Average nut size	Kernel %	Moisture %	Wafer/air pockets	Unsound Kernel (% pops)	Stick tights %
Control	165 ± 4.43a	L	59.8 ± 1.13a	3.0 ± 0.29a	6.6 ± 0.94a	2.5 ± 0.90a	5.1 ± 1.78a
Flowering and nut set	160 ± 5.19a	L	60.0 ± 1.17a	3.1 ± 0.09a	6.1 ± 1.76a	2.0 ± 0.73a	5.0 ± 2.14a
Nut sizing	216 ± 6.34b	S	59.3 ± 1.96a	3.0 ± 0.13a	7.6 ± 2.20a	1.6 ± 0.40a	3.9 ± 1.07a
Nut filling	171 ± 1.77a	M	58.3 ± 2.12a	3.0 ± 0.05a	18.3 ± 3.57b	9.0 ± 0.54b	4.5 ± 2.85a
Shuck dehiscence	167 ± 1.41a	L	59.0 ± 2.66a	3.0 ± 0.05a	7.1 ± 2.31a	5.3 ± 1.02a	10.1 ± 1.03b

Treatments with the same letter are not significantly different form each other ($p < 0.05$).

5.2.6 Gross income as a result of imposing water stress at key phenological stages

Gross income per season was determined after the nuts were graded (Table 5.1, 5.2 and 5.3) and classified for pricing as per Elandsdraai Pecan Growers price breakdown for all three seasons (Table 5.4). However, a decline in nut price was observed in the 2019/20 season due to the world's pandemic (COVID-19) affecting the market. Nonetheless, the second season's overall income decreased, whilst income was increased in the third season, which might reflect the alternate bearing nature of the crop (Table 5.5). Stress imposed during each phenological stage resulted in a reduction in income in the first two seasons, which was particularly evident during flowering and nut set and nut filling stages for both seasons. This reflects the reduction in yield as a result of these treatments. In the third season gross income for the treatments that were imposed during flowering and nut set and shuck dehiscence was higher than the control, as a result of reduced yield in the control relative to these treatments. This again reflects the stress experienced in the control treatment during flowering and fruit set due to the breakdown of the irrigation system. However, income received for trees stressed during the nut filling stage was again reduced relative to the control, indicating the importance of avoiding stress during this period.

Table 5. 4: Nut grading (classification of nuts kg $^{-1}$) for 'Wichita' variety and price breakdown as provided by Elandsdraai Pecan Growers for the 2017/18, 2018/19 and 2019/20 seasons. Letters denotes size distribution (XS for extra small, S for small, M for medium, L for large, XL for extra large and OS for oversize)

SA-Size/Classification of nut kg^{-1}	Base price (50% kernel)	
	2017/18 & 2018/19 seasons	2019/20 season
10 (XS) >265	R32.00	R26.00
11 (XS) >265	R37.00	R33.00
12 (S) 211-264	R45.00	R41.00
13 (M) 171-210	R52.00	R47.00
14 (L) 141-170	R60.00	R51.00
15 (XL) 121-140	R64.00	R56.00
16 (OS) <120	R67.00	R61.00

Table 5.5: Gross income received as a result of imposing water stress at different phenological stages per hectare (ha^{-1}).

Season	2017/2018	2018/2019	2019/2020
Control	R102 150.00	R91 609.50	R136 680.00
Flowering and nut set	R53 700.00	R60 396.00	R141 780.00
Nut sizing	R82 350.00	R76 410.10	R130 790.00
Nut filling	R54 340.00	R59 386.60	R112 710.00
Shuck dehiscence	R99 900.00	R86 247.00	R143 820.00

5.3 Discussion

It is important to determine which stages are sensitive to water stress if water savings needs to be made during a growing season or if deficit irrigation strategies are to be employed. This involves determining which stages are sensitive to water stress in terms of yield and quality. In pecans, water stress during reproductive stages from flowering to nut growth and through to nut maturation tended to have negative effects on final yield and quality. Water stress at the flowering and nut set stage significantly reduced yield relative to the control, which could largely be attributed to a reduced number of nuts per cluster (fewer nuts) as a result great of flower abortion. This is, however, in contrast to the results of Wells (2015) who found no impact of water stress early in the season on yield and quality, which could have been due to climatic differences. Nonetheless, Miyamoto (1989) demonstrated water stress at fruit or nut set resulted in poor fertility, which ultimately led to fruit abortion reducing final nut set on pecans. The same has been noted in almonds (Goldhamer et al. 2006). In macadamia Stephenson et al. (2003) found that water stress at this stage reduced water potential of racemes, which led to damage on some flower parts, resulting in nut set failure and reduced nut set per raceme. A similar situation could have occurred in pecan, where water stress during flower and nut set resulted in damage to the small flowers thereby preventing pollination and nut set. Water stress at this stage (flowering and nut set) also resulted in a great nut drop %. However, despite the lower nut number on the tree, quality results seem to suggest that nut size was not increased in this treatment, as nut number per kg was the same as the control. According to Wells and Conner (2007) nut drop at this stage is as a result in low food reserves during the early growth due to unfavorable soil water conditions. Wells and Conner (2007) further illustrated that the degree of drop is often inversely proportional to the shoot length or stress level imposed on the tree during the previous season.

During the nut sizing stage, water stress did not necessarily reduce yield relative to the well-watered control, but the nuts tended to be smaller, possibly due to a reduction in nut elongation as a result of water stress. Importantly, there was very little impact of water stress on the increase in nut diameter in this study. These results supports those obtained by Sparks (2001) who showed that soil moisture availability during the fruit elongation and expansion phases influences the final size and shape of pecan nuts. Wells (2015) determined that drought stress caused the production of small sized, fat and misshapen nuts, thereby reducing the yield of marketable nuts. Again, Heaton et al. (1982) and Garrot et al. (1993) found that pecan nuts harvested from low or non-irrigated treatments tended to be smaller in size compared to fully irrigated treatments. This compromises nut quality as the nuts become small and may in turn reduce their market value, as price decreases with a decrease in nut size. Furthermore, water stress during this stage tended to have the second highest % nut drop, as compared to the other stages. This suggests that yield can also be impacted when the trees are water stressed during this stage. Byford and Herrera (2005b) established that nut drop occurs as a result of water stress due to embryo abortion.

Water stress during the nut filling stage reduced yield due to a greater percentage of poorly filled nuts, which could be attributed to lower photosynthetic rates as a result of water stress. Sparks (1995) reported that the impact of soil water availability on kernel percentage is dramatic and that kernel quality is a direct function of available soil water. Furthermore, subject to the level of soil water, kernel quality may range from poor to excellent. Sparks (2001), Valentini et al. (2015) and Wells (2016b) demonstrated that insufficient irrigation water or rainfall during this period leads to poorly filled nuts and shriveled kernels, which reduces both the final nut mass and nut quality. Sparks (1995) demonstrated a significantly higher kernel percentage (46.5%) in irrigated orchards compared to non-irrigated orchards (40.2%) for ‘Stuart’ pecans. Similar results were obtained in this study, where a reduction in final nut mass was observed, hence a greater number of nuts per kg, more wafers and a higher unsound kernel percentage relative to the well-watered control. This greatly compromised nut quality through poorly filled nuts, increasing the number of nuts per kg, hence reduced nut size and price.

Water stress at the final phenological stage (shuck dehiscence) did not impact the yield, especially during the first season. During this season (first season) there was high rainfall during this stage and as a result a water stress could not be reliably induced, as indicated by

the stem water potential values for this treatment, which were similar to the control (see Chapter 4, section 4.4.3). However, there was a greater percentage of stick tights in the next two seasons which made harvesting and processing more difficult, thereby impacting the nut quality. Yield from this stage (shuck dehiscence) was slightly reduced in the second season due to reduced starch reserves from the previous season as a result of water stress, reducing the number of female flowers. Carroll et al. (2015) reported water stress during this late stage to delay shuck split, this might be due to reduced photosynthesis and hastened canopy senescence. Therefore, this could impact the accumulation of starch reserves in the tree that are required for bud break in the next season, which could have contributed towards the start of an alternate bearing cycle. Over the first two seasons yield of the various treatments was more or less consistent, indicating little effect of water stress on alternate bearing. Though yield increased significantly in the last season, possibly due to alternate bearing and the trees maturing, consistency was observed between treatments relative to the previous seasons. Importantly water stress at any given stage reduced the gross income of the nuts relative to the control. However, this was most prominent for two stages; namely flowering and nut set and nut filling stages. The exception was during the last season (2019/20) when the breakdown of the irrigation to the control treatment early in the season compromised comparisons between the flowering and nut set stage and the well-watered control.

5.4 Conclusion

Water stress during the flowering and nut set stage led to significant flower abortion and nut fall, which reduced the final number of nuts by at least 60%, which ultimately reduced the final yield compared to well-water control. During the nut growth stage (nut sizing), water stress did not necessarily reduce the final yield at this stage, but rather reduced the nut size (many smaller nuts), thereby compromising the nut quality. This, however, suggests that more detailed irrigation scheduling work needs to be done to understand the thresholds for stress that will allow the implementation of deficit irrigation during specific phenological stages without impacting yield and quality. Water stress at the nut filling stage significantly reduced both the yield and nut quality, reducing the nut mass, due to a high percentage of wafers/air pockets as a result of poor nut filling. During the final phenological stage, shuck dehiscence, stress did not have a major impact on final yield or quality, but there was an increase in the number of stick-tights due to shucks failing to split open. However, over the three seasons the increase in the number of stick tights did not always translate into a significant reduction in yield when compared to the control, depending on the degree of stress imposed during this

stage. Therefore, this suggest that the nut sizing and shuck dehiscence to be less sensitive to water stress in terms of yield. Therefore, in seasons where water allocations are reduced, it may be possible to make some water savings during nut sizing and shuck dehiscence without compromising yield. Stress during these stages may, however, slightly reduce the gross income, due to a reduction in quality. Our results, therefore, present the flowering and nut set and nut filling stages to more sensitive to water stress, marked with significantly reduced yield, quality, and gross income. However, insights for the effect of water stress at the final stage (shuck dehiscence) on the carry over effects of stress creates a great platform for further research on these trees, since water stress at this stage is believed to affect starch reserves for the next season.

CHAPTER 6: GENERAL CONCLUSIONS AND RECOMMENDATIONS

Under South African climatic conditions, water scarcity is the most common concern threatening agricultural productivity. In addition, this scenario is predicted to worsen under climate change scenarios, with many parts of the country expected to become drier in future. This is particularly true for nut tree crops such as pecan, which consume a great amount of water and where yield and quality are sensitive to water stress. The production of good quality pecan nuts therefore depends on the adequate supply of water for optimal growth (Garrot et al. 1993, Sammis et al. 2004). Since pecans are considered an important cash crop in South Africa, contributing to economic growth and employment, ensuring their optimal productivity is very crucial. Statistics by the South African Pecan Nut Producers' Association (SAPPA) indicates a continual expansion of the pecan industry, resulting in South Africa becoming the third largest producer and exporter in the world (INC 2019/20). The rapid growth of this industry comes with a great need for irrigation water, creating pressure on this already scarce resource. This is exacerbated by the fact that the majority of pecans in South Africa are planted in semi-arid regions, where the dry climate limits the impact of scab on pecan production. In order to ensure that this industry remains sustainable there need to be continuous improvements in water management strategies. Growers therefore need to manage their irrigation water more judiciously and become more water use efficient, as emphasized by Steduto et al. (2012).

One manner in which this can be achieved is through the identification of water sensitive and non-sensitive phenological stages, which will assist farmers with planning and managing irrigation in times when water allocations are reduced below the full evapotranspiration requirements of an orchard. Furthermore, this strategy may even allow growers to make water savings during a normal rainfall season, which can potentially be used to expand the planted area of pecans or for other crops. Savings can be achieved by allocating sufficient water to the most sensitive phenological stages and reducing irrigation below the full evapotranspiration (ET) requirements of the crop during less sensitive stages. Since water stress sensitivity differs with phenological growth stage, an understanding is required of the physiological responses of the crop to water stress at each stage in relation to its growth and final yield (both quantity and quality). Again, regional climatic conditions (dry, hot and wet periods) will also influence the impact of withholding irrigation on yield and quality. In addition, it is also important to identify the point at which pecan trees start experiencing water stress by assessing photosynthesis in stressed and well-watered trees relative to both predawn and midday stem water potentials. This will aid in avoiding stress during sensitive stages and in inducing a slight stress when water savings are possible without an impact on yield or quality.

Water stress imposed during the flowering and nut set stage significantly reduced the final yield due to greater flower abortion and nut drop relative to the well-watered control. Stress during the nut sizing stage did not necessarily reduce final yield, but rather reduced the nut size (many smaller nuts), thus compromising the nut quality and gross income. During the nut filling stage, water stress significantly reduced both the yield and nut quality, reducing nut mass due to poor nut filling of a number of nuts, which was denoted by a greater percentage of wafers/air pockets compared to the well-watered control, which again impacted the gross income. Water stress during the maturation stage, when shuck dehiscence occurs, did not have a major impact on final yield or quality, however there was an increase in the number of stick-tights. This study, therefore, demonstrates the flowering and nut set, and nut filling stages, to be most sensitive to water in terms of both yield and quality, indicating that stress during these stages should be avoided at all cost. Although our transpiration results indicated that water demand at flowering and nut set stage was low, the demand increases quite rapidly following bud break, as the canopy develops quickly. Moreover, rainfall in this region at this time is very low, and reference evapotranspiration (ET_0) is quite high compared to the other stages. Trees therefore depend solely on irrigation for water at this time and our results demonstrated that even slight deficits negatively impact nut set, as well as canopy development. It is suggested that growers should irrigate by considering the rate of canopy development, and work is being done on deriving transpiration crop coefficients for this period of the season. The nut sizing and shuck dehiscence stages were less sensitive to water stress in terms of yield; however, quality and income were compromised especially during the nut sizing stage.

The reduction in yield follows the physiological effects on the trees as a result of water stress implemented at each phenological stage. The decline in plant water status of the trees during each phenological stage subsequently led to a decline in stomatal conductance (g_s), photosynthesis (A_n) and transpiration. The decline in assimilation rate was also associated with poor nut filling during the nut filling stage, which is a stage with high energy requirements as oil accumulates in the nut (Stephenson et al. 2003). The decline in plant water status also negatively impacted plant growth due to lack of turgor pressure impacting elongation growth at certain times, thus delaying canopy growth during the early stages of canopy development (bud break, flowering and nut set stage). This phenomenon was also observed during the final growth stage of the trees (shuck dehiscence), where water stress hastened leaf senescence, at a time when the tree accumulates reserves for the following season. This is also supported by Wood et al. (2002)'s carbohydrate theory "fruit-set is proportional to the dormant season carbohydrate pool and implies that pool size is proportional to late-season net photo assimilation capacity of the tree's canopy".

These changes in the trees' physiological processes of the water stressed treatments were evident when midday stem water potential fell beyond -0.90 MPa, indicating a decline in plant water status of the trees. This study also found a predawn value of -0.42 MPa corresponded to the published midday threshold value of -0.90 MPa. These are therefore the recommended threshold values to trigger irrigation, aiding irrigation scheduling in pecan orchards for water sensitive stages and as a baseline for inducing slight stress for water insensitive stages, in order to try and increase water use efficiency.

The main aim of the study was to determine how water stress at different phenological stages impacts pecan tree physiology, as well as yield and quality. The data obtained showed a decline in the trees' physiological responses including stomatal conductance, photosynthesis, transpiration, and growth (more especially during bud break). Water stress in different phenological stages produced differences in the final yield and quality, hence significantly reducing the final yield and compromising the nut quality. Finally, from this study, it may be concluded that all four phenological stages were sensitive to water stress when considering yield, quality and gross income. Preferably, pecans should not be subjected to mild water stress (as defined by Othman et al. (2014) if yield and quality are to be maintained at optimal levels. Since traditional irrigation scheduling is primarily based on applying water to the crop's full ET requirement, this therefore, provides an opportunity to reduced irrigation below the full ET requirement to induce a slight water stress during during nut sizing and shuck dehiscence, as it was demonstrated that these stages were able to tolerate moderate stress, though quality was slightly compromised. As water was withheld a slight water application during the stress period may as well maintain nut quality. Still, care should also be taken to adjust irrigation schedules during periods of low water demand, for example cloudy days and when the canopy is limited at the start and end of the season. However, insights for the effect of water stress at the final stage (shuck dehiscence) on the carry over effects of stress creates a great platform for further research on these trees. Furthermore, in-depth work needs to be done to define threshold levels for stress, in terms of water potential measurements and remotely sensed indices for drier more arid regions in South Africa. This will therefore avoid too much water use, which will also have an impact on yield and increase precision in irrigation water, hence improving water use efficiency. Lastly, results obtained from this study presented dark respiration (R_d) values for the water stressed treatments to be reduced relative to the well-watered control treatment. Flexas et al. (2005), highlighted that water stress tends to increase respiration. This give reasons further research in pecans.

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