WATER STRESS EFFECTS ON GROWTH, DEVELOPMENT, RESOURCE CAPTURE AND RESOURCE USE EFFICIENCY OF TWO CONTRASTING SUGARCANE GENOTYPES

by

SIVUYILE NGXALIWE

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School of Life Sciences

College of Agriculture, Engineering and Science

University of KwaZulu-Natal

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ABSTRACT

High-fibre sugarcane may be suitable for second generation biofuel production in marginal areas. However, quantitative information about its productivity, resource use, efficiency of resource conversion and drought tolerance is lacking. This study compared growth, development and resource capture of two contrasting sugarcane genotypes under well-watered and water stress conditions. A high-sucrose sugarcane cultivar (N19) and a high-fibre sugarcane hybrid (04G0073) were planted in October 2011 at the South African Sugarcane Research Institute rainshelter facility at Mount Edgecombe, Durban, South Africa. All treatments received adequate irrigation for five months. Thereafter, irrigation was withheld from stress treatments while control treatments continued receiving adequate water. This resulted in two periods of water stress for the stress treatments of 21 and 31 days respectively, interspersed by a period of 28 days with adequate soil water brought about through an unintended intrusion of storm water. Green leaf area index (GLAI), stalk growth, radiation interception, relative available soil water content (RASWC) and midday leaf water potential (Ψ_L) were measured regularly. Dry aboveground biomass and its components were measured at harvest. Evapotranspiration was derived from neutron water meter measurements. Water use efficiency (WUE, defined as biomass produced per unit evapotranspiration) and radiation use efficiency (RUE, defined as biomass produced per unit radiation intercepted) were determined at harvest.

Under well-watered conditions, 04G0073 grew rapidly, producing 33% more stalks at peak tillering and a higher number of green leaves per stalk, resulting in a 6% higher GLAI than N19. This enabled it to capture 3% and 5% more water and solar radiation, respectively, compared with N19. 04G0073 also converted resources more efficiently than N19 (WUE: 7.6 vs. 6.9 kg m^{-3}; RUE: 1.52 vs. 1.39 gMJ^{-1}) to produce a 12% higher aboveground dry biomass yield. 04G0073 partitioned significantly more stalk biomass to fibre (0.58 vs. 0.45) and significantly less to sucrose (0.24 vs. 0.36) than N19. In both genotypes, stalk elongation rates declined when RASWC dropped below 0.55. Stalk elongation of 04G0073 ceased at RASWC=0.3, compared to RASWC=0.4 for N19. Water stress reduced GLAI by 77% and 88% for N19 and 04G0073, respectively, due to decreased green leaf number (4 and 5 leaves) and decreased stalk population (18% and 6%). Water stressed 04G0073 used resources less efficiently than N19 (WUE=5.8 vs. 7.8 kg m^{-3}; RUE=0.95 vs. 1.36 g MJ^{-1}). This resulted in stressed 04G0073 producing significantly less (23% reduction) aboveground dry biomass than N19. Although 04G0073 used resources more efficiently to produce biomass under well-watered conditions, it was unable to tolerate severe water stress as well as N19 did. The information gathered in this study is useful for calibrating crop models for determining the feasibility of growing high-fibre cane in marginal areas.

Keywords: biomass, high-fibre cane, water stress, water use efficiency, radiation use efficiency

PREFACE

The experimental work described in this dissertation was carried out at the South African Sugarcane Research Institute (SASRI), Mount Edgecombe, from October 2011 to May 2012, under the supervision of Prof. Abraham Singels, Dr. Alana Eksteen and Prof. Norman Pammenter (UKZN).

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institute. Where use has been made of the work of others it is duly acknowledged in the text.

Signed: 

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DECLARATION 1 - PLAGIARISM

I, Sivuyile Ngxaliwe, declare that:

1. The research reported in this dissertation, except where otherwise indicated, is my original research.
2. This dissertation has not been submitted for any degree or examination at any other university.
3. This dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being source from other persons.
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DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis (include publications in preparation, submitted, *in press* and published and give details of the contributions of each author to the experimental work and writing of each publication).

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CONFERENCE PRESENTATIONS

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TABLE OF CONTENTS

1. INTRODUCTION

1.1. World sugarcane production

Sugarcane is currently grown in over 130 countries world-wide in tropical and sub-tropical regions between 37°N and 31°S (Geisler, 2012). Approximately 1.6 billion tons of sugarcane are produced globally (Chandel *et al.*, 2012) from 22 million hectares (Goldemberg & Guardbassi, 2009). The four biggest producers namely Brazil, India, China and Thailand account for more than half of the global production (Geisler, 2012).

1.2. Sugarcane production in South Africa

The South African sugar industry is ranked fifteenth in the world in terms of sugarcane production and is the main producer on the African continent (Muir *et al.*, 2010). However, sugarcane production has been declining since 2005 from 21 million tons (South African Sugar Association, 2011) to 17 million tons in the 2012/2013 season (Singels *et al*., 2013). This decline has been closely linked to corresponding decreasing area under cane from 424 900 ha in 2005 (South African Sugar Association, 2011) to 263 369 ha in 2012/2013 (Singels *et al*., 2013).

In South Africa, sugarcane production occurs mainly in the eastern regions of the country under a wide range of climatic and environmental conditions, including a hot dry northern region (Pongola in northern KwaZulu-Natal and the Lowveld area of Mpumalanga), a humid sub-tropical coastal belt and cool frost-prone Midlands areas of KwaZulu-Natal (KZN). In these regions the thermal time and cumulative evaporative demand varies from low on the coastal region (thermal time of 3184 ^{o}Cd and a long-term mean sugarcane reference evaporation of 1365 mm (McGlinchey & Inman-Bamber, 1996) to high in the northern KZN (4071 ^{o}Cd and 1777 mm) with the highest values achieved in the Lowveld (4524 ^{o}Cd and 1855 mm) (Inman-Bamber, 1995a). Rainfall patterns also vary considerably from high annual rainfall (1150 mm) in the south coast of KZN, decreasing towards the northern KZN (683 mm in Pongola) and lowest in the Lowveld (629 mm) (Inman-Bamber, 1995a). The south coastal areas of KZN receive adequate rainfall (>1000 mm) for sugarcane production. In the Umfolozi area in KZN, annual rainfall alone is well below 1000 mm thus supplementary irrigation is required to compensate for periods of low rainfall (dry periods). In the Pongola and Lowveld production areas, sugarcane is fully irrigated because of low rainfall and high evaporative demand (Van der Laan *et al*., 2012). Climatic conditions in these production areas dictate the length of harvest cycle. In the northern irrigated areas (Pongola and the Lowveld) and along the coastal belt of KZN, harvesting occurs at 12 months of age whereas in the cold Midlands area, harvest age ranges from 15 to 24 months (Ramburan, 2012).

1.3. Uses of sugarcane

A sugarcane crop consists of approximately 64% stalks (stem) and 36% green leaves and trash (dead leaves and tops) (Thompson, 1978). Trash is usually burnt or left in the field during harvest and the stalk is sent to the mills for sugar processing. Sugarcane received by the mills contains water, fibre, sugars and dirt (Diasa *et al.*, 2009). Cane stalks are cleaned and crushed to extract juice. Water is removed from the juice through evaporation leaving syrup that is separated into sugar and molasses (a dark syrup containing mixture of sugar, water and impurities) through centrifugation. Molasses is boiled and centrifuged several times to extract as much sugar as possible and the remaining molasses is used to manufacture other by-products (Tongaat Hulett, 2013). Globally, about 173 million tons of sugar is produced annually (Singh, 2010).

During the production process, a fibre rich bagasse is produced as a waste product. Bagasse can be burned to produce electricity for the sugar mills (Kim & Day, 2011; Hofsetz & Silva, 2012). Generally, one ton of sugarcane produces about 280 kg of humid bagasse (Soccol *et al.*, 2010 cited by Chandel *et al.*, 2012).

Sugarcane can also be used to produce bioethanol based on the fermentation of sugars in the juice and molasses (first generation biofuel). Bioethanol production from sugarcane began in the 1970s and has been increasing since (Zuubier & Vooren, 2008). First generation ethanol production is done by fermenting all the sugars in the juice using yeast cells (Diasa *et al.*, 2009). During fermentation, sucrose is "hydrolysed into fructose and glucose, which are converted into ethanol and carbon dioxide" (Diasa *et al.*, 2009). Brazil is the leading world producer of ethanol and produced up to 22.24 billion litres in 2008 (Canilha *et al.*, 2012).

It is anticipated that in the coming years, bagasse along with trash, will also be used as a feedstock source for electricity generation and/or second generation ethanol production. Both trash and bagasse are rich in fibre making sugarcane an ideal candidate for second generation ethanol (Canilha *et al.*, 2012). Bagasse consists of 38-45.5% cellulose, 22-27% hemicellulose, 21-32% lignin, 2.2-2.8% ash and other organics (% w/w, dry basis) (Saha, 2003; Da Silva *et al.*, 2010) while trash contains 33-36% cellulose, 26-28% hemicellulose, 25-31% lignin, 2.1-5.7% ash and other organics (Da Silva *et al.*, 2010; Costa *et al.*, 2013). Hydrolysis of the hemicellulose and cellulose to monomer sugars is the first step in the production of ethanol from fibres and can be achieved either by dilute acid, concentrated acid or enzymatically (Walter & Ensinas, 2010). However, cellulose is protected by hemicellulose and lignin which are extremely hard to break down, therefore pre-treatment (for example using steam, chemical or biological methods) is required to expose the cellulose to enzymes (Kumar *et al.*, 2009; Walter & Ensinas, 2010). The resulting sugars are then fermented to produce ethanol (Walter & Ensinas, 2010). Currently, second generation biofuels production techniques are non-commercial although the concept is well established (Naik *et al.*, 2010).

1.4. Types of sugarcane

Sugarcane cultivars can be grouped into two categories. These are traditional sugarcane and energy cane (cane used for energy production) which can be subdivided into type I and type II. The differences among the sugarcane types are based on stalk composition, in terms of sugar, fibre and water content (Tew & Cobill, 2008). The traditional sugarcane consists mainly of *Saccharum officinarum* genes and is characterised by its high levels of sucrose and low levels of fibre. Traditional sugarcane is grown mostly for sugar production and has approximately 13% sucrose, 12% fibre and about 75% water (Tew & Cobill, 2008). Energy cane is a hybrid cross of *Saccharum* spp. with wild relatives (*S. spontaneum*, *Erianthus* spp. and *Miscanthus* spp.) (Known as F1 generation) or crosses between F1 generation hybrids and *Saccharum* spp. (BC1 generation hybrids) (Bransby *et al.*, 2010; Kim & Day, 2011). Type I energy cane is bred and cultivated for both sucrose (13%) and fibre (17%) and has about 70% water (Tew & Cobill, 2008). Type II energy cane is bred only for fibre (30%) and has very low sucrose content (5%) and water content (65%) (Tew & Cobill, 2008; Kim & Day, 2011). Published data show that type II energy cane, also known as high-fibre cane, produces higher biomass compared with traditional sugarcane (Kim & Day, 2011). There are also implications that the energy cane could survive more harsh environmental conditions and produce higher biomass yields than the traditional sugarcane (Tew & Cobill, 2008).

1.5. Mechanisms of increased biomass yield

- *Canopy development and radiation capture*

Aboveground dry matter and crop growth rates are dependent on the ability of the crop canopy to intercept and convert photosynthetically active radiation to dry matter (Varlet-Grancher *et al.*, 1993; Sinclair & Muchow, 1999; Singels *et al*., 2005). Leaf area index (LAI), a total one-sided area of a leaf per unit ground surface area (Breda, 2003), determines the ability of a crop to intercept solar radiation and photosynthesis. LAI and solar radiation interception are low during the early developmental stages of a crop and increase as more leaves and stalks are produced. LAI reaches a maximum at approximately six months after planting depending on cultivar and growing conditions, and then slowly declines due to self-shading (Bull & Glasziou, 1975). Factors that govern LAI development such as stalk population and green leaf area will determine the amount of radiation intercepted and ultimately biomass yield. Genotypes with high LAI can intercept more solar radiation thus increasing the energy available to drive photosynthesis resulting in higher dry matter accumulation.

LAI is also related to crop evapotranspiration (ETc, sum of water lost from the soil surface via evaporation and from the leaf canopy by transpiration) by determining (a) the evaporative soil surface and (b) the size of the transpiring surface. Blum (2011) reported that ETc increases with LAI until LAI reaches a maximum threshold beyond which transpiration does not increase.

- *Root development and water uptake*

After planting, sugarcane setts develop roots from the root primordia around the nodes (van Dillewijn, 1952). Sett roots are the first to emerge within 24 hours of planting and last for about 90 days. These roots are thin and highly branched and are important in maintaining the moisture in the sett (van Dillewijn, 1952). Shoot roots are the second type of roots to emerge usually 5–7 days after planting (Glover, 1967; van Antwerpen, 1999; Smith *et al.* 2005). Shoot roots emerge from the base of new stalks and are thicker and fleshier than sett roots and develop into the main root system of the plant (van Dillewijn, 1952). Shoot roots can elongate at a mean rate of 40 mm d^{-1} in light soils and at 28 mm d^{-1} in heavy soils showing that root penetration is affected by soil texture (Glover, 1967). Distribution of the root system is strongly determined by the distribution and availability of soil water, causing differences in the ability of crops to exploit deeper soil resources (Smith *et al.,* 2005). Well-irrigated crops tend to accumulate roots in the upper soil layers whereas rainfed crops experiencing water deficit at times usually possess a deep root system which enables access to water deep in the soil profile (Glover, 1967). Rooting depth is considered important in determining water uptake and drought tolerance. Deep root systems reduce the susceptibility of crops to soil water deficits by providing increased capacity for uptake of deep reserves of soil water.

- *Resource use efficiency*

The ability of a crop to convert fractional intercepted photosynthetic active radiation (FIPAR) to dry matter is known as radiation use efficiency (RUE). RUE is generally defined as the ratio of biomass accumulated to intercepted global solar radiation (Donaldson *et al.,* 2008). Maximum sugarcane RUE values range between 1.25 to 2.09 g MJ^{-1} (Muchow *et al.*, 1994; Sinclair & Muchow, 1999; Singels & Smit, 2002; Donaldson *et al.*, 2008; De Silva & De Costa, 2012). These values are higher than the maximum RUE values reported for maize (1.6 and 1.8 g MJ^{-1}) (Muchow & Davis, 1988; Kiniry *et al.*, 1998) and sorghum (1.34 g MJ^{-1}) (George-Jaeggli *et al.*, 2004) showing that sugarcane is more efficient at converting solar radiation into biomass.

The definition of water use efficiency (WUE) depends upon the time scale or context at which it is being discussed (Bacon, 2004). At a leaf scale, WUE (µmol CO_2 mol^{-1} H_2O) is the ratio of instantaneous net CO_2 assimilation rate to transpiration rate (Jones, 2004; Saseendran *et al*., 2008). At crop level, WUE (kg m^{-3} or kg ha^{-1} mm^{-1}or g kg^{-1} or t/100 mm) is the ratio of dry matter accumulated to the amount of water evapotranspired by the crop over a certain period (weeks, months, season) (Chaves *et al.,* 2004). Sugarcane WUE values that are in the range of 6 to 12 kg m^{-3}, have been cited in the literature (Thompson, 1976; Kingston, 1994; Keating *et al*., 1999; Inman-Bamber *et al*., 1999), showing that sugarcane can use water more efficiently than other bioenergy candidates such as maize (2.4 to 6.9 kg m^{-3}) (Liu & Zhang, 2007; Gao *et al.,* 2009; Yi *et al.,* 2010) and sorghum (1.42 to 9.6 kg m^{-3}) (Curt *et al*., 1995; Mastrorilli *et al.,* 1999; Tolk & Howell, 2003).

1.6. Sugarcane drought tolerance

Water stress causes water deficit, which limits plant growth and survival because plants absorb insufficient water to replenish water lost through transpiration (Blum, 1996). When sugarcane is subjected to water stress, the first physiological process to be affected is the expansive growth of leaves and stalks through a reduction in cell turgor thus restricting cell expansion (Inman-Bamber & de Jager, 1986a; Inman-Bamber & Smith, 2005; Koonjah *et al.*, 2006; Smit & Singels, 2006; Begum *et al.*, 2012). Previous studies showed that water stress also reduces stalk population, number of green leaves per stalk, stalk and leaf appearance rates and accelerates leaf and stalk senescence rates (Inman-Bamber & de Jager, 1986a; Inman-Bamber & Smith, 2005; Koonjah *et al.*, 2006; Smit & Singels, 2006). Moreover, water stress tolerant genotypes adapt to drying conditions by limiting transpiration through early stomatal closure in that way sustaining water and allowing plants to sustain critical physiological and biochemical processes for a longer time (Blum, 1996; Chaves *et al*., 2003). Stomatal closure often leads to a decline in biomass accumulation because carbon assimilation (photosynthesis) is reduced (Sinclair *et al.*, 1984). Photosynthesis is also reduced by reducing the photosynthetic area (reduced leaf area and number of green leaves) thus negatively affecting biomass accumulation and yield (Chaves *et al.*, 2003). Significant reductions in the amount of radiation intercepted by green leaves (through reduced green leaf area and the slower development of new leaves) together with reduced photosynthetic rates intensify the reduction in radiation use efficiency (RUE) (Robertson *et al.*, 1999; De Silva & De Costa, 2012). Even though root penetration rate is reduced by water stress, it has been observed that roots grown in drying areas tend to grow deep into the soil thus extracting more water from deep soil layers (Smith *et al.*, 2005). Deeper root systems combined with reduced plant water use often improve water use efficiency provided that water use reduction has no deleterious effects on yield (Fereres & Soriano, 2007; Jangpromma *et al.*, 2012; Singh *et al.*, 2012).

Although water stress effects on sugarcane growth, development and yield is thoroughly researched and published (Hsiao, 1973; Inman-Bamber & de Jager, 1986a, b; Abayomi & Lawal, 1998; Ali *et al*, 1999; Singels *et al.*, 2000; Singels & Inman-Bamber, 2002; Inman-Bamber & Smith, 2005; Koonjah *et al.*, 2006; Smit & Singels, 2006), there is very little information published regarding the effects of water stress on structural growth and development of the high-fibre sugarcane.

1.7. Problem statement

There is an indication that energy cane genotypes, also known as high-fibre sugarcane, could survive well in more xeric environmental conditions than the current sugarcane growing areas (Tew & Cobill, 2008). This provides an opportunity to grow high-fibre cane for biofuels in marginal areas to avoid competition with other food crops. It is not known though how the high-fibre sugarcane achieves its higher biomass yields and how this is affected by water stress, compared with the traditional

genotypes. There is very little quantitative information available for dedicated high-fibre sugarcane genotypes with respect to their resource (radiation and water) capture, or the efficiency of resource use, and their reaction to water-deficit stress.

This study is aimed at investigating the differences in structural growth and development between two contrasting sugarcane genotypes (traditional and high-fibre) and how these are affected by water stress. The study will also quantify resource use (capture) and conversion efficiency of the two genotypes grown under well-watered and drought conditions. The information collected will be used to determine crop parameters for crop simulation models i.e. CANEGRO (Singels *et al.*, 2008) and CANESIM (Singels, 2007), which could then be used to select suitable locations where high-fibre sugarcane could grow, and their predicted yields under different climatic conditions. This information is necessary to identify marginal areas where high-fibre sugarcane can be grown in South Africa.

1.7.1. Objectives

The objectives of this study are to quantify:

- structural growth and development parameters (stalk population, number of green and dead leaves, leaf area, canopy cover, stalk and leaf elongation rate, root length density and biomass partition fractions) between the traditional and the high-fibre sugarcane genotypes,
- the response of these parameters to water stress, for each genotype,
- The relationship between biomass yield; water use and radiation capture; and water and radiation use efficiencies of each genotype and how these are affected by water stress.

1.7.2. Hypotheses

High-fibre sugarcane produces higher biomass yields because it captures more resources (water and radiation) under well-watered and water stressed conditions, than traditional sugarcane. Higher biomass yields are achieved by investing a larger proportion of assimilate to structural growth (of the roots, tillers and leaves) and less to sucrose storage, resulting in a more dense rooting system and canopy cover, than traditional sugarcane.

1.7.3. Research questions

This study will answer the following questions:

- How is biomass yield related to water use and radiation capture?
- How does this relationship differ between genotypes and how is it affected by water stress?
- How does water stress affect crop structural growth and development (leaf and tiller appearance, senescence rate, stalk and leaf elongation rate, stalk diameter and leaf width and root length density)?
- How do the structural growth and development processes determine crop water use and radiation capture under well watered and water stressed conditions?

1.8. Dissertation outline

Chapter 1--Introduction is the introductory chapter and it looks at the world and South African cane production, important uses of sugarcane, different types of sugarcane, how sugarcane yield could be increased, sugarcane drought tolerance, problem statement, objectives, hypotheses, and research questions.

Chapter 2—Literature review reviews the existing literature related to the effects of water stress on plant growth and development and modelling of water stress effects on plant growth, development, resource capture, and resource use efficiency. The review also covers modelling water stress effects on plant growth and development. Lastly, a detailed description of different methods used to quantify water stress, their potential and limitations are also reviewed.

Chapter 3—Materials and methods describes the study area and the materials and methods used to collect data. This chapter is subdivided into two sections. The first section deals with materials used to collect data on weather, soil and plant water status, plant growth, plant development and biomass yield. The second section describes calculation of relative soil water content, thermal time, radiation interception, rooting depth, and resource use efficiency.

Chapter 4—Results and discussions discusses results obtained comparing results with existing literature on the response of two contrasting sugarcane genotypes to water stress in terms of plant growth, development, resource capture, biomass yield and resource use efficiency.

Chapter 5—Conclusions is the concluding chapter and sums up the study, as well as suggesting some recommendations.

Chapter 6—References is the list of cited literature.

Chapter 7—Appendix is the appendix.

2. LITERATURE REVIEW

The aim of this review is to collate relevant information from literature on sugarcane response to water stress, with emphasis on resource capture and conversion efficiencies of different types of sugarcane. This will enable the identification of knowledge gaps and research priorities, as well as the suitability of research approaches and experimental techniques for achieving the study objectives. The review covers: (i) effects of water stress on sugarcane growth, development and resource capture and use efficiency; (ii) modelling water stress effects on sugarcane growth, development and resource capture and use efficiency; (iii) measurement techniques.

2.1. Water flow in plants

According to the cohesion-tension theory water within the xylem of plants form a continuous liquid column from the roots to the leaves. This allows water to be moved from the soil through the plant xylem to the atmosphere. Water flow in plants begins with transpiration, loss of water from plant leaves to the atmosphere in the form of vapour. During transpiration, a surface tension is created at the leaf cell surface where it lowers the water potential of the xylem (Tyree, 1997). "This tension can also be described as a negative pressure that is quantitatively referred to as the water potential (Ψ) and typically measured in Pascals (Pa) such that increasingly negative water potentials indicate greater tension imposed on the water column" (Pittermann, 2010). The tension created in the leaves reduces the Ψ of the roots below that of the soil (Ψs). This result to water movement along the decreasing Ψ gradient, from the soil to the roots and from the roots to the leaves to replace water transpired at the surface of the leaves. This movement of through the soil-plant-atmosphere-continuum (SPAC) contain resistances to water flow as shown in Figure 2.1.

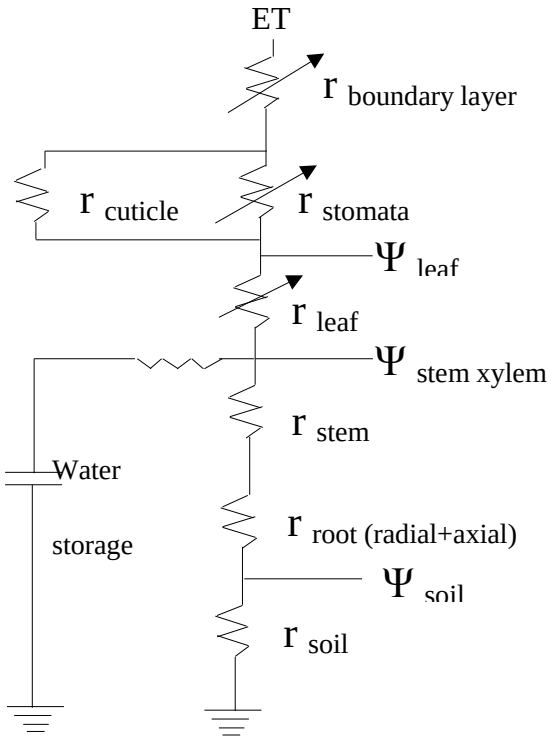


Figure 2.1. A schematic representation of the soil-plant-atmosphere continuum represented using Ohm**'**s Law**.** Arrows represent variable resistances, Ψ represents water potential and r indicates resistance. Water flow is driven by the differences between the Ψsoil and Ψleaf. (Re-drawn from Blum, 2011).

Plants are able to grow and extract water from the soil due to the soil's ability to retain water, which is highly influenced by soil texture and structure. Fine heavy clay soils with numerous smaller pores hold more water than coarse soils with larger pores (Plaster, 1997; Bardgett, 2005). Plants can only absorb available water between the drained upper limit (field capacity, FC) and the lower limit (permanent wilting point, PWP) of soil water storage. The PWP is when the "soil moisture is too low for plants to absorb water then the plant is permanently wilted and will die if water is not provided" (Smith & Mullins, 2000). Rainfall or irrigation events saturating the soil will cause a downward movement of water (drainage) due to gravitational force (Ψg) for some time. When drainage rate approaches zero, the soil is said to be at field capacity (Smith & Mullins, 2000). Maximum soil water uptake by plants generally occurs when the soil water content is near field capacity.

Lastly, the rate at which water is absorbed by plants is also dependent on transpiration, which in turn, is affected by weather conditions (relative humidity, solar radiation, temperature, and wind), plant physiological factors (root, xylem, stomatal and cuticular resistances) and the water status of the soil (soil water potential). Increasing solar radiation results in increased air temperatures, lower air humidity and increased vapour pressure deficit (VPD) between the leaf and the atmosphere. High VPD increases Ψ gradients between the air and the leaf causing rapid loss of water from the leaves to the atmosphere. This results in increased soil water depletion, especially when water is freely available (Belko *et al.,* 2013).

2.2. Plant water relations

Plants can only access water from the available soil water storage at the root zone which is decreased by transpiration and/or soil evaporation. As the soil water storage decreases, water deficit develops as plants lose more water than they can replenish (Blum, 2011). Saseendran *et al*. (2008) defined water stress as the "condition in which plant cells and tissues have less than full turgor due to transpiration demand exceeding water uptake by roots". The intensity of water stress in plants can be caused by rapid water loss from the soil through the plant to the atmosphere or slow water absorption by plants (Kozlowski & Pallardy, 1997). The occurrence of water stress triggers a series of plant responses that are either adaptive or harmful in nature. The ability of a plant to withstand water stress is determined by the duration and intensity of soil water depletion, changes in atmospheric demand, environmental conditions, plant growth and the phenological state in which water deficit has developed. Moderate or mild water stress can cause significant morphological and physiological changes (Chaves *et al.*, 2009), whereas severe water stress may lead to plant death.

Leaf water potential (Ψ_L) describes the energy state of water in leaves. Under unstressed conditions, Ψ_L is almost zero, however, under water stress conditions Ψ_L decreases with decreasing Ψs and soil water content (Blum, 2011). The degree to which Ψ_L is reduced depends on the sensitivity of the crop to water stress. For example, water stress-resistant crops reduce Ψ_L less than water stress prone crops as was shown by Carter & Patterson (1985) on Soybean and Schonfeld *et al*. (1988) on wheat. In sugarcane cultivars grown in a rainshelter, the Ψ_L of well-irrigated treatments varied from -0.3 MPa (Inman-Bamber & de Jager, 1986b) to -0.5 MPa (Koonjah *et al*., 2006). These values decreased with decreasing soil moisture and reached -1.5 MPa in NCo376 and -1.7 MPa in N12 within two weeks of withholding irrigation (Inman-Bamber & de Jager, 1986b). Similar observations were made by Koonjah *et al*. (2006) whereby Ψ_L of NCo376 decreased from -0.5 MPa in well-irrigated treatments to -1.57 MPa after 25 days of withholding irrigation. Inman-Bamber & de Jager (1986a) reported lower thresholds (*cv.* NCo376) of -0.9 MPa under well irrigated conditions which decreased to -2.3 MPa under water stress.

Ψ_L usually follows a diurnal variation, declining in the morning due to increased transpiration rates and rising in the late afternoon due to decreasing transpiration rates (Inman-Bamber & Smith, 2005). The Ψ_L of irrigated sugarcane was maximum at night (-0.05 MPa) and started decreasing after sunrise reaching a minimum of -1.0 MPa at midday thereafter recovered in the late afternoon (Inman-Bamber & Smith, 2005). The late recovery of Ψ_L in irrigated crops could be ascribed to a reduction in solar radiation (Kozlowski & Pallardy, 1997). However, rainfed sugarcane crops experiencing water stress had much lower Ψ_L values ranging from -0.2 MPa at night to -1.5 MPa at midday (Roberts *et al.*, 1990). Therefore, the diurnal variation of Ψ_L is reduced as water stress progresses and little variation from morning to evening could be observed under severe water stress conditions (Kozlowski &

Pallardy, 1997). Presumably this is because stomata are closed and so there is little transpiration to pull water potential down at midday.

As mentioned previously, water movement from the soil through the plant to the atmosphere is mainly a function of the water potential gradient between the soil and the atmosphere, and the resistance along the path of flow. Water leaves the plant to the atmosphere through the leaves through transpiration which is regulated by microscopic 'pores' known as the stomata. A plant can exercise control over transpiration by adjusting the size of the pores. The size of the stomata is controlled by turgor of surrounding guard cells, increasing with increased turgor and decreasing with decreased turgor of guard cells. The ability of the stomata to regulate transpiration is an important part of a plant's response to water stress. Under decreasing soil water potential, or increasing atmospheric evaporative demand (decreasing atmospheric water potential) stomata may begin to close due to decreasing cell turgor thus restricting transpiration rate (Warren *et al.*, 2004). Smit & Singels (2006) showed that stomatal conductance in sugarcane grown under water stress conditions dropped when the relative soil water content dropped below values of 0.73 and 0.55 in N22 and NCo376 cultivars, respectively. This confirmed the findings of Inman-Bamber & De Jager (1986a) who reported that stomatal conductance is highly sensitive to drying soils. They showed that stomatal conductance in NCo376 cultivar began declining at Ψ_L of -0.8 to -1.0 MPa. Turner (1990) illustrated the sensitivity of stomatal conductance in sugarcane under both clear sky and cloudy conditions, and reported that full stomatal closure occurred at Ψ_L of -1.8 MPa. Stomatal conductance can be also regulated by abscisic acid (ABA). ABA is often released by dehydrating roots even before guard cells turgidity is affected by decreasing soil moisture (Kozlowski & Pallardy, 1997; Inman-Bamber & Smith, 2005; Chaves *et al*., 2009; Blum, 2011). The ability of cultivars to close their stomata early is often associated with drought resistance (Inman-Bamber & Smith, 2005).

2.3. Effects of water stress

2.3.1. Plant development

Leaf appearance and senescence

Leaf appearance begins with the formation of leaf primordia after the bud, a miniature stem with its growing point and primordia of leaves and roots, has acquired certain critical water content required to drive cell division (van Dillewijn, 1952; Scarpella *et al.*, 2010). Leaves appear from the buds located at the nodes of a sugarcane stalk. This process is "driven by cell division, but, once a leaf establishes its shape, cell expansion drives leaf growth" (Scarpella *et al.*, 2010). Sugarcane leaves grow successively, senescing old mature leaves being replaced with young new leaves (van Dillewijn, 1952).

Leaf senescence is a natural process characterised by yellowing of old leaves located at the bottom of the crop canopy where they will be shaded. The balance between leaf appearance and senescence rates determines the number of green leaves per stalk (van Dillewijn, 1952). In crops grown under irrigated, high-input conditions in the field, Robertson *et al.* (1998) observed that sugarcane leaves appeared continuously and leaf senescence started when stalks had approximately seven leaves. Inman-Bamber (1994) observed that in NCo376 and N12 sugarcane cultivars, the number of green leaves was 11 and 10, respectively. This balance between leaf appearance and senescence is affected by water stress thus affecting the number of green leaves per stalk.

Inman-Bamber (2004) noted that soon after imposing water stress, leaf appearance rate in Q96 and Q134 sugarcane cultivars decreased rapidly whereas leaf senescence increased, consequently reducing the number of green leaves per stalk. Smit & Singels (2006) found that the number of green leaves per stalk in N22 and NCo376 sugarcane cultivars was reduced by 62% and 77%, respectively, after 42 days of withholding irrigation. This decrease was associated with a combined reduction in leaf appearance rate and increased leaf senescence (Inman-Bamber & de Jager, 1986a). However, the number of green leaves per stalk can recover from brief periods of water stress, and seemingly compensate for lost growth when soil moisture conditions improve (Wiedenfeld, 2000). This is because during the times of stress, "leaves tend to accumulate in the whorl and can recover rapidly to that of the non-stressed control following release from stress" (Inman-Bamber & Smith, 2005).

Modelling leaf development

Leaf appearance is simulated in the CANEGRO sugarcane model as a function of temperature (thermal time, ^{o}Cd) and is based on the phyllochron concept (Inman-Bamber, 1994). The phyllochron interval (PI) is defined as the thermal time between the appearances of two successive leaves (Singels *et al*., 2008). In sugarcane, the appearance rate for the first 14 leaves is significantly lower than the emergence rate of subsequent leaves (Inman-Bamber, 1994; Bonnett, 1998; Singels *et al*., 2005). Therefore, two phyllochrons apply to leaves below and above a cultivar-specific threshold (PSWITCH) respectively (Singels *et al*., 2008). Inman-Bamber (1994) determined 10 °C as the base temperature (temperature below which leaves fail to emerge, Tbase) for leaf development of South African sugarcane cultivars. Several base temperatures for sugarcane leaf development ranging from 8 ^{o}C (Smit & Singels, 2007) to 10 ^{o}C (Singels *et al.*, 2008) have been reported in the literature. Leaf senescence in CANGEGRO is simulated based on the assumption that unstressed plants cannot have more than a variety-specific number of green leaves. "Each new leaf is accompanied by the senescence of the oldest leaf on the tiller" and this process accelerated by water stress (Singels *et al.*, 2008).

Stalk appearance and senescence

After planting setts, meristematic cells in buds acquire a critical water content required for sprouting (van Dillewijn, 1952). Thereafter, the buds sprout to form primary stalks. Secondary tillers sprout from the buds located at the base of the primary stalks and from the buds of the secondary stalks (i.e. tertiary tillers) and the process is called tillering (van Dillewijn, 1952; Jones *et al.*, 1990). During the early stages of crop growth there is an overproduction of stalks/tillers reaching peak stalk/tiller population about 3-5 months after planting. However, up to 50% of the stalks senesce and the stalk population stabilises before 9 months (Bull & Glasziou, 1975). This negative correlation between the tiller density and the number of surviving tillers per primary stalk is associated with shading of young stalks by older stalks in competition for light (van Dillewijn, 1952; Jones *et al.*, 1990; Inman-Bamber, 1994). The tillering rate is cultivar dependent (Zhou *et al.*, 2003; Singels *et al.*, 2005) and can also be influenced by other factors including temperature (Inman-Bamber, 1994), crop age (Smit, 2009) and row spacing (Smit & Singels, 2006).

Tillering relies heavily on cell division which in turn depends on soil moisture. Therefore, the occurrence of water stress reducing soil water content could directly delay buds from achieving the necessary critical water content thus reducing stalk appearance rate. Smit & Singels (2006) showed that water stress imposed for 42 days increased stalk senescence rates by 8 and 4 stalks m^{-2} in N22 and NCo376, respectively. Lastly, De Silva & De Costa (2004) observed that water stress delayed sugarcane tillering and resulted in well-irrigated sugarcane crops achieving peak tiller population one month earlier than rainfed crops experiencing periods of water stress.

Modelling stalk development

In the CANEGRO (Singels *et al.*, 2008) and APSIM (Keating *et al.*, 1999) sugarcane models, primary stalk appearance is driven by the thermal time accumulated since planting. The appearance of primary stalks after a certain cultivar-specific thermal time has lapsed (e.g. 428 ^{o}Cd, base temperature 10 ^{o}C, for NCo376 cultivar) marks the end of the 'germination' phase. Stalk population increases due to tillering and reach peak stalk populations after a cultivar-specific thermal time has lapsed (Inman-Bamber, 1994), subsequently declining to reach a stable population (Singels *et al.*, 2008). In CANEGRO, stalk appearance rate is inversely proportional to row spacing and is reduced by water stress (Singels *et al.*, 2008).

Leaf area index

Green leaf area index (GLAI), the total one-sided area of the green leaf per unit ground surface area, is an important aspect used to measure canopy development (Cowling & Field, 2003). GLAI starts at zero in recently emerged crops, increasing over time up to a peak of 5 to 8 m^2 m^{-2}, before decreasing towards the end of the season (Hunsigi, 2001; Zhou *et al.*, 2003; Smit & Singels, 2006). However,

GLAI values between 4 and 5 seem to be the optimum (95% of solar radiation intercepted) (Hunsigi, 2001). GLAI development is cultivar specific (Singels & Donaldson, 2000) and is influenced by planting time (Inman-Bamber, 1994; Singels *et al*., 2005), row spacing (Singels & Smit, 2002) and planting density (Bell & Garside, 2005). GLAI is largely dependent on the rates of tillering, leaf appearance, leaf extension and the size of each leaf (Robertson *et al*., 1998; Inman-Bamber & Smith, 2005). Therefore, cultivars which develop a higher GLAI more rapidly could intercept more solar radiation thereby maximizing yield (Sinclair & Muchow, 1999; Singels *et al*., 2005).

Bull & Glasziou (1975) studied sugarcane grown under different environmental conditions and found that GLAI increased gradually to approximately 4 due to increased stalk senescence in the stressed crop, whereas irrigated crops continued to increase GLAI up to about 6. Similarly, water stress imposed for 42 days reduced GLAI from 6 to 2 by reducing stalk population, number of green leaves and leaf area per stalk (Smit & Singels, 2006). De Silva & De Costa (2004) observed that irrigated sugarcane crops achieved maximum GLAI two months earlier that rainfed crops experiencing periods of stress at times. The reduction in GLAI under water stress conditions is due to a reduced stalk population, number of green leaves, leaf expansion rate, leaf appearance rate, increased leaf rolling and leaf senescence rate (Inman-Bamber & de Jager, 1986a; Inman-Bamber, 2004; Smit & Singels, 2006). The stalk population is relatively insensitive to water stress; therefore, GLAI strongly depends on leaf appearance and senescence under mild water stress conditions.

Modelling effects of water stress on leaf area index

In CANEGRO, leaf area index (LAI) is calculated as the product of the mean area of green leaves per stalk (see section 2.3.3.2) and the tiller population (see section 2.3.2.2). LAI can be determined for green leaves only (GLAI), or for all leaves, including dead leaves that are still attached to the plant. In CANEGRO, GLAI is reduced by water stress conditions through a reduction in mean area per stalk as well as a reduction in stalk population.

2.3.2. Plant growth and yield

Root growth

In sugarcane, root primordia need to attain a certain critical water content and 96% relative humidity before they can sprout (van Dillewjn, 1952; Glover, 1967). Once these conditions are met, root primordia around the nodes of setts produce thin and highly branched sett roots (Smith *et al.* 2005). Under optimal conditions, these roots emerge within 24 hours after planting and are not attached to the primary shoot but are important in maintaining the moisture in the sett (van Dillewijn, 1952; Glover, 1967). On average, sett roots elongate at 24 mm d^{-1} (Glover, 1967) and will grow for 6-15

days after planting (Glover, 1967; Smith *et al.,* 2005). Shoot roots, which grow 5-7 days after planting, are thicker and fleshier than sett roots and develop into the main root system of the plant (van Dillewijn, 1952; Glover, 1967; van Antwerpen, 1999; Smith *et al.* 2005). Shoot root elongation responds to soil texture and roots elongate at 40 mm d^{-1} in sandy soils and at 28 mm d^{-1} in clay soils (Glover, 1967). The growth pattern of the developed root system of sugarcane is also dependent on water availability at different depths of soil during the root development, severity of soil drying and the interaction between soil moisture status and cultivars (De Silva *et al.,* 2011).

Rainfed sugarcane crops experiencing periods of water stress promoted root growth and possessed a deep root system which enabled access to water deep in the soil profile (Glover, 1967; Thompson & de Robillard, 1968). Gascho & Shih (1983) reported that well-irrigated sugarcane crops promoted root growth in the upper 20 cm of the soil. Similarly, Evensen *et al.* (1997) observed that the root system of irrigated sugarcane crops was limited to the top 46 cm of the soil profile. However, water deficit in the upper layer promoted root elongation of rainfed crops in deep layers from 10 to 20 mm d^{-1} in 1.6 m soil depth (Smith *et al.,* 2005). Laclau & Laclau (2009) found that root length density (RLD) of rainfed crops was highest in deep layers (below a depth of 0.6 m) from 125 days after planting onwards. Gascho & Shih (1983) found deeper rooting depths whereby irrigated sugarcane on clay soil exploited soil water to a depth of 0.9 m while rainfed cane on the same soil removed water from a depth of at least 1.2 m. Therefore, rainfed sugarcane cultivars experiencing periods of water stress will have a greater RLD in the deep soil layers and extract more moisture from the soil than the cultivars grown under well irrigated conditions (De Silva *et al.,* 2011).

Modelling root growth

In CANEGRO, an increase in rooting depth and density of roots per soil layer is dependent on daily partitioning of biomass to roots. The fraction of daily biomass partitioned to roots changes with crop age and is highest in young crops and decreases as the crop ages (Singels *et al.,* 2008). The actual increments in rooting depth are simulated as a function of thermal time (base temperature of 16 ^{o}C) assuming a potential penetration rate of 2.2 mm $(^{o}Cd)^{-1}$. Root length density per layer is then simulated based on soil water content (Ritchie *et al*., 1998). The penetration rate will continue at this rate until the maximum rooting depth is reached (Singels *et al.,* 2008). The actual penetration rate is however reduced below the potential rate when water stress occurs (Singels *et al*., 2008).

Leaf and stalk elongation

Following leaf emergence, leaves expand until a maximum leaf size is reached. Continued leaf elongation or expansion determines the area of individual leaves. "Area per leaf increases linearly with leaf number along the stalk until a maximum leaf size is reached and increases little thereafter" (Robertson *et al.,* 1998; Sinclair *et al.,* 2004). Previous workers on sugarcane reported a maximum

area per leaf of 350 to 650 cm^2 at leaf number 15 (Inman-Bamber, 1994; Nayamuth, 1997, cited in Robertson *et al.*, 1998). In contrast, sugarcane cultivars in America reached maximum area per leaf at leaf number 20 to 25 (Sinclair *et al.*, 2004).

Stalks begin elongating when the primary stalk emerges and continues until harvest (van Dillewijn, 1952). The expansive growth of stalks is important for cane yield. The expansive growth of leaves and stalks is driven by cell division which takes place in the meristem of the growing point (van Dillewijn, 1952). Water is needed to support cell division and elongation with necessary water content and cell turgor (van Dillewijn, 1952). The required water is absorbed from the soil through roots to the growing points. This means, any occurrence of water stress could reduce leaf elongation rate (LER) and stalk elongation rate (SER) by depriving cells of the necessary critical water content to drive cell growth.

According to Inman-Bamber (2004), LER is one of the most water stress sensitive plant processes. Inman-Bamber & de Jager (1986a) found that sugarcane plant extension rate (PER, a combination of LER and SER) declined from 40 mm d^{-1} at a Ψ_L of -0.5 MPa to almost 0 mm d^{-1} at a Ψ_L of -1.3 MPa. Koonjah *et al*. (2006) found higher thresholds whereby PER began decreasing when Ψ_L reached -0.8 MPa and ceased Ψ_L of -1.2 MPa. In Hawaii, relative stalk elongation rate (RSER, SER of stressed plants divided by SER of unstressed plants) of cultivar H62-4671 rapidly decreased when Ψ_L dropped below -0.6 MPa and was approximately zero when Ψ_L was -1.3 MPa (Koehler *et al*., 1982). The reduced growth rates are due to insufficient quantity of water to support cell division and cell elongation. Inman-Bamber (2004) reported that area per leaf of stressed cane was significantly reduced by water stress because of a "reduction of new and larger leaves entering the pool of mature green leaves".

The effects of water stress on expansive growth of leaves and stalks are reversible. According to Roberts *et al*. (1990), "the activity of water stressed plants after re-wetting has often shown higher rates than treatments remaining fully-irrigated throughout and may contribute to compensatory growth between treatments". They further observed that LER was close to zero in water stressed cane but quickly rose above that of well-watered treatments within three days, after 30 mm of rainfall. In a pot experiment by Inman-Bamber & de Jager (1986a), LER of stressed cane increased up to 60% of the unstressed treatments within three days when re-watered.

Modelling effects of water stress on leaf and stalk elongation

In the CANEGRO model, leaf and stalk expansive growth of unstressed cane are simulated as a function of thermal time (Inman-Bamber, 1991: 1994). SER does not start until a cultivar-specific thermal time has lapsed since the emergence of a primary stalk. Under conditions of water stress, CANEGRO reduces LER and SER through a soil water deficit factor ($SWDF_2$).

$SWDF$ is calculated from potential transpiration (EOP) and root water uptake (RWU, mm d^{-1}) as follows:

$$SWDF_1 = MIN\left(1, f_1 \cdot \frac{RWU}{EOP}\right) \qquad \text{(Equation 2.1)}$$

$$SWDF_2 = MIN\left(1, f_2 \cdot \frac{RWU}{EOP}\right) \qquad \text{(Equation 2.2)}$$

Where f_1 = 1, f_2 = 0.5. $SWDF_2$ is more sensitive to water stress than $SWDF_1$ and reduces SER and LER when water uptake by roots drops two times the atmospheric evaporative demand.

Yield

The quantity of biomass partitioned to the stalk and to sucrose controls the productivity of sugarcane (Inman-Bamber *et al.,* 2002). Following the emergence of a primary stalk, assimilates distribution shifts towards roots and canopy development. Thereafter, "assimilates are diverted towards structural growth, starting with leaf expansion and then to stalk elongation" (Singels & Inman-Bamber, 2011). The stalk fraction starts at zero prior to the emergence of a primary stalk and then increases with aboveground biomass to a maximum value at crop maturity (Robertson *et al*., 1996; Inman-Bamber *et al*., 2002; Singels *et al.*, 2005). The stalk fraction can be as high as 0.85 in mature crops (Robertson *et al.,* 1996; Inman-Bamber *et al*., 2002) or as low as 0.65-0.69 (Rostron, 1972; Thompson, 1988) depending on temperature (Singels & Inman-Bamber, 2002), cultivar and water availability (Inman-Bamber *et al*., 2002). Water stress reduces cane yield by decreasing the number of millable stalks (Robertson *et al*., 1999; De Silva & De Costa, 2004), stalk height (Thomas *et al.,* 1978; Inman-Bamber & de Jager, 1986a; Singels & Inman-Bamber, 2002), stalk diameter and stalk mass (De Silva & De Costa, 2004).

Secondly, the stalk could be separated into sucrose, non-sucrose and fibre fractions. The sucrose fraction is influenced by time of harvest, crop age and cultivar (Evensen *et al*., 1997; Redshaw & Nuss, 2001; Inman-Bamber *et al*., 2002). Moreover, previous workers showed that sucrose content tends to increase under low temperatures and soil water content (Inman-Bamber *et al*., 2002; Singels *et al*., 2005). Evidence shows that water stress may increase sucrose yield and sugarcane farmers are advised to withhold irrigation prior to harvest, a procedure known as drying-off (Robertson & Donaldson, 1998; Robertson *et al.,* 1999; Singels & Inman-Bamber, 2002; Inman-Bamber, 2004; Inman-Bamber & Smith, 2005). This is because water stress rapidly inhibits the expansive growth while photosynthesis continues resulting in translocation of photosynthates to storage tissues rather than to structural growth (Bull & Glasziou, 1975; Chaves *et al*., 2009). Robertson & Donaldson (1998) found that drying-off improved sucrose yields by 0.5 to 2.5 t ha^{-1}. However, under severe water stress conditions, stomata tend to close restricting photosynthesis and inhibiting photosynthate translocation to sucrose storage tissues resulting in decreased sucrose yields.

The increased sucrose yield under water stress conditions is at the expense of both fibre and non-sucrose fractions (Botha *et al.*, 1996; Inman-Bamber & Smith, 2005). Therefore, the fibre fraction may only increase by improving soil water status (Singels & Inman-Bamber, 2011).

The trash (dead leaves plus growing tops) fraction is low in young crops increasing with leaf senescence. Inman-Bamber *et al.* (2002) reported that "approximately 15-20% of sugarcane dry mass accumulated ends up in trash under moderate to high rainfall or irrigated conditions". Under water stress conditions, about 35% of the accumulated dry biomass ends up in the trash (Inman-Bamber *et al.*, 2002). The trash fraction of NCo376 and N16 sugarcane genotypes was approximately 0.2 in well-watered conditions and increased up to 0.31 and 0.36 in dry conditions for the two cultivars, respectively.

Modelling effects of water stress on yield

In the CANEGRO model, daily biomass increments (ΔTOT) is affected by temperature. The model simulates ΔTOT using PAR conversion efficiency ($PARCE$ in g MJ^{-1}):

$$\Delta TOT = (1 - g)(PARCE \times FIPAR - mTOT)SWDF_1 \qquad \text{(Equation 2.3)}$$

Where g and m are the coefficients for growth respiration (0.242 t/t) and maintenance respiration (0.004 t/t), respectively. $FIPAR$ is the amount of PAR intercepted by leaf canopy in MJ ha^{-1}. TOT is the size of the crop (t ha^{-1}). Biomass partitioning is less sensitive to water stress and continues under stress conditions which would affect expansive growth. Biomass partitioning to stalks is simulated as a function of thermal time and partitioning does not occur until a certain cultivar specific thermal time has lapsed (Singels & Bezuidenhout, 2002).

2.3.3. Resource capture

Evapotranspiration

Crop water use or evapotranspiration (ETc) refers to a combination of water evaporated from the soil surface and transpired through the plant. Potential evapotranspiration (PET) can be defined as the maximum ET that could occur for a given crop if it had an unlimited water supply (Allen *et al.*, 1998). Actual evapotranspiration (AET) is the amount of water that is actually lost via evaporation and transpiration for a given crop. AET determination is based on reference evapotranspiration (ETo) defined as the rate of ET from a hypothetical reference crop, usually a short grass. The hypothetical reference crop has a height of 0.12 m, surface resistance of 70 s m^{-1}, albedo of 0.23, completely shading the ground and has adequate water (Allen *et al.*, 1998).

Leaf transpiration is dependent on crop characteristics (crop height, crop roughness, reflection and climatic conditions (solar radiation, temperature, wind, vapour pressure deficit (VPD) between the leaf and the air). ETc is best explained through the Penman-Monteith equation:

$$ETo = \frac{\Delta(Rn-G)+\rho a\ cp\frac{(es-ea)}{ra}}{\Delta+\gamma(1-\frac{ra}{rs})} \qquad \text{(Equation 2.4)}$$

Where R_n: net radiation (MJ m^{-2} d^{-1}); G: soil heat flux (MJ m^{-2} d^{-1}); (e_s-e_a): difference between the saturated and actual vapour pressure of the air (i.e. VPD) (kPa); ρa: mean air density at a constant pressure (kg m^{-3}); cp: constant heat capacity of the air (J kg^{-1} $^oC^{-1}$); Δ represents the slope of saturation vapour pressure curve at a given temperature (kPa $^oC^{-1}$); γ: psychrometric constant (kPa $^oC^{-1}$); and r_s: stomatal resistance and r_a: surface resistance and aerodynamic resistance (m s^{-1}).

Stomatal resistance (r_s) to transpiration is controlled by guard cells which in return are controlled by turgor pressure (Blum, 2011). Increasing solar radiation results in increased air temperatures, lower air humidity and increased vapour pressure deficit (VPD) between the leaf and the atmosphere. Increased leaf-air VPD decreases turgor of guard cells, thus resulting in increased stomatal resistance (r_s) as stomata close. However, under well-watered conditions, where the water potential of the soil is high, guard cells remain turgid and open regardless of transpiration rate. This is because under these conditions, the water potential gradient between the soil and the leaf is so small that there is enough moisture to maintain Ψ_L at higher levels needed to keep the stomata open. Under water stress conditions, Ψ_L decreases due to insufficient water supply from the soil and the plant will transpire more water than it can replenish. This result in guard cells remaining flaccid and stomata remain closed thus reducing transpiration.

Modelling effects of water stress on evapotranspiration

The CANEGRO sugarcane model uses soil, plant and atmosphere inputs to calculate daily soil evaporation and plant transpiration. The first step is to calculate potential ET (PET) as the product of ETo and the crop coefficient.

PET is then partitioned into potential evaporation ($Epot$) and potential transpiration ($Tpot$) (mm d^{-1}) based on the fraction of solar energy ($FIPAR$) reaching the soil surface (Jones *et al*., 2003):

$$Tpot = ETmax \times FIPAR \qquad \text{(Equation 2.5)}$$

$$Epot = ETmax \times (1 - FIPAR) \qquad \text{(Equation 2.6)}$$

Secondly, the model simulates how the water content of the soil changes by calculating the actual daily water lost from the soil surface through evaporation. This is done following a two-step process. The first step calculates actual evaporation (AE) for a wet surface after a rainfall or irrigation event.

AE under these conditions occurs at $Epot$ rate until a cumulative soil evaporation amount since wetting is reached (Jones *et al*., 2003). Thereafter, the evaporation rate is driven by the conductivity of the soil. If the evaporative demand is higher than the potential water uptake, then water stress occurs and actual water uptake is equal to potential water uptake. During water stress when AE is less than *Epot*, or the ratio of AE to *Epot* is less than 1.0, ETo is increased by adding the difference between AE and *Epot* (Ritchie, 1972).

Radiation interception

Solar radiation plays an important role in plant growth by providing the energy needed to drive photosynthesis and biomass accumulation (De Silva & De Costa, 2012). Plants absorb only 50% of the global solar radiation for photosynthesis. This spectrum absorbed by plants is known as photosynthetically active radiation (PAR, 400 to 700 nm wavelengths) (Spitters *et al*., 1986). The ability of the crop canopy to intercept solar radiation is a function of leaf area (Varlet-Grancher *et al.*, 1993). Koonjah *et al.* (2006) found that NCo376 sugarcane cultivar grown under water stress conditions for 20 days intercepted 25% less PAR compared with control treatments. However, when severely stressed, less than 60% PAR was intercepted by the leaf canopy (Inman-Bamber & de Jager, 1986b). This decline was associated with a reduction in canopy cover as a result of reduced leaf and stalk appearance rates, and increased leaf and stalk senescence rate (Inman-Bamber, 2004; Smit & Singels, 2006).

Modelling radiation interception

The CANEGRO sugarcane model simulates the fraction (FI) of PAR intercepted by the crop canopy using Beer's law of radiation extinction in plant canopies.

$$FI = 1 - \exp(-Kc * FIPAR) \qquad \text{(Equation 2.7)}$$

where Kc is the PAR extinction coefficient. According to Inman-Bamber (1991), a coefficient of 0.58 is used when less than 15 leaves have appeared and 0.86 for more than 15 leaves per stalk. If FIPAR is derived using green LAI only, it is used to simulate transpiration and total LAI (green and dead leaves) for soil evaporation. Water stress reduces $FIPAR$ through a reduction in LAI.

2.3.4. Resource use efficiency

Water use efficiency

Water use efficiency (WUE) has different definitions depending on the scale or context being considered. At a leaf scale WUE is defined as the ratio of instantaneous net CO_2 assimilation rate (A) to transpiration rate (T) (Bacon, 2004; Jones, 2004; Saseendran *et al*., 2008). WUE at leaf level is also known as the intrinsic water use efficiency. At crop level, WUE is defined as the net gain in dry

matter over a period of time per unit of water used over the same period (Chaves *et al.*, 2004). Partitioning water use (ETc) into transpiration (T) and soil evaporation (E) will enable determination of transpiration efficiency (TE) (Zhang *et al.,* 1998). TE, at leaf level, is defined as the production of dry matter per unit of water consumed in transpiration (Thevar *et al*., 2010). WUE seems to be affected by row spacing, cultivar and soil moisture (Olivier & Singels, 2003; Jangpromma *et al*., 2012). Alternative terms for WUE were suggested, e.g. 'biomass water ratio' (Morison *et al*., 2008) and 'crop water productivity' (Ali & Talukder, 2008). The biomass is usually determined as dry weight rather than as fresh weight. WUE is expressed as follows:

$$\mathrm{WUE} = \frac{\mathrm{Y}}{\mathrm{ET}} \qquad \text{(Equation 2.8)}$$

Where Y is the biomass yield produced per unit area over a given period (kg m^{-2}); ET is the amount of evapotranspired over the same period (mm).

Passioura (2006) suggested that WUE could be effectively improved by (i) minimizing unwanted water loss, (ii) increasing transpiration efficiency (TE) and (iii) converting most of the biomass into harvestable products. TE could be improved by decreasing stomatal conductance (Passioura, 2006; Inman-Bamber *et al.,* 2012; Songsri *et al.,* 2013). Unwanted water loss could be restricted by improving canopy development thus minimizing soil evaporation (Passioura, 2006). Lastly, restricting irrigation or imposing water stress at certain stages of crop development could also improve WUE by promoting rooting depth thus increasing water capture (Fereres & Soriano, 2007). Olivier & Singels (2003) found that the WUE of N25, N22 and N14 sugarcane cultivars grown under water stress conditions (irrigated 50% of the potential evapotranspiration) was highest reaching 14.0, 10.4, and 13.4 t/100 mm whereas the control treatments had WUE of 9.4, 9.5, and 10.3 t/100 mm, respectively. This improvement in WUE of crops grown under stressed conditions could be directly linked with increased rooting depth.

Radiation use efficiency

The ability of a crop to convert intercepted photosynthetically active radiation (FIPAR) to dry matter is known as radiation use efficiency (RUE). RUE is generally defined as the ratio of aboveground biomass accumulated to intercepted global solar radiation (Donaldson *et al.,* 2008). Crop models usually assume that RUE is constant for a given crop species (Yi *et al*., 2010). RUE values for sugarcane cultivars range from 1.25 to 2.09 g MJ^{-1} (Muchow *et al.,* 1994; Sinclair & Muchow, 1999; Singels & Smit, 2002; Donaldson *et al.,* 2008; De Silva & De Costa, 2012) whereas that of maize crops ranges from 1.6 to 1.8 g MJ^{-1} (Muchow & Davis, 1988; Kiniry *et al.,* 1998). Nevertheless, RUE may be influenced by growing season (Inman-Bamber, 1994; Singels *et al.,* 2005) and is also

influenced by other factors such as row spacing (Liu *et al.*, 2012). Climatic factors determine the interception of radiation and hence RUE (Jamieson *et al.*, 1995; De Silva & De Costa, 2012).

During water stress, crops conserve soil moisture by closing their stomata (thus reducing CO_2 diffusion) and reduce leaf area (through increased senescence or rolling). Consequently, these reductions may reduce crop productivity as biomass accumulation is reduced (Robertson *et al.*, 1999). De Silva & De Costa (2012) studied the growth and RUE of eight commercial sugarcane cultivars under irrigated and rainfed conditions. They found that, on average RUE of cultivars grown under water stress conditions decreased by 50%.

Modelling radiation use efficiency

The CANEGRO model simulates RUE (g MJ^{-1}) as a function of temperature (Jones, 2013):

$$RUE = RUE_{MAX}\left[1 - e^{KRUE\ (T_{MEAN} - B_{photos})}\right] \cdot SWDF_1 \qquad \text{(Equation 2.9)}$$

Where RUE_{MAX} is the theoretical maximum RUE, $KRUE$ is the air temperature sensitive coefficient (equals to -0.08), T_{MEAN} is the daily mean air temperature (^{o}C) and B_{photos} is the base temperature for photosynthesis (7 ^{o}C). Under water stress conditions, RUE is reduced below RUE_{MAX} by a stress index ($SWDF_1$).

2.4. Measurement techniques

2.4.1. Crop water use

In sugarcane experiments, various methods are used to quantify crop water use or evapotranspiration (ETc). These include, but are not limited to, weighing lysimeter, soil water balance, energy balance, Penman-Monteith Equation and the sap flow.

Weighing lysimeter

ETc can be determined directly through weighing of the cropped surface including the soil zone supplying water to crop, over a given time interval (Thompson, 1971; Fisher, 2012). When using weighing lysimeters, crops are usually grown in isolated tanks filled with soil resting on top of mass determining sensors. These tanks resemble the surrounding cropped surface in all aspects. In the absence of rainfall or irrigation, the changes in mass are due to ETc (Allen *et al.*, 1998). During rainfall or irrigation events, drainage water is collected at the bottom of the lysimeters, thus allowing accurate estimates of ETc by subtracting the mass of the drainage. One disadvantage of lysimeter systems is that the soil profiles are commonly too shallow compared to the surrounding natural soil profile. As a result, the water inside the lysimeter may differ from that outside and subsequently the ETc process (Allen *et al.*, 2011).

Soil water balance

ETc can also be estimated by calculating the soil water balance from measurements of its components: the balance between the inflow and outflow of water or water remaining in the soil over a given time interval (Allen *et al*., 1998).

$$ETc = P + I \pm \Delta SW - D - R - CR \qquad \text{(Equation 2.10)}$$

Where P is the precipitation (mm), I is the irrigation (mm), ΔSW is the change in soil water storage (mm), D is drainage (mm), R is the surface runoff (mm) and CR is the capillary rise from the groundwater table. Precipitation and irrigation can easy be measured with rainfall gauges and flow meters.

Soil water content can be measured gravimetrically or with a neutron water meter or capacitance soil moisture sensors.

Gravimetrically measured soil water content (SWC_g) is expressed as mass ratio (g g^{-1}) or volume ratio (m^3 m^{-3}) (Gardner *et al.*, 2000). The gravimetric method involves weighing wet soil samples and heating them to 105 ^oC to evaporate water before dry mass is determined. This method is used as the standard method to calibrate other techniques. SWC_g can be determined as follows:

$$SWC_g = \frac{mass\ of\ wet\ soil - mass\ of\ dry\ soil}{mass\ of\ dry\ soil} \qquad \text{(Equation 2.11)}$$

If soil volume is known (determined from core soil samples), then volumetric soil water content (SWC) (m^3 m^{-3} or %) can be directly calculated from:

$$\text{SWC} = \frac{Volume\ of\ water}{Soil\ volume} \qquad \text{(Equation 2.12)}$$

If soil bulk density is known, then SWC can be directly calculated as follows:

$$\text{SWC} = mass\ of\ water \times \frac{soil\ bulk\ density}{water\ density} \qquad \text{(Equation 2.13)}$$

Soil bulk density is calculated by dividing soil mass by the volume of soil (g cm^{-3}). The gravimetric method is simple and inexpensive but it is time consuming. Because of its destructive nature, repeated measurements at the same point are not possible. Results are only known after 24 hours.

SWC can also be estimated through capacitance soil moisture sensors. These sensors determine soil water content by measuring dielectric permittivity of the surrounding soil. “The dielectric permittivity of the soil is directly related to the water content. The capacitance soil moisture sensors output a voltage proportional to the dielectric permittivity, and therefore the water content of the soil” (ICT International Pty Ltd, 2013). Capacitance soil moisture sensors are quick and accurate, but the readings are influenced by soil texture and temperature. The major drawback of capacitance soil moisture sensors is their sensitivity to soil temperatures.

SWC can also be estimated through a technique called time domain reflectometry time domain reflectometry (TDR). TDR determines the dielectric permittivity of a medium by measuring the time it takes for a voltage pulse to travel along a transmission line that is surrounded by the medium (Bittelli *et al.*, 2008). The time and speed at which the reflected pulse travels from the end of the transmission line or probe depends on the dielectric value of the soil, which is related to the water content of the soil (Menziani *et al.*, 1996).

Unlike TDR, the frequency domain reflectometry (FDR) consists of two or more capacitors that are inserted into the soil. These capacitors are connected to an oscillator to form an electric circuit such that the changes in soil water can be detected by changes in the circuit's operating frequency (Rossel *et al.*, 2011).

The neutron water meter (NWM) has been widely used to determine SWC in field experiments (Singels *et al*., 2000; Li *et al*., 2003; Smit & Singels, 2006). According to Gardner *et al.* (2000), the main advantages of the neutron method compared to the gravimetric method are; (1) it is non-destructive, (2) it is fast, and (3) repeated measurements can be carried out *in situ*. NWM is not affected by soil salinity and air gaps. One major disadvantage of NWM is that it contains radioactive substance which could be hazardous to human health.

A major drawback in ETc determined by the soil water balance method is uncertainty in drainage from the zone sampled. However, when using a rainshelter constructed with drainage pipes, drainage water can be collected in containers and quantified with accuracy.

Energy Balance

The Bowen Ratio Energy Balance (BREB) method is a micrometeorological method based on the characteristics of the energy balance of a surface. This method estimates ETc as the latent heat flux density (λE) from a surface by measuring air temperature and humidity gradients above the crop, net radiation (Rn, MJ m^{-2} d^{-1}) and soil heat flux density (G, MJ m^{-2} d^{-1}) (Todd *et al*., 2000). Latent heat flux density (in W m^{-2}) can be calculated by substituting the measurements into a rearranged surface energy balance equation:

$$\lambda E = \frac{Rn-G}{1+\beta} \qquad \text{(Equation 2.14)}$$

Where, β is the ratio of sensible heat flux to latent heat flux derived from temperature and humidity measurements (Inman-Bamber & McGlinchey, 2003).

ETc can also be determined through the eddy covariance (EC) method. The EC method is a micrometeorological technique which measures surface to atmosphere fluxes of heat and water vapour (Mengistu, 2008). The system measures flux by measuring individual rotational eddies within

the air, allowing for the determination of the net vertical turbulent flux. Such measurements permit the determination of latent heat fluxes, allowing for the ETc to be calculated. This method is accurate in large fields. The major disadvantage of the energy balance methods is that they require a big fetch for accurate measurements. That means the EC method may not applicable for experiments in small plots e.g. a rainshelter.

Penman-Monteith Equation

The Penman-Monteith equation is regarded by the United Nations Food and Agriculture Organisation (FAO) as the standard method for estimating ET (Inman-Bamber & McGlinchey, 2003). The Penman-Monteith method (Eq. 2.4) utilises weather components - solar radiation, relative humidity, wind run, air temperature and crop characteristics - to estimate ETo (mm d^{-1}) see Eq. 2.4.

Crop ET (ETc, mm d^{-1}) is determined by multiplying ETo (Eq. 2.4) by a sugarcane crop coefficient (*Kc*). *Kc* depends on the extent of canopy cover, canopy properties, aerodynamics and ranges from 0.3-1.25 for the low to mid canopy development to 0.7 for mature sugarcane canopies (Allen *et al.*, 1998). According to Allen *et al.* (1998), *Kc* can be determined as either single or dual. The single crop coefficient approach combines crop and soil evaporation effects into a single coefficient. The dual crop coefficient approach splits crop and soil evaporation into separate coefficients in order to account for the effect of wetting events on the value of *Kc*. The basal crop coefficient (K_{cb}) characterizes crop canopy and *Ke* characterizes soil surface evaporation. To account for water stress, K_{cb} or *Kc* are multiplied by a coefficient K_s which is equal to 1.0 until half the available water is used and which then declines linearly to zero when all the available water in the rooting zone has been used . Hence:

$$ETc = (K_{cb} \times K_s + K_e)ET_o \qquad \text{(Equation 2.15)}$$

McGlinchey & Inman-Bamber (1996) modified the Penman-Monteith equation to accommodate the influence of increasing sugarcane height on aerodynamics resistance and hence sugarcane ET (Inman-Bamber & McGlinchey, 2003). Their approach was then modified to a reference cane ET (ETcane) from a 3 m height with a LAI of 3.5.

Sap flow

The sap flow method measures the movement of sap in the stem xylem. Therefore, the sap flow method quantifies only the transpiration component of ET. Sap flow methods calculate the amount of heat dissipated from a given section of the stem downstream or the velocity of a heat pulse carried away from the heat source in the transpiration stream (Granier, 1987; Allen *et al*., 2011). The velocity of the sap flow is then related to water lost by the crop via transpiration. Three main methods are

used: heat pulse-sap velocity method (HPV), heat dissipating method (HD) and the tissue heat balance method (THB) (Granier, 1987). These methods involve inserting needle-like heating thermocouples into the trunk of trees (HPV and HD) or wrapping stalks with gauges containing a set of thermocouples (THB) (Allen *et al.*, 2011). Although the sap flow method directly measures transpiration, it has not been widely applied in sugarcane studies.

2.4.2. Radiation interception

Plant leaves reduce the intensity of PAR due to the absorbance of PAR for photosynthesis (Bakker, 1999). Plants with greater green leaf area index (see Section 2.3.1.3) absorb more PAR than those with a smaller surface area. This absorbance can be measured with radiation sensors. Line quantum sensors (e.g. ceptometer) are used to quantify PAR absorbed by plants. By measuring PAR (µmol m^{-2} s^{-1}) above (Ra) and below (Rb) the leaf canopy, radiation interception ($FIPAR, in\ \%$) can be calculated as follows:

$$FIPAR = \left[1 - \left(\frac{Rb}{Ra}\right)\right] 100\% \qquad \text{(Equation 2.16)}$$

Solarimeters and pyranometers could also be used to determine solar radiation interception by the leaf canopy of different wavelength range. These sensors measure shortwave irradiance below and above the leaf canopy and data loggers are used to collect data. Then $FIPAR$ is estimated as the difference between above and below the leaf canopy readings. Several solarimeters and pyranometers sensors must be installed to measure variations in leaf canopy and these sensors are often costly.

2.4.3. Plant water status

Among the symptoms of a plant suffering from water stress are leaf rolling and leaf wilting. However, a more sensitive plant-water-stress-indicator than leaf rolling or wilting is needed because by the time plants have wilted, growth has already been reduced. Leaf water potential (Inman-Bamber & de Jager, 1986b; Koonjah *et al*., 2006) and plant elongation rate (PER) are early indications of plant water stress (Inman-Bamber, 1986b; Singels & Inman-Bamber, 2002).

Leaf water potential

Measurement of leaf water potential (Ψ_L) has gained wide acceptance as a plant water stress indicator (Inman-Bamber & de Jager, 1986b; Koonjah *et al.*, 2006; Govender *et al*., 2009) and has also been used to describe the water status of different species (Scholander *et al*., 1965). Predawn and midday leaf water potential measurements are usually performed with a pressure chamber (Koonjah *et al.*, 2006; Smit & Singels, 2006). Using this method, leaves are cut and inserted into the pressure chamber and pressure (kPa, MPa) is applied around the leaf, forcing out the xylem sap. The pressure at which xylem sap is first expressed is known as the balance pressure. This corresponding pressure recorded

when the xylem sap first appeared is equivalent to the water potential of the leaf. Water stressed plants usually require more pressure for balance pressure to be measured.

Another method used to measure leaf water potential is the thermocouple psychrometer. This method involves enclosing a small leaf sample in a small air-tight chamber with a thermocouple and droplet of solution with known vapour pressure. Once the humidity is in equilibrium, a steady pressure which is proportional to leaf water potential develops inside the chamber. A cooling current is then applied to the thermocouples thereby causing water to condense around the measuring junction. The "amount of water on the junction is proportional to the vapour pressure in the chamber which in turn is proportional to leaf water potential" (Blum, 2011).

Stomatal conductance

Stomatal conductance can be measured with a leaf porometer using the leaf diffusion principle (ICT international, 2008). Stomatal conductance can also be estimated from gas exchange measurements measured with an infrared gas analyser (IRGA) (LiCor Biosciences, 2008). However, none of these measurements were done in this study.

Plant elongation rate

The literature has shown that plant, stalk and leaf elongation rates (PER, SER, LER) are affected by declining Ψ_L (Inman-Bamber & de Jager, 1986b). Inman-Bamber & de Jager, (1986b), Singels & Inman-Bamber (2002), Inman-Bamber (2004), Koonjah *et al.* (2006) have all shown the negative effects of water stress on PER supporting the use of PER as the plant stress indicator.

Stalk height is measured manually with a ruler or tape measure from the soil surface to the TVD leaf, "the uppermost fully expanded leaf that has a visible dewlap or distinct collar" (McCray *et al.*, 2005). Leaf length is measured from the base to the tip of the TVD leaf. Height differentials between consecutive measurements will allow the calculation of SER and LER. This method is laborious and does not allow for estimates of diurnal patterns.

SER and LER (mm h^{-1}) can also be measured electronically with growth transducers or potentiometers (Inman-Bamber, 1995a). Potentiometers are used to convert plant extension to electrical resistance (Inman-Bamber, 1995a) and a data logger is used to capture data (Smit *et al.*, 2005). To measure SER a fishhook is secured to the collar of the TVD leaf and to the leaf tip for LER (Smit *et al.*, 2005). As the leaf/stalk grows, the string is stretched causing changes in resistance. Growth transducers are accurate up to 0.006 mm or 0.00002% (Smit *et al.*, 2005).

2.4.4. Biomass measurements

According to Inman-Bamber *et al.* (2002), biomass yield is determined by cutting standing sugarcane at the soil surface of known area, then weighing it before partitioning into subsamples of green leaves, stalks, trash (dead leaves plus tops). Subsamples are weighed to determine fresh aboveground biomass yield (t ha^{-1} or kg m^{-2}) and dried in an oven at 80 °C. Subsamples are then removed from the oven and weighed to determine dry biomass yield of different biomass components (t ha^{-1} or kg m^{-2}).

2.4.5. Recommended measurement techniques

Frequent monitoring of soil water status is necessary for water relation studies hence accurate and robust methods that enable repeated SWC measurements are required. That means the gravimetric method would not be suitable for this purpose because it does not accommodate repeated measurements on single points and is destructive in nature. Rather, the gravimetric method could be used for calibrating other methods. A neutron water meter (NWM) seems to be the most reliable method for monitoring SWC due to its insensitivity to soil temperatures compared with capacitance moisture sensors.

The size of the experimental site and costs of the method will determine which method to use for estimating ETc. It is clear that the energy balance methods would not be suitable for studies under rainshelter facilities. This is because rainshelter facilities are usually small in nature and the energy balance methods need a large uniform fetch for accuracy. Weighing lysimeters are expensive to construct. It seems that the soil water balance (SWB) method combined with Penman-Monteith (PM) method would be more suitable for a rainshelter facility study. This could be done by replacing ETc estimated via the SWB method for days with uncertainties with ETc estimated though the PM method.

Radiation intercepted by the crop canopy is not uniform due to gaps caused by germination failure and other factors. Therefore, a portable ceptometer seems to be more suitable in determining radiation interception as repeated measurements can be taken at different sampling points thus quantifying this variation.

Leaf water potential is accurately performed with a pressure chamber. Growth transducers are more accurate in determining stalk and leaf elongation rates and consume less time compared with manual methods. Lastly, the method for determining biomass yield described in Section 2.4.4 is accurate, less expensive and is widely used across the South African sugarcane industry.

2.5. Concluding remarks

This literature review showed that the effects of water stress on plant growth and development are well studied. Several aspects of sugarcane were studied but most of the work was done on traditional or high-sucrose sugarcane cultivars. There is a lack of information on other classes of sugarcane such as the high-fibre sugarcane hybrids. In this study, the focus will be on the growth and development of a high-fibre sugarcane cultivar compared with a traditional sugarcane cultivar and how the two genotypes respond to water stress. More precisely, the growth and development process, resource capture, biomass yield and resource use efficiency of the two contrasting cultivars will be compared under well-watered and water stress conditions. This information would be useful for calibrating the existing crop models and for determining the feasibility of growing high fibre sugarcane cultivars in marginal areas.

3. MATERIALS AND METHODS

3.1. Study area

This study was conducted at the South African Sugarcane Research Institute (SASRI) rainshelter facility in Mount Edgecombe (29°42'40"S; 31°02'35"E and 96 m above mean sea level) (Figure 3.1). Drainage pipes covered with coarse gravel stones and sand were installed underneath the rainshelter soil to promote drainage. Waterproof plastic sheeting was installed underneath the soil profile at a depth of 1 m to limit rooting depth and water movement from below. The total area of the facility was 30 m x 8 m.

Figure 3.1. Rainshelter facility with primary shoots emerging from the soil 36 days after planting.

3.1.1. Soil properties

The rainshelter soil properties are presented in Table 3.1. The soil profile is artificial and homogenous with depth, as it was removed from the top soil of a nearby open field in June 2011. Soil was mixed and packed to a depth of 1 m. Particle size category (soil texture) was determined following the method of Bouyoucos (1962). Field capacity (FC), stress point (SP), available soil water content (ASWC), bulk density (BD) and permanent wilting point (PWP) were determined in the laboratory using the methods of Peters (1967). The methods of Schulze *et al*. (1985), Hutson (1986) and van Antwerpen *et al*. (1994) were also used to estimate FC, PWP & ASWC and the results compared to that of Peters' (1967) method (Table 3.2). Each plot had three sampling points and at each point four soil samples were taken at different soil depths. Sampling points within plots were 1.9 m apart on average and 8m away from the sampling point of the next plot.

Table 3.1. Rainshelter soil properties: Clay, silt and sand content, soil bulk density (BD), field capacity (FC), stress point (SP), available soil water content (ASWC) and permanent wilting point (PWP) per soil depth. Average and standard deviation of 48 soil samples for each plot are also shown.

		Soil particle size category				Soil moisture characteristics				
Plot #	**Depth**	**Clay**	**Silt**	**Sand**	**Silt+Clay**	**BD***	**FC**	**SP**	**ASWC**	**PWP**
	cm	%	%	%	%	g/cm³	m³/m³	m³/m³	m³/m³	m³/m³
1	0-20	10.8	4.5	84.7	15.3	1.523	0.174	0.141	0.090	0.084
	21-40	10.7	4.9	84.4	15.6	1.518	0.177	0.150	0.095	0.083
	41-60	10.7	4.5	84.8	15.2	1.524	0.173	0.133	0.089	0.084
	61-80	10.8	5.1	84.1	15.9	1.514	0.182	0.148	0.098	0.084
	Ave.	10.7	4.8	84.5	15.5	1.520	0.177	0.143	0.093	0.083
	Std. Error	0.08	0.27	0.30	0.30	0.005	0.004	0.008	0.004	0.001
2	0-20	11.1	4.5	84.4	15.6	1.513	0.181	0.147	0.097	0.084
	21-40	11.1	5.5	83.5	16.5	1.520	0.179	0.146	0.096	0.083
	41-60	10.7	5.1	84.3	15.7	1.511	0.182	0.151	0.096	0.086
	61-80	10.8	5.2	84.0	16.0	1.504	0.187	0.147	0.100	0.087
	Ave.	10.9	5.1	84.0	16.0	1.512	0.182	0.148	0.097	0.085
	Std. Error	0.20	0.39	0.41	0.41	0.006	0.004	0.002	0.002	0.002
3	0-20	10.8	4.5	84.7	15.3	1.514	0.183	0.146	0.098	0.085
	21-40	10.7	4.8	84.5	15.5	1.519	0.180	0.146	0.097	0.083
	41-60	10.3	4.9	84.8	15.2	1.522	0.174	0.131	0.089	0.085
	61-80	10.8	5.1	84.1	15.9	1.513	0.184	0.147	0.099	0.085
	Ave.	10.6	4.8	84.5	15.5	1.517	0.180	0.143	0.096	0.084
	Std. Error	0.25	0.23	0.29	0.29	0.004	0.005	0.008	0.005	0.001
4	0-20	10.4	4.5	85.1	14.9	1.512	0.181	0.150	0.097	0.084
	21-40	10.4	4.7	84.9	15.1	1.513	0.177	0.153	0.099	0.078
	41-60	10.0	5.2	84.8	15.2	1.524	0.174	0.136	0.090	0.084
	61-80	10.5	4.8	84.7	15.3	1.510	0.177	0.155	0.099	0.078
	Ave.	10.3	4.8	84.9	15.1	1.515	0.177	0.148	0.097	0.081
	Std. Error	0.23	0.29	0.17	0.17	0.006	0.003	0.008	0.004	0.003

***** Soil bulk density (BD) is calculated from the dry mass of core soil samples with known volume. BD (in g cm^{-3}) is then calculated by dividing soil mass by the volume of the soil samples.

Table 3.2 Rainshelter field capacity (FC), plant available soil water content (ASWC) and permanent wilting point (PWP) for the whole soil profile calculated using different methods. The method of Peters (1967) was used in this study.

Method	**FC (m^3m^{-3})**	**PWP (m^3m^{-3})**	**ASWC (m^3m^{-3})**
Peters (1967)	0.180	0.083	0.097
Hutson (1986)	0.171	0.071	0.100
van Antwerpen *et al.* (1994)	0.166	0.067	0.099
Schultze *et al.* (1985)	0.167	0.086	0.081

3.1.2. Fertilizer application

Fertilizer was applied according to SASRI Fertilizer Advisory Service (FAS) recommendations to ensure sufficient nutrient availability. Fertilizer was applied twice; at planting and 18 days after planting. Application details are shown in Table 3.3. Plots were irrigated immediately after fertilizing.

Table 3.3. Fertilizer application details according to FAS recommendations and application dates at the rainshelter facility.

	Application date	**Element**	**Fertiliser form**	**Element content, %**	**Fertiliser application rate, kg/ha**
First fertilizer application (applied into furrows at planting)	2011/10/05	N	NPK (1:0:1)	24:0:24	625
		P	Superphosphate	10.5	571.43
		K	NPK (1:0:1)	24:0:24	625
Second fertilizer application	2011/10/24	N	LAN (limestone ammonium nitrate)	28	142.86
		K	KCl	50	300
		Zn	Zinc sulphate heptahydrate	22	68.18

3.2. Experimental design

The rainshelter was divided into four plots such that each genotype had a combination of genotype x water treatment (Figure 3.2a). Each plot had six rows (7.7 m long each spaced at 1.25 m) covering an area of 55.4 m^2 with one plot per genotype used as the control (well-watered) and the other plot used for water stress treatment. Two guard rows were planted to prevent possible monkey damage and the edge effect on either sides of the rainshelter facility. In each plot, the third and fourth row (each 6 meters long) had 20 randomly selected tagged plants assigned for non-destructive measurements. Weeding was done manually or using hoes whenever weeds emerged. The irrigation system was

designed such that each treatment was irrigated independently (Figure 3.2b). Water was applied by drip irrigation scheduled to maintain soil water content above 80% of FC.

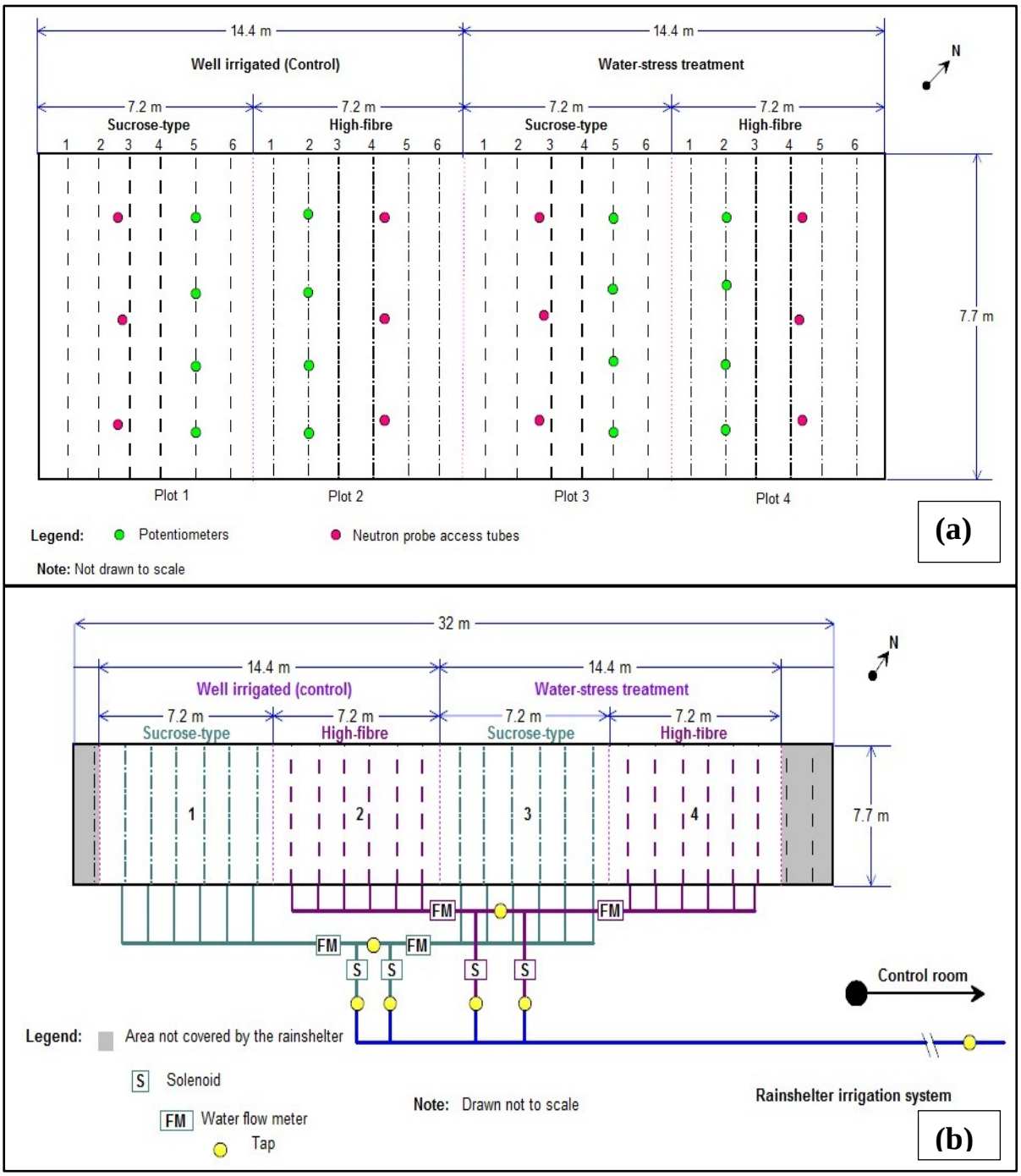


Figure 3.2. Rainshelter trial layout showing four treatments (a) and irrigation system design (b) (modified from Tsupko, 2010).

3.2.1. Treatments

Varieties

Two varieties, N19 (high sucrose) and 04G0073 (high-fibre) sugarcane genotypes were used for the study.

N19 (*Saccharum officinarum*) is a cross between NCo376 and CB40/35 and is commonly grown in the northern irrigated regions of KwaZulu-Natal (McIntyre & Nuss, 1996; SASRI, 2006). It is classified as having high sucrose and moderate fibre contents (McIntyre & Nuss, 1996; SASRI, 2006). The genotype is susceptible to water stress with poor stress recovery and has a medium stalk population of up to 112 000 ha^{-1} (SASRI, 2006). This genotype is resistant to smut, rust and leaf scald (SASRI, 2006).

Genotype 04G0073 was selected as a high fibre genotype based on SASRI plant breeding screening trials (unpublished data). It is a type II *Saccharum* hybrid cross between 88M0287 and US56158S ([1]Zhou, pers. com.). This genotype is characterised by its thin stalks and numerous long and narrow leaves.

Water treatments

From 10 February 2012 onwards i.e. 128 days after planting, water was withheld from the two water stress plots, while the other two plots were irrigated to prevent water stress. The two treatments were referred to as "stressed" (water stress) and "well-watered" (well irrigated) treatments. At 22 days after last irrigation (2-3 March 2012), a tropical storm occurred causing water to intrude into rainshelter soil from below through the drainage system. This resulted in 28 days of stress relief but later plants were stressed again for 29 days. The experiment therefore consisted of two stress events interspersed by period of favourable water status.

Plants were believed to have been water stressed when the volumetric soil water content (SWC) fell below the stress point value of 0.155 $m^3\ m^{-3}$, determined in the laboratory using the method of Peters (1962). The first stress period was defined from 10 February to 01 March 2012. The second stress event commenced from 01 April to 03 May 2012.

Well-watered treatments were irrigated whenever the average SWC dropped below 0.155 $m^3\ m^{-3}$. The required irrigation amount for the well-watered treatments were determined based on sugarcane reference evapotranspiration losses (ETcane, 3 m tall, fully canopied and well watered crop) (McGlinchey & Inman-Bamber, 1996) since last irrigation. The Penman-Monteith equation (Eq. 2.4) has been widely used as a standard for estimating ETcane in the South African sugar industry (Singels *et al.*, 1998; Singels *et al.*, 1999; Olivier & Singels, 2012).

[1] Zhou, M. 2013. SASRI, Plant breeding department, P/bag X02, Mount Edgecombe, 4300.

3.3. Measurements

A summary of all variables measured (with measurement time intervals) for this study is presented in Figure 3.3. The measurements carried out during the experiment can be categorised into weather, soil, non-destructive (plant growth and development) and destructive (biomass sampling) measurements.

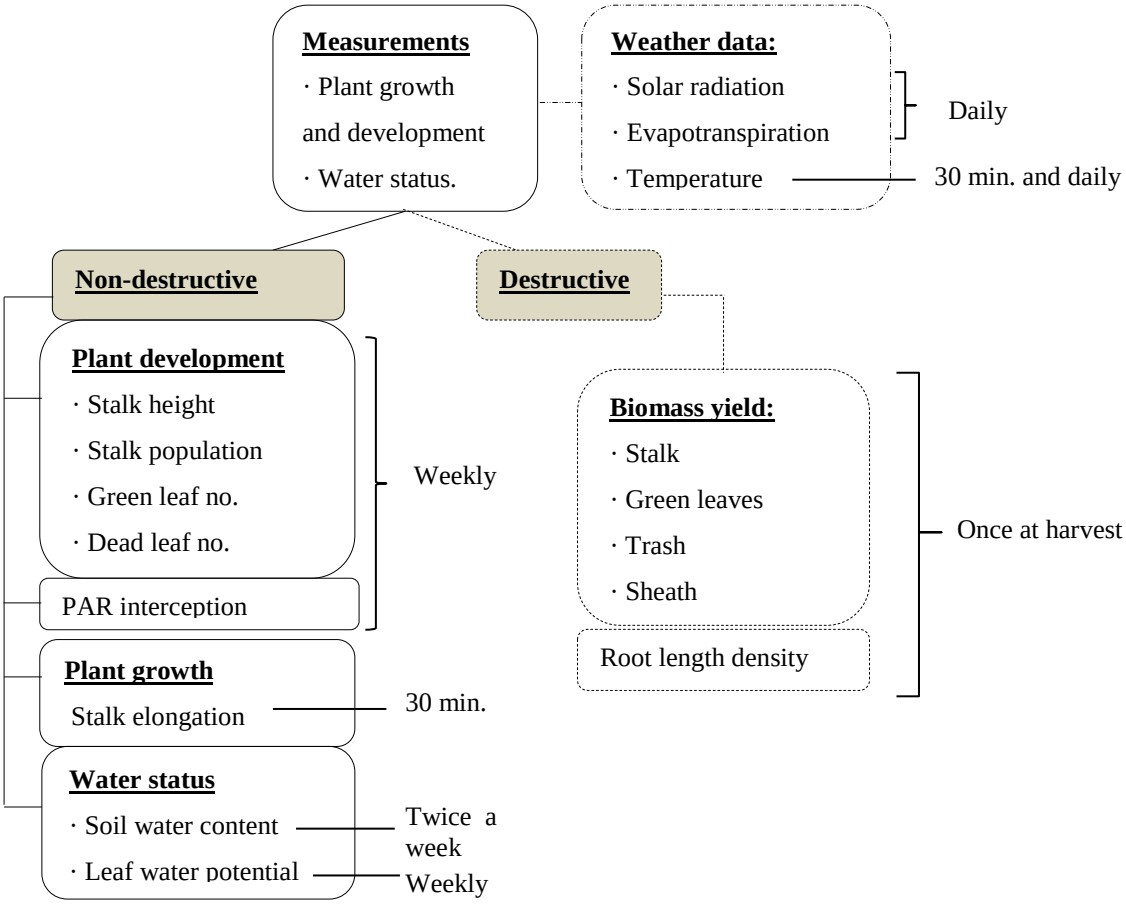


Figure 3.3. Summary of all rainshelter experiment measurements with intervals.

3.3.1. Weather

An automated weather system located 20 m from the experimental site was used to measure 30 minute interval temperatures from which average hourly temperature was derived. The time between 18h30 and 05h30 was used to calculate average night temperatures. Daily minimum and maximum, mean temperature (°C), Solar radiation (SRAD, MJ m^{-2} d^{-1}) and sugarcane reference evapotranspiration (ETcane, mm d^{-1}) were also recorded. A total of two rain gauges were installed on steel poles underneath the rainshelter, above the crop canopy, to allow for accurate estimate of rainfall in case the rainshelter failed to close. The weather data were used to calculate seasonal intercepted solar radiation, thermal time, and for irrigation scheduling.

3.3.2. Soil water content

Neutron water meter (503DR CPN Hydro probe, Campbell Pacific Nuclear, CA, USA) readings were taken in three aluminium access tubes per plot in order to determine volumetric soil water content (SWC in m^3 m^{-3}) (Fig. 3.2). SWC measurements were taken twice a week (from planting to harvest) at different soil depths of 12.5, 37.5, 62.5 and 82.5 cm. SWC data were later used to derive seasonal crop evapotranspiration (ETc).

During neutron water meter (NWM) calibration, gravimetric soil water content was multiplied by measured soil bulk density (see Table 3.1 and Appendix 2) to obtain SWCg and a linear regression was then fitted to SWCg vs. NWM counts (X) data (R^2 = 0.85, see Figure 3.4) (see Table A2 in Appendix 2 for the relevant data). The calibration equation was:

$$SWC = 0.00001193x + 0.03435 \quad (n=48) \qquad \text{(Equation 3.1)}$$

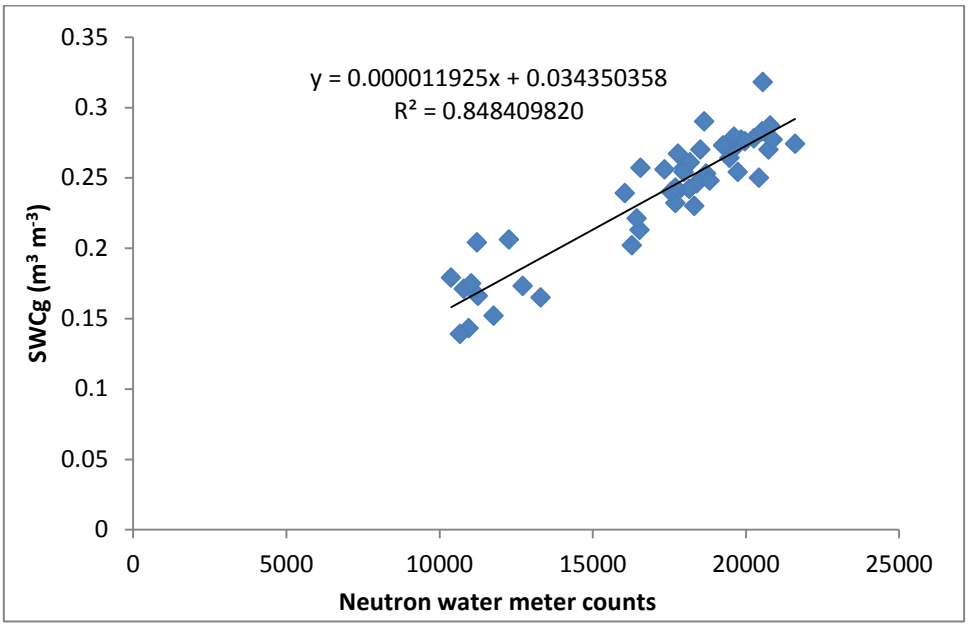


Figure 3.4. Volumetric soil water content (SWCg) plotted against neutron water meter counts.

3.3.3. Evapotranspiration

Crop evapotranspiration (ETc) was determined from soil water content measurements following a water balance approach (Eq. 2.10 in section 2.4.1) on days with no drainage.

Drainage was assumed to be zero when the SWC in the bottom layer was below FC. For days when the bottom layer SWC was above FC, ETc was estimated as the product of fractional interception (FI) and ETcane (McGlinchey & Inman-Bamber, 1996). This method was also used for days with suspect data, such as negative ETc values and values that were 30% higher than ETcane. In total, 10%, 14%,

19% and 24% of the ETc data were replaced in this way for the N19 control, 04G0073 control, N19 stressed and 04G0073 stressed treatments, respectively.

3.3.4. Plant development

Plant development measurements included non-destructive determination of stalk population and height, number of green leaves per stalk, leaf width and length, and canopy interception of radiation. All developmental measurements were done on the two net rows (6 m each) in each plot.

Leaf development

A total of 20 tillers were randomly selected in each of the two designated rows (row 3 and 4, in each plot) and tagged. The top visible dewlap (TVD) leaf width and length were measured with a tape measure for each of the tagged tillers from leaf emergence (17 Nov. 2011) until harvest (May 2012). The TVD leaf is defined by McCray *et al.* (2005) as the "uppermost fully expanded leaf that has a visible dewlap or distinct collar" (Figure 3.5). The number of green and dead leaves was counted on the tagged stalks starting from the stalk base upwards to the TVD leaf. The tag was moved to a nearby healthy and similar sized stalk when it was clear that the tagged stalk was dying.

Leaf area per leaf was calculated as a product of leaf width, leaf length and a shape factor (Sinclair *et al.*, 2004). A mathematical relationship between leaf area per leaf and leaf number, determined from measurements, was used to calculate total leaf area per primary stalk on a given date. Green leaf area per primary stalk was then taken as the difference between total leaf area and dead leaf area per primary stalk. Green leaf area index (GLAI) was calculated as the product of green leaf area per primary stalk and stalk population per unit ground area. It should be noted that GLAI was probably overestimated as all stalks were assumed to be primary stalks of the same age and leaf area, thereby overestimating the contribution of younger tillers with lower leaf areas. Also, the leaf area of expanding leaves was not included.

Stalk population and height

Stalk population (SKpop) was determined weekly by counting the number of stalks over two 6 m rows in each plot from 17 November 2011 until harvest (03 May 2012). Stalk height was measured with a 2m ruler from the soil surface to the TVD leaf on the 20 tagged stalks.

Fractional interception

A ceptometer (Decagon devices, AccuPAR model LP-80) was used to measure weekly photosynthetic active radiation (PAR) intercepted by green leaves between 11h30 and 13h00 on cloudless days. Two readings were taken above-canopy and 20 readings below the green leaf canopy (above all dead leaves) in each plot. The ceptometer was held horizontally at an angle to the rows such that one end

of the device was in the centre of the row and the other midway between rows (centre of inter-rows). From above and below canopy readings, FIPAR was computed using Eq. 2.16 in section 2.4.2.

3.3.5. Plant growth

Stalk elongation

Potentiometers were used to measure stalk elongation (Figure 3.5). The calibration coefficient was determined by adjusting the potentiometer string attached to a vernier calliper by 5 mm intervals recording equivalent resistance until 140 mm. The slope (a), intercept (b) and r^2 for the stalk potentiometer calibrations are summarised in Table 3.4.

Table 3.4. Summary of the slope (a, mm ohm^{-1}, mean ± standard deviation), intercept (b, mm, mean ± standard deviation) and r^2 (mean ± standard deviation) obtained from the calibration of stalk potentiometers (Pot). Mean and standard deviation (std dev) of 16 stalk potentiometers are also shown.

Pot #	**a (mm $ohm^{-1)}$**	**b (mm)**	**r^2**
1	0.0338 ± 3E-05	4.7181 ± 0.291	0.9999 ± 3E-05
2	0.0334 ± 2E-04	4.2521 ± 0.491	1 ± 0
3	0.0330 ± 1E-04	3.9728 ± 0.327	1 ± 0
4	0.0328 ± 6E-05	4.5314 ± 0.141	1 ± 0
5	0.0326 ± 3E-04	3.9784 ± 0.327	1 ± 0
6	0.0310 ± 2E-04	4.5321 ± 0.236	1 ± 0
7	0.0326 ± 0	4.64 ± 0.291	0.9999 ± 6E-05
8	0.0328 ± 0	4.5901 ± 0.092	1 ± 0
9	0.0328 ± 6E-05	4.2308 ± 0.171	1 ± 0
10	0.0324 ± 0	4.4673 ± 0.069	1 ± 0
11	0.0333 ± 0	4.5086 ± 0.107	1 ± 0
12	0.0329 ± 4E-04	4.0691 ± 0.054	1 ± 0
13	0.0324 ± 6E-05	4.7549 ± 0.182	1 ± 0
14	0.0334 ± 2E-04	4.9125 ± 0.041	0.9999 ± 6E-05
15	0.0331 ± 3E-04	4.8894 ± 0.294	1 ± 0
16	0.0323 ± 2E-04	4.5418 ± 0.101	1 ± 0
Mean	**0.051098333**	**4.47395375**	**0.999991458**
Std dev	**0.070463458**	**0.28854008**	**1.89285E-05**

A Spectrol 10 kΩ potentiometer (Smit *et al.*, 2005) was mounted on a lightweight, 10 mm aluminium tube with clamps to hold onto the sugarcane stalk. A wheel and a string were then attached to the pipe with one end of the string hooked onto the TVD leaf such that when plants grow the string is pulled turning the wheel which causes a change in resistance (Figure 3.5). The change in resistance was recorded with a data logger at 30 minutes intervals. All the potentiometers were installed a week before water treatments were applied until harvest in May 2012.

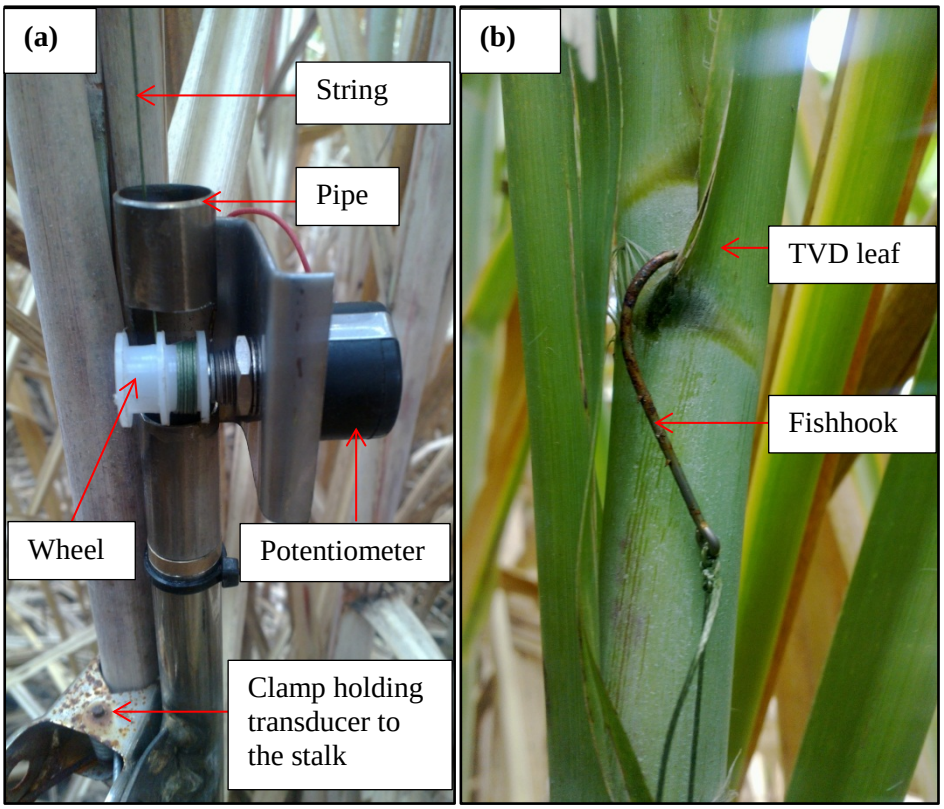


Figure 3.5. Growth transducer attached to the stalk (a) and a fishhook (with string) attached to the TVD leaf (b).

A data filter was used to reject data points that showed a difference in stalk height of more than 1 mm or less than 0 mm from the preceding data point recorded 30 min earlier. Consequently, readings that were measured when the string was hooked onto new leaves were eliminated. Hourly stalk elongation rate (SER_h) was determined by summing up the differences in stalk height between two 30 minutes intervals. Daily stalk elongation rate (SER_d) was determined by cumulating hourly stalk elongation over 24 hours. SER_d values were divided by daily thermal time (using a base temperature of 12.5 ^oC) in order to eliminate the strong temperature effect on it. The resultant variable was named normalized daily stalk elongation rate [$NSER_d$, mm $(^oCd)^{-1}$]. SER_d of stressed treatments were also expressed as a fraction of the well-watered treatments, and named relative stalk elongation rate ($RSER_d$).

Root length density

After harvesting the trial, roots were sampled using a root core sampler. In each plot, eight soil cores were extracted, with each divided into three samples namely 0-25, 25-50, and 50-75 cm. A total of 90 root samples were collected, washed (thus removing excess soil leaving roots only) and air dried. Dried roots were placed on a blank A4 paper and scanned using hp Scanjet 4570c scanner and the images were saved in the tiff format at 600 dots per inch (dpi) (Wiwart *et al.*, 2006). The images were analysed using APS ASSESS 2.0 Image Analysis Software for Plant Disease Quantification (American Phytopathological Society Press, St. Paul, MN, USA) (Brazelton *et al.*, 2008). The

software was simply calibrated by scanning a hand-drawn straight line of known length (5 cm). The actual length of the line was then divided by the software determined value to get a correction factor for each sample. After roots were scanned, root length (cm) (determined by the software) was multiplied by the correction factor to get corrected root length of each sample. Root length density (Lv, cm cm^{-3}) was then determined by dividing corrected root length (cm) by the sample volume (cm^3).

3.3.6. Leaf water potential

Midday (12h00 to 13h00) leaf water potentials (Ψ_L in MPa) were destructively determined weekly from 10 February until harvest (May 2012). This was done on leaf strips/segments of the two topmost, fully expanded leaves using a Scholander pressure chamber following a protocol by Saliendra *et al.* (1990). Leaf segments about 15 cm long were cut from the leaf margin to the midrib then immediately placed into a wet paper towel to prevent water loss. The strips were then put into the pressure chamber such that the cut end protruded through a hard rubber seal (Hsiao, 1990). The chamber was pressurised with compressed air until xylem water returned to the cut end. The pressure at this stage was recorded as the negative total leaf water potential.

3.3.7. Biomass sampling

Destructive sampling of aboveground biomass was done once at harvest on 07 and 08 May 2012. In each plot, 2 m rows were sampled destructively in four out of six rows (excluding the two 6m rows where non-destructive measurements had been done) by hand cutting stalks at the soil surface. After cutting the cane, the whole fresh sample including trash was weighed before sub samples of stalk, green leaves, dead leaves (trash), sheath and meristem were taken.

Sub samples were taken from the sample and weighed to determine their fresh mass before they were dried in an oven at 80 °C (until the sub samples dry mass remained constant) to determine the dry mass of the different plant components. The mean mass per unit area of different biomass components was determined by averaging the product of the sample mass per stalk and the average stalk population of the sample. Stalk material was analysed for fibre, sucrose and non-sucrose contents and the mass of each component determined accordingly by the SASRI Mill-room.

3.4. Calculation of parameters

3.4.1. Relative soil water content

Relative available soil water content (RASWC) was calculated as:

$$\text{RASWC} = \frac{\text{SWC}-\text{PWP}}{\text{FC}-\text{PWP}} \qquad \text{(Equation 3.2)}$$

where SWC was the average volumetric soil water content of the profile (m^3 m^{-3}), PWP was the permanent wilting point (m^3 m^{-3}) and FC was the field capacity (m^3 m^{-3}). RASWC was used to indicate the influence of soil water status on various plant parameters.

3.4.2. Seasonal radiation interception

Seasonal (planting to harvest) total radiation intercepted (MJ m^{-2}) was calculated as the sum of the product of cumulative daily solar radiation between two consecutive fractional interception (FI) measurements and FI over a two week period. This data was used to calculate radiation use efficiency.

3.4.3. Thermal time

Cumulative thermal time on a given day n (TT, in ^{o}C days) was calculated as follows (McMaster & Wilhelm, 1997):

$$TT = \sum_{d=1}^{d=n} \left[\left(\frac{Tmax+Tmin}{2}\right) - Tb\right] \qquad \text{(Equation 3.3)}$$

Where $Tmax$ and $Tmin$ are the daily maximum and minimum temperature on day d, respectively, and Tb is the base temperature (in ^{o}C). Tb for leaf and stalk phenology were taken as 10 ^{o}C and 16 ^{o}C, respectively (Inman-Bamber, 1994). A base temperature of 12.5 ^{o}C was used for SER_d based on Figure 4.14. This information was necessary for the determination of crop parameters for crop models and to normalize stalk elongation rate.

3.4.4. Resource use efficiency

In this study, WUE was defined as the net gain in total aboveground dry matter over a period divided by the amount of water evapotranspired over the same period (Chaves *et al.*, 2004). Only seasonal WUE was determined for this study. WUE was calculated as the ratio of total dry biomass yield to total seasonal ETc in units of kg m^{-3} for each of the treatments.

Radiation use efficiency (RUE) was calculated as the ratio of the total aboveground dry biomass yield to intercepted global short wave radiation in units of g MJ^{-1}. RUE was only determined once at harvest for the whole season for each treatment.

3.5. Data processing and analysis

All data collected were tested for significance using a t-test statistic with GENSTAT® *v.14* statistical package where possible.

4. RESULTS AND DISCUSSION

4.1. Weather data

Weather data graphs with a summary of trends are given in Appendix 1.

4.2. Water status

4.2.1. Soil water status

The trend in volumetric soil water content (SWC) for the rainshelter soil profile is shown in Figure 4.1, while results for each layer are shown in Figures 4.2a-d. After planting, the SWC of all treatments remained well above field capacity (FC) with no drainage observed (Figure 4.2). This was ascribed to changes in soil structure due to soil disturbance as the soil used for this study was obtained from the top soil of a nearby open field. Published data showed that macroporosity, which promote free drainage, is altered when soil is disturbed, thus resulting in negligible or no drainage (Tuli *et al.*, 2005). This often leads to a rise in SWC of disturbed soils (Perrier & Evans, 1961 cited by Shaykewich, 1970) until the roots of a crop planted on the disturbed soil start to extract excess water. In this study, the profile SWC returned to FC at -38 days after last irrigation (DALI).

The FC and permanent wilting point (PWP) for the soil profile (determined *in situ*) were 0.180 and 0.131 m^3m^{-3}, respectively. Plants were considered to have been water stressed whenever SWC dropped below the stress point value of 0.155 m^3m^{-3}. There were at least 11 days where SWC of the well-watered 04G0073 treatment dropped below 0.155 m^3m^{-3}. This was due to the use of an irrigation threshold derived from laboratory measurements that was subsequently found to be too low. Well-watered treatments nonetheless maintained a higher SWC compared with stress treatments.

Under both water treatments, N19 had a higher SWC than 04G0073. The SWC for stressed 04G0073 treatment dropped below 0.155 m^3m^{-3} three days sooner and remained lower than that of the stressed N19 treatment for the duration of the experiment (Figure 4.2). SWC continued to decrease with progressing stress, reaching low values of 0.137 m^3m^{-3} in N19 and 0.135 m^3m^{-3} in 04G0073 at the end of the first stress period i.e. at DALI=21.

The intrusion of water into the rainshelter on DALI=22 relieved plants from stress and increased SWC to well above FC. Thereafter SWC decreased, reaching stress point at DALI=50. From this point, SWC for the stress treatments decreased rapidly for ten days and then more gradually later on. The final SWC recorded at harvest for stress treatments were 0.131 and 0.134 m^3m^{-3} in 04G0073 and N19, respectively.

These results show that 04G0073 extracted water more rapidly in both water treatments, suggesting that it had higher evapotranspiration rates compared with N19 (see Table 4.1).

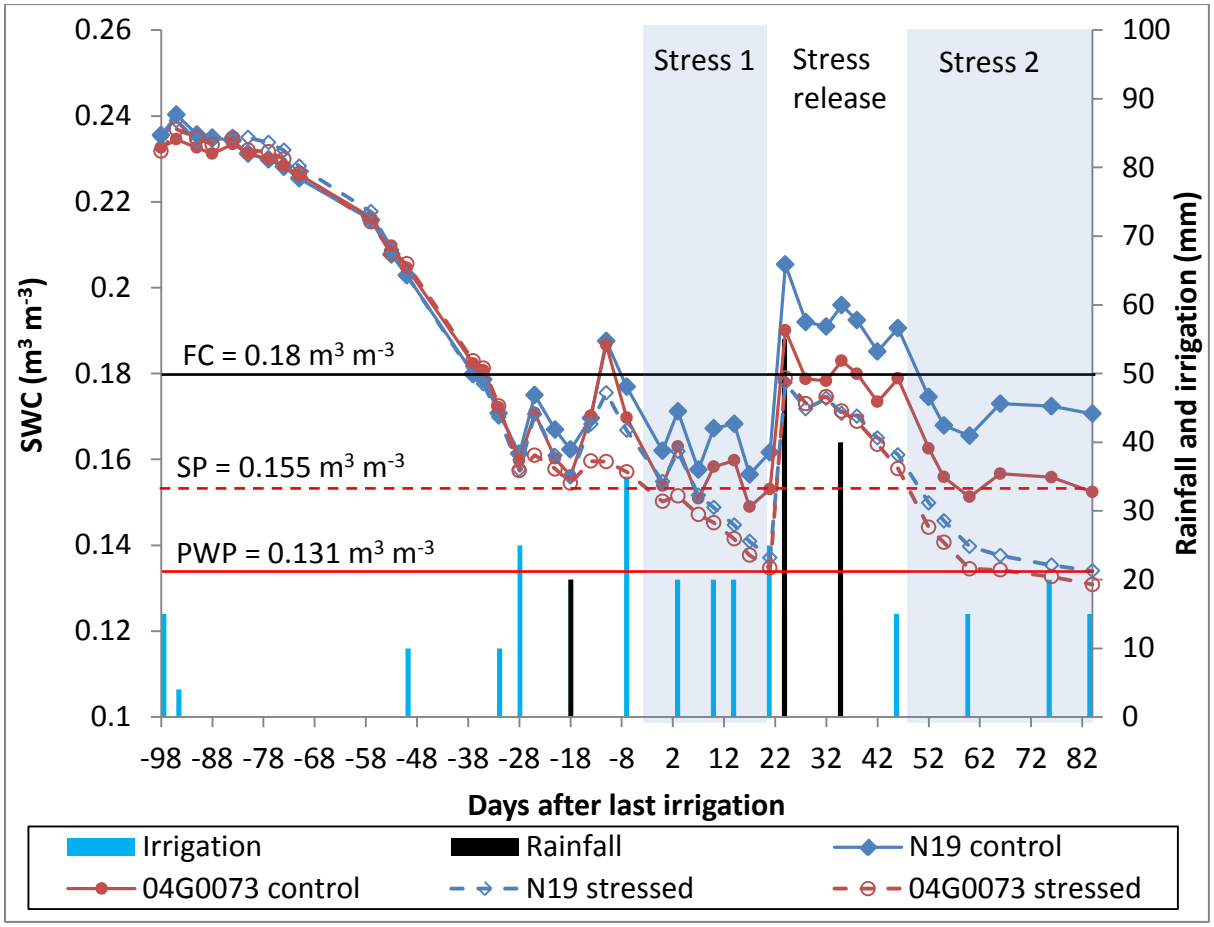
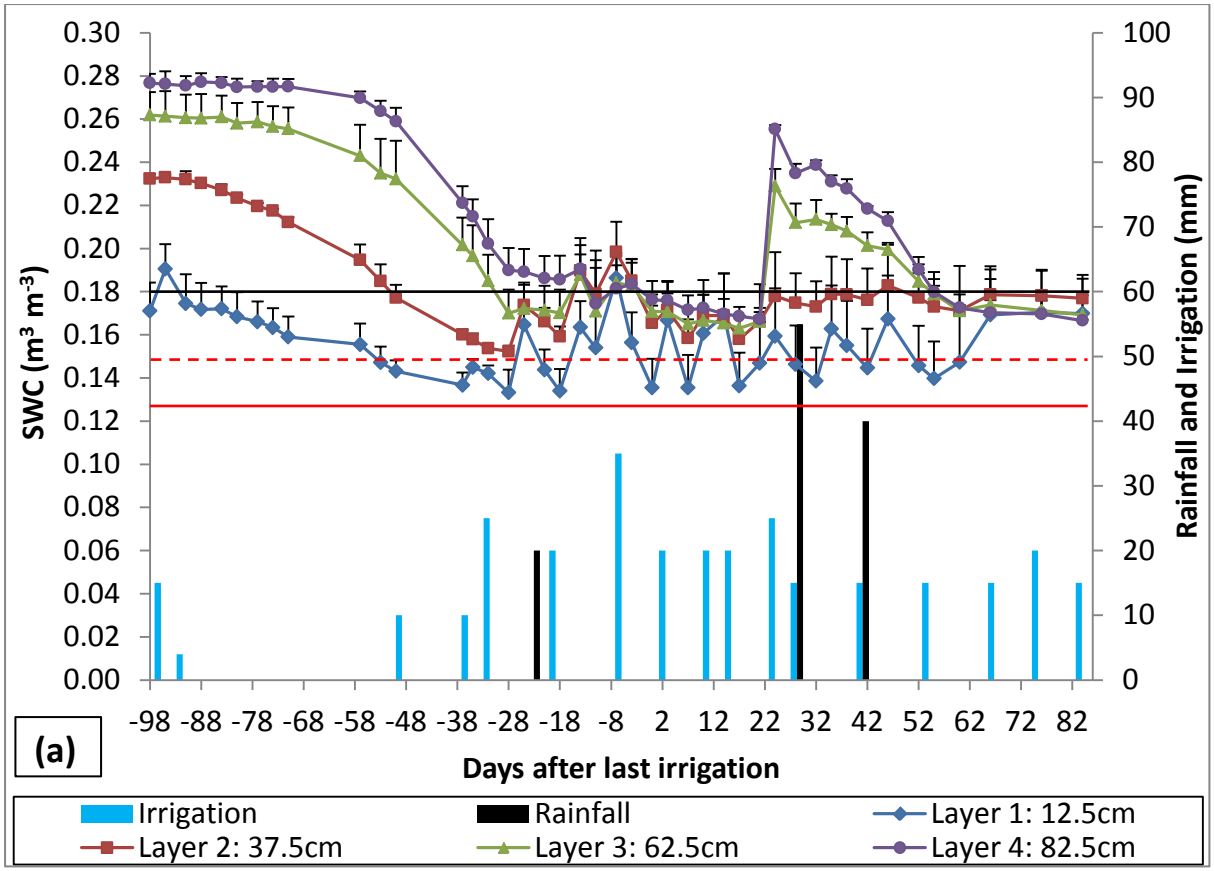
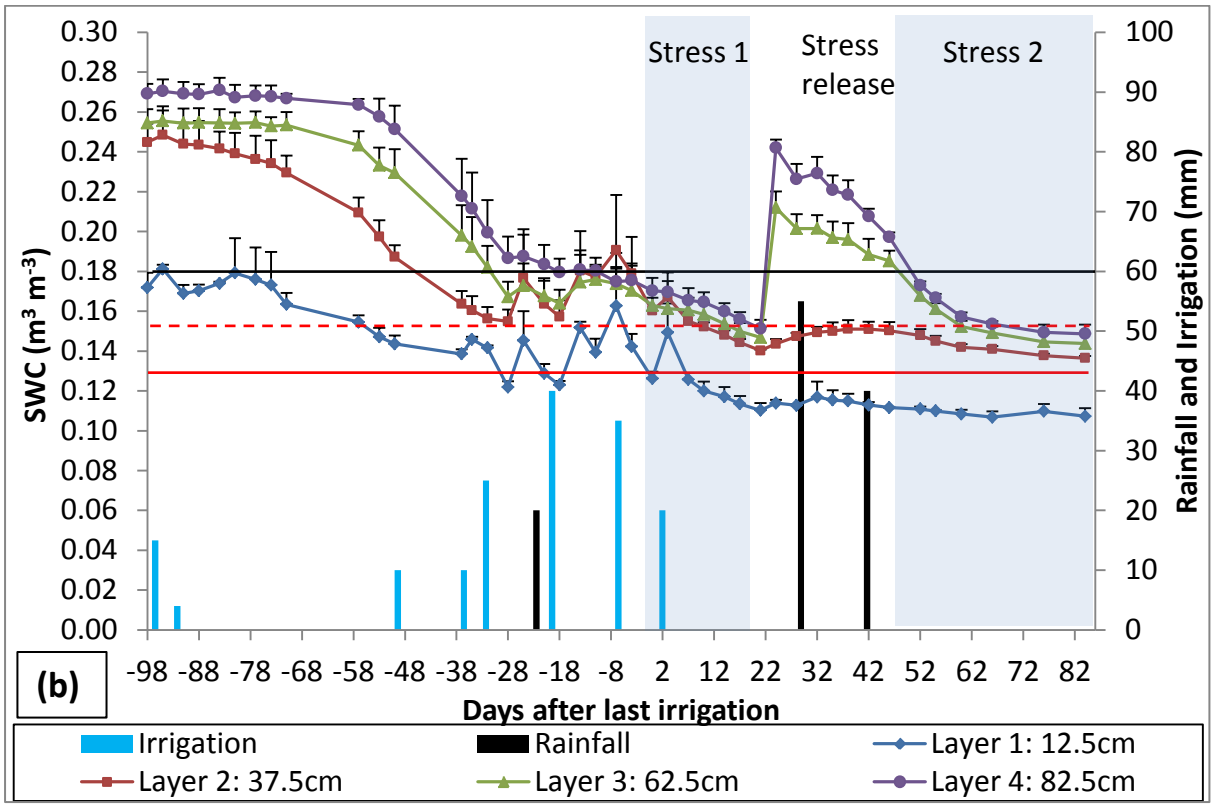


Figure 4.1. Time course of profile average soil water content (SWC) for the four treatments. Field capacity (FC) and permanent wilting point (PWP) are shown as a horizontal black and red lines and stress point (SP) as a dashed horizontal redline. Periods where plants in the stress treatments were believed to have been stressed (SWC<0.155 m^3 m^{-3}) are shown as blue shaded areas (stress 1 and stress 2).


SWC (m³ m⁻³)
Rainfall and Irrigation (mm)
Days after last irrigation
(a)
Irrigation
Rainfall
Layer 1: 12.5cm
Layer 2: 37.5cm
Layer 3: 62.5cm
Layer 4: 82.5cm


Stress 1
Stress release
Stress 2
SWC (m³ m⁻³)
Rainfall and Irrigation (mm)
Days after last irrigation
(b)
Irrigation
Rainfall
Layer 1: 12.5cm
Layer 2: 37.5cm
Layer 3: 62.5cm
Layer 4: 82.5cm

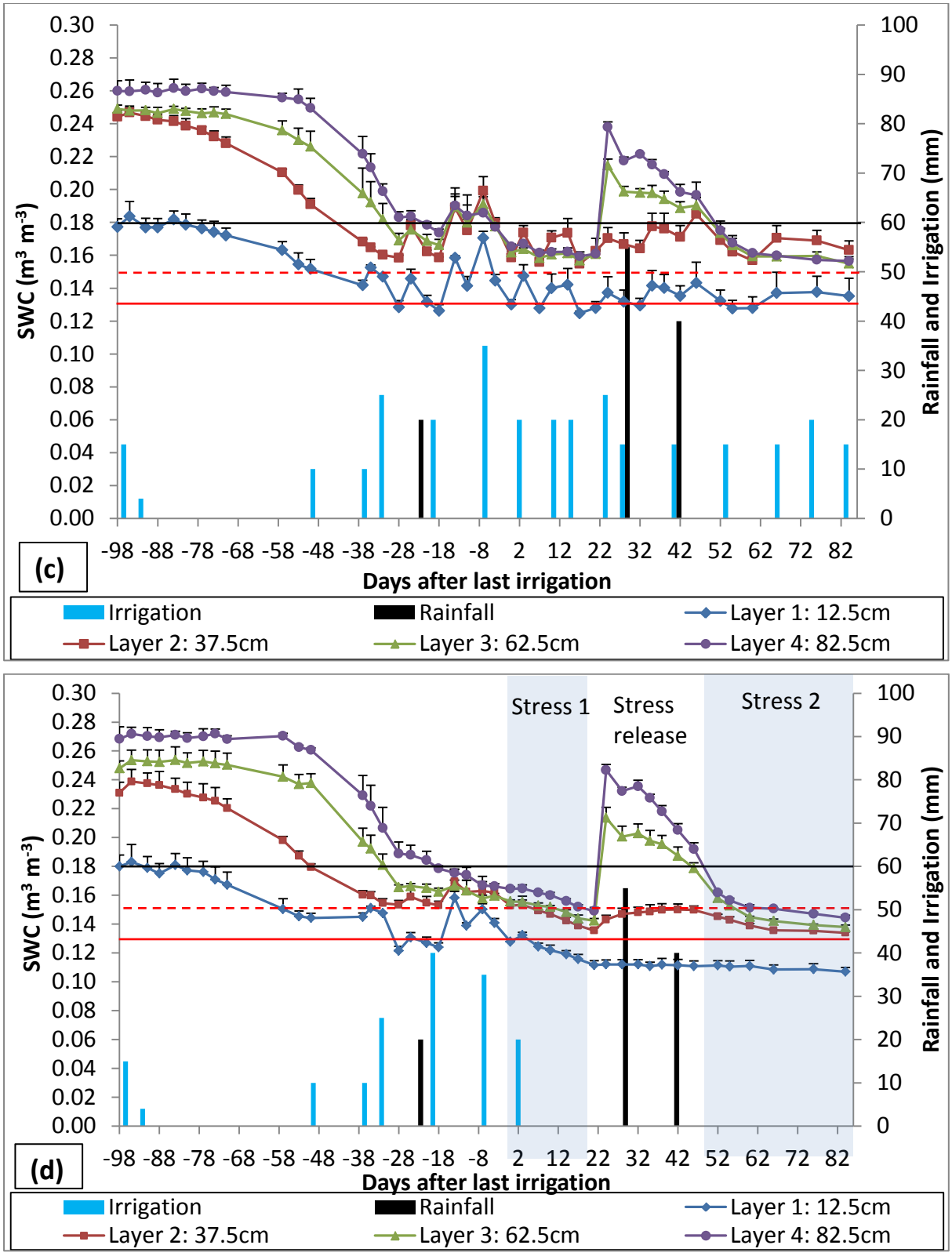


Figure 4.2. Time course of soil water content (SWC) for four different layers for N19 control (a), N19 water stress (b) 04G0073 control (c), and 04G0073 water stress (d) treatments. Rainfall and water intrusions (black bars) and irrigation (blue bars) are also shown. Field capacity, permanent wilting point and stress point are shown as horizontal black-solid, red-solid and red-dashed lines, respectively. Vertical bars show the standard deviation of SWC measurements for N19 treatment.

Seasonal total values of crop evapotranspiration (ETc) derived from SWC measurements are shown in Table 4.1. 04G0073 used 3% more water compared with N19 in both water treatments. Water stress reduced seasonal ETc of both genotypes by 27%.

Table 4.1. Seasonal total crop evapotranspiration (ETc) for the different treatments.

Treatment	ETc (mm)
N19 control	428.8
04G0073 control	443.3
N19 stressed	314.4
04G0073 stressed	322.7

4.2.2. Leaf water potential

Midday leaf water potential (Ψ_L) results are shown in Figure 4.3. In the well-watered treatments, genotypic differences in Ψ_L were mostly non-significant (Table A3.1, Appendix 3) except at DALI=60 and 75 when N19 had higher Ψ_L than 04G0073. The significantly lower Ψ_L of the well-watered 04G0073 treatment at these days could have been due to a low SWC caused by unintended stress and probably experienced some stress as the profile SWC was close to the stress point. The Ψ_L of well-watered treatments was generally above -0.5 MPa except for three cases on DALI=5, 60 and 75. The low Ψ_L on these days was caused by SWC dropping, unintentionally, below the stress point (Figure 4.1). The Ψ_L value of -0.5 MPa observed here was comparable with results in the literature (Inman-Bamber & de Jager, 1986b; Koonjah *et al*., 2006; Smit & Singels, 2006).

A rapid decline in Ψ_L was noticeable as soon as the SWC of stress treatments dropped below the stress point. From DALI=0 up to DALI=13, stressed N19 treatment had a significantly (Table A2.1) higher Ψ_L than 04G0073. Progressing stress reduced Ψ_L of 04G0073 and N19 to about -2.15 and -2.10 MPa at the end of the first stress period, respectively, which was similar to the maximum degree of stress (-2.3 MPa) that can be tolerated by sugarcane (Inman-Bamber & De Jager, 1986a).

During stress relief, Ψ_L of both genotypes increased and was similar to that of well-watered treatments within 13-17 days after wetting. However, the Ψ_L of stressed 04G0073 was always lower throughout the recovery period, presumably due to a lower profile SWC (Figure 4.1).

Ψ_L decreased gradually with decreasing SWC and more rapidly when profile SWC dropped below 0.155 m^3m^{-3} (Figure 4.3). Figure 4.4 clearly shows that, Ψ_L was higher for both genotypes during the second stress period than during the first stress period, despite having similar SWC. This could be due to lower evaporative demand compared with that in the first stress period (Average ETo for second stress period was 2.9 mm d^{-1} compared to 4.2 mm d^{-1} of the first stress period) (see Appendix

1). This could also be a result of osmotic adjustment in response to the first stress periods. Under stress conditions, plants tend to accumulate solutes to maintain high turgor pressure and allow for stress sensitive processes such as cell elongation to continue despite decreasing Ψ_L (Inman-Bamber & Smith, 2005). Inman-Bamber & de Jager (1986a) found that sugarcane experiencing severe stress for the fourth time had a lower osmotic potential compared with well-watered treatments.

The data presented in Figure 4.4 show a strong correlation (R^2 = 0.98 in N19 and 0.97 in 04G0073) between Ψ_L and relative available soil water content (RASWC). Ψ_L of both genotypes remained above -1.0 MPa until RASWC dropped below approximately 0.4.

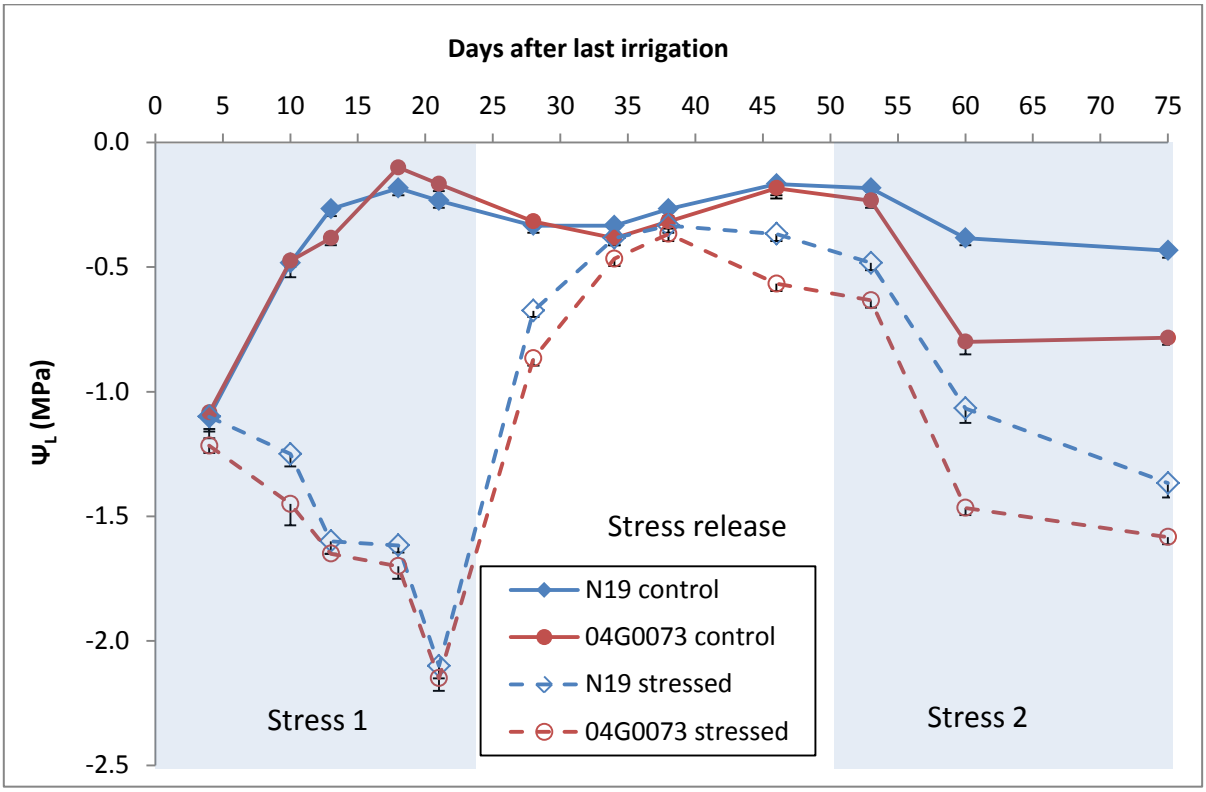


Figure 4.3. Midday leaf water potential (Ψ_L) over time for the different treatments. Periods where plants in the stress treatments were believed to have been stressed (SWC<0.155 m^3 m^{-3}) are shown as blue shaded areas. Vertical bars show one standard deviation of the means.

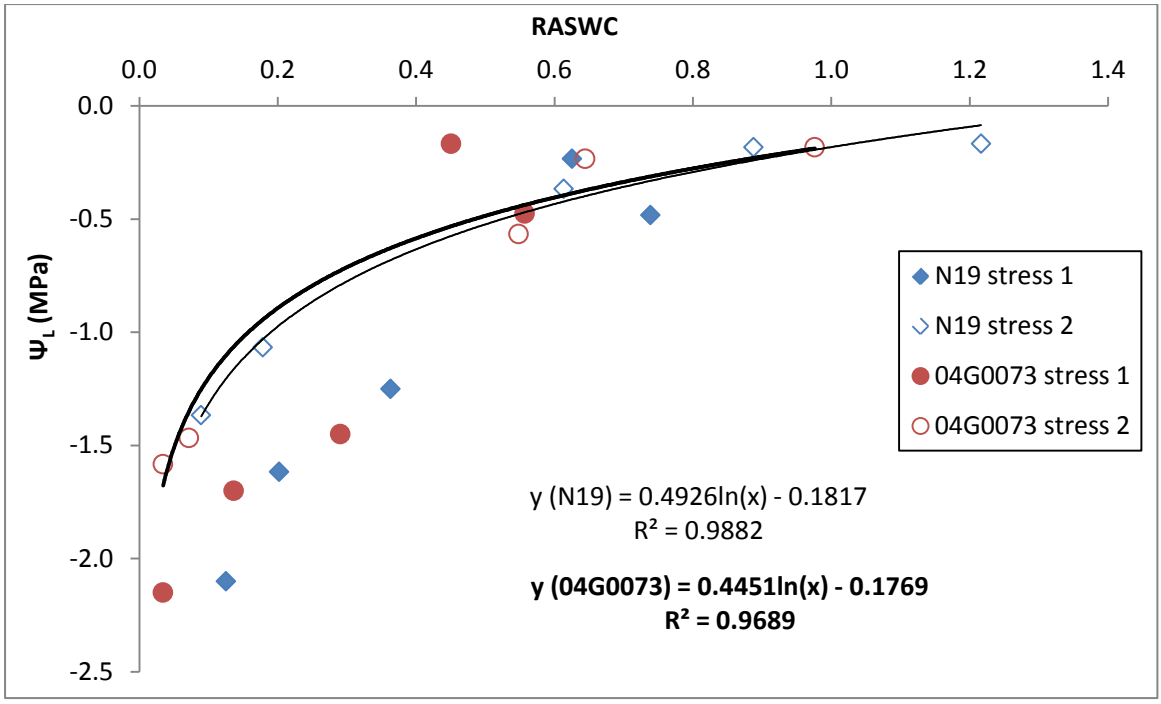


Figure 4.4. Midday leaf water potential (Ψ_L) in relation to relative available soil water content (RASWC) for the four treatments during the first and the second stress periods. Regression lines were also fitted in second stress period data.

4.3. Plant development

4.3.1. Stalk population

Development of stalk population is shown in (Figure 4.5). Poor germination in the well-watered 04G0073 treatment at the beginning of the experiment resulted in slow tiller production but later increased and exceeded N19 treatments. 04G0073 and N19 stressed treatments had similar tillering rates until after DALI=-60, after which 04G0073 produced more stalks than N19.

Although all treatments reached peak stalk population at the same time, well-watered 04G0073 treatment had a 15% higher peak stalk population than the well-watered N19 treatment (Figure 4.5). However, about 50% of the stalks produced were senesced, resulting in final stalk populations of 15 and 11 stalks m^{-2} for 04G0073 and N19, respectively. This behaviour is well documented for sugarcane and is associated with shading of young stalks by the leaf canopy (van Dillewijn, 1952; Bull & Glasziou, 1975; Inman-Bamber, 1994). It possible that this behaviour could have been exacerbated by unintended water stress experienced by the well-watered 04G0073 treatment during both stress events.

Stress reduced stalk population of 04G0073 and N19 by 9 and 2 stalks m^{-2}, respectively, within DALI=13. At the end of the first stress period, a total of 11 and 9 stalks m^{-2} for 04G0073 and N19, respectively were lost due to stress. At this point, well-watered treatments had 6% (04G0073) and 17% (N19) more stalks than stress treatments. Other studies specific to sugarcane also found that water stress reduced stalk population by accelerating stalk senescence (Robertson *et al.*, 1999; Inman-Bamber & Smith, 2005; Smit & Singels, 2006).

During the stress relief period, stalk population of stressed 04G0073 treatment increased by at least one stalk m^{-2}. Over the same period, senescence of stressed N19 treatment stalks stopped but the stalk population remained unchanged.

During the second stress period, stalk population of both genotypes decreased again 13 days after the SWC fell below the stress point (<0.155 m^3 m^{-3}). The rate of decline of stressed 04G0073 treatment was similar to that of well-watered 04G0073 treatment while the stalk population of stressed N19 treatment decreased quicker than that of its well-watered treatment. The rapid decline in stalk population of the well-watered 04G0073 treatment was presumably due to the unintended stress.

These results showed that 04G0073 produced stalks at a quicker rate than N19.

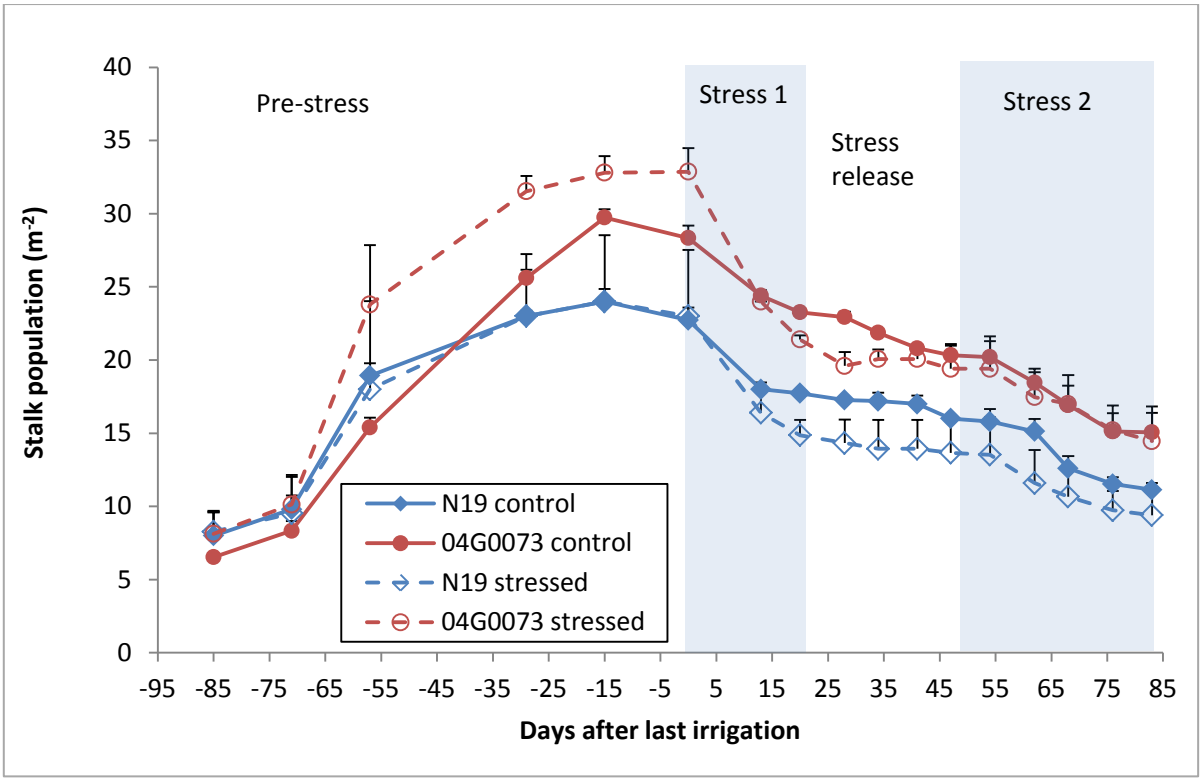


Figure 4.5. Stalk population in relation to time. Periods where plants in the stress treatments were believed to have been stressed (SWC<0.155 m^3 m^{-3}) are shown as blue shaded areas. Vertical bars indicate one standard deviation of the mean.

4.3.2. Stalk height

The stalk height for all four treatments increased gradually at first and then more rapidly after DALI = -57 (Figure 4.6). It was only after DALI = -15 that significant differences (Table A2.2) were observed between genotypes. From this point onwards, the well-watered 04G0073 treatment had significantly ($p<0.01$) taller stalks than the well-watered N19 treatment. The stalks of well-watered 04G0073 treatment at harvest were significantly taller (by 31 cm) than the well-watered N19 treatment. The stalk height of well-watered 04G0073 treatment levelled off during the second stress period, presumably due to a combined effect of unintended stress and cooler night-time temperatures (Figure 4.1 and Figure A1 in the Appendix).

Results suggest that stalk elongation by plants subjected to stress treatments stopped when the SWC fell below the stress point (<0.155 m^3 m^{-3}) resulting in stress treatments having significantly shorter stalks than their well-watered counterparts (Figure 4.6). At the end of the first stress period, stalk height for stressed 04G0073 and N19 treatments were respectively 42 cm (24%) and 22 cm (15%) shorter than well-watered treatments (Figure 4.6). Previous studies showed that water stress severely reduced stalk elongation of cane under water stress conditions resulting in significantly shorter stalks than that of unstressed irrigated cane (Silva & Costa, 2004; Silva *et al.*, 2008).

Upon releasing crops from stress, stalk height of stress treatments began increasing after seven days of wetting due to resumed stalk elongation (Figure 4.6). The stalk height of stress treatments was similar to those of well-watered N19 treatment within 13 days after wetting i.e. DALI=34. Despite this recovery, stalks of the well-watered 04G0073 treatment remained significant taller than any of the treatments for the duration of the experiment.

The onset of the second stress period again inhibited stalk elongation resulting in shorter stalks compared with well-watered treatments (Figure 4.6). Genotypic differences in stalk height of the stress treatments were not observed. Nevertheless, significant differences in stalk height between water treatments persisted until harvest. At harvest, stressed 04G0073 and N19 stalks were 53 (21%) and 27 cm (13%) shorter than well-watered treatments, respectively.

These results show that 04G0073 stalks grew more rapidly than those N19 under both well-watered and water stress conditions.

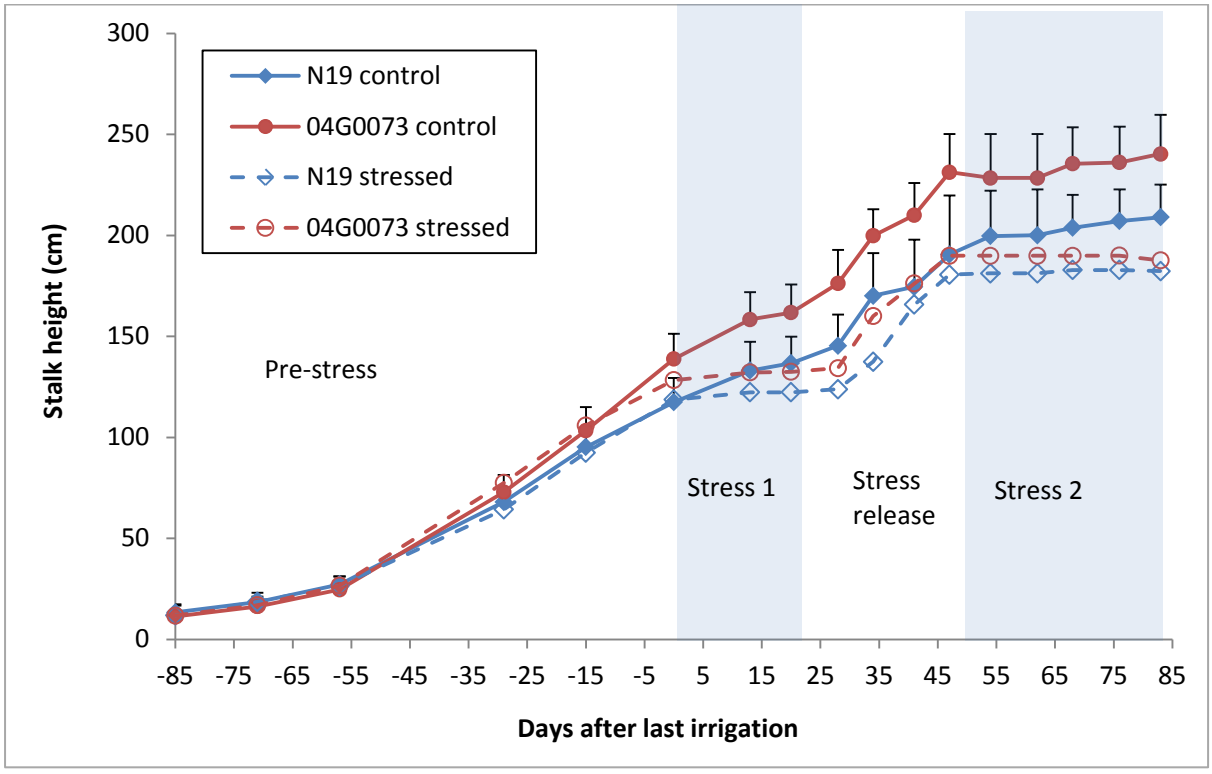


Figure 4.6. Stalk height measured from primary stalk emergence until harvest in May 2012. Periods where plants in the stress treatments were believed to have been stressed (SWC<0.155 m^3 m^{-3}) are shown as blue shaded areas. Vertical bars indicate standard deviation of the mean for well-watered treatments.

4.3.3. Number of green leaves

The balance between leaf appearance and senescence determines the number of green leaves (Robertson *et al.*, 1998).

Well-watered treatments had more green leaves than stress treatments (Figure 4.7). Generally, the well-watered 04G0073 treatment had more green leaves than the well-watered N19 treatment. This difference was mostly significant (Table A2.3) except for four cases at DALI=28, 47, 54 and 83, due to accelerated leaf senescence in well-watered 04G0073 treatment caused by unintended stress (Figure 4.7).

There were no genotypic differences in the number of green leaves (GLN) observed between the stress treatments. GLN decreased with progressing stress and at the end of the first stress period both stress treatments had significantly (Appendix 3) fewer (35%) green leaves than the well-watered treatments. Inman-Bamber (2004) and Smit & Singels (2006) found that the reductions in the number of green leaves was associated with synchronized reduction in leaf appearance rate and increased senescence rate.

Re-watering stress treatments increased GLN for both genotypes. During the 28 day recovery period, GLN for stressed 04G0073 treatment increased at a higher rate, reaching a maximum of 10 leaves (38% increase) compared with a value of 9 for stressed N19 (18% increase). The increase in GLN could be due to increased leaf appearance. Inman-Bamber (2004) reported that leaves tend to accumulate in the whorl during water stress and appear rapidly when relieved from stress, thus recovering GLN to that of unstressed treatments in a short period of time.

During the second stress period, stressed 04G0073 treatment showed a reduction in GLN three days sooner than N19. This early response was due to stress rather than genetics as the SWC of 04G0073 was also decreased below the stress point three days sooner than N19 (Figure 4.1). At harvest, stressed 04G0073 had an average of 3.3 green leaves (60% reduction) compared with a value of 4.7 for N19 (47% reduction). It should be noted that the reduction in GLN of well-watered 04G0073 treatment during the second stress period could have been accelerated by the occurrence of unintended stress at times.

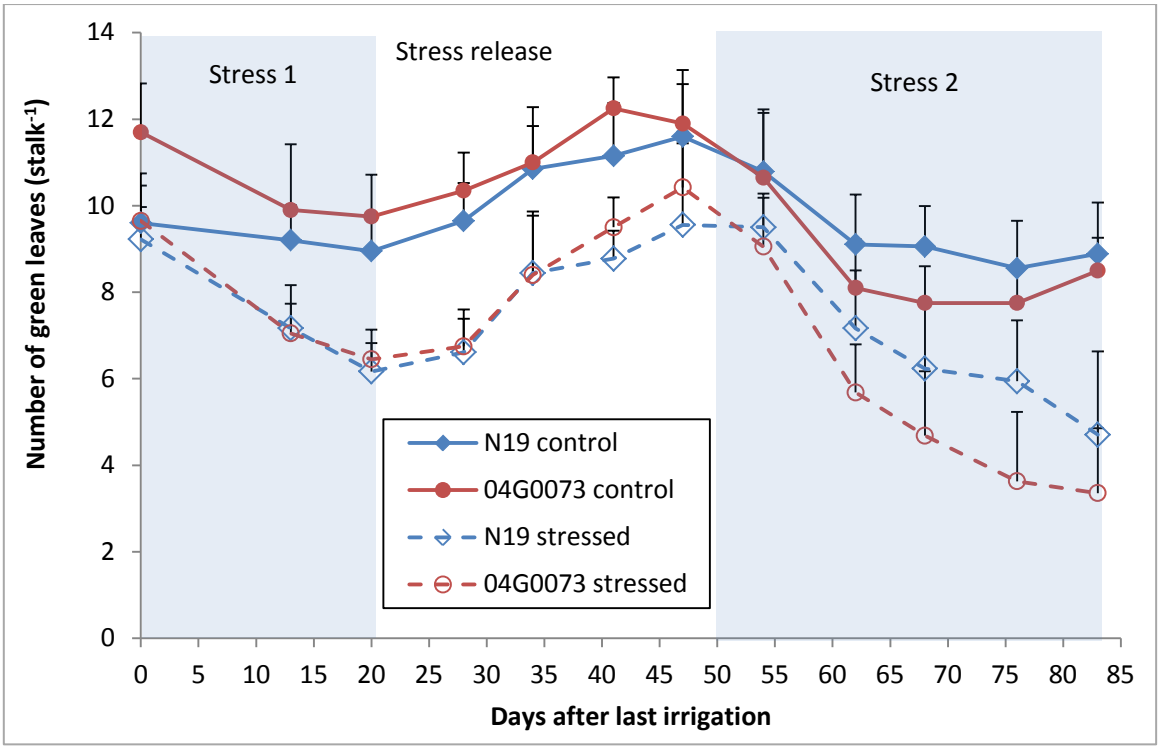


Figure 4.7. Number of fully expanded green leaves per stalk over time. Periods where plants in the stress treatments were believed to have been stressed (SWC<0.155 m^3 m^{-3}) are shown as blue shaded areas. Vertical bars indicate one standard deviation of the mean.

4.3.4. Leaf width

Leaf width increased with leaf position and stabilized after leaf number 20 (N19) and 23 (04G0073) for both water treatments (Figure 4.8). There were clear significant genotypic differences in leaf width between 04G0073 (maximum width of 2.9 cm) and N19 (maximum width of 4.0 cm).

Water stress had no effect on leaf width of the two genotypes.

The results suggest that genetics and not environmental conditions, is the primary factor determining leaf width.

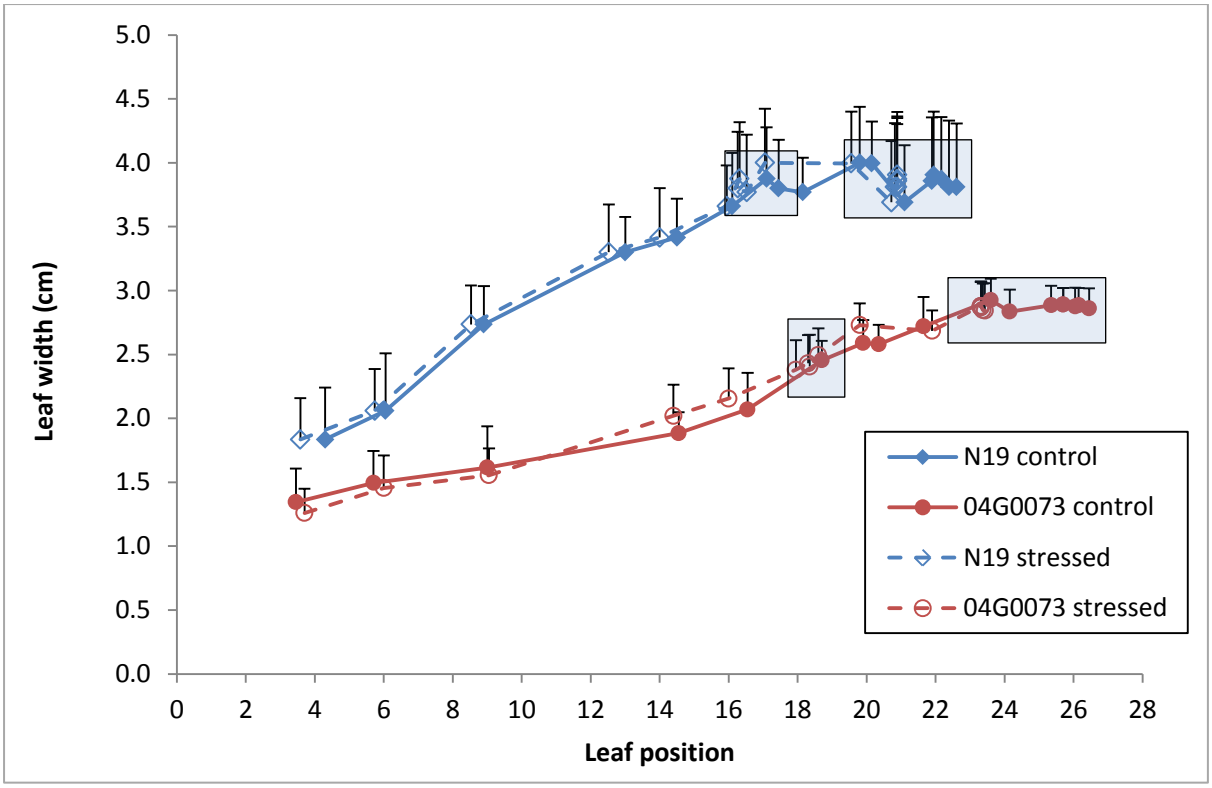


Figure 4.8. Leaf width of individual leaves at each node up the stalk counting from the lowest (oldest) leaf for each treatment. Blue shaded areas indicate periods when plants in the stress treatments were believed to have been water stressed (SWC<0.155 m^3 m^{-3}). Vertical bars indicate standard deviation of the mean.

4.3.5. Leaf length

Leaf length increased with leaf position and stabilized after leaf number 17 and 18 for N19 and 04G0073, respectively (Figure 4.9). The maximum leaf lengths reached at this point were 164 and 182 cm for N19 and 04G0073 genotypes, respectively (Figure 4.9).

Stress stopped leaf elongation resulting in successively shorter leaves compared with well-watered treatments.

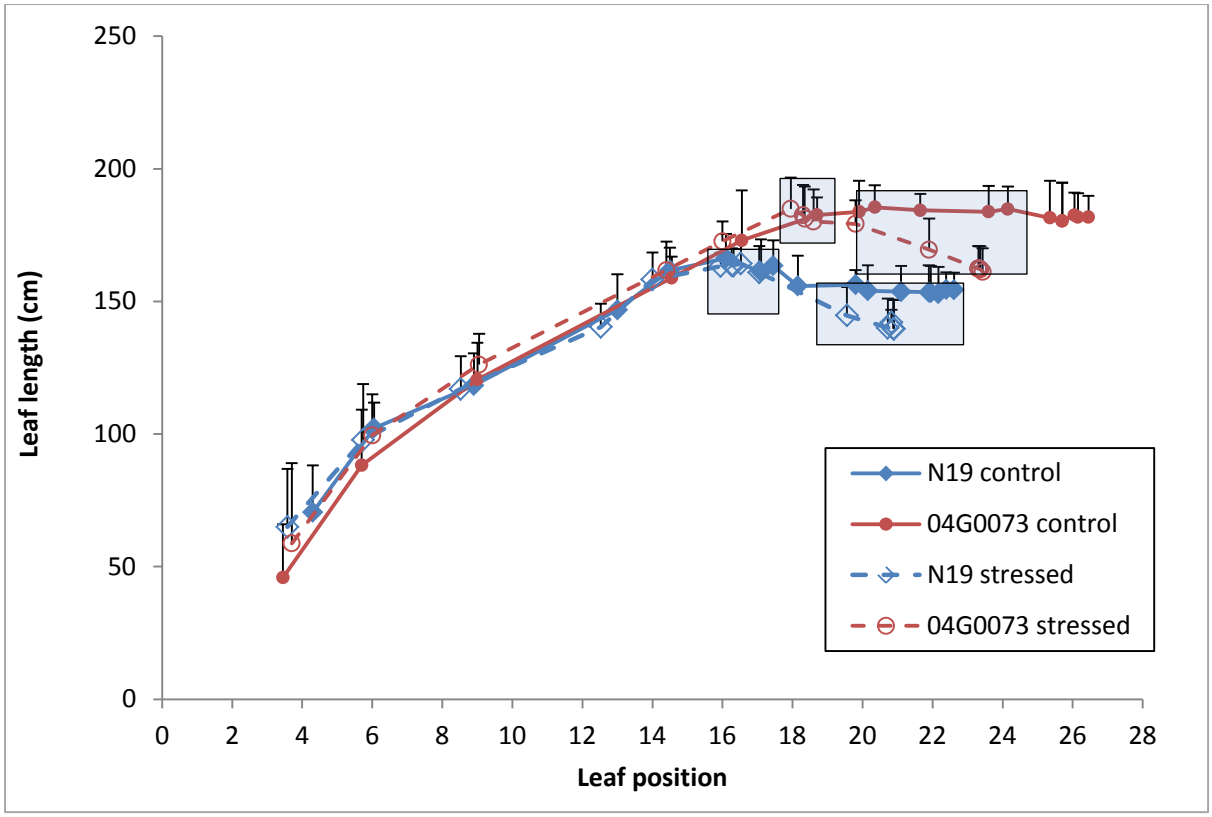


Figure 4.9. Length of individual leaves at each node up the stalk counting from the lowest (oldest) leaf for the four treatments. Blue shaded areas indicate periods when plants in the stress treatments were believed to have been water stressed (SWC<0.155 m^3 m^{-3}). Vertical bars indicate one standard deviation of the mean.

4.3.6. Area per leaf

Area per leaf increased with leaf position and stabilized after leaf number 17 and 24 in N19 and 04G0073, respectively (Figure 4.11). The area of the largest leaf was 450 and 382 cm^2 for well-watered 04G0073 and N19, respectively. Inman-Bamber (1994) showed that NCo376 and N12 cultivars reached a maximum leaf size of 350 and 420 cm^2, respectively at leaf number 15. Sinclair *et al.* (2004) found that other sugarcane cultivars achieved maximum area per leaf at higher leaf numbers of 20 to 25.

In the stress treatment, N19 treatment had noticeably larger leaves than 04G0073 (Figure 4.10). The results suggest that the area per leaf for both stress treatments was not affected by the first water stress (Figure 4.9).

The second stress period reduced the area of leaves appearing during that period, due mainly to reduced leaf length (Figure 4.9). Inman-Bamber (2004) found that the decrease in area per leaf under water stress conditions was caused by "new smaller leaves entering the pool of mature leaves".

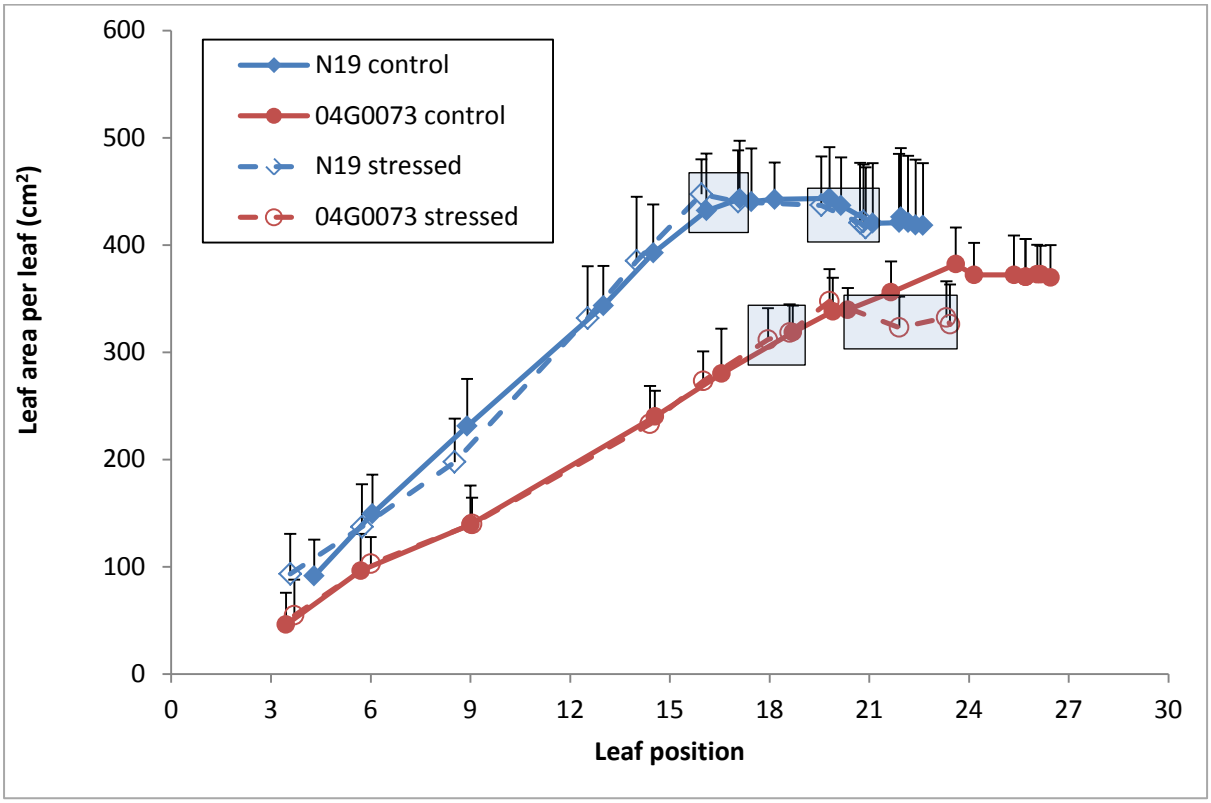


Figure 4.10. Area of individual fully expanded green leaves at each node of the stalk counting from the lowest (oldest) leaf for the four treatments. Blue shaded areas indicate periods when plants in the stress treatments were believed to have been water stressed (SWC<0.155 m^3 m^{-3}). Vertical bars indicate standard deviation of the mean.

4.3.7. Green leaf area index

Canopy development expressed as green leaf area index (GLAI) derived from primary stalks is shown in Figure 4.11. Sugarcane GLAI generally peaks after the tillering phase then declines towards harvest as small immature tillers die due to shading (Bull & Glasziou, 1975; Zhou *et al.*, 2003; Inman-Bamber, 2004; Smit & Singels, 2006). In this study, GLAI was measured just after the tillering phase when natural senescence was already occurring, thus explaining the decline in GLAI of well-watered treatments. However, the decline was exacerbated by the unintended stress experienced during both stress periods. It should be noted that GLAI was probably overestimated because all tillers were assumed to have the same properties than those of primary tillers, while in reality younger tillers with lower leaf area per tiller existed.

The results show that sugarcane genotypes with more green leaves and higher stalk population have the potential to achieve a higher GLAI. Well-watered 04G0073 treatment produced more green leaves (Figure 4.7) and had a higher stalk population (Figure 4.5) to produce a mean GLAI of 6.2 which was 6% higher than the value of 5.9 for well-watered N19 treatment. The reduction in GLAI of well-watered treatments was caused by a decrease in number of green leaves due to leaf shading.

Stress reduced GLAI of stressed 04G0073 and N19 treatments to 3.6 and 3.2, respectively at the end of the first stress period. The reduction of GLAI was primarily due to a 35% reduction in GLN (Figure 4.7), a 15% reduction in area per leaf for 04G0073 and 17% reduction in stalk population for N19 (Figure 4.5a). These results conformed to findings in the literature whereby water stress reduced GLAI by reducing number of green leaves through increased leaf senescence and reduced area per leaf (Inman-Bamber, 2004; Smit & Singels, 2006). No genotypic differences were observed in the stress treatments.

When relieved from stress, GLAI of stress treatments rapidly increased over a two week period mainly due to increased number of green leaves (Figure 4.7). GLAI of 04G0073 increased slightly more rapidly reaching a maximum of 7.7, compared with a value of 7.5 for N19. This was ascribed to a more rapid increase (38%) in the number of green leaves for 04G0073 (Figure 4.7).

The second stress period reduced GLAI more severely than the first stress period. At the end of this period, GLAI of 04G0073 and N19 were reduced by 64% and 54% respectively, compared to the well-watered treatments. The severe reduction was due to large reductions in the number of green leaves (60% and 47% for 04G0073 and N19, respectively).

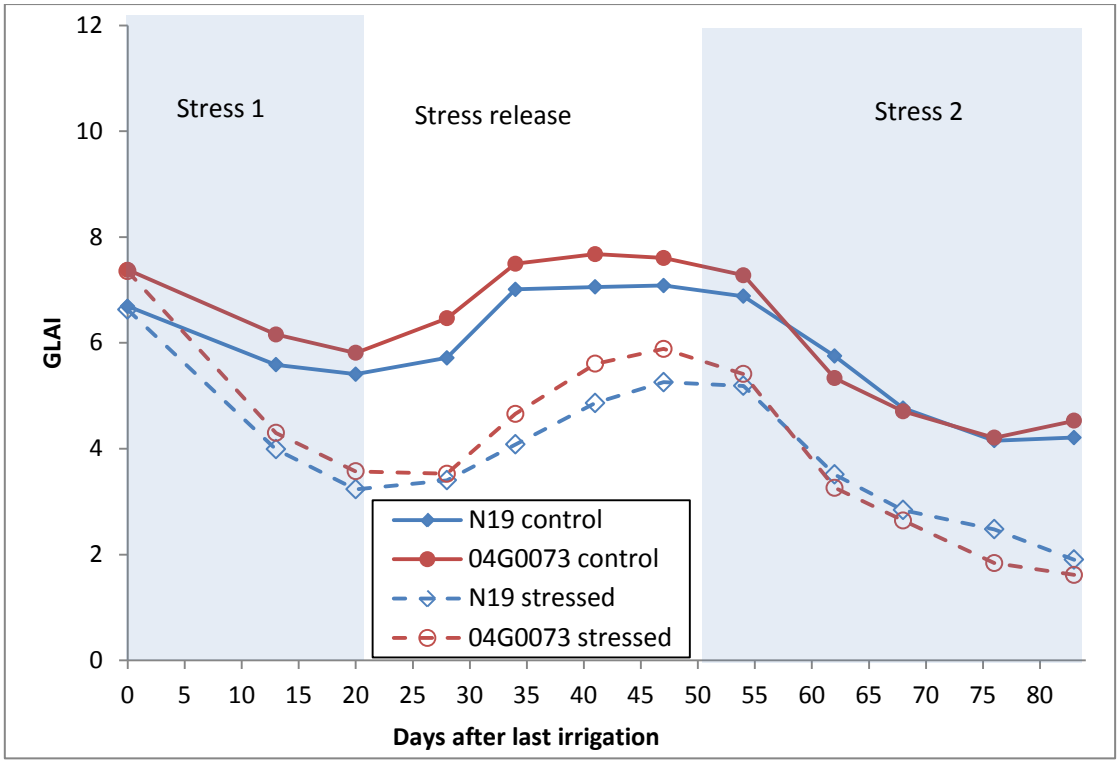


Figure 4.11. Time course of green leaf area index (GLAI) over time. Periods where plants in the stress treatments were believed to have been stressed (SWC<0.155 m^3 m^{-3}) are shown as blue shaded areas.

4.3.8. Fractional interception

Fractional interception of photosynthetically active radiation (FIPAR) is shown in Figure 4.12. FIPAR is considered a good measure of crop canopy cover. In the well-watered treatment, 04G0073 intercepted 9% (0.92) more solar radiation than N19 (0.84) presumably because of a higher GLAI. As expected, well-watered treatments intercepted more solar radiation than stress treatments.

In the stress treatments, FIPAR of 04G0073 was higher than N19 for the duration of the first stress period. FIPAR was more stress tolerant and was not affected by stress until after RASWC fell below 0.5 and 0.4 in 04G0073 and N19, respectively (Figure 4.12). This was in agreement with Smit & Singels (2006), who found that radiation interception (*cv.* N22 and NCo376) was more resilient to water stress and was not affected until RASWC dropped below 0.6. They further explained that this was the result of water stress promoting senescence of leaves located at the bottom of the canopy which have negligible contribution to radiation interception.

When RASWC for stressed 04G0073 treatment dropped below 0.4, FIPAR of stressed 04G0073 decreased from 0.91 to 0.76 and to 0.72 at DALI=13 and 21, respectively. The corresponding decrease in stressed N19 treatment occurred when RASWC dropped below 0.5 and was more severe

decreasing from 0.84 to 0.65 and to 0.56 over the same periods. The reduction of FIPAR was ascribed to a decrease in GLAI through increased leaf senescence and reduced stalk population (Figure 4.5). The severe reduction in FIPAR of N19 was exacerbated by leaf rolling under stress conditions (data not shown). In other sugarcane trials, when the NCo376 genotype was stressed for 20 days, it intercepted 20% less solar radiation than well-irrigated treatments (Koonjah *et al*., 2006). However, when severely stressed, less than 60% solar radiation was intercepted (Inman-Bamber & De Jager, 1986b).

When stress was relieved, FIPAR of stressed N19 treatment increased rapidly reaching 0.85 after 28 days of wetting. Over the same period, the FIPAR of stressed 04G0073 treatment recovered to 0.88 or 90% of the well-watered treatment.

The onset of the second stress period reduced FIPAR only after RASWC dropped below 0.5 for N19 and below 0.4 for 04G0073. The FIPAR at final harvest for stress treatments was 41% (04G0073) and 39% (N19) lower than the well-watered treatments.

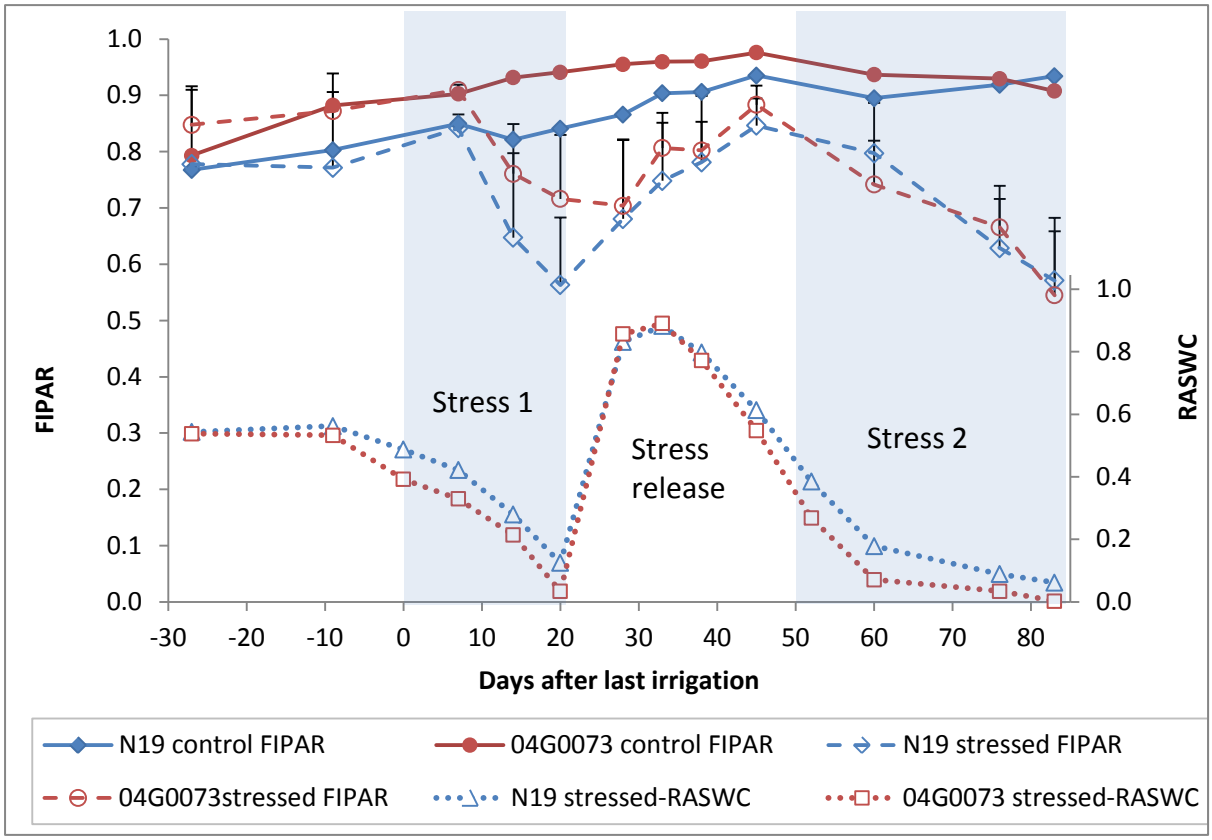


Figure 4.12. Time course of fractional interception of photosynthetically active radiation (FIPAR) for the different treatments, as well as relative available soil water content (RASWC) of the stress treatments. Periods where plants in the stress treatments were believed to have been stressed (SWC<0.155 m^3 m^{-3}) are shown as blue shaded areas. Vertical bars indicate one standard deviation of the mean for stressed treatments FIPAR.

4.4. Plant growth

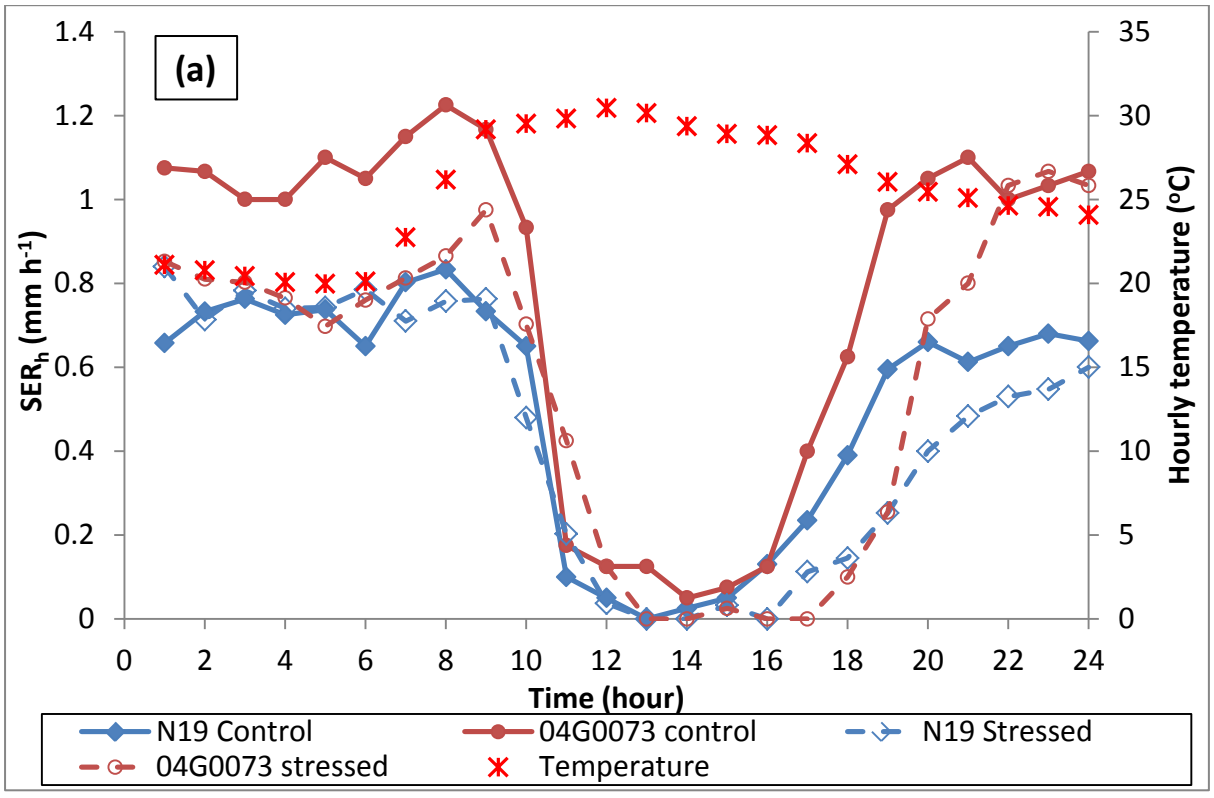
4.4.1. Stalk elongation

Hourly stalk elongation rate

At DALI=4, hourly mean stalk elongation rate (SER_h) of well-watered treatments was highest at 08h00 (SER_h =1.2 and 0.8 mm d^{-1} for 04G0073 and N19, respectively), thereafter decreased reaching lowest values at midday (SER_h =0.05 and 0 mm d^{-1} for 04G0073 and N19, respectively) before increasing again in the late afternoon at about 16h00 (Figure 4.13). Inman-Bamber & De Jager (1986a) also found that hourly plant elongation rate (PER) of potted sugarcane was zero at midday and increased in the late afternoon and remained high through the night.

Mean SER_h of stress treatments followed a similar diurnal pattern but was lower than that of well-watered treatments from DALI=4 onwards. Mean SER_h of stress treatments began increasing two hours later than well-watered treatments (Figure 4.13a). This was ascribed to delayed recovery in Ψ_L of stress treatments.

From DALI=8 onwards, SER_h of stress treatments was practically zero throughout the 24 hours (Figure 4.13b). Similarly, Inman-Bamber (1995b) found that water withheld for four days sharply decreased hourly PER at midday while stress imposed for longer periods completely stopped PER. Boyer (1968) found that water withheld longer than four days reduced pre-dawn Ψ_L such that cells were not able to resuscitate overnight and deprived cells of the necessary turgor to drive elongation.



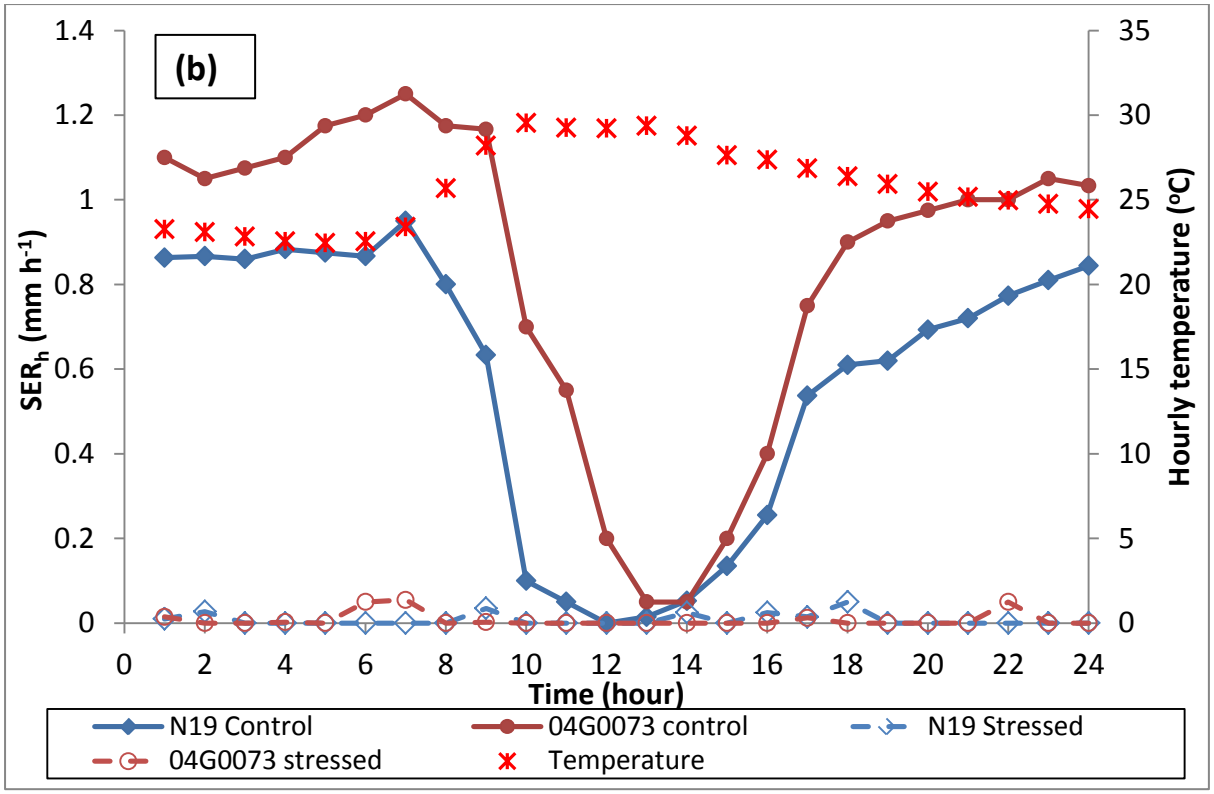


Figure 4.13. Hourly stalk elongation rate (SER_h) of the different treatments on (a) 4 and (b) 8 days after last irrigation. Air temperature is also shown.

Stalk elongation rate and air temperature

The relationship between daily stalk elongation rate (SER_d) and air temperature is shown in Figure 4.14. Generally, SER_d correlated well to daily minimum (Tmin), daily mean (Tmean) and mean night (Tnight) temperatures. Based on Figure 4.13 above, it is clear that stalks grew more during the night and in the early morning than during the day, which is why SER_d was related to Tnight and Tmin. In this study, SER_d was more strongly correlated to Tmin (R^2=0.84 in 04G0073 and R^2=0.78 in N19) than to Tmean or Tnight. (Figure 4.14a, b). This confirmed reports by van Dillewijn (1952) that the elongation of stalks is governed by Tmin.

The SER_d/Tmin relationship had a base temperature of 12.5 ^oC compared with 17 ^oC and 14.9 ^oC for Tmean and Tnight respectively. These base temperatures were all higher than the base temperature of 10 ^oC used for Tmean in the CANEGRO sugarcane model. The base temperature for the SER_d/Tmin relationship calculated here was higher than the base temperature of 10.6 ^oC reported by Smit *et al.* (2005).

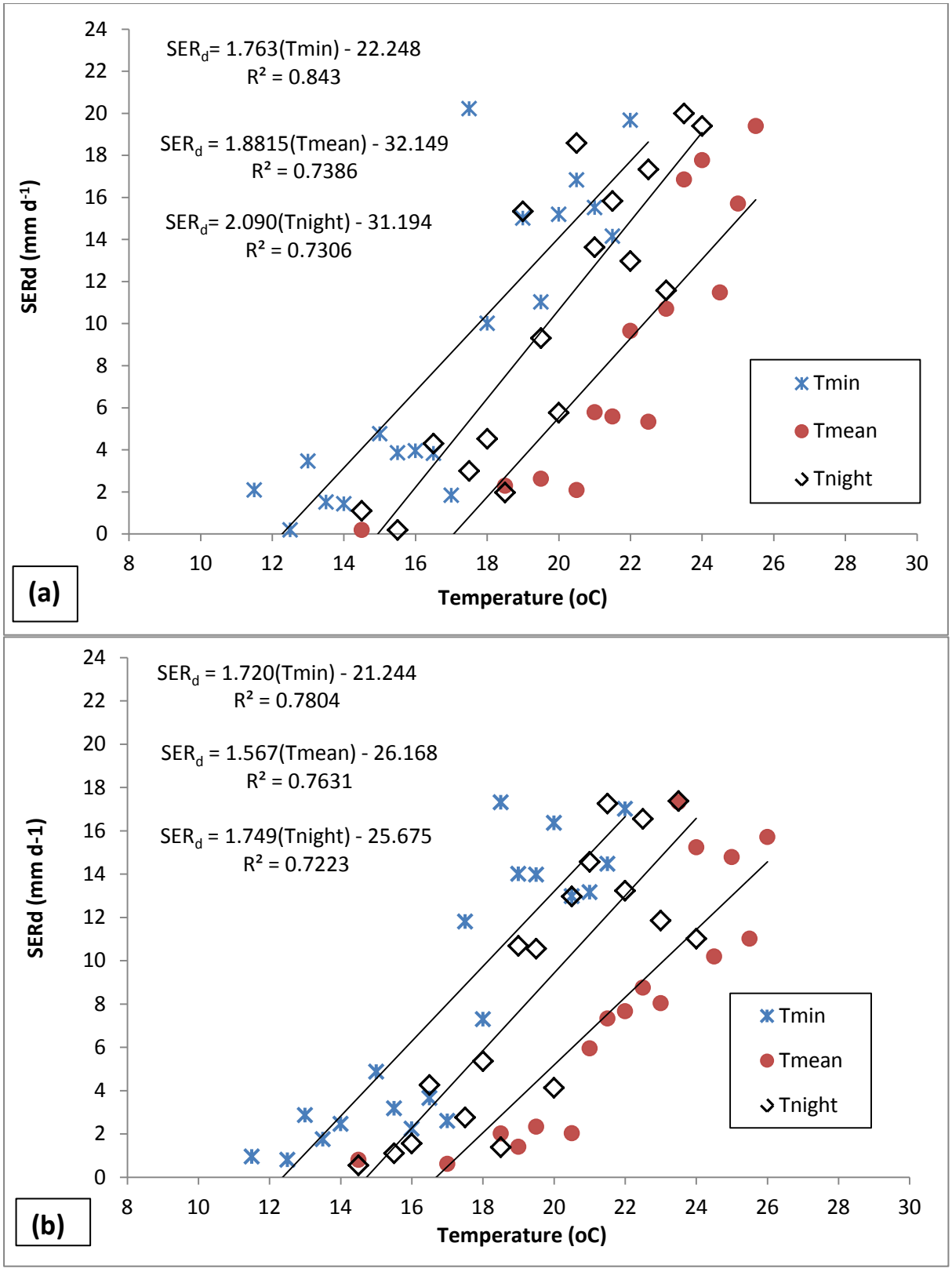


Figure 4.14. Daily stalk elongation rate (SER_d) for well-watered 04G0073 (a) and N19 (b) as a function of daily min (Tmin), daily mean (Tmean) and night (Tnight) temperature. Night temperature was taken as the average temperature during the period from 18h30 to 05h30. Linear regressions were fitted to the data.

Normalized and relative daily stalk elongation rate

SER_d is a good indicator of plant water status, however, care must be taken when using SER_d as it is also strongly affected by air temperature particularly daily minimum temperature as showed above. Therefore, dividing SER_d by daily thermal time (using a base temperature of 12.5 ^oC) was an attempt to eliminate the temperature effect on stalk elongation. The resultant variable was named normalized daily stalk elongation rate ($NSER_d$).

The average $NSER_d$ for unstressed days was 1.9 and 1.5 mm $(^oCd)^{-1}$ for 04G0073 and N19 respectively. These results suggest that unstressed stalk elongation rate is higher in 04G0073 than in N19 (Figure 4.15).

Results suggest that there were no clear differences in $NSER_d$ between the two genotypes under stress conditions (Figure 4.15). During the first stress period, stalks in the stress treatments were elongating at rates of between 80% and 60% of the well-watered treatments when the RASWC was above 0.6 (Figure 4.17). The elongation of stalks in the stress treatments decreased sharply when the RASWC fell below this value and stopped completely at DALI=8 when the RASWC below 0.4 (Figure 4.17). It appears from Figure 4.16 that N19 stalks were more sensitive to stress and stopped elongating at higher RASWC of 0.4 compared with the value of 0.3 for 04G0073.

When stress was relieved, SER_d of the stress treatments increased and exceeded that of the well-watered treatments three days after the wetting event (Figure 4.17). This suggests that stalks were able to compensate for the slow growth during the stress period. Similar results were reported by Inman-Bamber & De Jager (1986a) whereby the daily SER of stressed crops exceeded that of unstressed crops within 3-4 days after re-watering. Inman-Bamber (1995b) associated this compensatory growth to increased turgor of cells.

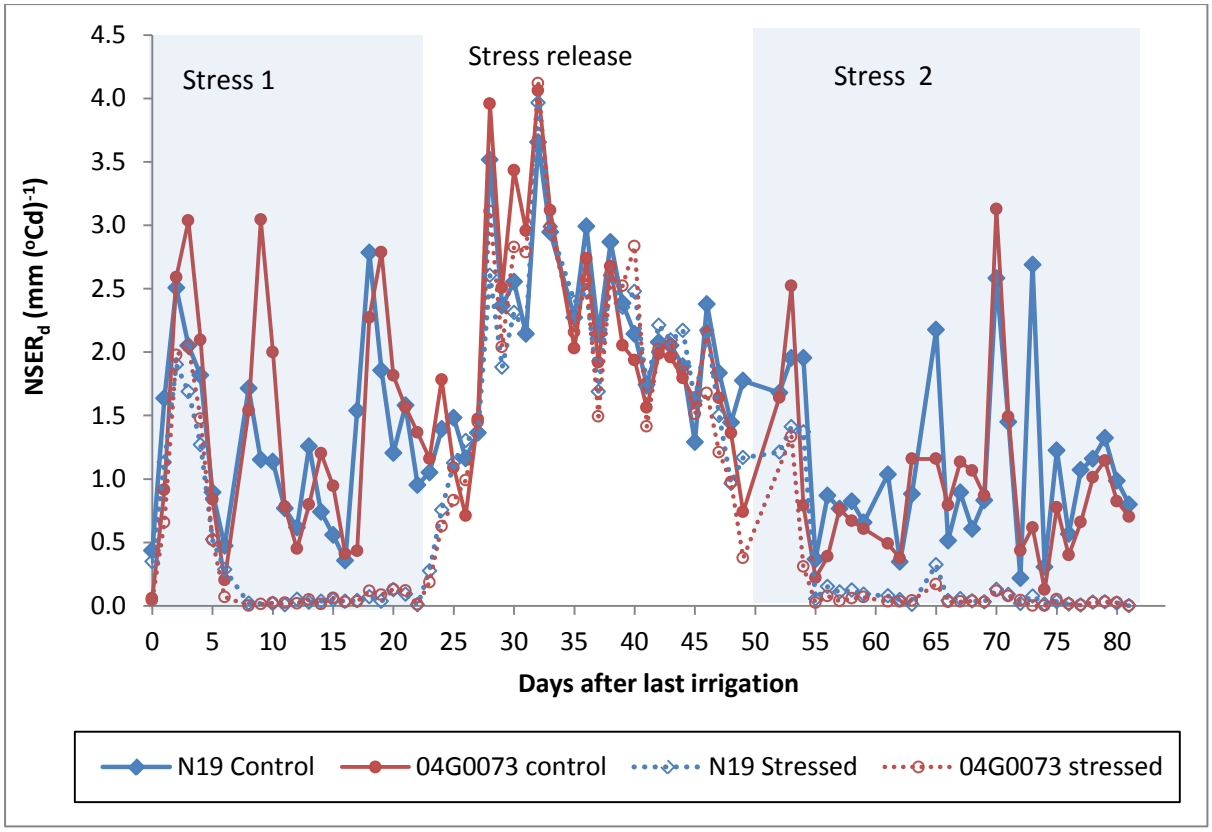


Figure 4.15. Normalized daily stalk elongation rate (NSER$_d$) over time. Periods where plants in stress treatments were believed to have been stressed (SWC<0.155 m^3 m^{-3}) are shown as blue shaded areas.

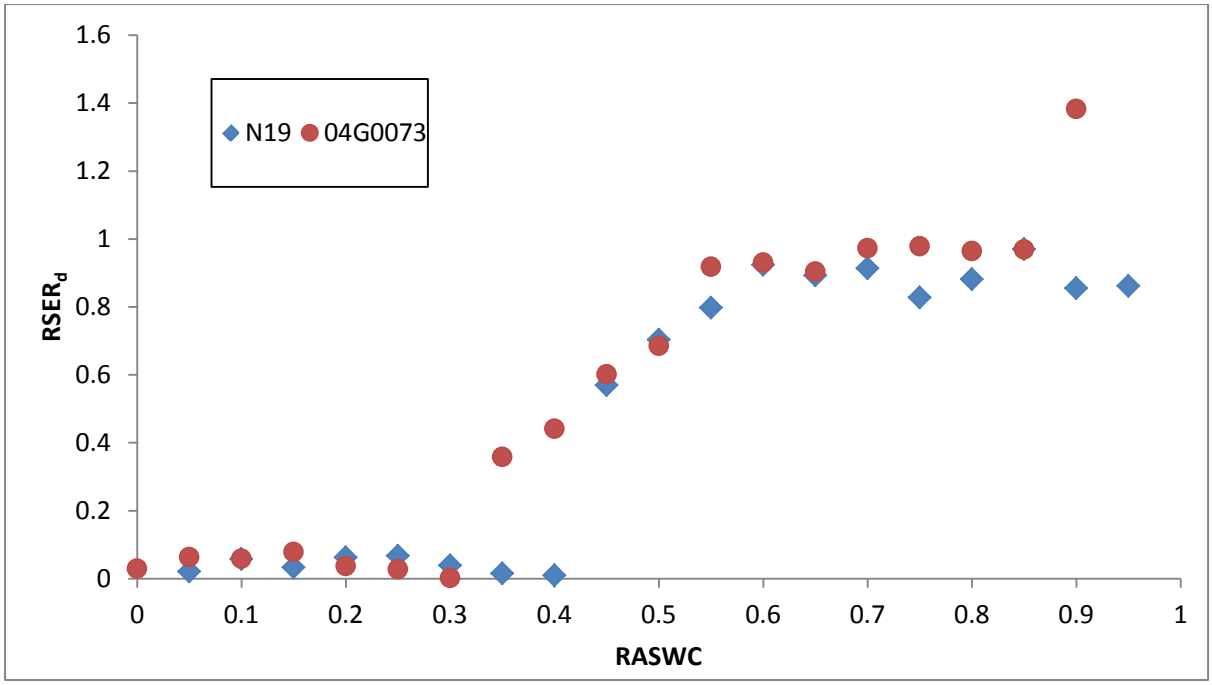


Figure 4.16. Relationship between relative stalk elongation rate (RSER$_d$, defined as SER$_d$ of stress treatments expressed as a fraction of SER$_d$ of the corresponding well-watered treatment) and relative soil water content (RASWC) for stress treatments.

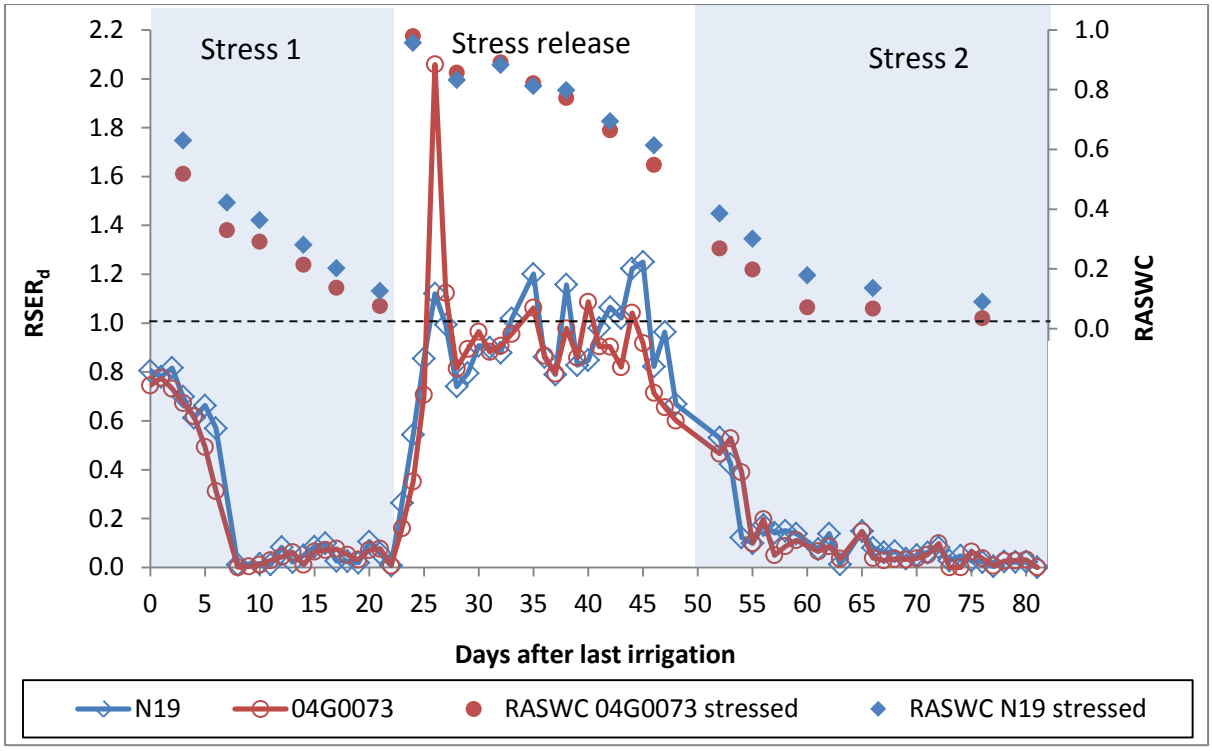


Figure 4.17. Relative stalk elongation rate (RSER$_d$, defined as SER$_d$ of stress treatments expressed as a fraction of SER$_d$ of the corresponding well-watered treatment) and relative available soil water content (RASWC) over time. Periods where plants were believed to have been stressed (SWC<0.155 m^3 m^{-3}) are shown as blue shaded areas. The black horizontal dotted line indicate RSER$_d$ = 1.

4.4.2. Root length density

Root length density (Lv) results are presented in Figure 4.18. Lv was highest in the top 0-25 cm layer and declined with increasing soil depth. Previous workers also found that well-watered sugarcane promoted root growth in the top 20 cm (Gascho & Shih, 1983) and 46 cm soil depth (Evensen *et al.*, 1997). For the well-watered treatments, 04G0073 had higher Lv than N19 in the top and bottom layers, while the opposite was true for the middle layer.

Stressed 04G0073 had higher (by 29%) Lv than stressed N19 in the top and middle layers, while the opposite was true for the bottom layer. The results obtained here suggest that under water stress conditions, N19 may stimulate root growth in deeper soil layers where more water could be extracted, while 04G0073 did not show this response. The N19 response is similar to that reported in the literature that sugarcane experiencing water stress tends to have a higher Lv in deeper soil layers than unstressed cane (Van Antwerpen, 1999; Laclau & Laclau, 2009; De Silva *et al.*, 2011). This occurrence is often associated with improved water use efficiency (De Silva *et al.*, 2011).

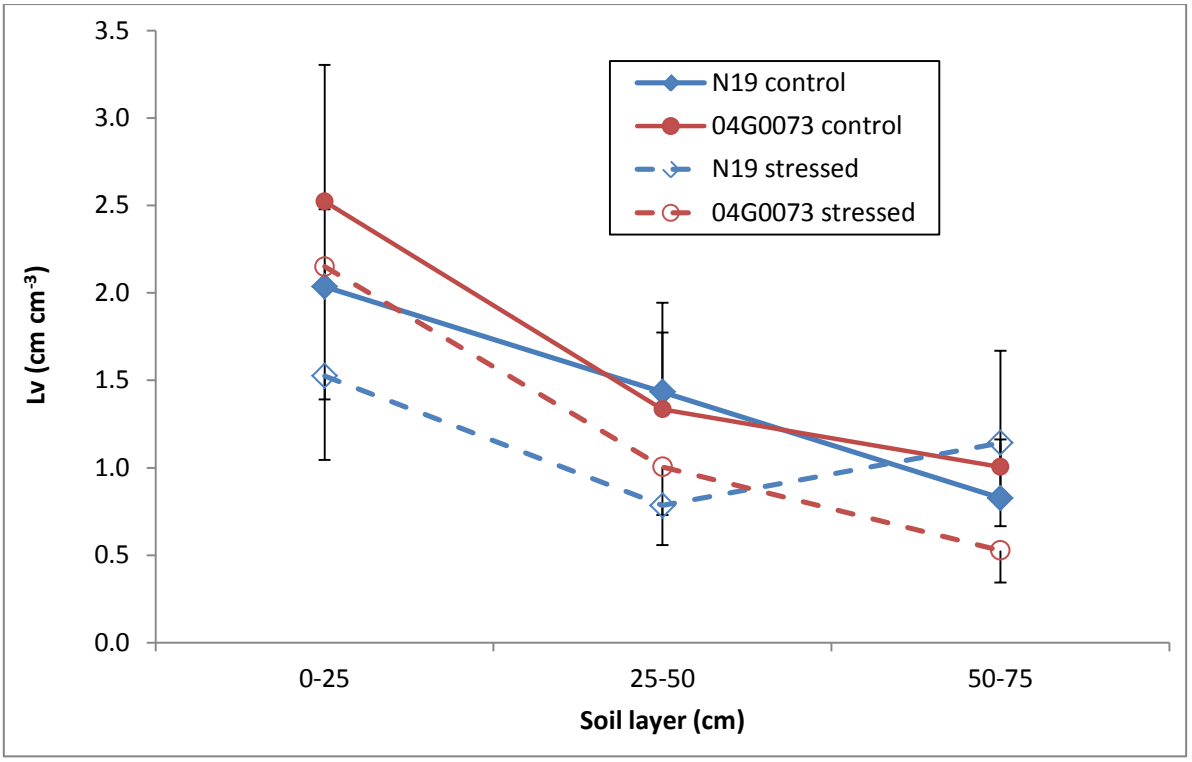


Figure 4.18. Root length density (Lv) at final harvest as a function of soil depth. Vertical bars indicate the standard error of the mean of 8 samples.

4.5. Plant yield

4.5.1. Dry biomass yield

Biomass yield of unstressed crops is closely determined by the amount of PAR intercepted by the leaf canopy (Singels & Donaldson, 2000). In this study, well-watered 04G0073 treatment maintained a higher green area index (GLAI) (Figure 4.11), intercepted 9% more solar radiation (Figure 4.12) and produced 12% higher dry biomass compared with N19 (Table 4.2). However this difference in dry biomass yield between genotypes was not significant.

Stress reduced dry biomass yield of the stressed 04G0073 treatment by 44% (statistically significant) and that of N19 by 17% (statistically non-significant) (Table 4.2). The severe reduction in the dry biomass of 04G0073 stress treatment is ascribed to a more severe stress experienced due to continued rapid soil water extraction at low soil water content levels (see Figure 4.1). This reduced biomass components such as leaf area (Figure 4.10) and stalk height (Figure 4.6) more severely compared with stressed N19 treatment. Secondly, the 04G0073 stress treatment intercepted 6% less solar radiation (Table 4.2), thus it had less energy to drive photosynthesis. Previous studies found that water stress reduced cane yield by reducing biomass components such as stalk height (Thomas *et al.*, 1978; De Silva & De Costa, 2004). Robertson *et al*. (1999) ascribed biomass yield reduction of stressed cane to a decrease in the fraction of seasonal radiation intercepted and radiation use efficiency (RUE). It should be noted that the yield obtained in the control treatment could not be considered as the maximum yield attainable for either of the two genotypes due to the periodic unintended stress.

4.5.2. Biomass fractions

Dry biomass fractions are presented in Table 4.2. Well-watered treatments had similar stalk, tops (leaf blade, sheath and meristem) and trash (includes dead leaves and dead stalks) fractions. The stalk fraction observed in this study (0.55 and 0.59 for 04G0073 and N19, respectively) was well below the value of 0.85 reported by Inman-Bamber *et al.* (2002) because the crop was harvested at seven months age. The stalk fraction observed in this study could have been greater if the crop was harvested at 12 months old as stalk fraction increases with crop age (Robertson *et al*., 1996; Inman-Bamber *et al*., 2002).

Water stress reduced stalk fraction by 11% and 2%, the tops fraction by 38% and 37%, and increased the trash fraction by 27% and 37% for 04G0073 and N19, respectively. The fraction of biomass in stalks for the stress treatments was not significantly different from their well-watered counterparts. In the stress treatments, N19 had a significantly higher (16%) fraction of biomass in stalks and a significantly lower (21%) fraction of biomass in trash than did 04G0073 (Table 4.2).

These results show that stress only decreased the tops fractions and increased the trash fraction due to increased senescence (Table 4.2).

4.5.3. Stalk fractions

Well-watered 04G0073 treatment partitioned significantly more stalk biomass to fibre and significantly less to sucrose compared with N19 well-watered treatment, confirming its status as high-fibre cultivar (Table 4.2).

Water stress had no significant effect on stalk fractions. Significant genotypic differences persisted in the stress treatment whereby 04G0073 had a 19% higher fibre fraction and a 27% lower sucrose fraction compared with N19 stress treatment (Table 4.2). Stress non-significantly increased the fibre fraction by 2% and 6% in 04G0073 and N19, respectively. The sucrose fraction for 04G0073 stress treatment was not affected by stress whereas that of N19 was non-significantly reduced by 8% under stress conditions. Despite the effects of stress on stalk fractions, significant differences between water treatments were not observed (Table 4.2).

Research showed that sucrose yield may be increased under stress conditions (Inman-Bamber & de Jager, 1988b; Robertson *et al.*, 1999; Singels & Inman-Bamber *et al*., 2002). However, in this study the sucrose fraction was slightly reduced by water stress for N19. An explanation for this could be that, at harvest, the cane was severely stressed therefore photosynthesis and translocation of assimilates to sucrose storage organs were inhibited. Similarly, Robertson & Donaldson (1998) reported that the concept of increased sucrose content under drying conditions is only true provided that the photosynthesis is maintained at sufficient high rates.

4.6. Resource use efficiency

4.6.1. Water use efficiency

Water use efficiency (WUE) results are shown in Table 4.2. Well-watered 04G0073 had a 12% higher WUE compared with N19 (7.6 vs. 6.9 kg m^{-3}). The WUE values obtained in this study fall within the range of 6 to 12 kg m^{-3} reported in the literature (Thompson, 1976; Kingston, 1994; Keating *et al*., 1999; Inman-Bamber *et al*., 1999).

Stress increased WUE of N19 by 15% and reduced it by 25% in 04G0073. The increase in WUE of stressed N19 was ascribed to a proportionally smaller decrease in ET (Table 4.1) than the 17% decrease in biomass yield.

These results show that 04G0073 captured slightly more water and used it more efficiently to produce more biomass under well-watered conditions. However, 04G0073 was unable to achieve this under the stress conditions imposed in this study.

4.6.2. Radiation use efficiency

Radiation use efficiency (RUE) results are shown in Table 4.2. Well-watered 04G0073 had a 9% (1.52 g MJ^{-1}) higher RUE than well-watered N19 (1.39 g MJ^{-1}), but this difference was not significant. These values were within the range of 1.39 to 2.09 g MJ^{-1} reported in the literature (Muchow *et al.*, 1997; Donaldson *et al*., 2008; De Silva & De Costa, 2012).

Stress reduced RUE of 04G0073 much more severely (38% reduction, statistically significant) than N19 (2%), because of the much larger reduction biomass yield in 04G0073 than in N19 (Table 4.2).

These results therefore show that 04G0073 captured more solar radiation (Table 4.2) and used it more efficiently than N19 to produce more biomass, but only under well-watered conditions.

Table 4.2. Dry biomass yield, biomass and stalk fractions, water use efficiency (WUE) and radiation use efficiency (RUE) at final harvest. The green leaf component includes leaf blade, sheath and meristem whereas trash includes dead leaves and dead stalks.

Parameter		**Well-watered treatment**		**Stress treatment**		**Well-watered x stress treatment**	
		04G0073	**N19**	**04G0073**	**N19**	**04G0073**	**N19**
Total dry biomass yield (kg m^{-2})		3.39	2.97^{NS}	1.89	2.47*	**	NS
Biomass fractions	Stalk	0.55	0.59^{NS}	0.49	0.58**	NS	NS
	Trash	0.24	0.22^{NS}	0.38	0.3*	**	*
	Tops	0.21	0.19^{NS}	0.13	0.12^{NS}	**	**
Stalk fractions	Fibre	0.58	0.45**	0.59	0.48**	NS	NS
	Sucrose	0.24	0.36**	0.24	0.33**	NS	NS
	Non-sucrose	0.18	0.19^{NS}	0.17	0.19^{NS}	NS	NS
WUE (kg m^{-3})		7.6	6.9^{NS}	5.8	7.8*	*	NS
RUE (g MJ^{-1})		1.52	1.39^{NS}	0.95	1.36*	**	NS
Fraction of intercepted seasonal global shortwave radiation		0.66	0.62	0.54	0.59		

*indicates significance at $p \leq 0.05$, ** indicates significance at $p \leq 0.01$ and NS indicates non-significance of treatment differences.

4.7. Summary

Well-watered treatments

There were no genotypic differences observed in Ψ_L which was mostly above -0.5 MPa. 04G0073 developed more rapidly, producing 15% more stalks at peak stalk population than N19. The stalks of 04G0073 elongated faster (1.9 vs. 1.5 mm $(^oCd)^{-1}$) and were 31 cm (13%) taller than those of N19 at harvest. Daily stalk elongation for both genotypes was more strongly driven by daily minimum temperature (R^2=0.84 and 0.78 in 04G0073 and N19, respectively) rather than by daily mean temperature (R^2=0.73 and 0.76 in 04G0073 and N19, respectively).

Rapid production of 04G0073 green leaves and stalks resulted in a denser canopy cover (10% and 9% higher GLAI and fractional interception, respectively) compared with N19. Consequently 04G0073 captured 3% and 4% more water and seasonal solar radiation, respectively, compared with N19. 04G0073 used resources more efficiently (WUE: 7.6 vs. 6.9 kg m^{-3}; RUE: 1.52 vs. 1.39 g MJ^{-1}) to produce 12% more aboveground dry biomass compared with N19. 04G0073 partitioned 7% less biomass to stalks and slightly more to trash and tops, compared with N19. 04G0073 treatment partitioned significantly more stalk biomass to fibre (0.58 vs. 0.45) and significantly less to sucrose (0.24 vs. 0.36) compared with N19, confirming its status as high-fibre cultivar.

Effects of water stress

Plants experienced two periods of water stress; the first stress period lasted about 21 days, followed by a 28 day stress recovery period and another 28 days of stress. 04G0073 continued extracting soil water for longer and at lower RASWC than N19. In both genotypes stalk elongation rates declined when RASWC dropped below 0.55. Stalk elongation of 04G0073 ceased at RASWC=0.3, compared to RASWC=0.4 for N19. This resulted in 21% (53 cm) and 13% (27 cm) shorter stalks for 04G0073 and N19 stress treatments compared with well-watered treatments, respectively.

Most plant growth and development processes of 04G0073 were reduced more severely than those of N19 due to a severe stress. At harvest, the stalk population of 04G0073 and N19 was reduced by 7% and 18%, GLN by 4 and 3 leaves, GLAI by 64% and 58%, and canopy cover by 41% and 39%, respectively, compared with well-watered treatments. Secondly, stressed 04G0073 used resources less efficiently than N19 (WUE=5.8 kg m^{-3}; RUE=0.95 g MJ^{-1}) while N19 improved its WUE from 6.9 to 7.80 kg m^{-3} and slightly reduced RUE from 1.39 to 1.36 g MJ^{-1}. Poor resource conversion efficiency resulted in 04G0073 producing significantly less (23% reduction) aerial dry biomass than N19. This could be a result of a complete shutdown of photosynthesis in 04G0073 while N19 possibly continued photosynthesising for longer even at lower soil water contents than the stressed 04G0073 treatment.

5. CONCLUSIONS

In the past few decades there has been growing interest in cultivating sugarcane as feedstock for electricity generation and/or second generation ethanol production. Tew & Cobill (2008) reported the existence of high-fibre sugarcane genotypes that are more suitable for second generation bioethanol production. They also reported that these genotypes may have the potential to grow and produce higher yields even under more harsh environmental conditions compared with the existing high-sucrose sugarcane genotypes. Based on the literature reviewed to date, it is not clear how the high-fibre sugarcane achieves its high biomass yields or how growth and yield is affected by water stress. The current study was therefore aimed at evaluating differences in growth, development, resource capture and resource conversion efficiency between high-sucrose (N19) and high-fibre (04G0073) sugarcane genotypes. Secondly, the effects of water stress on the above-mentioned parameters between N19 and 04G0073 sugarcane genotypes were also investigated.

The study indicated the following contrasting genotypic growth characteristics for the two genotypes under well-watered conditions: 04G0073 'germinated' more slowly, but later produced stalks (Figure 4.6a), and leaves (Figure 4.8) more rapidly, allowing it to establish a canopy more rapidly. This resulted in a 6% higher seasonal mean green leaf area index (GLAI) (Figure 4.13) and 4% higher seasonal mean solar radiation interception for well-watered crops (Figure 4.14). 04G0073 also transpired at higher rates presumably because of a higher GLAI than N19. 04G0073 had a 12% and 9% higher WUE and RUE, respectively, thus 04G0073 was more efficient at converting resources into biomass than N19. 04G0073 partitioned a larger proportion of its stalk biomass to fibre (32%) than N19 (26%). N19, however diverted a significantly higher proportion of its assimilates to sucrose, thereby producing 38% more sucrose than 04G0073 (Table 4.2). A strong relationship existed between stalk elongation rate (SER) and air temperature for both genotypes. SER related better to daily minimum temperature (R^2 = 0.84 and 0.78 in 04G0073 and N19, respectively) than to daily mean temperature (R^2 = 0.73 and 0.76 in 04G0073 and N19, respectively).

Under conditions of water stress, 04G0073 continued extracting water as soil water content declined, while N19 reduced water use as soil water content declined, presumably due to stomatal closure. 04G0073 stalks also continued elongating for longer than N19, but at reduced rates, as soil water content declined. Stalk elongation of 04G0073 ceased at a lower RASWC compared with N19 (0.3 vs. 0.4) (Figure 4.16). The continued water extraction by 04G0073 eventually led to a more severe stress compared to N19. Stress reduced stalk population, the number of green leaves per stalk and solar radiation interception of both genotypes. Water stressed 04G0073 converted solar radiation into biomass less efficiently than N19 (RUE=0.95 vs. 1.36 g MJ^{-1}). WUE of 04G0073 declined by 25% in response to water stress, while that of N19 increased by 15%.

The hypothesis stating that high-fibre sugarcane produces higher biomass yields by capturing more resources (water and radiation) than the traditional sugarcane genotype is supported only under well-watered conditions. This hypothesis is not supported under conditions of severe water stress as the high-fibre sugarcane captured less resources and produced less biomass yield compared with the traditional genotype. The hypothesis stating that the high-fibre sugarcane achieves it higher biomass yields are achieved by investing a larger proportion of assimilate to structural growth and less to sucrose storage, resulting in a more dense rooting system and canopy cover, than traditional sugarcane was found to be true under well-watered conditions. The high-fibre genotype achieved higher biomass yields by investing more assimilates to structural growth of roots, tillers and leaves, resulting in a more dense rooting system and canopy cover compared with the traditional sugarcane genotype.

The information collected in this study can be used to derive parameters that capture genetic control of crop growth and development such as thermal time requirements for shoot emergence and, timing peak stalk population, phyllochron intervals for leaf development and leaf elongation rates per unit thermal time, and leaf dimensions. For example, based on preliminary estimated crop parameter values, the high-fibre cultivar produced stalks and leaves quicker, elongated stalks more rapidly, produced a higher peak and final stalk population and had higher RUE compared with the traditional sugarcane genotype (see Table A5 in the Appendix). This information will assist crop modellers to simulate growth, development and resource use of high-fibre sugarcane cultivars and identify suitable areas for cultivation. This study showed that daily minimum temperature is the principal limiting factor behind stalk elongation rather than mean daily temperature. Therefore, it is recommended that the CANEGRO crop model (Singels *et al.*, 2008) be refined to simulate stalk extension as a function of daily minimum temperature using a base temperature of 12.5 ^{o}C, instead of the mean daily temperature that is currently used.

There is a possibility that 04G0073 would not respond in the same manner observed here under natural conditions. Therefore, detailed analyses subjecting 04G0073 and one traditional sugarcane genotype to different water stress levels is recommended under natural conditions where drought stress is frequently occurring. This investigation is recommended because the severe water stress imposed in this experiment is unlikely to occur in a real field situation.

The high fibre genotype, 04G0073, which was used in this study, was chosen based on very limited information. It is likely that other high fibre genotypes could perform better than 04G0073 and that their response to water stress could be different from that of 04G0073.

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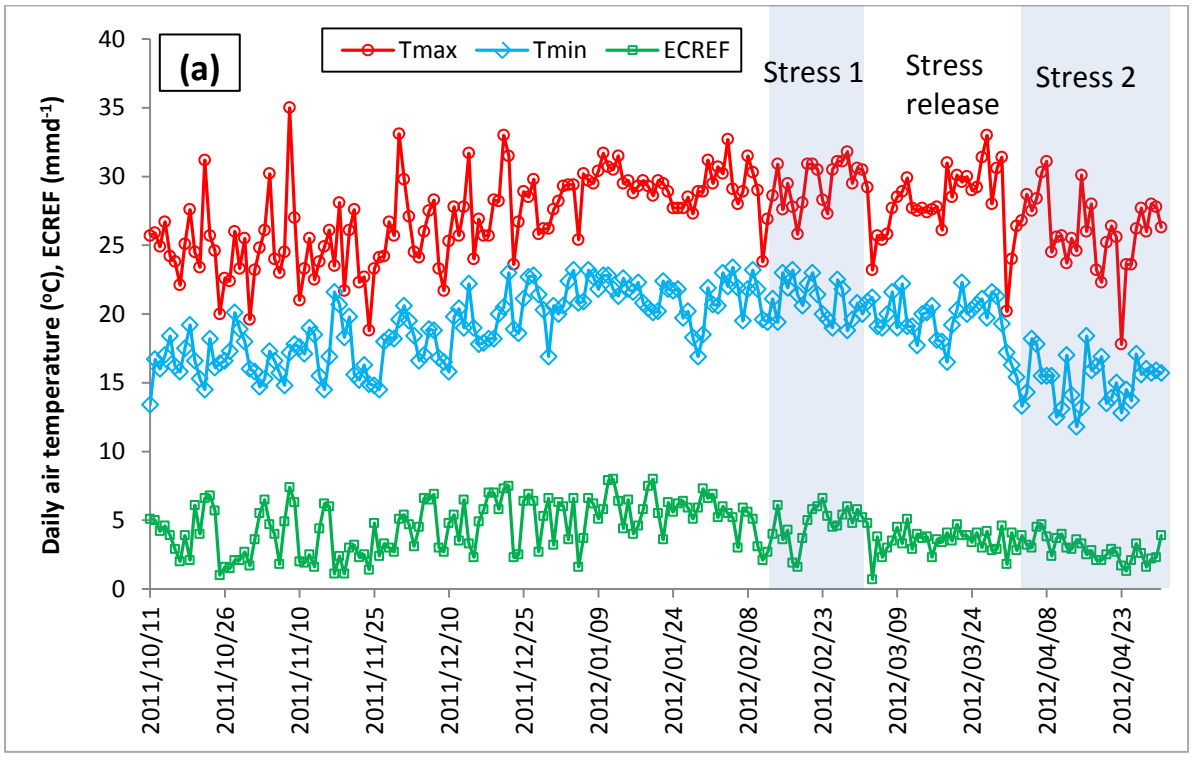
7. APPENDICES

APPENDIX 1: WEATHER DATA

Weather data such as minimum (Tmin) and maximum daily temperature (Tmax), hourly temperature, daily solar radiation (Srad), reference sugarcane evapotranspiration (ECREF) downloaded from an automatic weather station are presented in Figure A1.

The maximum and minimum temperatures ranged from 17.8 to 35 °C and 11.8 and 23.4 °C, respectively. The average minimum temperature was 18.9 °C and was above the base temperature required for stalk elongation (Liu *et al.*, 1998). Reference sugarcane evapotranspiration mean was 4.2 ± 1.7 mm d^{-1}. Solar radiation fluctuated due to frequent cloudy days. The seasonal global solar radiation available for a crop was 3367 MJ m^{-2} with a mean of 17.4 MJ m^{-2} d^{-1}. During the first water stress period (10 February to 01 March 2012), average daily Tmin, Tmax and Srad were 21.1 °C, 29.5 °C, and 19 MJ m^{-2} d^{-1} respectively. The corresponding daily averages during the second water stress period (04 April to 03 May 2012) were 15.3 °C, 26.4 °C and 13.8 MJ m^{-2} d^{-1}, respectively.



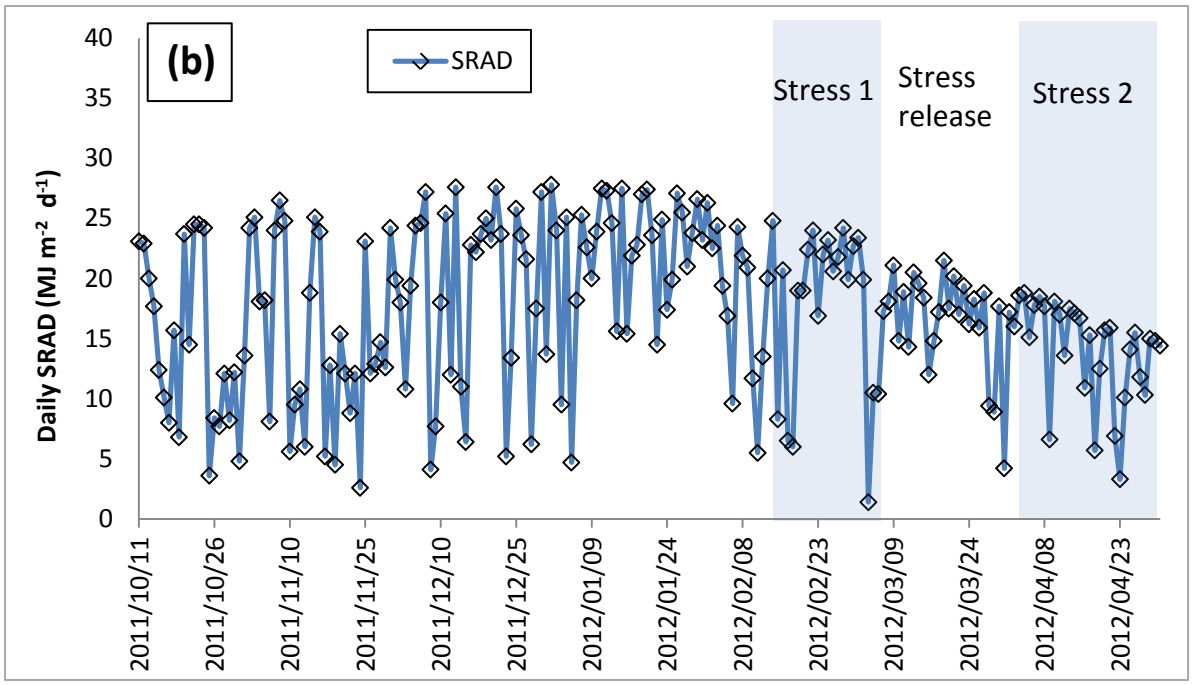


Figure A1. Minimum (Tmin) and maximum (Tmax) daily air temperature, reference sugarcane evapotranspiration (ECREF) (a) and daily solar radiation (SRAD) (b) for the duration of the experiment. Shaded portions indicate periods of water stress (SWC <0.155 m^3 m^{-3}).

APPENDIX 2: NEUTRON WATER METER CALIBRATION DATA

Neutron water meter calibration data is listed in Table A2. Gravimetric soil samples and neutron water meter readings were taken in each plot at three sampling points with four soil samples per point at depths of 0-20, 21-40, 41-60 and 61-80 cm. Sampling points within plots were 1.9 m apart on average and 8 m away from the sampling point of the next plot. Soil bulk density was calculated from the mass of core soil samples of known volume. The dry mass of soil samples was divided by the volume of soil to get soil bulk density (BD in g cm^{-3}).

Table A2. Neutron water meter calibration data: gravimetric soil water content (SWC_g), dry soil bulk density (BD), neutron probe counts, and volumetric soil water content (SWC) derived from probe counts. The slope and the constants obtained from this calibration were 0.00001193 $m^3\ m^{-3}$/count and 0.03435 m^3m^{-3}, respectively.

Plot #	Reps	Soil depth	SWC_g	BD	Neutron probe counts	SWC
		cm	m^3/m^3	g/cm^3		m^3/m^3
1	1	0-20	0.165	1.609	13301	0.203
		21-40	0.213	1.563	16529	0.234
		41-60	0.257	1.586	17985	0.248
		61-80	0.25	1.586	20421	0.271
	2	0-20	0.152	1.678	11770	0.188
		21-40	0.221	1.653	16431	0.233
		41-60	0.278	1.665	20260	0.27
		61-80	0.274	1.659	21602	0.283
	3	0-20	0.143	1.553	10947	0.18
		21-40	0.202	1.627	16278	0.232
		41-60	0.277	1.59	19845	0.266
		61-80	0.27	1.608	20734	0.275
2	1	0-20	0.206	1.606	12268	0.193
		21-40	0.232	1.65	17699	0.245
		41-60	0.261	1.628	18165	0.25
		61-80	0.264	1.639	19462	0.262
	2	0-20	0.204	1.57	11218	0.183
		21-40	0.23	1.592	18315	0.251
		41-60	0.241	1.581	17790	0.246
		61-80	0.246	1.587	18386	0.252
	3	21-40	0.24	1.647	17555	0.244
		41-60	0.254	1.647	17966	0.248
		61-80	0.254	1.647	19739	0.265
3	1	0-20	0.171	1.603	10794	0.179
		21-40	0.253	1.683	18703	0.255
		41-60	0.27	1.643	18512	0.253
		61-80	0.283	1.663	20528	0.273

	2	0-20	0.175	1.657	11036	0.181
		21-40	0.257	1.629	16557	0.234
		41-60	0.242	1.643	18151	0.25
		61-80	0.271	1.636	19615	0.264
	3	0-20	0.166	1.631	11253	0.183
		21-40	0.248	1.653	18820	0.256
		41-60	0.279	1.642	19611	0.264
		61-80	0.287	1.647	20789	0.275
4	1	0-20	0.179	1.705	10379	0.175
		21-40	0.256	1.744	17339	0.242
		41-60	0.267	1.725	17785	0.246
		61-80	0.318	1.734	20551	0.273
	2	0-20	0.139	1.624	10668	0.178
		21-40	0.239	1.672	16049	0.229
		41-60	0.29	1.648	18634	0.254
		61-80	0.276	1.66	19961	0.267
	3	0-20	0.173	1.637	12712	0.197
		21-40	0.243	1.651	17694	0.245
		41-60	0.273	1.644	19256	0.26
		61-80	0.277	1.647	20878	0.276

APPENDIX 3: STATISTICAL TABLES

Table A3.1. T-test statistical results for leaf water potential of two genotypes in two water treatments for different periods of the experiment. DALI denotes days after last irrigation.

		Well-watered treatments			**Water stress treatments**			**Reduction due to water stress**	
	DALI	**04G0073**	**N19**	**Sig**	**04G0073**	**N19**	**Sig**	**04G0073**	**N19**
First stress period	4	-1.108±0.087	-1.10±0.075	NS	-1.22±0.029	-1.10±0.05	*	*	NS
	10	-0.47±0.025	-0.48±0.058	NS	-1.45±0.087	-1.25±0.05	*	**	**
	13	-0.38±0.029	0.27±0.029	**	-1.65±0.000	-1.60±0.05	NS	**	**
	18	-0.10±0.000	-0.18±0.029	**	-1.70±0.05	-1.62±0.028	NS	**	**
	21	-0.17±0.029	-0.23±0.029	*	-2.15±0.05	-2.10±0.05	NS	**	**
Stress recovery	28	-0.32±0.029	-0.33±0.028	NS	-0.87±0.029	-0.68±0.025	**	**	**
	34	-0.38±0.029	-0.33±0.029	NS	-0.47±0.029	-0.38±0.029	*	*	NS
	38	-0.32±0.029	-0.27±0.029	NS	-0.37±0.029	-0.33±0.029	NS	NS	NS
	46	-0.18±0.029	-0.17±0.058	NS	-0.57±0.029	-0.37±0.028	**	**	**
Second stress period	53	-0.23±0.029	-0.18±0.029	NS	-0.63±0.029	-0.48±0.029	**	**	**
	60	-0.79±0.060	-0.38±0.025	**	-1.35±0.218	-0.95±0.18	NS	*	*
	75	-0.78±0.029	-0.43±0.05	**	-1.5±0.173	-1.2±0.346	NS	**	**

*indicate $p \leq 0.05$, ** indicate $p \leq 0.01$ and **NS** indicate non-significant differences. **Sig** indicate Significance of the difference between well watered treatments, between stress treatments and between treatments of the same genotype.

Table A3.2. T-test statistical results for stalk height (cm) for the different treatments during different periods of the experiment. DALI denotes days after last irrigation.

		Well-watered treatments			**Water stress treatments**			**Well-watered x stress treatment**	
	DALI	**04G0073**	**N19**	**Sig**	**04G0073**	**N19**	**Sig**	**04G0073**	**N19**
Pre-stress period	-85	11.64 ±0.73	12.66 ±0.53	NS	11.8 ±0.59	11.88 ±0.54	NS	NS	NS
	-71	16.33 ±1.09	18.55 ±1.00	NS	16.94 ±0.98	18.61 ±0.84	NS	NS	NS
	-29	72.9 ±1.9	68.08 ±1.4	NS	77.5 ±1.83	64.3 ±1.904	**	NS	NS
	-15	103 ±2.59	95 ±1.92	*	105.9 ±2.05	91.6 ±2.54	**	NS	NS
	-1	138.8 ±2.82	117.4 ±2.69	**	128.4 ±2.4	116.5 ±3.33	**	NS	NS
First stress period	13	158.4 ±3.02	133.1 ±3.17	**	132.2 ±2.52	120.7 ±3.39	*	**	*
	20	161.8 ±3.10	136.7 ±2.93	**	132.5 ±2.51	122.3 ±3.14	*	**	**
Stress recovery	28	176.2 ±3.72	145 ±3.46	**	134.3 ±2.71	123.8 ±3.39	*	**	**
	34	199.8 ±2.94	170.1 ±4.7	**	160 ±3.37	137.5 ±3.88	**	**	**
	41	210.1 ±3.54	174.6 ±5.2	**	172.2 ±3.30	165.7 ±2.69	*	**	NS
	47	231.3 ±4.22	190.5 ±6.55	**	189.9 ±3.63	180.6 ±2.87	NS	**	NS
Second stress period	54	228 ±4.88	199.6 ±5.15	**	189.9 ±3.63	181.2 ±2.7	NS	**	**
	62	228.4 ±4.88	200.1 ±5.21	**	189.9 ±3.63	181.2 ±2.7	NS	**	**
	76	236.1 ±3.97	207 ±3.70	**	190 ±3.64	182.9 ±2.84	NS	**	**
	83	240.4 ±4.32	209 ±3.79	**	190 ±4.49	182.9 ±9.6	NS	**	**

*indicate p ≤0.05, ** indicate p ≤0.01 and **NS** indicate non-significant differences. **Sig** indicate Significance of the difference between well watered treatments, between stress treatments and between treatments of the same genotype.

Table A3.3. T-test statistical results for number of green leaves of two genotypes in two water treatments for different periods of the experiment. DALI denotes days after last irrigation.

		Well-watered treatments			**Water stress treatments**			**Reduction**	
	DALI	**04G0073**	**N19**	**Sig**	**04G0073**	**N19**	**Sig**	**04G0073**	**N19**
First stress period	0	12±1.13	10±1.15	**	10±0.81	9±0.75	**	**	NS
	13	10±1.52	9±0.81	*	8±0.69	8±1.00	NS	**	**
	20	10±0.97	9±0.83	*	6.5±0.69	6±0.66	NS	**	**
Stress recovery	28	10±0.87	9.7±1.0	NS	6.8±0.85	6.5±0.77	NS	**	**
	34	13±1.28	11±1.0	**	8±1.47	8±1.33	NS	**	**
	41	12±0.72	11±1.23	**	9.5±0.69	9±.065	NS	**	**
	47	12±0.91	12±1.54	NS	10±1.02	9±2.155	*	**	**
Second stress period	54	11±1.5	11±1.44	NS	9±1.13	9.6±0.78	NS	**	**
	62	8±1.1	9±1.5	**	6±1.11	7±1.34	**	**	**
	68	8±0.85	9±0.94	**	5±1.49	6±1.61	*	**	**
	76	8±0.79	9±1.1	*	4±1.61	6±1.41	**	**	**
	83	9±0.76	9±1.18	NS	3±1.5	5±1.93	*	**	**

*indicate p ≤0.05, ** indicate p ≤0.01 and **NS** indicate non-significant differences. **Sig.** denotes significant differences.

APPENDIX 4: CROP PHENOLOGY RELATIONSHIPS

Crop growth and development are strongly dependent on weather conditions. Under ideal conditions, air temperature becomes the driving factor of crop development. Crop development can occur over a range of temperatures. A base temperature (Tbase) is the lower limit whereby conditions are too cold and if reached development will fail, whereas the optimum temperature is the upper limit which, if exceeded, development will still occur, but at a slower rate. “Every phase of development requires a minimum accumulation of temperature before that stage can be complete and the plant can move to the next stage” (Rawson & MacPherson, 2000). This accumulation of temperature is called thermal time and is measured in degree days (°Cd). Crop modellers use these relationships (Figure A4.1 to A4.3) to simulate crop performance.

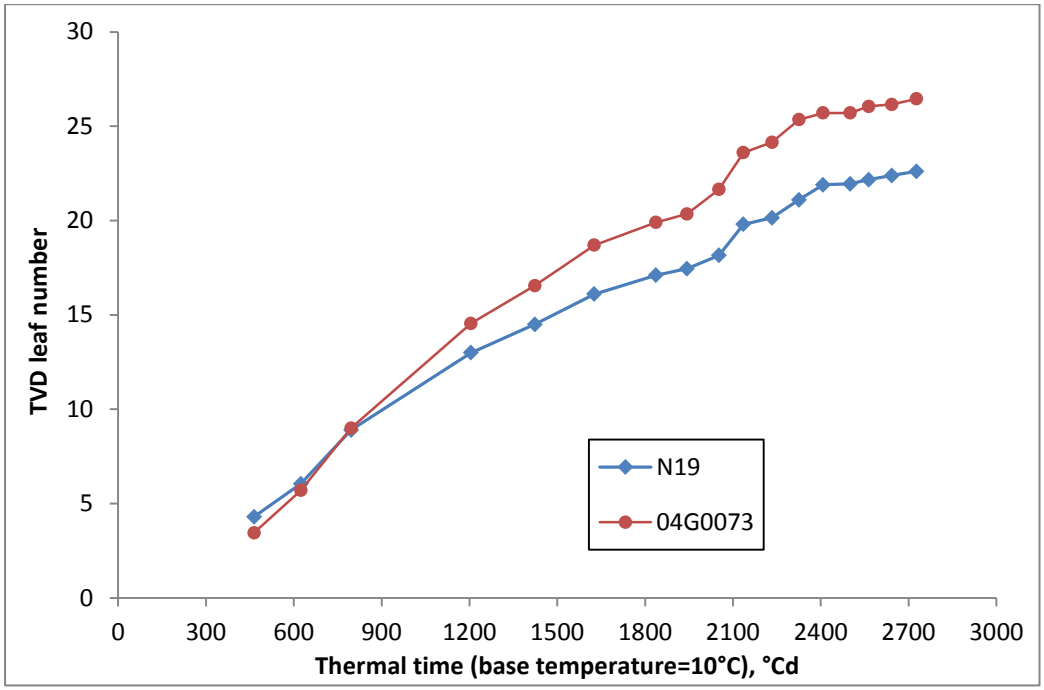


Figure A4.1. Top visible dewlap **(**TVD) leaf number over thermal time of N19 and 04G0073 under well watered treatments. The TVD leaf was defined as the “uppermost fully expanded leaf that has a visible dewlap or distinct collar” (McCray *et al.*, 2005).

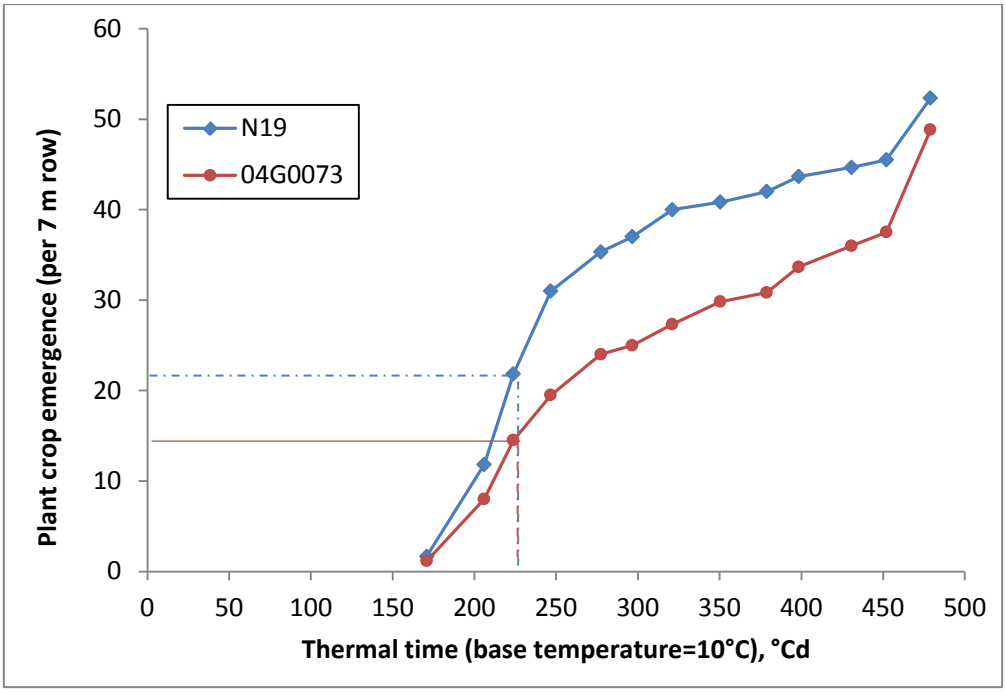


Figure A4.2. Plant crop emergence over thermal time for the well watered treatment. Red solid and the blue dashed lines indicate 50% emergence for 04G0073 and N19 respectively.

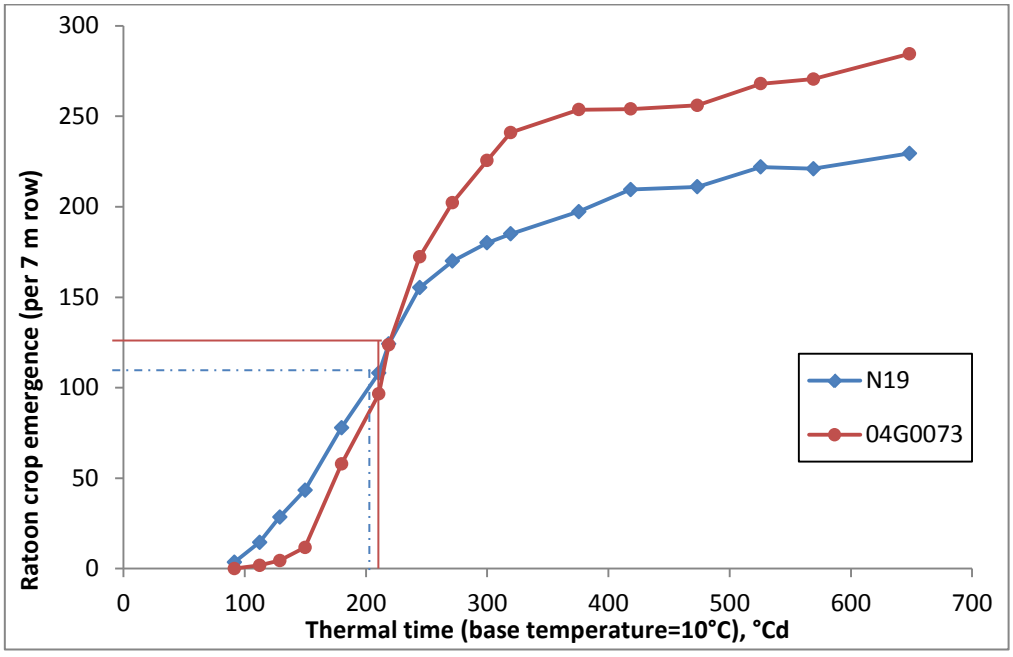


Figure A4.3. Ratoon crop emergence over thermal time for the well watered treatment. Red solid and the blue dashed lines indicate 50% emergence for 04G0073 and N19 respectively.

APPENDIX 5: PRELIMINARY ESTIMATES OF CROP PARAMETER VALUES.

Crop growth models are computer programs that run a series of mathematical equations to represent or mimic real plant growth and development processes in the soil-plant-atmosphere system (Jones, 2013). These models could be used to forecast water use and yield (Singels *et al.*, 2005), and for supporting management practices e.g. irrigation scheduling (Singels & Smit, 2009). Crop growth models can also be used to simulate the sensitivity of crops to certain environmental conditions such as water stress (Singels & Inman-Bamber, 2002; Singels *et al.*, 2010).

Currently, the South African sugar industry uses two crop growth models namely; DSSAT-CANEGRO (Singels *et al*., 2008) and CANESIM (Singels, 2007). The accuracy of these models relies heavily on accurate soil, weather and crop parameters (Bezuidenhout & Singels, 2003). Crop parameter values needed by these models may differ between cultivars. For example, Zhou *et al.* (2003) found that sugarcane cultivar ZN7 developed leaf canopy quicker than cultivar NCo376 which is an important factor determining the amount of solar radiation intercepted by leaves for biomass accumulation.

The CANEGRO model has been found capable of simulating other cultivar traits, however, it has only been calibrated for NCo376 cultivar. Thus the CANEGRO model needs to be calibrated for N19 and 04G0073 cultivars so that suitable areas for 04G0073 cultivation and its predicted yield under different climatic condition can be accurately simulated. The information presented in Table A5 was used to simulate the performance of N19 and 04G0073 cultivars under different environmental scenarios.

Table A5. Genotype parameters for the CANEGRO and CANESIM models as listed and defined by Singels *et al.* 2008. Parameter values for cultivar NCo376 are shown for comparison.

CANEGRO parameter name	**CANESIM parameter name**	**Description**	**NCo376**	**N19**	**04G0073**	**Reference**
dPERdt		Change in plant extension rate (mm/h) per unit change in effective temperature (°C)	0.176	0.18	0.20	Figure 4.14
MXLFAREA		Max leaf area assigned to all leaves above leaf number MXLFARNO (cm²)	360	442	382	Figure 4.10
MXLFARNO		Leaf number above which leaf area is limited to MXLFAREA	14	17	24	Figure 4.10
PI1		Phyllocron interval 1 (for leaf numbers below Pswitch, °C.d (base TTBASELFEX))	69	71	59	Figure A4.1
PI2		Phyllocron interval 2 (for leaf numbers above Pswitch, °C.d	169	146	117	Figure A4.1

		(base TTBASELFEX))				
PSWITCH		Leaf number at which the phyllocron changes.	18	12	12	Figure A4.1
MAX_POP		Maximum stalk population (stalks/m²) (Ranges between 20 to 80)	30	24	32	Figure 4.7
POPTT16		Stalk population at/after 1600 degree days (/m²)	13.3	10	14	Figure 4.7
TTPLNTEM	TT_PLANT	Thermal time (base 10) to emergence for a plant crop	428	214	223	Figure A4.2
TTRANTNEM		Thermal time (base 10) to emergence for a ratoon crop	203	219	211	Figure A4.3
CHUPIBASE	TT_STALK_START	Thermal time (base 10) from emergence to start of stalk growth	1050 or 1000	1423	1423	Figure 4.7
TT_POPGROWTH		Thermal time from emergence to peak stalk population (°C·d) base 16) (Ranges between 400 to 800),	600	739	605	Figure 4.5
	TT_ RATOON	Thermal time required from cut back to emergence of primary stalks (^{o}C.d)	100	219	211	Figure A4.3
	TT_ PLANT	Thermal time required from plant to emergence of primary stalks	300	214	223	Figure A4.3
	RADIATION_USE	Radiation use efficiency – aboveground biomass produced per unit of intercepted radiation (g/MJ)	1.7	1.39	1.52	Table 4.2

APPENDIX 6. DATA FILE LOCATIONS AND CONTENTS

Data from this study is stored on the **H drive** under the folder "**Sivuyile**" on the SASRI network at 170 Flanders drive, Mount Edgecombe, 4300, South Africa.

Folder name	**Excel spread sheet**	**Excel sheet tab name**	**Comments**
Sivuyile	MSc data	Trial details	Planting date, harvest date, row spacing, varieties,
		Fertilizer	fertilizer application rates
		Texture & moisture	Soil physics determined sand, silt and clay; Lab determined field capacity, permanent wilting point, available soil water content, soil density
		Weather data	Daily ETo, solar radiation, min and max temperature for the duration of the experiment
		Neutron probe	Neutron probe calibration data
		Equation R2 calibration	Neutron probe calibration graphs
		Actual vs. calculated graph	
		Irrigation	Irrigation amounts and soil water content
		ET & WUE from SWC	Seasonal evapotranspiration derived from SWC; water use efficiencies
		RASWC	Relative available soil water content
		Leaf water potential	Pre-dawn and midday leaf water potential data and graphs
		Thermal time	Accumulated thermal time for leaf and stalk appearance
		Stalk population	Stalk population per 7 m row and per m^2
		Phenology	Stalk height, leaf number, number of green and dead leaves, leaf length and width, area per leaf, green leaf area index
		Phenology-Graphs	Graphs for area per leaf vs. leaf number; Stalk height vs. DALI; Leaf number vs. Thermal time; green leaf number vs. DALI; stalk population vs. thermal time; leaf width vs. leaf position; Leaf length vs. leaf position; Green leaf area index vs. DALI.
		FIPAR	Fractional interception data and graph
		Yield	Dry and fresh aerial biomass yield; Dry and fresh biomass fractions; Stalk fractions
		RUE	Seasonal radiation interception data and radiation use efficiencies
		Brix data	Brix readings along the stalk profiles
		Root length density	Root length density per soil depth
		Potentiometer data	Stalk potentiometer raw data and filtered data; Raw data for hourly and daily stalk elongation rates

		Hourly SER_h	Hourly stalk elongation graphs
		SER_d vs. Temp	Relationship between stalk elongation, daily minimum, mean and night temperature
		$NSER_d$-$RSER_d$	Stalk elongation rate normalized with air temperature; Relative stalk elongation rate
		$NSER_d$-$RSER_d$-Graphs	Graphs for normalized and relative stalk elongation rates
		SER_d vs. RASWC	Relationship between normalized stalk elongation rate and relative soil water content
		$RSER_d$ vs. RASWC	Relationship between relative stalk elongation rate and relative soil water content
		SER_d vs. LWP	Relationship between stalk elongation rate and leaf water potential