POT TRIAL PHENOTYPING TO PREDICT SUGARCANE GENOTYPE FIELD PERFORMANCE WITH THE CANEGRO MODEL

by

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ABSTRACT

Crop models are valuable tools in scientific research and crop management. Models integrate knowledge across research disciplines, thus providing a tool for analysing and predicting the effects of genetic (G), environmental (E) and management (M) factors on crop performance. Crop models are also used as a decision making tool in crop management for optimising practices and predicting the effects of agronomic conditions on yield. The Canegro sugarcane model has the potential to support plant breeding by identifying desirable genetic traits for improved crop performance. The suitability of Canegro for this application has not been tested because genetic trait parameter (TP) values for genotypes other than cultivar NCo376 are lacking. Appropriate phenotyping methods for obtaining these data also have not been developed. The study tested the ability of the Canegro model to (1) simulate genetic differences in crop growth and yield observed in field trials using TP values estimated from pot trial data, and (2) identify a set of ideal TP values for a target environment.

Phenotyping was conducted in a well-watered pot trial at Mount Edgecombe comprising 14 genotypes replicated five times. TP values were estimated from monthly measurements of leaf and stalk development, leaf gas exchange measured on two occasions, and destructive sampling of biomass components at harvest. TP values for the different genotypes were determined directly from phenotypic data where possible, or through normalization with respect to the reference genotype (NCo376), for which statistically calibrated TP values were already available. Reference stalk elongation rate (SERo) showed the greatest genetic variation (range of 78% of the mean), followed by maximum leaf area (MXLFAREA, 73%), the leaf number at which MXLFAREA occurs (MXLFARNO, 63%) and phyllochron interval 2 (PI2, 52%). Maximum PAR conversion efficiency (PARCEmax) also showed significant variation with a range of 47% of the mean. Phyllochron interval 1 (PI1, 24%), maximum number of green leaves (LFMAX, 23%) and maximum sucrose content (SUCA, 15%) showed less variation, although these were still significant. A range of 17% for the maximum stalk partitioning fraction (STKPFMAX) was not statistically significant. The range for thermal time required to the start of stalk elongation (CHUPIBASE) was 30%, although this could not be tested for significance.

Effective phenotyping procedures were developed for generating data required for TP estimation. The study recommends phenotyping before the onset of flowering, as follows:

(1) three measurements of fully expanded leaf number before leaf number 14, followed by three bi-monthly measurements, for estimation of PI1 and PI2 values, respectively; (2) measurements of green leaf number shortly after transplanting, and again after a thermal time of 720°Cd (base10), for estimation of LFMAX; (3) measurements of leaf size and number of all green fully expanded leaves shortly after transplanting, and at intervals of 900°Cd (base10) thereafter, for estimation of MXLFAREA and MXLFARNO; (4) stalk height once every two months for estimation of SERo; (5) stomatal conductance with a leaf porometer measured between 10:00 and 13:00 on all genotypes within a replicate, for estimation of PARCEmax; (6) biomass fractions at harvest for estimation of STKPFMAX and SUCA. In particular, the proposed protocol for measuring stomatal conductance could be used to develop high-throughput phenotyping technologies in future.

The model showed some potential for simulating genetic differences observed in field trials using TP values estimated from pot trial data. It predicted the genotype rankings for stalk dry mass (SDM) observed for an irrigated field trial in Pongola well (r=0.75*). There were no significant genotype differences in observed SDM in three other field trials at Komatipoort and Mount Edgecombe, and model simulations also showed small differences in SDM for these. The model was unable to predict genotype differences in canopy development observed in field trials and seemed to over-emphasize the influence of PI1 and under-estimate the influence of MXLFAREA.

Impacts of six traits (PI1, PI2, CHUPIBASE, PARCEmax, STKPFMAX and SUCA) were assessed by comparing simulated SDM for 24 hypothetical genotypes with different trait values (four levels per trait), with SDM of the baseline genotype (trait values set at pot trial means). Trait impacts were also assessed for 32 genotypes with multiple trait parameter values generated with LP-TAU algorithm.

Results for single trait changes showed that PARCEmax, STKPFMAX and CHUPIBASE had the largest impacts on SDM. The importance of PARCEmax and STKPFMAX in determining irrigated SDM was confirmed by a path coefficient analysis on the multiple trait genotype model output.

The ideotyping study suggested that by combining optimal values for these three traits, long term mean SDM could be increased 8 and 12 t ha^{-1} compared to the highest-yielding multiple and single trait variant genotypes, respectively. Indications are that these three traits could be candidates for screening in early breeding stages, especially if reliable high-throughput phenotyping methods could be developed for them.

The study confirmed that Canegro in its present form is not suitable for exploring trait impacts on canopy development, a key process in sugarcane yield formation. The canopy development algorithm is too empirical, it is disconnected from the biomass growth and partitioning algorithm and is therefore unable to simulate interactions between these processes.

The knowledge generated in this study will be useful for improving the suitability of the Canegro model for supporting sugarcane breeding, and for developing procedures for screening sugarcane populations for desirable traits.

Keywords: Canegro, genetic trait, parameter estimation, phenotyping, photosynthetic efficiency, simulation model, sugarcane, stomatal conductance, trait impact

PREFACE

The experimental work described in this dissertation was carried out at the South African Sugarcane Research Institute (SASRI), Mount Edgecombe, from October 2014 to March 2017, under the supervision of Prof. Abraham Singels and Dr. Alana Patton.

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 institution. Where use has been made of the work of others it is duly acknowledged in the text.

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I, Natalie Hoffman, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.

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Crop physiologist: South African Sugarcane Research Institute

DECLARATION 2 - PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis.

1. Hoffman, N., Singels, A., Patton, A. & Jones, M.R. 2016. Oral presentation. Phenotyping sugarcane genotypes for radiation use efficiency. *Combined Congress of the South African Society for Crop Production, Soil Science Society of South Africa, Southern African Society for Horticultural Sciences and Southern African Weed Science Society held from 18-21 January 2016 in Bloemfontein*

2. Singels, A., Hoffman, N., Jones, M.R., Ramburan, S. and Eksteen, A. 2016. Genetic trait modelling: Achievements and lessons from sugarcane. *Combined Congress of the South African Society for Crop Production, Soil Science Society of South Africa, Southern African Society for Horticultural Sciences and Southern African Weed Science Society held from 18-21 January 2016 in Bloemfontein*

3. Hoffman, N., Singels, A., Patton, A. & Jones, M.R. 2016. Pot trial phenotyping to predict genotype field performance with the Canegro model. *Proceedings of the South African Sugar Technologists Association 89: 149-153*

4. Singels, A., Hoffman, N., Paraskevopoulos, A. & Ramburan, S. 2016. Sugarcane genetic trait parameter estimation. Conference presentation, *Proceedings of the iCROPM2016 International Crop Modelling Symposium held from 15 to 17 March 2016 in Berlin, 143-144*

Signed: …  ……

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LIST OF SYMBOLS / ABBREVIATIONS

$\Delta SP_{p,d}$	Actual number of primary shoots appearing on day *d* (shoots linear m^{-1})
$\Delta SP_{pot,d}$	Potential number of primary shoots appearing on day *d* (shoots linear m^{-1})
A	Leaf-level photosynthesis rate: net carbon fixation rate per unit leaf area (µmol m^{-2} s^{-1})
ADMPF	Fraction of daily biomass growth partitioned to aerial components (DSSAT-Canegro parameter)
ANOVA	Analysis of variance
APFMX	Aerial biomass partitioning coefficient: maximum fraction of daily dry biomass growth partitioned to aerial parts (t t^{-1}) (DSSAT-Canegro parameter)
Cane_fraction	Fraction of daily dry biomass growth partitioned to cane (g g^{-1}) (APSIM-sugarcane parameter)
CHUPIBASE	Thermal time from shoot emergence to the start of stalk elongation (°Cd) (DSSAT-Canegro parameter)
DSSAT	Decision Support System for Agrotechnology Transfer
dSuc	Daily sucrose accumulation in the stalk (t ha^{-1})
$dTillers_n$	Tillering rate: number of new tillers that appear on a given day per primary shoot cohort *n* (tillers $cohort^{-1}$ d^{-1})
dTOT	Daily dry biomass accumulation (t ha^{-1})
DTT_{SP}	Daily thermal time during the germination phase driving the emergence of primary shoots (°Cd)
$DTT_{tillering}$	Daily thermal time driving the tillering phase (°Cd)
E	Leaf-level transpiration rate (mmol m^{-2} s^{-1})

Ecref	Sugarcane reference evapotranspiration rate (µm h^{-1})
FC	Field capacity: soil water holding characteristic (m^3 m^{-3})
F_{CO2}	Atmospheric carbon dioxide concentration factor which controls the conversion of photosynthetically active radiation to biomass growth
FI	Fractional interception of photosynthetically active radiation by the crop canopy (%)
Fi_{intra}	Intra-row (within stool) fractional interception of photosynthetically active radiation by the crop canopy (%)
FI_{PC}	Fractional interception of photosynthetically active radiation at partial canopy (FI_{PC})
FT	Temperature control factor for photosynthesis and respiration
g_c	Canopy conductance for gaseous exchange (m s^{-1})
GLUE	Generalized likelihood uncertainty estimation
g_s	Leaf-level stomatal conductance for gaseous exchange (mmol m^{-2} s^{-1})
g_{sLicor}	Leaf-level stomatal conductance measured using the LiCor-6400 gas analyzer (mmol m^{-2} s^{-1})
g_{sPoro}	Leaf-level stomatal conductance measured using the Decagon porometer (mmol m^{-2} s^{-1})
GxExM	Genotype x Environment x Management interaction
GxH	Genotype x Hour interaction
HTP	High-throughput phenotyping
IPAR	Photosynthetically active radiation intercepted by the crop canopy (MJ m^{-2})

IRGA	Infrared gas analyser
LAI	Leaf area index (m^2 m^{-2})
Leaf_size	Surface area of individual leaves (mm^2) (APSIM-sugarcane parameter)
Leaf_size_no	Leaf number of individual leaves for which surface area (Leaf_size) is specified (APSIM-sugarcane parameter)
LERo	Reference leaf elongation rate per unit thermal time (cm $(°Cd)^{-1}$) (DSSAT-Canegro parameter)
LFMAX	Maximum number of fully expanded green leaves per primary stalk on a healthy plant under optimal conditions (DSSAT-Canegro parameter)
LG_AMBASE	Aerial fresh mass at which lodging starts (t ha^{-1}) (DSSAT-Canegro parameter)
LSD	Least significant difference
MAXPOP	Maximum tiller population (tillers m^{-2}) (DSSAT-Canegro parameter)
MCMC	Markov Chain Monte Carlo
Min_sstem_sucrose	Minimum dry stalk biomass required before partitioning to sucrose commences (g) (APSIM-sugarcane parameter)
MSE	Mean square error: mean of the squared differences between simulated and observed values
MXLFAREA	Leaf area of the largest fully expanded leaf (cm^2) (DSSAT-Canegro parameter)
MXLFARNO	Leaf number at which MXLFAREA occurs (DSSAT-Canegro parameter)
N_{buds}	Number of viable buds in the ground (buds m^{-1} of row)

PAR	Photosynthetically active radiation (µmol photons m^{-2} s^{-1})
PARCE	PAR conversion efficiency on a given day (g MJ^{-1})
PARCEmax	Maximum PAR conversion efficiency: the gross photosynthate produced per unit of intercepted PAR under ideal temperature and water status (g MJ^{-1}) (DSSAT-Canegro parameter)
PI	Phyllochron interval: Thermal time elapsed between the appearance of successive fully expanded leaves
PI1	Phyllochron interval 1 (°Cd) (DSSAT-Canegro parameter)
PI2	Phyllochron interval 2 (°Cd) (DSSAT-Canegro parameter)
POPTT16	Final tiller population at a thermal time of 1600°Cd (tillers m^{-2}) (DSSAT-Canegro parameter)
PSWITCH	Leaf number at which PI changes from PI1 to PI2 (DSSAT-Canegro parameter)
PWP	Permanent wilting point: soil water holding characteristic (m^3 m^{-3})
$Respcon_i$	Fraction of biomass lost through maintenance respiration for biomass pool *i*
RespQ10	Fractional increase in maintenance respiration rate per 10 °C rise in air temperature
Respcf	Fraction of daily structural growth lost through growth respiration
Rg	Daily growth respiration rate (t ha^{-1})
RH	Relative humidity of the air (%)
Rm	Daily biomass lost to maintenance respiration (t ha^{-1})

RMSE	Root mean square error: square root of the mean of the squared differences between simulated and observed values
RTPF	Fraction of daily dry biomass growth partitioned to roots
RUE	Radiation conversion efficiency (g MJ^{-1}) : daily aboveground biomass produced per unit of intercepted shortwave radiation for a crop that is water and nutrient-stress free (APSIM-sugarcane parameter)
SASRI	South African Sugarcane Research Institute
SDM	Stalk dry mass (t ha^{-1})
SERo	Reference stalk elongation rate per unit thermal time (cm $(°Cd)^{-1}$)
SLA	Specific leaf area: surface area of leaf per unit fresh mass (m^2 kg^{-1})
SP	Stress point: soil water holding characteristic (m^3 m^{-3})
SP_d	Primary shoot population for a given day *d* (shoots linear m^{-1})
SRAD	Solar radiation (W m^{-2} or MJ m^{-2} d^{-1})
STKPFMAX	Stalk partitioning coefficient: maximum fraction of aerial dry biomass growth partitioned to stalks (t t^{-1}) (DSSAT-Canegro parameter)
Stress_factor_stalk	Stress factor for sucrose accumulation (APSIM-sugarcane parameter)
SUCA	Sucrose partitioning coefficient: maximum sucrose content in the bottom of a mature stalk (t t^{-1}) (DSSAT-Canegro parameter)
SUCM	Stalk sucrose yield (t ha^{-1})
Sucrose_fraction_stalk	Fraction of biomass growth partitioned to sucrose (g g^{-1}) (APSIM-sugarcane parameter)

SUCROWS	Sugar-cane growth simulator
$SWDF_{30}$	Soil water deficit factor that reflects soil water status in the top 30 cm of the soil profile and regulates tillering rate
SWSI	Crop water status factor
TARo	Reference tiller appearance rate per unit thermal time (tillers $°Cd^{-1}$) (DSSAT-Canegro parameter)
Tb	Base temperature used to calculate thermal time for a given plant process (°C)
TBFT	Temperature at which 50% of stalk mass increments is partitioned to sucrose under reference conditions
Tmax	Daily maximum temperature (°C)
Tmean	Daily mean temperature (°C)
Tmin	Daily minimum temperature (°C)
To	Optimal temperature for a given plant process (°C)
TOT	Total dry biomass (above and below ground) of the crop (t ha^{-1})
TP	Trait parameter
TT	Thermal time (°Cd)
TT_{EM}	Thermal time accumulated since shoot emergence (°Cd) (DSSAT-Canegro parameter)
TTPLTEM	Thermal time required for shoot emergence of a plant crop (°Cd) (DSSAT-Canegro parameter)
TTPOPGROWTH	Thermal time window during which tillers develop (°Cd) (DSSAT-Canegro parameter)

TTRATNEM	Thermal time required for shoot emergence of a ratoon crop (°Cd) (DSSAT-Canegro parameter)
Tu	Upper temperature above which the rate of a given plant process is zero (°C)
TVD	Top visible dewlap
VPD	Vapour pressure deficit (Pa)
VWC	Soil volumetric water content (m^3 m^{-3})

1. INTRODUCTION

Sugarcane is one of the principal agricultural crops grown world-wide under tropical and subtropical conditions (Chandel *et al*. 2012; Geisler 2012), with the largest producers being Brazil, India, China and Thailand which together account for more than half of the global production (Geisler 2012). The annual world production of sugarcane is estimated to be 1.6 billion tons (Chandel *et al*. 2012), accounting for approximately 75% of the world's sugar (Geisler 2012). Sugarcane is the second largest (based on gross value) field crop in South Africa, surpassed only by maize. The approximately 22 500 registered sugarcane growers in South Africa, of which 21 100 are small-scale growers, annually produce an average of 19 million tons of sugarcane across KwaZulu-Natal to the Mpumalanga Lowveld. The South African sugar industry contributes significantly to the country's economic growth by generating an estimated direct income of R12 billion annually and creating employment opportunities for over one million people directly and indirectly, with some in deep rural areas where few other opportunities are available.

The sugarcane industry both locally and globally is faced with a supply/demand imbalance, where increasingly limited resources (e.g. land and water) challenge an increasing demand for sugar with a growing population. In addition, climate change has been identified as a key factor which threatens future agricultural production and food security globally. Future climate projections show increased temperatures and elevated CO_2 conditions with more frequent and more severe dry spells in irrigated and dryland production areas (Schulze & Kunz 2010). One way to mitigate these impacts on agricultural production is to use high-yielding, resilient crop cultivars. There is an increasing need for sugarcane genotypes which not only produce high sugar and biomass yields in high and low potential environments, but are also tolerant to hot and dry climates in the face of climate change.

Traditional sugarcane breeding consists of evaluating the offspring of designed crosses of parents with successful track records over many cycles in target environments. Due to sugarcane's polyploidy, many individuals of a given cross enter selection programs to avoid losing genotypes that perform well. The South African Sugarcane Research Institute is responsible for producing high-yielding, disease and pest resistant varieties which are adapted to five major agro-climatic regions. The breeding cycle starts off with the rearing of 250 000 clones from parental crosses, of which about 175 000 will be planted in the field at

each of the five selection sites (Parfitt 2005). These are visually assessed for growth vigor and disease resistance before about 20 000 of these will progress to the next stage. Indirect selection criteria are used during these stages. The first quantitative yield assessment will only occur in the next stage on about 3 000 clones grown in replicated plots. Selection for yield (and other selection targets) will continue for another three stages before one to three varieties will be released for commercial production. The typical duration of the breeding cycle from crossing of the parents to release of a variety is about 13 years. It is clear from this that sugarcane breeding in South Africa is resource-intensive and time-consuming. It also does not use physiological knowledge to apply selection pressure, such as screening for traits that are associated with high yields.

Crop simulation models may have the potential to assist plant breeding in this regard. Crop simulation modelling as a scientific discipline has been in development for approximately 50 years, and can be described as "the dynamic simulation of crop growth by numerical integration of constituent processes with the aid of computers" (Sinclair & Seligman 1996). In essence, crop simulation models are computer programs which simulate crop growth and development as a function of genetic traits (G), environmental conditions (E) and management inputs (M) by using mathematical analogues representative of the fundamental processes within the dynamic soil-plant-atmosphere system. Models that are capable of simulating intrinsic plant processes and their interaction with the environment can predict complex trait behaviour such as yield. This could be useful in assisting plant breeding, which is hindered by the difficulties associated with segregating genetic, environmental and management influences when a large number of possible GxExM combinations is being evaluated (Hammer *et al*. 2006; Hammer *et al*. 2010). A better understanding of trait impacts on crop growth and yield and its interactions with environmental and management factors could help with identifying key traits for improved crop performance, and with designing ideal genotypes ("ideotypes") with optimal traits for target environments.

Crop models need to meet certain criteria for credible application in this area. The physiological mechanisms of GxExM interactions should be well understood and simulated (Hammer *et al*. 2006), and models should be tested across a wide range of environments to evaluate their robustness and accuracy (Rötter *et al*. 2015). Model trait parameters should also represent genetic controls of crop growth adequately (Boote *et al*. 2001). Sugarcane crop models have not yet been used to assist breeding programs. Sexton *et al*. (2014) pointed

out the limited ability of APSIM-sugarcane (Keating *et al*. 1999) to simulate cultivar differences in yield due to inadequate cultivar parameter definitions. The situation can be addressed by (1) accurately quantifying appropriately defined genetic trait parameters using appropriate phenotyping procedures; and (2) evaluating and improving model capabilities for simulating observed genotypic differences in crop growth and yield.

This study considered the DSSAT-Canegro crop simulation model (Inman-Bamber 1991; Singels *et al*. 2008), which has been used extensively in research and management (e.g. Singels *et al*. 1998; Singels & Bezuidenhout 2002; Singels *et al*. 2013) and can be regarded as one of the leading sugarcane crop simulation models. The Canegro model has a long-standing history with the South African Sugarcane Research Institute (SASRI) where it was first developed (Inman-Bamber 1991), and has been continually improved since (Inman-Bamber 1995b; Inman-Bamber & Kiker 1997; Singels & Bezuidenhout 2002; Singels *et al*. 2005; Singels *et al*. 2008, Jones *et al*. 2011). This model was therefore chosen for this study over other leading sugarcane models such as APSIM-sugarcane (Keating *et al*. 1999), MOSICAS (Martiné *et al*. 1999), QCANE (Liu & Bull 2001) and CASUPRO (Villegas *et al*. 2005).

1.1 Problem statement

It is unknown whether the DSSAT-Canegro model has the simulation capabilities for assisting sugarcane plant breeding. There has been little research into evaluating whether the model simulates physiological processes and their interactions realistically, and represents genetics well in trait parameters. This has not been tested because trait parameter values for genotypes other than NCo376 are lacking. Appropriate phenotyping methods for obtaining these data also have not been developed. The study aims to address these knowledge gaps.

1.1.1 Hypotheses

The study aimed to test the following hypotheses:

1. The Canegro model can simulate genetic differences in crop growth and yield observed in field trials accurately using trait parameter values estimated from data collected in a pot trial.
2. The Canegro model can be used to identify a set of ideal trait parameter values for a target environment.

1.1.2 Objectives

The overall goal of the study was to evaluate the suitability of the Canegro model to support sugarcane breeding by predicting the impacts of genetic traits on yield.

This study has the following objectives:

1. To determine trait parameter values for selected genotypes in a pot trial.
2. To determine the accuracy of simulated genotypic differences in canopy cover, stalk dry mass and sucrose yield for selected genotypes grown in irrigated field trials using the trait parameter values estimated from data collected in the pot trial.
3. To develop a phenotyping protocol for estimating trait parameter values.
4. To determine trait impacts by evaluating the sensitivity of simulated stalk dry mass to changes in single and multiple trait values for a selected irrigated environment.
5. To identify through simulation, a set of ideal trait values for a selected irrigated environment.

1.1.3 Dissertation outline

Chapter 1 introduces the sugarcane industry and the challenges it faces. It briefly discusses the South African sugarcane plant breeding program and the difficulties associated with the current approaches used and how crop models can be used to assist plant breeding. The problem statement, hypotheses and research objectives are also described.

Chapter 2 reviews the existing literature needed to form recommendations for the study. This includes general crop modelling concepts and a detailed description of the DSSAT-Canegro model processes, briefly comparing to those simulated in the APSIM-Sugarcane model. Approaches to phenotyping and deriving trait parameter values from measurements of plant growth are discussed. Methods of evaluating trait impacts on simulated yield are also reviewed.

Chapter 3 describes the trial details and phenotyping methods of the pot trial, followed by a description of the methodology for deriving trait parameter values and assessing trait impacts on stalk dry mass.

Chapter 4 consists of two sections. The first presents results from measurements of leaf and stalk development, gas exchange and biomass components in the phenotyping pot trial. The trait parameter values derived from these measurements, and their use in field trial simulations with the DSSAT-Canegro model, is described. The second section describes the assessment of trait impacts on simulated yield and the estimation of potential yield gain from combining optimal trait values.

Chapter 5 summarises the main findings of the study and presents recommendations for future research.

2. LITERATURE REVIEW

2.1 Introduction

The aim of this review was to collate information about (1) sugarcane crop simulation modelling approaches, model parametrization and validation methods; (2) methods of phenotyping in sugarcane; and (3) trait impacts modelling. This will be used to identify knowledge gaps and successful approaches and to formulate recommendations for the current study.

2.2 Crop models and parameters

2.2.1 General overview of model concepts

Crop models simulate plant growth and predict crop yield as a function of genetic, weather, soil and management information (Figure 2.1).

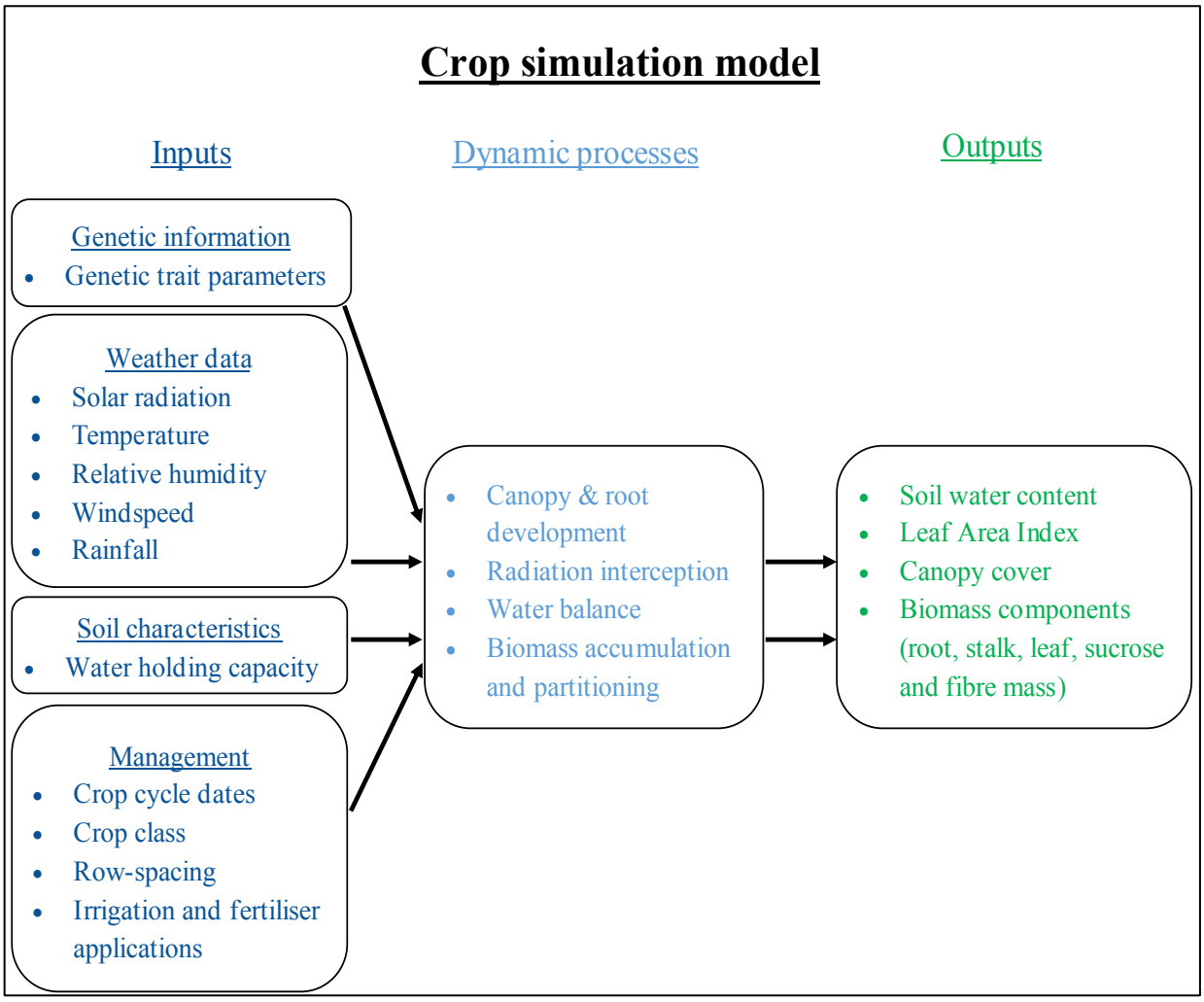


Figure 2.1. Diagram to illustrate data types and flow in crop simulation.

2.2.2 Sugarcane modelling

Sugarcane crop modelling started with the development of the SUgar-Cane gROWth Simulator (SUCROWS) by Tovey & Bull (1977). The study proposed that a sugarcane model may be used to (1) predict sugarcane performance and estimate potential yields under a range of agronomic and climatic conditions; (2) identify factors limiting productivity; and (3) infer information which could be used to aid genotype selection.

Several process-based sugarcane models have been developed since, including DSSAT-Canegro (Inman-Bamber 1991), APSIM-Sugarcane (Keating *et al*. 1999), MOSICAS (Martiné *et al*. 1999), QCANE (Liu & Bull 2001), CASUPRO (Villegas *et al*. 2005) and Canesim (Singels 2007).

The DSSAT-Canegro model

The Canegro model has been in development since the early 1990s (Inman-Bamber 1991). It was first incorporated as a standalone program into the Decision Support System for Agrotechnology Transfer (DSSAT) (IBSNAT 1989) crop modelling software package v3.1 (Inman-Bamber & Kiker 1997). The model was further developed by Singels & Bezuidenhout (2002) and Singels *et al*. (2005), and was modularized to operate as a plant module within DSSAT v4.5 (Singels *et al*. 2008). Thereafter, Jones *et al*. (2011) developed the GTP-Canegro model version in an attempt to address shortcomings identified in DSSAT-Canegro v4.5. These included the fact that the simulation of biomass growth and partitioning is disconnected from that of plant organ development. The version of Canegro used in this study (v4.5_C2.0, Singels *et al*. 2016a) was derived from DSSAT-Canegro v4.5 (Singels *et al*. 2008) with modifications to the tillering, photosynthesis and respiration and water balance sub-models (Singels *et al*. 2016a). Canegro simulation of the main crop processes are now described, and briefly compared to that of APSIM-Sugarcane (Keating *et al*. 1999), the other widely used sugarcane crop model.

Model processes

Many of the processes in the Canegro model are driven by thermal time (TT, °Cd). TT can be considered to be a measure of the temperatures experienced for a given time period. Daily TT is calculated from temperature (T) using three cardinal values, namely the base temperature (Tb) below which the specific plant process ceases, the optimal temperature

(To) where the process rate is at a maximum, and the upper temperature (Tu) above which the process rate is zero:

$$TT = 0 \text{ when } T < Tb \text{ or } > Tu \quad \text{(Equation 2.1)}$$

$$TT = T - Tb \text{ when } Tb < T < To \quad \text{(Equation 2.2)}$$

$$TT = \frac{(T-Tb)(Tu-T)}{Tu-To} \text{ when } To < T < Tu \quad \text{(Equation 2.3)}$$

The Tb, To and Tu values are process-specific. By comparison, the APSIM-Sugarcane model (Keating *et al*. 1999) does not consider cardinal temperatures to be process-specific, and uses generic Tb, To and Tu values of 9, 32 and 45°C, respectively. Neither models consider cardinal temperatures to be genotype-specific. Previous studies on other crops have shown genotype variation in cardinal temperatures (e.g. Slafer & Rawson 1995).

Germination and emergence

Bud germination is governed by trait parameters TTPLTEM and TTRATNEM (°Cd), defined as the TT required for the completion of the germination phase of plant and ratoon crops respectively (using Tb, To and Tu values of 16, 28 and 41°C, respectively). The end of the phase marks the emergence of the first primary shoot from the ground.

The actual number of primary shoots appearing on day *d* ($\Delta SP_{p,d}$, shoots linear m^{-1}) is calculated as a function of the potential change ($\Delta SP_{pot,d}$, i.e. the difference between SP_{pot} for today and yesterday) and a soil water stress factor for tillering ($SWDF_{30}$).

$$\Delta SP_{p,d} = \Delta SP_{pot,d} * SWDF_{30} \quad \text{(Equation 2.4)}$$

The potential number of primary shoots appearing on a given day ($\Delta SP_{pot,d}$, shoots linear m^{-1}) is calculated as a function of the TT for that day (DTT_{SP}), the TT accumulated since emergence (TT_{EM}), and the number of viable buds in the ground ($N_{buds,}$ buds m^{-1} of row),

$$\Delta SP_{pot,d} = DTT_{SP}.m.Nbuds.e^{-m.TT_{EM}}, \quad TT_{EM} \leq 600 \quad \text{(Equation 2.5)}$$

where *m* is an empirical parameter (*m* = 0.00707).

Primary shoot population for a given day (SP_d, shoots linear m^{-1}) is calculated as the sum of the primary shoots on the previous day and the daily change.

$$SP_d = SP_{d-1} + \Delta SP_p \quad \text{(Equation 2.6)}$$

Two examples of simulated primary shoot population are shown in Figure 2.2

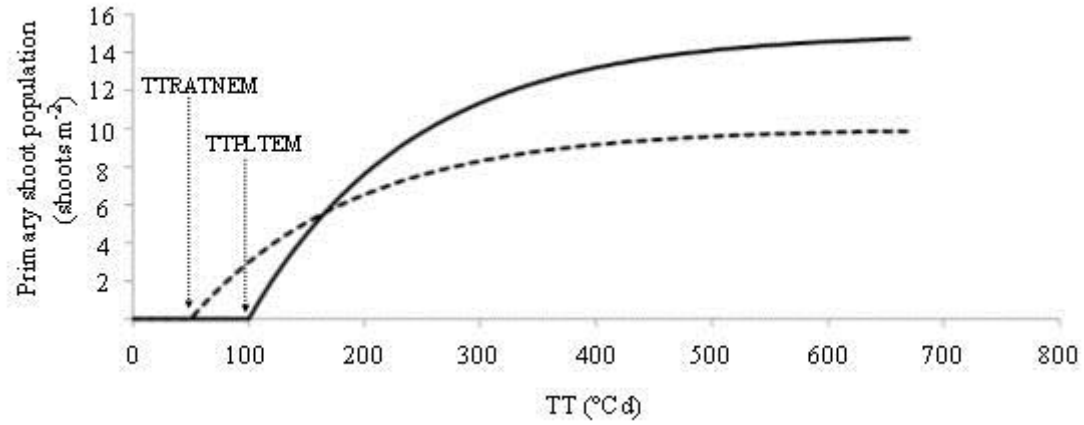


Figure 2.2. Simulated primary shoot population against thermal time (TT) using bud populations of 15 (solid line) and 10 buds m^{-2} (dashed line), where the TT to emergence was specified as 50 and 100°Cd for ratoon (TTRATNEM) and plant (TTPLTEM) crops, respectively (Singels *et al.* 2016a).

The simulation of bud germination and primary shoot emergence differs from that of the APSIM-Sugarcane model, where sprouting occurs after a specified lag phase (350°Cd and 100°Cd for plant and ratoon crops, respectively) after which shoots elongate at 0.8mm°Cd^{-1} (Keating *et al*. 1999).

Tillering

Tillering rate, defined as the number of new tillers that appear on a given day per primary shoot cohort ($dTillers_n$, tillers $cohort^{-1}$ d^{-1}), is calculated as a function of the reference tiller appearance rate per unit TT (trait parameter TARo, tillers $shoot^{-1}$ °Cd^{-1}) and the TT on the given day ($DTT_{tillering}$, °Cd) using Tb, To and Tu values of 16, 35 and 48°C, respectively. It is also influenced by intra-row fractional interception of light (Fi_{intra}), water stress factor for tillering ($SWDF_{30}$) and the number of primary shoots in primary shoot cohort *n*.

$$\mathrm{dTillers_n} = \mathrm{SP_n} * \mathrm{DTT_{tillering}} * \mathrm{TAR_0} * \max\left(0, 1 - \left(\frac{\mathrm{Fi_{intra}}}{0.9}\right)\right) * \mathrm{SWDF_{30}} \quad \text{(Equation 2.7)}$$

Tiller senescence is initiated after a specified TT (trait parameter TTPOPGROWTH) has elapsed. Tiller senescence is accelerated under water stress, and cohort senescence occurs when the shoot cohort has fewer than 3 green leaves per shoot. No tiller senescence occurs after a specified TT (trait parameter POPTT16) has elapsed.

The model uses various trait parameters to characterise tiller development, namely reference tiller appearance rate per unit TT (TARo, tillers °Cd^{-1}), the TT window during which tillers develop (TT_POPGROWTH, °Cd), maximum tiller population (MAX_POP) and final tiller population after TT of 1600°Cd (POPTT16, tillers m^{-2}) (Figure 2.3).

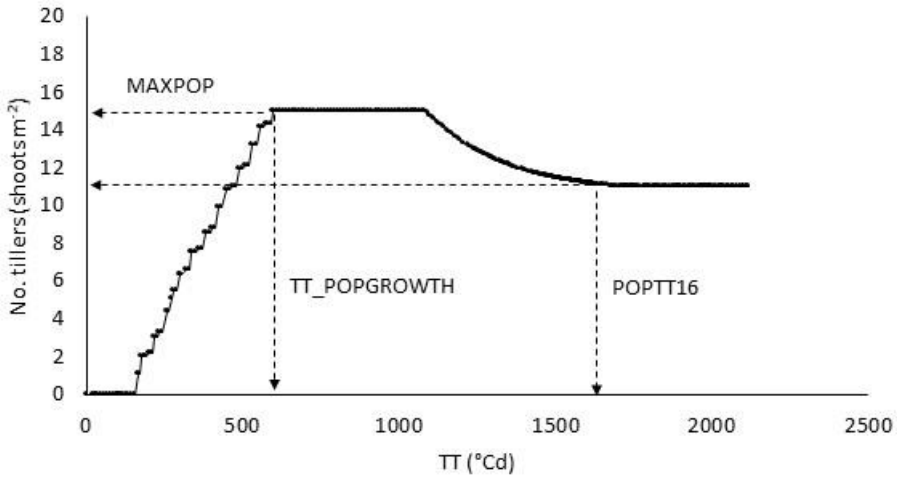


Figure 2.3. An example of simulated tiller population as a function of thermal time (TT) since planting. The genotype-specific trait parameters governing the simulation of tillering phases in the Canegro model are the thermal time window during which tillers develop (TT_POPGROWTH), maximum tiller population (MAX_POP) and final tiller population after a TT of 1600°Cd (POPTT16).

APSIM-Sugarcane assumes a user-specified notional constant stalk number for the duration of crop growth. In the early stages of development, a calibrated tillering factor increases the area of leaves on tillers which appear and subsequently senescence. In this way, the model is able to calculate leaf area index (the total green leaf area expressed per unit ground area) during early growth without simulating the complexities associated with tillering.

Leaf development

Leaf appearance is driven by TT using Tb, To and Tu values of 10, 30 and 43°C, respectively. Inman-Bamber (1994) described leaf development as a linear bi-phasic process, i.e. two distinct leaf appearance rates per unit TT exists for the two phases. The phyllochron interval is defined as the TT between the emergence of fully expanded leaves on a given tiller. Phyllochron intervals (PI1 and PI2, °Cd) characterise the two phases of leaf development, which occur before and after a specified leaf number (PSWITCH).

Total green leaf area of the crop is calculated as the sum of the area of all green leaves for all tiller cohorts. Area of individual leaves when fully expanded, increases with leaf number up to a specified leaf number (trait parameter MXLFARNO), which is defined as the youngest leaf to reach the maximum fully expanded area (trait parameter MXLFAREA, cm^2). The model assigns the MXLFAREA value to all leaves that develop thereafter (Figure 2.4). The area of individual leaves that are not fully expanded yet is determined by the rate at which leaves elongate, which is described by the reference leaf elongation rate per unit TT (trait parameter LERo, cm $°Cd^{-1}$).

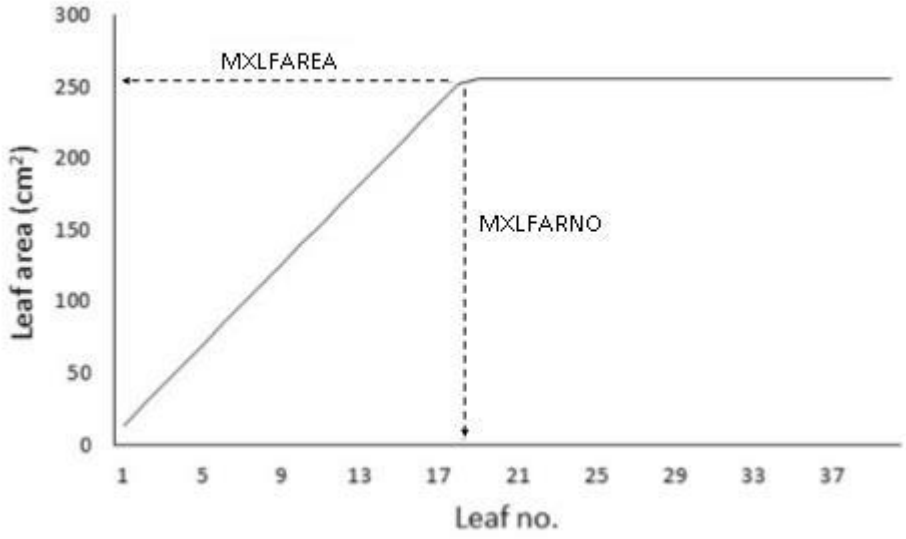


Figure 2.4. An example of simulated fully expanded leaf area as a function of leaf number. The genotype-specific trait parameters governing the simulation of leaf size the maximum fully expanded area (MXLFAREA) and the number of the youngest leaf to which it applies (MXLFARNO).

Leaf senescence commences when the number of green leaves for a given cohort equals the specified maximum number (trait parameter LFMAX). Thereafter, leaf senescence rate equals leaf appearance rate under well-watered conditions. Leaf senescence is accelerated under water stress conditions.

In APSIM-Sugarcane, leaf appearance rate gradually declines with TT. Green leaf area per stalk is calculated as the sum of the area of individual fully expanded leaves, using a correction factor for the area of expanding leaves. Individual leaf area is calculated from genotype-specific trait parameters which specify the leaf area (trait parameter leaf_size) and corresponding leaf number (trait parameter leaf_size_no). Leaf senescence commences after 13 fully expanded green leaves have appeared, and is affected by light competition, water stress and frost (Keating *et al*. 1999).

Stalk elongation

Stalk elongation is initiated once a genotype-specific TT period (trait parameter CHUPIBASE, ^{o}Cd) has elapsed since primary shoot emergence (using Tb, To and Tu values of 16, 35 and 48°C, respectively), similar to the approach used in APSIM-Sugarcane (Keating *et al*. 1999). The rate of stalk elongation is governed by a genotype-specific stalk elongation rate per unit TT (trait parameter SERo, cm °Cd^{-1}). It should be noted that stalk elongation has no downstream effects in the model apart from determining stalk height.

Canopy interception of radiation

Fractional interception of photosynthetically active radiation (PAR) is calculated as a function of leaf area at a whole crop level using a hedgerow model (Beer's law). This equation uses a radiation extinction coefficient to reflect the influence of canopy architecture on radiation interception. This parameter is not considered to be genotype-specific in the Canegro model. Simulated PAR interception also responds to any factor that affects crop leaf area, namely row spacing, bud density, final leaf size and senescence. This is comparable with APSIM-Sugarcane, which also uses Beer's law at the canopy level, although global shortwave radiation is used instead of PAR.

Photosynthesis and respiration

The model calculates daily increments in dry biomass (dTOT, t ha^{-1}) as the product of intercepted PAR (IPAR, MJ m^{-2}) and the PAR conversion efficiency (PARCE, g MJ^{-1}) minus growth (Rg) and maintenance (Rm) respiration.

$$dTOT = PARCE * IPAR * 10^6 - Rg - Rm \quad \text{(Equation 2.8)}$$

PARCE is calculated as a function of maximum PAR conversion efficiency (trait parameter PARCEmax, g MJ^{-1}, defined as the amount of gross photosynthate produced per unit of intercepted PAR under ideal temperature and water status), a temperature control factor (FT), crop water status factor (SWSI) and an atmospheric CO_2 concentration factor (F_{CO2}):

$$PARCE = PARCEmax * FT * SWSI * F_{CO2} \quad \text{(Equation 2.9)}$$

FT ranges from zero to one and is calculated using the mean (Tmean), Tb, To and Tu temperatures (10, 20 and 40, and 47°C, respectively) for photosynthesis, as well as the lower (To1) and upper (To2) values of the optimal temperature range for photosynthesis, as follows:

$$FT = 1 \text{ when } To1 < Tmean < To2 \quad \text{(Equation 2.10)}$$

$$FT = MAX\left(0, 1 - \frac{Tmean - To2}{Tu - To2}\right), Tmean > To2 \quad \text{(Equation 2.11)}$$

$$FT = 1 - \frac{To1 - Tmean}{To1 - Tb}, Tmean < To1 \quad \text{(Equation 2.12)}$$

Daily growth respiration (Rg, t ha^{-1}) is calculated assuming that a constant fraction (Respcf) of structural growth is lost through respiration. Structural growth is assumed to be the difference between daily biomass accumulation (dTOT, t ha^{-1}) and daily sucrose accumulation in the stalk (dSuc, t ha^{-1}).

$$Rg = Respcf * (dTOT - dSuc) \quad \text{(Equation 2.13)}$$

Daily maintenance respiration (Rm, t ha^{-1}) is calculated for the viable biomass pools of the plant, i.e. live roots, green leaves and meristem, as well as for stored sucrose. It is determined

by the mass of the pool (t ha^{-1}) assuming a fraction of biomass is lost through maintenance respiration for biomass pool *i* ($Respcon_i$) at the reference temperature, and a temperature (Tmean) dependent function (RespQ10, defined as the fractional increase in maintenance respiration rate per 10 °C rise in air temperature).

$$Rm = Mass * Respcon_i * RespQ10^{\left(\frac{Tmean-10}{10}\right)}, Tmean < To \quad \text{(Equation 2.14)}$$

$$Rm = Mass * Respcon_i * RespQ10^{\left(\frac{To-10}{10}\right)\left(1-\frac{Tmean-To}{Tu-To}\right)}, Tmean > To \quad \text{(Equation 2.15)}$$

In APSIM-Sugarcane, radiation conversion efficiency (RUE, g MJ^{-1}, defined as the daily aboveground biomass produced per unit of intercepted shortwave radiation for a crop that is water and nutrient stress free) is considered to be a species parameter which differs only between plant and ratoon crops (Keating *et al*. 1999). APSIM RUE is sensitive to temperature, water and nitrogen status (Singels 2014). The APSIM-Sugarcane model does not simulate respiration.

Biomass partitioning

Biomass partitioning in the Canegro model is described by Singels & Bezuidenhout (2002). The fraction of daily biomass growth partitioned to aerial components (ADMPF) depends on the amount of biomass (TOT) and a genotype specific parameter APFMX (defined as the maximum fraction of daily dry biomass growth partitioned to aerial parts). The empirical parameter b determines the rate of decline in ADMPF with increasing biomass. The fraction partitioned to roots is then calculated as the complement of ADMPF.

$$ADMPF = APFMX(1 - e^{-bTOT}) \quad \text{(Equation 2.16)}$$

$$RTPF = 1 - ADMPF \quad \text{(Equation 2.17)}$$

The model does not partition aerial biomass to stalks until a genotype-specific TT period is reached (trait parameter CHUPIBASE, defined as the TT from shoot emergence to the start of stalk elongation). Thereafter, a constant fraction of aerial biomass is partitioned to stalks (trait parameter STKPFMAX, defined as the maximum fraction of aerial dry biomass growth partitioned to stalks).

APSIM-Sugarcane uses a comparable trait parameter (cane_fraction) to characterise the maximum fraction of daily dry biomass growth partitioned to cane.

The partitioning of stalk mass to the sucrose and fibre plus non-sucrose pools is determined by source and sink strength, where the source is considered to be the daily mass allocated to stalks (as determined by crop development stage, radiation, temperature and water status) and the sink is considered to be the capacity to store sucrose and build fibre (as determined by temperature and water status). Sucrose accumulation is governed by the trait parameters which specify the maximum sucrose content in the base of a mature stalk (trait parameter SUCA, t t^{-1}) and the temperature at which 50% of stalk mass increments is allocated to sucrose under reference conditions (TBFT).

APSIM-Sugarcane simulates sucrose accumulation using trait parameters that represent the maximum fraction of daily biomass growth allocated to sucrose storage (trait parameter sucrose_fraction_stalk) and the stem dry biomass required before sucrose accumulation commences (min_sstem_sucrose). Sucrose partitioning under water stress conditions is governed by a stalk growth stress factor (trait parameter stress_factor_stalk) which limits photosynthesis and restricts the partitioning of assimilate to sucrose at the expense of the stem.

The DSSAT-Canegro trait parameters are described in Table 2.1.

Table 2.1. Genetic trait parameters of the DSSAT-Canegro model.

Category	Parameter	Description
Germination and emergence	TTPLTEM, TTRATNEM	Thermal time (TT) required for shoot emergence of plant and ratoon crops, respectively (°Cd)
Leaf development	PI1, PI2	Phyllochron interval (PI): TT elapsed between the appearance of successive fully expanded leaves (°Cd)
	PSWITCH	Leaf number at which PI changes
	LFMAX	Maximum number of fully expanded green leaves per primary stalk on a healthy plant under optimal conditions
	MXLFAREA	Leaf area of the largest fully expanded leaf (cm^2)
	MXLFARNO	Leaf number at which MXLFAREA occurs
Tiller development	TARo	Reference tiller appearance rate per unit TT (tillers $°Cd^{-1}$)
	TTPOPGROWTH	TT window during which tillers develop (°Cd)
	MAXPOP	Maximum tiller population (tillers m^{-2})
	POPTT16	Final tiller population at a TT of 1600°Cd (tillers m^{-2})
Stalk development	CHUPIBASE	TT from shoot emergence to the start of stalk elongation (°Cd)
	SERo	Reference stalk elongation rate per unit TT (cm $°Cd^{-1}$)
Photosynthetic efficiency	PARCEmax	Maximum PAR conversion efficiency: the gross photosynthate produced per unit of intercepted PAR under ideal temperature and water status (g MJ^{-1})
Biomass partitioning	APFMX	Aerial biomass partitioning coefficient: maximum fraction of daily dry biomass growth partitioned to aerial parts (t t^{-1})
	STKPFMAX	Stalk partitioning coefficient: maximum fraction of aerial dry biomass growth partitioned to stalks (t t^{-1})
	SUCA	Sucrose partitioning coefficient: sucrose content in the bottom of a mature stalk (t t^{-1})
	TBFT	Temperature at which 50% of stalk mass increments is partitioned to sucrose under reference conditions
Lodging	LG_AMBASE	Aerial fresh mass at which lodging starts (t ha^{-1})

2.2.3 Estimation of trait parameter values

Three approaches could be used to estimate trait parameter values, namely through (1) direct determination from closely related phenotypic data; (2) the use of statistical methods on indirect phenotypic data; and (3) a combination of these two approaches.

An example of direct determination is deriving the phyllochron interval (Table 2.1) from leaf appearance and TT data collected under well-watered conditions.

Statistical methods estimate trait parameter values by minimising the difference between simulated and observed data of selected variables such as yield. Makowski *et al*. (2006) discussed several methods for estimating trait parameter values statistically. Generally, two approaches are used, i.e. frequentist and Bayesian approaches.

The frequentist approach produces a single estimate for a given parameter, rather than a distribution of possible values. The maximum likelihood method estimates parameter values which maximize the probability of generating simulated values that match observed data, whereas the least squares method estimates parameter values which minimize the sum of the squares of the differences between simulated and observed data. These methods do not take into account prior information about trait parameter values, i.e. the likely distribution of parameter values (Makowski *et al*. 2006).

The Bayesian approach uses experimental data, as well as prior knowledge of the likely distribution of parameter values based on literature and expert opinion, to estimate the posterior parameter probability distribution. Bayesian approaches include the generalized likelihood uncertainty estimation (GLUE, Beven & Binley 1992) and Markov Chain Monte Carlo (MCMC, Gelman *et al*. 1997) methods which have been used to parameterize crop models for major crops such as maize (He *et al*. 2010; Tao *et al*. 2009), wheat (Mo & Beven 2004; Dumont *et al*. 2014), and to a lesser extent, sugarcane (Marin *et al*. 2011). Sexton *et al*. (2016) found that the GLUE and MCMC methods for estimating trait parameter values in both theoretical and real world evaluations yielded similar results when calibrating sugarcane varieties in APSIM-sugarcane. It should be noted that one of the main difficulties with statistical parameterization is “equifinality”, i.e. different parameter sets may produce

simulated data that fit the observed data equally well, but may not be realistic from a biological point of view (Beven & Freer 2001).

The third approach is to use a combination of the direct and indirect (statistical) approaches. For example, trait parameter PARCEmax (Table 2.1) would be extremely difficult to measure directly because (1) respiration and photosynthesis occurs simultaneously and cannot easily be separated; and (2) it is difficult to measure photosynthesis at whole crop level under ideal conditions. Singels & Bezuidenhout (2002) manually implemented the least squares statistical method to estimate the PARCEmax value from aerial dry biomass and radiation data collected in field experiments. PARCEmax could be also estimated by (1) measuring leaf-level CO_2 fixation rate under reference conditions and normalising genotypic values relative to that of a reference genotype (e.g. NCo376 in Canegro); and (2) applying the normalized (relative) value to the value of the reference genotype that was statistically derived from field experimental data. This approach assumes that genotypic differences in leaf-level radiation conversion efficiency are similar to that of maximum radiation conversion efficiency at the crop canopy level.

2.2.4 Simulation accuracy

Once trait parameter values have been estimated, it is necessary to quantify simulation accuracy using statistical comparison of simulated and observed data. In the context of this study, evaluating simulation accuracy is also a means of indirectly determining how reliable the trait parameter values are, assuming that the soil, management, and weather inputs are realistic.

Wallach (2006) and Bennet *et al*. (2013) proposed several statistical parameters for quantifying simulation accuracy. The basis of these measures is the difference between simulated and observed values.

The mean difference between simulated and observed values is the simulation bias, which is a measure of overall under or overestimation. Using bias alone to quantify simulation accuracy is inadequate because severe over- and under-predictions in the same dataset will counteract to produce a low bias value, thereby masking poor model performance.

Another measure of simulation accuracy is the mean square error (MSE), defined as the mean of the squared differences between simulated and observed values. The square root of the mean square error (RMSE) is a convenient measure of model accuracy because it has the same units as the variable been assessed, and it is used very widely for crop model evaluations.

Linear regression analysis may also be used to assess simulation performance. The gradient and intercept of the regression between simulated and observed values convey useful information about model performance across the range of observed values, while the correlation coefficient reflects the strength and nature (positive/negative) of the relationship between simulated and observed values.

Other measures include the index of agreement of Willmott (1981) and modelling efficiency (Wallach 2006), where model deviations are normalized so that index values range between upper and lower bounds to enable comparison between datasets and models.

These statistical parameters can be used to evaluate modelling capability for predicting key crop parameters such as leaf area index or cane yield, as these are influenced by the environment (and management), as well as by genetic factors. Modelling capability can also be evaluated by statistically comparing simulated and observed genotype rankings for a given variable.

2.3 Phenotyping

2.3.1 Manual measurements

Leaf development and growth

Leaf parameters (e.g. PI1 and PI2, Table 2.1) can be estimated from monitoring leaf development over time in association with temperature. Leaf numbers are typically measured on tagged sugarcane stalks and numbered chronologically from the oldest leaf at the base (leaf 1) to the youngest fully expanded leaf with a visible dewlap (TVD – top visible dewlap; McCray *et al*. 2005). Leaf dimensions (length and width) are usually measured on the TVD leaf, and leaf area calculated by multiplying length and width with a leaf shape coefficient (Robertson *et al*. 1998; Sinclair *et al*. 2004). Leaf area can also be measured with a leaf area meter (LiCor Biosciences, Nebraska, USA).

Stalk development and growth

Stalk parameters (e.g. SERo, Table 2.1) can be estimated from monitoring stalk height over time in association with temperature. Stalk height is mostly taken as the distance from the base of the stalk to the collar of the TVD leaf. Stalk extension rate can be determined from sequential measurements of stalk height either manually with a tape measure or electronically with potentiometers (Inman-Bamber 1995a; Smit *et al*. 2005; Smit & Singels 2007; Ngxaliwe 2014).

Photosynthetic efficiency

Maximum radiation conversion efficiency before respiration (PARCEmax, Table 2.1) cannot be estimated directly from phenotypic observations. It can be estimated through statistical calibration on measurements of aerial dry biomass and intercepted radiation (e.g. Singels & Bezuidenhout 2002). These data may not always be available, especially for numerous genotypes. PARCEmax could possibly also be estimated from leaf-level gas exchange measurements, such as instantaneous photosynthetic rate (*A*, defined as the net carbon fixation rate per unit leaf area, µmol m^{-2} s^{-1}) and stomatal conductance for gaseous exchange (g_s, defined as the rate at which water vapour is lost from the leaf, mmol m^{-2} s^{-1}). This is based on the assumption that genotypic differences in leaf-level radiation conversion efficiency are similar to that of maximum radiation conversion efficiency at the crop canopy level. Leaf-level gas exchange can be measured with an infrared gas analyser (IRGA) (LiCor Biosciences, Nebraska, USA; PP Systems, USA) which measures the flow rate of carbon dioxide and water vapour using sample and reference gas analysers. The open system of the IRGA also allows user-specified control of environmental variables (leaf chamber CO_2, humidity, temperature and light). Stomatal conductance can also be measured using a leaf porometer (Decagon Devices, USA). In this steady-state design, vapour flux through stomata is measured by comparing the humidity at two points in a fixed diffusion path within the sensor head, without modifying cuvette conditions.

Three key aspects should be considered when measuring gas exchange: (1) the time of day during which measurements are conducted; (2) the leaves that are chosen for measurement; and (3) the choice of instrument and settings. Basnayake *et al*. (2015) measured stomatal conductance using the Decagon leaf porometer on well-lit topmost fully expanded leaves of three plants per plot between the hours of 10:00 and 14:00. Jackson *et al*. (2016) measured

leaf gas exchange with the LiCor-6400 on the youngest fully expanded leaves of clones grown in a pot trial between the hours of 9:00 and 15:00, based on procedures developed by Inman-Bamber *et al*. (2011).

Biomass accumulation and partitioning

Biomass partitioning parameters (e.g. STKPFMAX and SUCA, Table 2.1) can be estimated from sequential measurements of biomass components. STKPFMAX can be estimated directly as the gradient of the linear regression between stalk dry mass and aerial dry biomass measurements, while SUCA requires statistical calibration using stalk dry mass and sucrose mass measurements (e.g. Singels & Bezuidenhout 2002). STKPFMAX and SUCA could also be estimated from dry biomass components measured at harvest only, based on the assumption that this measurement can be normalized relative to that of a reference genotype calibrated using sequential measurements. Inman-Bamber *et al*. (2002) outlined the process of harvesting sugarcane and determining biomass yield by partitioning into stalk, trash, green leaves and meristem. Samples of biomass components are weighed to determine the fresh above-ground mass, following which sub-samples are dried and weighed to determine dry matter content of each component. The sucrose (pol) and total sugar (brix) content of stalk juice samples can be measured using a saccharimeter and refractometer. Alternatively, pol% and brix% can be measured using near infrared spectroscopy on shredded cane samples.

2.3.2 High-throughput phenotyping

High-throughput phenotyping (HTP) is the use of modern technologies such as spectroscopy to non-destructively measure plant properties in a very short space of time, which is otherwise not feasible using conventional methods (adapted from Araus & Cairns 2014). Examples of plant properties that can easily be measured are crop height, canopy cover and photosynthetic efficiency (Araus & Cairns 2014). HTP provides a means of screening for drought tolerant or high-yielding genotypes in a rapid, cost-effective manner and has progressed in a number of crops (Munns *et al*. 2010; Yang *et al*. 2013) although little research has been done for sugarcane.

Canopy conductance of gas exchange (g_c) is a key characteristic determining the capacity of crops to fix carbon. It comprises green leaf area index (LAI) and g_s (Basnayake *et al*. 2012; Jackson *et al*. 2016). LAI and g_s could for example, be estimated rapidly through proximal sensing of surface reflectance (Munns *et al*. 2010; Yang *et al*. 2013).

2.4 Trait impacts modelling

A crop ideotype was first described by Donald (1968) as "a plant model, which is expected to yield a greater quantity or quality of grain, oil or other useful product when developed as a cultivar". Process-based models which are able to capture genotypic differences in crop development and growth could be used to identify traits which confer advantages under target environments (Jeuffroy *et al*. 2006), and can therefore assist in crop ideotyping. Within the context of crop modelling, a crop ideotype can be considered to be a set of trait parameter values that produces high simulated yields for a particular environment. Crop models have been used to design ideotypes for key crops such as wheat (Donald 1968; Semenov & Stratonovich 2013), soybean (Boote & Tollenaar 1994; Boote *et al.* 2001), maize (Boote & Tollenaar 1994; Boote *et al.* 2001), rice (Aggarwal *et al*. 1997; Peng *et al*. 2008) and peanut (Boote & Jones 1986; Suriharn *et al.* 2011).

Trait impact assessment is a necessary step towards designing ideotypes. This entails assessing the yield response to changes in parameter values. An important aspect in trait modelling is filling the parameter space. This involves (1) choosing the traits of interest; (2) deciding on the range of values to be explored for each trait; and (3) deciding on the modelling approach, i.e. varying single traits at a time, or varying a combination of traits. If numerous traits are varied at once, the method of generating combinations of trait parameter values also needs to be considered.

Secondly, the model configuration should be considered, taking into account the simulation period, crop cycles, environmental and management levels. Thereafter, the method of analysing and quantifying trait impacts should be considered. Model adequacy for simulating trait impacts should also be evaluated.

A common thread in the literature is the interest in traits related to rooting, development phase duration, photosynthesis and biomass partitioning. Most studies varied trait parameter values by about 10-20%. In some cases, this range has been supported by literature, and in others, the choice was arbitrary. For example, Aggarwal *et al*. (1997) used a range of 20% in trait parameters governing crop duration, whilst recognising that this range does not fully cover the variability observed. The range of trait parameter values could also be based on experimental data (e.g. Boote *et al*. 2001).

Saltelli *et al*. (2008) described various approaches to trait impacts modelling. Some studies varied single parameters at a time (local sensitivity analysis) (e.g. Boote & Jones 1986; Aggarwal *et al*. 1997; Boote *et al.* 2001; Suriharn *et al*. 2011), while others vary a set of parameters simultaneously (global sensitivity analysis) (e.g. Khan 2012; Sexton & Everingham 2014; Sexton *et al*. 2015; Casadebaig *et al*. 2016). Various methods have been used to generate combinations of trait parameter values in global sensitivity analysis to reduce computational requirements. These include the Random Input Generator for the Analysis of Uncertainty in Simulation (RIGAUS) program (Bouman & Jansen 1993), Morris method (Casadebaig *et al*. 2016) and LP-TAU method in the GEM-SA software package (Sexton *et al*. 2015).

The main considerations when deciding on the model configuration are the availability of weather data, and the computational requirements. The simulation periods of trait impact assessment varied greatly between studies, ranging from three (Aggarwal *et al*. 1997) to 125 years (Casadebaig *et al*. 2016). Most included multiple cropping cycles, depending on the crop under consideration. Most also investigated trait impacts for different environmental and management conditions. This appears to account for the fact that trait expression has been shown to vary depending on the environmental and management conditions simulated (e.g. Aggarwal *et al*. 1997).

The main consideration for quantifying trait impacts on yield is the type of dataset that is generated, which depends on the modelling approach. Trait impacts are quantified as differences in yield between a given genotype and the baseline genotype, expressed as a percentage (Boote *et al*. 2001) or ratio (Aggarwal *et al*. 1997) of the baseline value. The entire yield distribution in the form of the cumulative distribution frequency can be compared (Inman-Bamber *et al*. 2012), or key points of the distribution, such as the mean, minimum and maximum yields, can be compared (Boote & Jones 1986). These methods are more suited to local sensitivity analysis when impacts of a single trait parameter at a time are assessed.

Khan (2012) used a path coefficient analysis, which is an extension of multiple regression analysis, to estimate the direct and indirect effects of traits on yield. Casadebaig *et al*. (2016) and Sexton *et al*. (2015) used sensitivity indices which partitions the variation in yield

generated by changes in multiple trait parameters to the variation generated by changes in a given trait. These methods are more suited to global sensitivity analysis when multiple trait parameters have been varied at a time.

Boote & Jones (1986) suggested that candidate models for trait impact studies (and breeding applications) should be well validated and sensitive to the trait parameters of interest. Boote *et al*. (2001) expressed the belief that crop models do not adequately represent the genotypic specificity required for breeding applications. For example, trait parameters often represent a mixed genetic and environmental signal, resulting in values that are not always stable across environments (e.g. Zhou *et al*. 2003; Singels *et al*. 2005; Ngobese 2015).

Literature presented several ways in which model capabilities for plant breeding applications can be improved. For example, Casadebaig *et al*. (2016) concluded that further research is needed to determine the genetic variability and heritability of traits before models can be used in plant breeding. The APSIM-Sugarcane model simulates genotypic differences based primarily on traits related to biomass partitioning (cane and sucrose) and the leaf area profile on the stalk. Sexton *et al*. (2014) showed that APSIM-Sugarcane could not simulate genotypic responses to stress very well. This could be improved by investigating traits not currently considered to be genotypic-specific. For example, increased transpiration efficiency has been shown to confer a yield advantage under water-limited conditions (Inman-Bamber *et al*. 2012) and this trait has been shown to differ significantly between genotypes (Jackson *et al*. 2016). The ability of models to simulate genotypic differences and identify beneficial traits could also be improved by investigating the expression of traits under different nutrient conditions (Aggarwal *et al*. 1997).

Despite these challenges, crop models have the potential to generate very useful information on trait impacts to support plant breeding.

2.5 Conclusions and recommendations

This review described (1) approaches to sugarcane crop modelling of genetic and environmental interactions, including methods for estimating trait parameter values and assessing simulation accuracy; (2) phenotyping methods for sugarcane; and (3) approaches to trait impact modelling. This will be used to formulate recommendations for this study.

2.5.1 Parameter estimation and phenotyping

Semi-automated methods for high-throughput phenotyping are still in the early developmental stages, and further research in HTP technologies, particularly in sugarcane, is required. For this reason, manual measurements are recommended.

The following measurements are proposed to provide data for parameter estimations:

(1) Periodic measurements of leaf number and TVD leaf dimensions for estimating leaf and TT requirement parameters (PI1, PI2, LFMAX, MXLFAREA, MXLFARNO and CHUPIBASE;
(2) Periodic measurements of stalk height for estimating stalk parameters (SERo);
(3) Measurements of leaf gas exchange with a leaf porometer and infrared gas analyser for estimating photosynthetic efficiency (PARCEmax);
(4) Measurements of biomass components at harvest to estimate biomass partitioning fractions (STKPFMAX and SUCA).

The study should investigate whether the sampling frequency of leaf and stalk measurements can be reduced by comparing values of PI1, PI2 and SERo estimated from the full complement of data, with values estimated from reduced datasets.

Leaf-level gas exchange measurements (A and g_s) should be optimised in order to gain a better understanding of diurnal variation in measured variables and to refine measurement protocols using both instruments.

Biomass components should be measured and analysed at harvest using the methods discussed in section 2.3.1 in order to derive partitioning parameters (STKPFMAX and SUCA).

This study recommends using direct determination of parameter values from closely related phenotypic data where possible (i.e. leaf and stalk parameters), as well as the combined approach for those parameters that cannot be determined directly, such as PARCEmax, STKPFMAX and SUCA.

2.5.2 Simulation accuracy

The following measures are proposed to evaluate simulation accuracy:

(1) Gradient and intercept of the linear regression between simulated and observed values and rankings;

(2) Correlation coefficient between simulated and observed values and rankings;

(3) RMSE of simulated and observed values.

2.5.3 Trait impacts

The range of trait parameter values to be explored in the tract impact study should be dictated by data collected in this study as well as data published in the literature. Investigation of yield responses to changes in single and multiple trait parameters are suggested, as this will provide insight into single trait effects as well as the effect of interacting traits. The method recommended for filling the multiple parameter space is the LP-TAU program in the freely available GEM-SA package (Sexton *et al*. 2015). Quantifying single trait impacts on yield should be done by comparing yield distribution changes to that of the baseline trait value as this is widely used. Multiple trait impacts and interactions should be analysed using path coefficient analysis, which is a simple statistical method that is easy to implement and that is suitable for small-scale trait impact studies.

3. METHODOLOGY

3.1 Overview

This methodology section outlines the approaches used to (1) phenotype selected sugarcane genotypes in a pot trial and derive trait parameter (TP) values from measurements of plant growth and development; (2) assess the accuracy of simulated genotypic differences in canopy cover, stalk dry mass (SDM) and sucrose yield (SUCM) predictions for selected genotypes grown in four well-watered field trials; and (3) assess trait impacts on simulated SDM and estimate the potential SDM gain from optimising TP values for a target environment.

3.2 Phenotyping

A pilot pot trial was conducted to optimize trial management and measurement procedures. Methodology and results of this pot trial are reported in Appendix A.

The objectives of the phenotyping pot trial were to (1) determine TP values for selected genotypes from measurements of leaf and stalk development, leaf-level gas exchange and of biomass components at harvest; and (2) develop a phenotyping protocol for estimating Canegro TP values.

3.2.1 Trial design

The trial was established at the rainshelter facility located at the South African Sugarcane Research Institute in Mount Edgecombe, KwaZulu-Natal (29°42’40”S; 31°02’0”E). The randomised complete block design consisted of five replications of 14 genotypes, with guard pots placed around the perimeter to minimise edge effects (Figure 3.1). The genotypes were selected to represent a wide range of genetic diversity. Genotype selection was also based on availability of suitable field trial data for hypothesis testing.

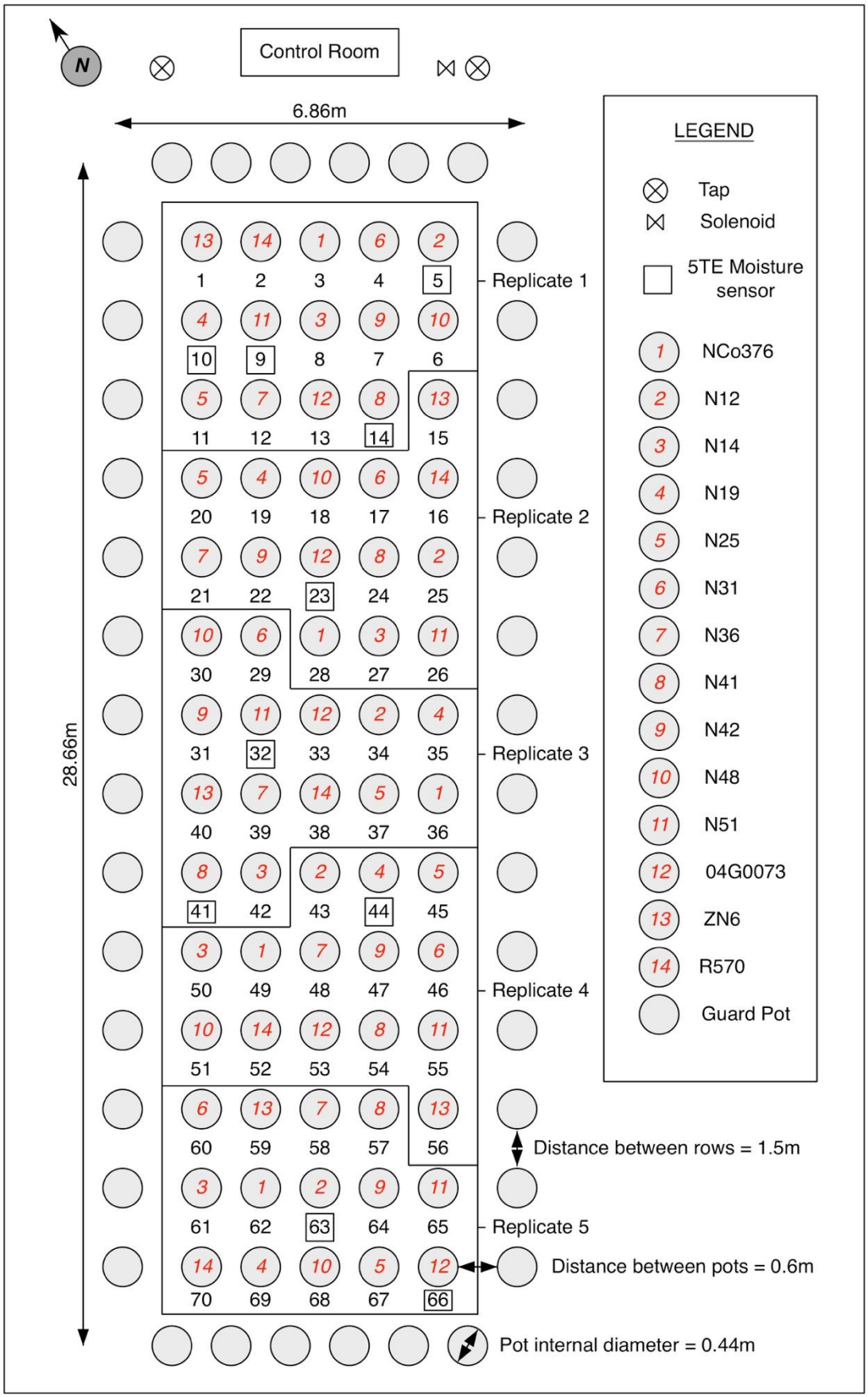


Figure 3.1. Layout of the phenotyping pot trial at the SASRI rainshelter facility.

3.2.2 Trial operations

Single-budded setts were pre-treated with a cold water dip of Benomyl® (Arysta LifeScience, South Africa), and planted into Styrofoam germination trays containing a synthetic medium of river sand, sugarcane filter press and vermiculite (4:2:1). The setts were pre-germinated in a controlled room with an average daily temperature of 30°C, and shoots emerged after seven days. Thereafter, the speedlings were transferred to the SASRI nursery at Mount Edgecombe and allowed to acclimatise to ambient conditions.

Four speedlings were transplanted into pots (80l capacity; internal diameter of 0.44m and height of 0.64m) containing the same synthetic medium used for pre-germination on 3 October 2014. Pots were fertilised according to SASRI recommendations (330 kg ha^{-1} N; 200 kg ha^{-1} P; 720 kg ha^{-1} K). Plants were sprayed with (1) Dursban® (1ml $litre^{-1}$) (Dow AgroSciences, UK), (2) Mectic and Breakthru® (0.2 ml $litre^{-1}$) (Evonik, Germany), and (3) Fastac® (0.003 ml $litre^{-1}$) (BASF Crop Protection, UK) on two occasions, to combat aphids, thrips and eldana, respectively.

Harvesting was carried out from 29 June to 3 July 2015.

3.2.3 Measurements

<u>Weather conditions</u>

Hourly average solar radiation (SRAD, Wm^{-2}), temperature (°C), relative humidity (RH, %), rainfall (mm) wind speed (m s^{-1}) and vapour pressure deficit (VPD, Pa) were recorded from samples taken at ten second intervals using an automatic weather station located at the SASRI rainshelter. Hourly sugarcane reference evapotranspiration data (Ecref, µm h^{-1}) (McGlinchey & Inman-Bamber 1996) were calculated from weather data recorded at the Mount Edgecombe weather station, located approximately 340m from the rainshelter.

Thermal time (TT, °Cd) was calculated as follows:

$$TT = \sum_{d=i}^{n} Max\left(0, \left(\frac{Tmax+Tmin}{2} - T_b\right)\right) \qquad \text{(Equation 3.1)}$$

where Tmax and Tmin are the daily maximum and minimum temperatures respectively, and Tb is the base temperature, taken as 10°C and 16°C for leaf and stalk development (Singels *et al*. 2016a), respectively.

The weather data collected during the phenotyping pot trial are shown in Appendix B.

Crop water status

Volumetric soil water content (VWC, m^3 m^{-3}) was measured using 20 5TE soil moisture sensors (Decagon Devices, Washington, USA) installed at depths of 15-20cm and 35-40cm. One sensor was placed at each depth in ten pots containing genotypes with diverse canopy development characteristics (based on SASRI information sheets, SASRI, 2015). The genotypes and pot numbers (shown in Figure 3.1) were as follows:

- N12 (slow canopy formation); pots 5 and 63
- N19 (rapid canopy formation); pots 10 and 44
- N41 (slow canopy formation); pots 14 and 41
- N51 (rapid canopy formation); pots 9 and 32
- 04G0073 (rapid canopy formation); pots 23 and 66

Sensor output (dielectric permittivity logged at 30-minute intervals with a CR1000 data logger, Campbell Scientific, Inc.) were converted to VWC values with the Topp equation (Topp *et al*. 1980). Field capacity (FC, m^3 m^{-3}) and permanent wilting point (PWP, m^3 m^{-3}) of the synthetic medium were determined by the pressure plate technique. Values of VWC were then adjusted by a sensor-specific conversion factor so that the sensor-estimated PWP value corresponded with the laboratory-determined PWP value. Stress point (SP, m^3 m^{-3}) was taken as 50% of the difference between FC and PWP.

The crop water status data for the phenotyping pot trial are shown in Appendix B. Irrigation was applied with dripper lines up to three times daily, with amounts ranging from 335ml to 1000ml per irrigation event (up to 3000ml per pot, per day). Based on the findings of the pilot pot trial (Appendix A), it was necessary to apply smaller amounts of irrigation more frequently in order to prevent crop water stress and drainage of water out of the pot.

Leaf and stalk development

One primary shoot in each pot was tagged for leaf and stalk measurements. The number of fully expanded leaves were recorded monthly from shortly after planting (November 2014) to harvest (June 2015). Similarly, the number of green and senesced leaves were recorded, where leaves were considered senesced when more than 50% of the leaf surface was brown or yellow. The length and maximum width of the topmost fully expanded leaf associated with the top visible dewlap (TVD) were measured using a tape measure, and a leaf shape coefficient of 0.7 used for the estimation of TVD leaf area (Sinclair *et al*. 2004).

Stalk height was measured monthly as the distance from the base of the stalk up to collar of the TVD leaf.

Leaf and stalk development data collected after 31 March 2015 were excluded from analyses for genotypes N36, N42 and 04G0073 due to flowering.

Stomatal conductance and photosynthetic rate

Instrumentation

A leaf porometer (Decagon Devices, Washington, USA) was used to measure stomatal conductance for gaseous exchange (g_{sPoro}). A LiCor-6400 infrared gas analyser (LiCor Biosciences, Nebraska, USA) was used to measure leaf-level stomatal conductance (g_{sLicor}) and photosynthetic rate (A).

Diurnal experiments

Two experiments were conducted to better understand diurnal variation in A and g_s and to refine measurement protocols.

The first experiment was performed on 18th February 2015 on genotype R570, from 8:00 – 17:00. Within a given hour, three measurements on three different TVD leaves were taken in each of the five pots (Figure 3.1). This procedure was repeated every hour except for 11:00-12:00 when battery power was insufficient.

The second experiment was performed on 4th March 2015 on genotype N48 in the same manner as for the first experiment. No data could be collected for hour 10:00-11:00 due to insufficient battery power.

LiCor-6400 settings were as follows:

(1) red/blue light source = 1500µmol quanta m^{-2} s^{-1};
(2) Flow rate = 500 µmol s^{-1};
(3) Sample CO_2 concentration set to the reference values of 400ppm at the beginning of each hourly set of measurements;
(4) Sample RH maintained between 50 and 90% by adjusting scrubbing mechanism;
(5) Leaf and block temperature = 26°C.

The porometer was equilibrated once before the start of measurements in both experiments. More frequent calibrations were not needed because changes in RH and temperature did not exceed 20% and 15°C, respectively.

The diurnal variation in plant variables (*A*, g_s and transpiration, E) was related to that of weather variables (SRAD, temperature, RH, VPD and Ecref).

Phenotyping experiments

Two experiments were carried out at a crop age of eight (13-24th April 2015) and nine months (26th May-10th June 2015), respectively. Each experiment consisted of five measuring days, with one replicate (14 pots) measured on a given day. Measurements of gas exchange were performed between 10:00 and 13:00 on two well-lit leaves with the LiCor-6400 and Decagon porometer concurrently. The same LiCor-6400 instrument parameters were used as previously described for the diurnal experiments.

Data were filtered using the following exclusion criteria:

(1) negative intercellular CO_2 concentration (C_i) readings;
(2) transpiration (E) rates of <1 and >5 mmol m^{-2} s^{-1};
(3) RH readings <50 and >90%;

Hourly *A* and g_s readings were expressed as a percentage of that of the reference genotype NCo376 for the given hour, to minimize time-of-day effects. Hourly normalized values of

A and g_s (A^* and g_s^*) were tested for Genotype (G) x Hour (H) interactions using a two-way analysis of variance (ANOVA), and where not significantly different (F.pr. > 0.05), the hourly values were combined for each genotype. Thereafter, a one-way ANOVA identified significant differences between genotypes using the Tukey's post-hoc analysis, where possible. Subsequently, the two experimental datasets were tested for Genotype x Experiment interactions, and where possible, combined and analysed for significant genotypic differences using ANOVA.

Biomass components

Destructive sampling of biomass components in each replicate was carried out from 29 June – 3 July 2015 (one replicate per day). In each pot, stalks were cut at the base and total above-ground fresh biomass recorded. Each sample was divided into senesced leaves (trash), millable stalk, leaf sheath, meristem (tops), green leaf and flower components and weighed prior to sub-sampling of each component. Sub-samples were weighed for fresh mass, before being dried in an oven at 80°C to constant mass, and weighed once again for dry weight. These data were used to estimate dry matter content (%), from which the dry mass of biomass components and of total above-ground biomass could be inferred. Analysis of stalk composition (fibre, sucrose and non-sucrose contents) were conducted by the SASRI mill-room. A sub-sample of 12 stalks from each pot was shredded in a blender, filtered, and juice samples assessed with a polarimeter and refractometer to determine pol and brix% (Schoonees-Muir *et al*. 2009) respectively. The percentage of stalk material that consisted of fibre, sucrose and non-sucrose (fresh mass basis) was determined according to established methods. Stalk dry mass was calculated as the product of stalk fresh mass and stalk dry matter content, while sucrose content on a dry mass basis was calculated from sucrose content fresh mass basis and stalk dry matter content.

Green leaf area per pot was calculated by multiplying specific leaf area (SLA, $m^2\ kg^{-1}$) with green leaf fresh mass per pot. SLA was determined for three sub-samples per pot consisting of 10 green leaves each, that was weighed and the leaf area recorded using the LI-3000C scanning head coupled to the LI-3050C desktop accessory (LiCor, Lincoln, NE).

Biomass data collected at harvest were excluded from analyses for genotypes N36, N42 and 04G0073 due to flowering.

3.2.4 Data analysis

Phenotypic data were analysed using the Microsoft Excel and Genstat® v14 software packages. The distribution of data was tested for normality using the Shapiro-Wilk test. Where possible, linear regressions were fitted and differences in slopes analysed using t-test statistics. Furthermore, ANOVA was carried out with a 95% confidence limit using the Fisher's protected post-hoc analysis, where appropriate.

3.2.5 Parameter estimation

Table 3.1 outlines the selected Canegro TPs that were estimated from pot trial phenotypic observations.

Table 3.1. Methods for determining Canegro trait parameter values from phenotypic data. Parameter descriptions are given in Table 2.1.

Category	Parameter	Measurement	Determination
Leaf development	PI1, PI2	No. fully expanded leaves per primary shoot	Inverse of the gradient of the linear regression of TVD leaf number against thermal time (TT)
	LFMAX	No. green leaves per primary shoot	Maximum green leaf number
	MXLFAREA	TVD leaf area	TVD leaf length and width multiplied by shape factor (0.7); Mean of the area of the biggest leaf in each pot
	MXLFARNO	TVD leaf area and number	Corresponding mean leaf no. of MXLFAREA
Stalk development	CHUPIBASE	No. fully expanded leaves per primary shoot	TT taken from shoot emergence to the appearance of leaf no. 10
	SERo	TVD height	Gradient of the linear regression of TVD height against TT
Photosynthetic efficiency	PARCEmax	Stomatal conductance (g_{sPoro}), photosynthetic rate (A)	Normalized g_{sPoro} and A, scaled using a field-calibrated value for NCo376 (5.7g MJ^{-1})
Biomass partitioning	STKPFMAX	Stalk dry mass and aerial dry biomass at harvest	Normalized stalk dry mass fraction of aerial dry biomass, scaled using a field-calibrated value for NCo376 (0.7 t t^{-1})
	SUCA	Sucrose content and stalk dry mass at harvest	Normalized sucrose fraction of stalk dry mass, scaled using a field-calibrated value for NCo376 (0.56 t t^{-1})

PI1 and PI2 values were calculated as the inverse of the gradient of the linear regression between leaf number and TT data. Variation in PSWITCH introduces unwanted variation in PI1 and PI2 due to their interdependency, and thus a constant value of 14 (Inman-Bamber 1994) was assumed for all genotypes. The feasibility of reducing sampling frequency was evaluated by comparing PI1 and PI2 estimates from monthly measurements to bi-monthly measurements where every second data point was removed to generate the reduced dataset. The sampling frequency was determined by evaluating the gradient of the limited experimental dataset, which was required to be within one standard error of the gradient of the full data set.

LFMAX values were estimated by recording the number of green leaves on each tagged stalk and determining the maximum number of green leaves reached for the duration of the trial. MXLFAREA was taken as the mean of the area of the biggest leaf in each pot. MXLFARNO was taken as the mean of the leaf number of the biggest leaf in each pot.

SERo values were calculated as the gradient of the linear regression between stalk height and TT data. The feasibility of reducing sampling frequency was evaluated by comparing SERo estimates from monthly measurements to three reduced sampling methods, namely (1) bi-monthly measurements; (2) measurements on three occasions (TT of 406, 1030 and 1814°Cd); and (4) measurements on two occasions (TT of 406 and 1030°Cd). The sampling frequency was determined by evaluating the gradient of the limited experimental dataset, which was required to be within one standard error of the gradient of the full data set.

PARCEmax values were calculated by multiplying A^* and g_s^* values with the field calibrated value for NCo376 (5.7g MJ^{-1}) as determined by Singels *et al*. (2016a).

STKPFMAX values were calculated by multiplying the normalized stalk fraction values with the field calibrated value for NCo376 (0.7 t t^{-1}) determined by Singels *et al*. (2016a).

SUCA values were calculated by multiplying the normalized sucrose fraction of SDM values with the field calibrated value for NCo376 (0.56 t t^{-1}) determined by Singels *et al*. (2016a).

3.2.6 Evaluation

The objective of the evaluation process was to test the hypothesis that TP values estimated in a pot trial can be used to accurately simulate genotypic differences in crop growth and development observed in field trials. This was achieved by determining the accuracy of fractional interception (FI) of photosynthetically active radiation (PAR) at partial canopy (FI_{PC}), SDM and SUCM predictions for selected genotypes grown in four well-watered field trials (Table 3.2).

Datasets were required to have observations of stalk population and canopy cover (represented by FI of PAR) for the duration of the trial, and SDM and SUCM (at harvest) measured under well-watered conditions for two or more genotypes which were included in the phenotyping pot trial. In addition, reliable soil, weather and management information were needed for accurate simulations.

The values of some TPs could not be determined in the pot trial. For example, STKPFMAX and SUCA values for genotypes N36 and 04G0073 were not available due to flowering. It was decided to use STKPFMAX and SUCA values for a generic, high-fibre genotype of 0.60 and 0.30 for 04G0073 (Singels *et al*. 2016a), respectively. STKPFMAX and SUCA values of 0.62 (Ngobese 2015) and 0.63 (Singels *et al*. 2016b) were used for N36, respectively.

The values of TPs related to tiller development (TARo, TTPOPGROWTH, MAXPOP and POPTT16) were also not determined in the pot trial due to the unusual spatial configuration of plants and the lack of typical canopy formation. Tiller parameter values were estimated by trial-and-error using the observed tiller population data for each experiment (Table 3.3, Figure 3.2). A best fit between simulated and observed values fit was attained in order to minimise the impact of simulated tiller population on model predictions of FI_{PC}, SDM and SUCM. This was mostly achieved, although the model was unable to simulate the very high peak tiller population observed for genotype 04G0073 in the Komatipoort_2012 trial, presumably due to the simulated shading effect of a very dense canopy.

Values and rankings of simulated FI_{PC} (%), SDM and SUCM were compared with measured values and rankings. FI_{PC} was defined as the FI at partial canopy (approximately 50%) where genotypes showed the largest differences in canopy development. The observed FI_{PC} value was taken as the mean of two consecutive FI measurements in the middle of the period of partial canopy. Observed FI_{PC} was not recorded in the Mount Edgecombe trial. Simulated FI_{PC} was taken as the mean of all daily FI values between the two measurement dates.

The simulation accuracy was quantified using the correlation coefficient (r) and root mean square error (RMSE) between simulated and observed values where possible. Model performance was further evaluated by comparing the correlation between parameter values for a given trait on one hand, and simulated and observed values of FI_{PC}, SDM and SUCM on the other hand.

Table 3.2. Experimental details of datasets used for hypothesis testing.

Site	Location	Genotypes	Planting date	Harvest date	Crop class[a]	Row-spacing (m)	Bud density (buds m^{-2})	Soil form and series[b]	Soil profile[c]		Irrigation				Reference
									LL	DUL	Method	Depth (cm)	Threshold (%)	Amount (mm)	
Pongola, KZN	27°24'0"S 31°35'0"E 308m	N12, N19, N25, N31, N36, N41, N48, N51, NCo376	11/11/2011	20/11/2012	P	1.4	20	Hutton Schorrocks	0.138	0.287	Drip (automatic when required)	20	80	40	Ngobese (2015)
Komatipoort, Mpumalanga	25°33'0"S 31°57'0"E 170m	N31,N19, 04G0073	12/10/2011	26/10/2012	P	1.5	60	Shortlands Glenrosa	0.170	0.330	Furrow (on reported dates)	30	50	10	Olivier *et al*. (2016)
Komatipoort, Mpumalanga	25°33'0"S 31°57'0"E 170m	N31,N19, 04G0073	28/10/2012	6/11/2013	R	1.5	80	Shortlands Glenrosa	0.170	0.330	Sprinkler (automatic when required)	50	60	10	Olivier *et al*. (2016)
Mount Edgecombe, KZN	29°42'0"S 31°2'0"E 96m	N19, 04G0073	05/10/2011	07/05/2012	P	1.25	9	Westleigh Rietvlei[d]	0.083	0.180	Furrow (on reported dates)	30	50	10	Ngxaliwe (2014)

[a] Crop class indicated as plant (P) or ratoon (R)
[b] SASEX (1999)
[c] Soil water holding characteristics: lower limit (LL) and drained upper limit (DUL)
[d] Artificially constituted

Table 3.3. Tillering parameter values estimated by trial-and-error using observed tiller population data for selected genotypes in four experiments conducted in Pongola, Komatipoort and Mount Edgecombe. Parameter descriptions are given in Table 2.1.

Experiment	Genotype	TARo (tillers °Cd^{-1})	TTPOPGROWTH (°Cd)	MAXPOP (tillers m^{-2})	POPTT16 (tillers m^{-2})
Pongola_2011	N12	0.0020	800	20	17
	N19	0.0040	400	17	13
	N25	0.0040	400	16	12
	N31	0.0040	450	20	13
	N36	0.0035	450	18	11
	N41	0.0015	700	15	12
	N48	0.0025	500	15	11
	N51	0.0025	550	17	14
	NCo376	0.0040	500	23	16
Komatipoort_2011	N19	0.0060	780	22	11
	N31	0.0060	700	24	14
	04G0073	0.0090	650	25	18
Komatipoort_2012	N19	0.0090	500	39	14
	N31	0.0100	500	41	16
	04G0073	0.0200	600	74	18
Mount Edgecombe_2011	N19	0.0160	600	25	14
	04G0073	0.0180	710	30	17

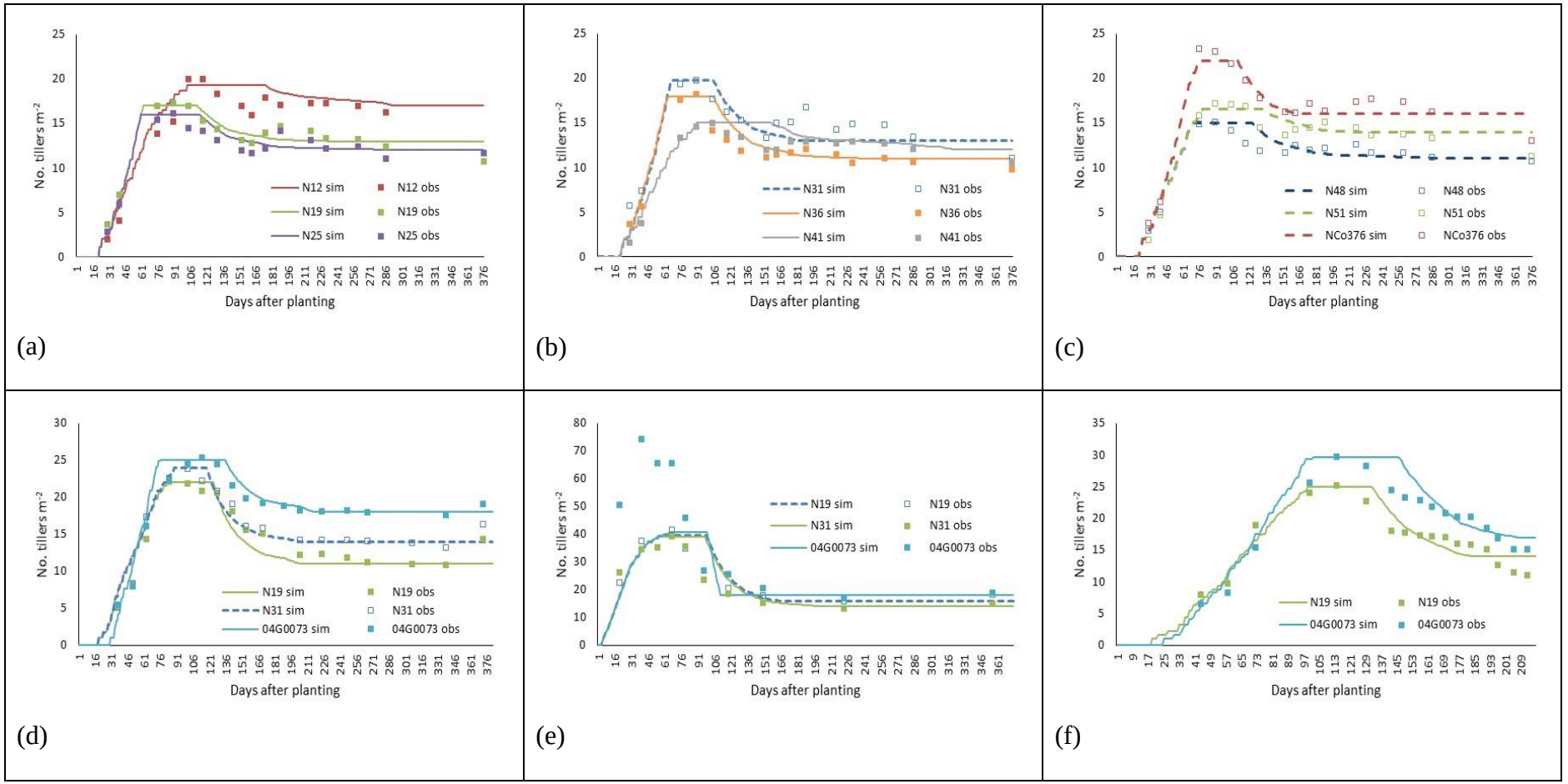


Figure 3.2. Simulated and observed tiller population for selected genotypes grown in Pongola (a, b & c), Komatipoort_2011 (d) and _2012 (e) and Mount Edgecombe (f)

3.3 Trait impact study

The objectives of the trait impact study were to (1) assess impacts of TPs on simulated SDM by evaluating the SDM of hypothetical genotypes that varied with respect to one or more of the traits investigated; and (2) investigate potential SDM gains by simulating an ideotype with optimal trait values under well-watered conditions.

Six traits were chosen for the impact study namely PI1, PI2, CHUPIBASE, PARCEmax, STKPFMAX and SUCA. This selection was based on the strength of the correlation between traits and with simulated FI_{PC}, SDM and SUCM as determined in the evaluation study.

Two approaches were used to assess trait impacts. Single trait variants were used to assess the impact of a single trait when all other TP values were identical. Multiple trait variants were used to assess the combined effects of changes in multiple TPs.

3.3.1 Single trait variants

Twenty-four hypothetical genotypes were defined, differing only with respect to one TP value (single trait variants), as shown in Table 3.4. Five values were tested for each parameter with equal intervals between values. This distance was taken as half of the range observed in the pot trial. The baseline value was taken as the mean value observed in the pot trial. This configuration ensured a range of 200% of that observed in the pot trial, with the lowest and highest value 50% below and above the minimum and maximum observed values, respectively. This catered for the fact that the pot trial may be a limited sample of the genetic variation in sugarcane germplasm.

Table 3.4. Trait parameter values used in the single trait impact study. Parameter descriptions are given in Table 2.1. The baseline genotype comprises the mean of the trait parameter values observed.

	Trait value				
Trait parameter	Extreme min[b]	Min[a]	Baseline	Max[a]	Extreme max[b]
PI1 (°Cd)	81	93	105	118	130
PI2 (°Cd)	54	83	112	141	170
CHUPIBASE (°Cd)	700	851	1002	1153	1304
PARCEmax (g MJ^{-1})	2.52	3.66	4.79	5.93	7.06
STKPFMAX (t t^{-1})	0.57	0.63	0.69	0.75	0.81
SUCA (t t^{-1})	0.50	0.55	0.59	0.64	0.68

[a] Maximum and minimum values observed in the pot trial

[b] Extreme maximum and minimum values to account for genetic variation not observed in the pot trial

3.3.2 Multiple trait variants

Thirty-two hypothetical genotypes were defined, differing with respect to six TPs (combined trait variants) (Table 3.5). The parameter values were generated using the LP-TAU efficient space filling design built into the GEM-SA package (Sexton *et al*. 2015). The same range of trait parameter values shown in Table 3.4 were used. Trait impacts on mean SDM were evaluated using a path coefficient analysis as described by Akintunde (2012).

The model was configured to simulate crop growth in Pongola (27°24’0”S, 31°35’0”E, 308m). Two 12 month crops were simulated starting in April and October of each year from 1980 to 2009. The irrigation settings were configured to simulate well-watered conditions (application of 40mm applied when the soil water content of the top 50 cm reached 60% of field capacity).

Trait impacts were evaluated by assessing changes in mean SDM responses to changes in trait values over the 30 seasons.

Table 3.5. Trait parameter values used in the multiple trait impact study. Parameter descriptions are given in Table 2.1. The minimum, maximum and mean values are shown.

Trait variant	PI1 (°Cd)	PI2 (°Cd)	CHUPIBASE (°Cd)	PARCEmax (g MJ^{-1})	STKPFMAX (t t^{-1})	SUCA (t t^{-1})
1	81	54	700	2.52	0.57	0.50
2	106	112	1002	4.79	0.69	0.59
3	93	141	851	5.93	0.63	0.64
4	118	83	1153	3.66	0.75	0.55
5	87	127	1229	6.49	0.72	0.52
6	112	69	927	4.22	0.60	0.61
7	99	98	1078	3.09	0.78	0.66
8	124	156	776	5.36	0.66	0.57
9	84	163	1115	3.94	0.62	0.51
10	109	105	813	6.21	0.74	0.60
11	96	76	1266	5.07	0.68	0.65
12	121	134	964	2.80	0.80	0.56
13	90	90	889	5.64	0.71	0.53
14	115	148	1192	3.37	0.59	0.62
15	102	119	738	4.51	0.77	0.67
16	127	61	1040	6.78	0.65	0.58
17	83	116	945	3.51	0.68	0.55
18	107	58	1247	5.78	0.80	0.64
19	95	87	794	6.92	0.62	0.60
20	119	145	1096	4.65	0.74	0.51
21	89	72	1021	6.35	0.77	0.57
22	113	130	719	4.08	0.65	0.66
23	101	159	1172	2.95	0.71	0.62
24	125	101	870	5.22	0.59	0.53
25	86	108	1210	4.36	0.64	0.56
26	110	166	908	6.63	0.76	0.65
27	98	137	1059	5.50	0.58	0.61
28	122	79	757	3.23	0.70	0.52
29	92	152	832	4.93	0.79	0.58
30	116	94	1134	2.66	0.67	0.67
31	104	65	983	3.80	0.73	0.63
32	128	123	1285	6.07	0.61	0.54
Min	81	54	700	2.52	0.57	0.50
Max	128	166	1285	6.92	0.80	0.67
Mean	105	110	993	4.72	0.69	0.59

3.3.1 Ideotyping

An ideotype was defined by assigning optimal values identified for CHUPIBASE, PARCEmax and STKFPMAX from the single trait study. Growth for this genotype was simulated as previously described in section 3.2.2 and SDM yield compared to that of the baseline genotype.

4. RESULTS AND DISCUSSION

Section 4.1 describes the results from the phenotyping pot trial, including the (1) plant measurements; (2) trait parameter (TP) values; and (3) evaluation of TP values in the simulation of field experiments.

Section 4.2 describes the trait impact study where the Canegro model was used to (1) assess trait impacts on simulated stalk dry mass (SDM) and (2) estimate the potential SDM gain of a crop ideotype.

4.1 Phenotyping

4.1.1 Measurements

Leaf development

Initial leaf number ranged from seven (N41) to 10 leaves (R570), with R570 being significantly higher than all other genotypes which did not differ significantly from one another (Table 4.1). The number of leaves that developed between the initial and pre-flowering measurements differed significantly between genotypes, ranging from 16 (N12) to 22 leaves (04G0073), with a mean value of 20 leaves.

Peak leaf length ranged from 137.8 (N51) to 175.9 cm (ZN6) (Table 4.2). ZN6 had significantly longer leaves than all other genotypes, except N42 and N12. Peak leaf length of N51 was not significantly different to that of R570, NCo376, N31, N14 and N25. Peak length was reached at a thermal time (TT) of 2060 °Cd for most genotypes, while occurring slightly earlier at 1805°Cd for ZN6, NCo376 and R570.

R570 had the widest leaves which were 32% higher than the mean peak leaf width, and 78% higher than 04G0073, which had the narrowest leaves. Peak leaf width for R570 was not significantly different to that of N36. Peak width was observed at TT of 2439°Cd for all genotypes.

The genotypic differences in maximum leaf length and width found in this study are comparable with that reported by Ngxaliwe (2014) for genotypes N19 and 04G0073 (leaves of 04G0073 were 5-10% longer, and about 25% narrower than that of N19). Leaf

development is largely driven by temperature (Inman-Bamber 1994). The smaller leaf sizes reported in this study compared to that of Ngxaliwe (2014) are ascribed to lower temperatures.

Table 4.1. Number of fully expanded leaves for different genotypes measured shortly after transplanting (initial leaf no.), just before flowering occurred in three of the genotypes (pre-flowering leaf no.) and at harvest (final leaf no.). The number of leaves developed between the initial measurement to just before the onset of flowering are shown. Standard error of the mean value is indicated in brackets. Values with common superscripted letters do not differ significantly at p=0.05. Final leaf number is not reported for genotypes N42, N36 and 04G0073 due to the effects of flowering.

Genotype	Initial leaf no.	Pre-flowering [a] leaf no.	No. leaves developed	Final leaf no.
04G0073	7.6^{b} (0.24)	29.4^{a} (0.24)	21.8^{a} (0.37)	-
N41	7.4^{b} (0.40)	29.0^{a} (1.05)	21.6^{ab} (0.68)	32.2^{a} (1.02)
N25	8.6^{b} (0.60)	30.0^{a} (1.14)	21.4^{abc} (0.60)	33.0^{a} (1.14)
NCo376	8.6^{b} (0.24)	29.8^{a} (0.66)	21.2^{abc} (0.49)	33.0^{a} (0.45)
N42	8.2^{b} (0.58)	29.4^{a} (0.93)	21.2^{abc} (0.37)	-
N51	8.2^{b} (0.20)	28.8^{a} (0.97)	20.6^{abc} (1.17)	32.4^{a} (0.51)
ZN6	8.0^{b} (0.45)	28.2^{abc} (0.49)	20.2^{abcd} (0.37)	31.8^{ab} (0.73)
N14	8.4^{b} (0.68)	28.6^{ab} (0.68)	20.2^{abcd} (0.37)	32.8^{a} (0.73)
R570	10.0^{a} (0.63)	30.0^{a} (0.84)	20.0^{bcd} (0.32)	32.4^{a} (1.03)
N19	8.4^{b} (0.51)	28.2^{abc} (0.86)	19.8^{cde} (0.80)	31.6^{ab} (0.40)
N48	7.8^{b} (0.49)	26.6^{bcd} (0.68)	18.8^{de} (0.58)	31.0^{ab} (0.89)
N31	7.8^{b} (0.37)	26.4^{cd} (0.24)	18.6^{de} (0.24)	29.8^{bc} (0.37)
N36	7.8^{b} (0.49)	26.0^{de} (0.71)	18.2^{e} (0.37)	-
N12	8.0^{b} (0.32)	24.0^{e} (0.84)	16.0^{f} (0.63)	27.8^{c} (0.49)
Mean	8.2 (0.13)	28.2 (0.28)	20.0 (0.23)	31.6 (2.16)
L.S.D.[b]	1.32	2.22	1.63	2.15

[a] Leaf measurements conducted on all genotypes before the onset of flowering, which occurred in three of the genotypes only

[b] L.S.D. – Least significant difference at p=0.05

Table 4.2. Length and width of fully expanded leaves for different genotypes, measured shortly after transplanting and when the peak value occurred. The thermal time (TT) at peak length and width is indicated. Standard error of the mean value is indicated in brackets. Values with common superscripted letters do not differ significantly at p=0.05.

Genotype	Leaf length (cm)		TT (°Cd) at peak length	Leaf width (cm)		TT (°Cd) at peak width
	Initial	Peak		Initial	Peak	
ZN6	107.2 ^{bcdef} (3.47)	175.9 ^{a} (4.88)	1805	2.44 ^{b} (0.06)	4.56 ^{b} (0.14)	2439
N42	102.5 ^{cdefg} (7.43)	168.6 ^{ab} (4.70)	2060	2.12 ^{cd} (0.13)	3.62 ^{cde} (0.19)	2439
N12	94.0 ^{ghi} (2.95)	163.4 ^{abc} (4.66)	2060	1.56 ^{g} (0.05)	3.58 ^{cde} (0.07)	2439
04G0073	118.6 ^{a} (2.94)	161.0 ^{bc} (3.41)	2060	1.30 ^{h} (0.04)	2.88 ^{f} (0.10)	2439
N48	111.3 ^{abcd} (3.36)	160.2 ^{bcd} (3.97)	2060	2.42 ^{b} (0.11)	4.58 ^{b} (0.04)	2439
N41	92.2 ^{ghi} (4.14)	158.6 ^{bcd} (1.57)	2060	1.72 ^{fg} (0.06)	3.24 ^{ef} (0.05)	2439
N36	114.6 ^{ab} (3.56)	156.2 ^{bcde} (2.91)	2060	1.88 ^{def} (0.10)	4.76 ^{ab} (0.10)	2439
N19	110.2 ^{abcde} (3.93)	152.6 ^{cdef} (3.91)	2060	1.88 ^{def} (0.12)	3.76 ^{cd} (0.16)	2439
N25	96.8 ^{fghi} (3.71)	146.4 ^{defg} (10.14)	2060	2.02 ^{cde} (0.13)	3.96 ^{c} (0.16)	2439
N14	89.4 ^{i} (2.11)	144.4 ^{efg} (2.94)	2060	1.64 ^{fg} (0.05)	3.62 ^{cde} (0.17)	2439
N31	112.2 ^{abc} (4.63)	142.6 ^{efg} (3.99)	2060	2.20 ^{bc} (0.04)	3.48 ^{de} (0.16)	2439
NCo376	100.4 ^{efgh} (2.46)	140.8 ^{fg} (4.38)	1805	1.88 ^{def} (0.08)	3.66 ^{cd} (0.21)	2439
R570	101.2 ^{defgh} (1.02)	140.5 ^{fg} (5.38)	1805	2.72 ^{a} (0.07)	5.14 ^{a} (0.19)	2439
N51	90.7 ^{hi} (3.64)	137.8 ^{g} (6.51)	2060	1.80 ^{efg} (0.09)	3.64 ^{cd} (0.07)	2439
Mean	103.0 (3.21)	153.5 (4.04)	-	1.97 (0.11)	3.89 (0.18)	-
L.S.D.[a]	10.74	13.95	-	0.25	0.40	-

[a] L.S.D. – Least significant difference at p=0.05.

Stalk height

The increase in stalk height between the initial and pre-flowering measurements was 1365 mm on average, with the highest increase for 04G0073 being approximately double that of the lowest increase observed in N14 (Table 4.3). N41 had the highest final stalk height of 2065mm, which differed significantly from values for all other genotypes, except N48 and

ZN6. N14 had the lowest final stalk height at 1548 mm, which differed significantly from all other genotypes.

Table 4.3. Stalk height of different genotypes measured shortly after transplanting (initial), just before flowering occurred in three of the genotypes (pre-flowering) and at harvest (final). The increase in stalk height from planting to just before flowering is shown. Standard error of the mean value is indicated in brackets. Values with common superscripted letters do not differ significantly at p=0.05. Final stalk height is not reported for genotypes N42, N36 and 04G0073 due to the effects of flowering.

	Stalk height (mm)			
Genotype	Initial	Pre-flowering [a]	Increase	Final
04G0073	258 [a] (13.4)	2118 [a] (30.1)	1860 [a] (37.9)	-
N41	229 [bcd] (5.6)	1778 [b] (21.0)	1549 [b] (24.0)	2065 [a] (16.9)
N36	213 [de] (7.9)	1690 [bc] (56.2)	1477 [bc] (56.8)	-
ZN6	224 [cde] (7.8)	1674 [bc] (66.2)	1450 [bc] (60.8)	1925 [abc] (74.9)
N48	210 [de] (10.2)	1635 [cd] (62.3)	1425 [bcd] (67.8)	1989 [ab] (95.8)
R570	172 [g] (4.7)	1572 [cdef] (28.3)	1400 [cde] (29.5)	1836 [bcd] (24.3)
N19	205 [de] (7.8)	1598 [cde] (33.7)	1393 [cdef] (38.7)	1875 [bcd] (41.1)
NCo376	173 [fg] (5.1)	1524 [defg] (34.4)	1351 [cdefg] (31.8)	1788 [cd] (42.6)
N51	153 [gh] (10.8)	1461 [fgh] (56.4)	1308 [defgh] (58.7)	1802 [cd] (64.7)
N31	253 [ab] (13.6)	1516 [defgh] (25.0)	1263 [efgh] (34.2)	1775 [cd] (31.5)
N42	214 [de] (11.6)	1472 [efgh] (73.0)	1258 [fgh] (74.1)	-
N12	142 [h] (6.0)	1389 [h] (42.0)	1247 [gh] (41.1)	1767 [cd] (62.5)
N25	246 [abc] (15.6)	1425 [gh] (60.3)	1179 [h] (71.9)	1751 [d] (70.8)
N14	200 [ef] (10.2)	1150 [i] (20.4)	950 [i] (26.8)	1548 [e] (38.2)
Mean	208	1572	1365	1832
L.S.D. [b]	28.0	133.0	140.8	163.3

[a] Stalk measurements conducted on all genotypes before the onset of flowering, which occurred in three of the genotypes only

[b] L.S.D. – Least significant difference at p=0.05.

Photosynthetic rate and stomatal conductance

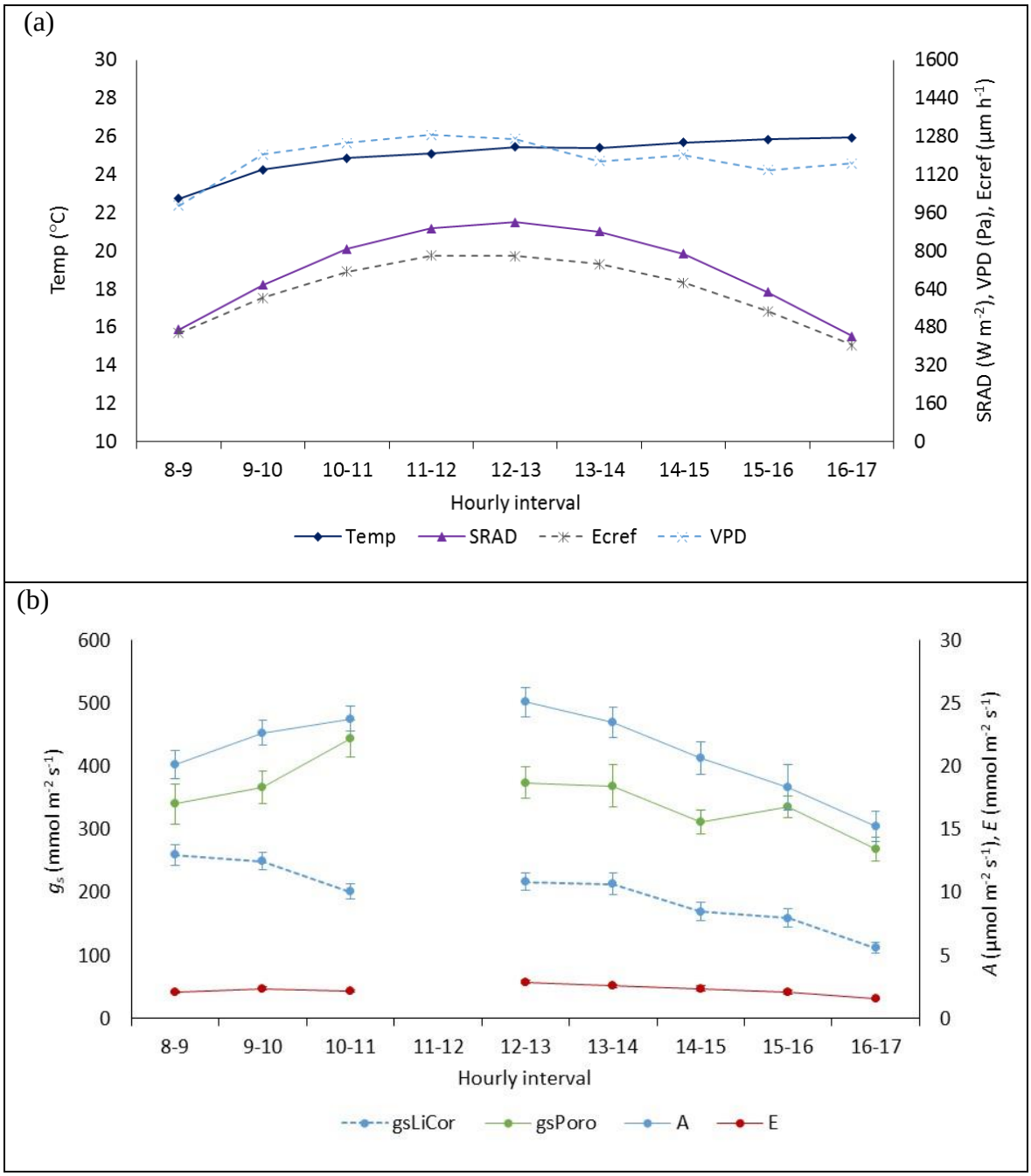
Diurnal experiments

SRAD followed the typical bell-shaped pattern expected in both experiments, being lowest in the morning (08:00) and late afternoon (17:00), and peaking between 12:00-13:00 at approximately 900 W m^{-2} (Figure 4.1). There was relatively little variation in vapour pressure deficit (VPD) in experiment 1, with high values occurring between 09:00 and 13:00 in experiment 1, and between 10:00 and 15:00 in experiment 2 (Figure 4.1). Temperature was lowest in the morning in both experiments, with peaks occurring later in the day (between 14:00-17:00).

Photosynthetic rate (*A*) was lowest in the morning in both experiments, with peak values occurring at 12:00-13:00 in experiment 1, and between 09:00 and 13:00 in experiment 2, declining thereafter (Figure 4.1). A similar trend was observed in stomatal conductance measured with the porometer (g_{sporo}), with high values occurring from 10:00-11:00 in experiment 1 and 09:00-13:00 in experiment 2, declining thereafter in both cases. Both *A* and g_{sporo} followed the curved trend of SRAD and Ecref, remaining high later in the morning and at midday, and declining thereafter. Stomatal conductance measured with the LiCor-6400 (g_{sLiCor}) showed contradictory trends to g_{sporo} early morning (Figure 4.1), for reasons unknown. In both experiments, g_{sLiCor} was high early in the morning (08:00-10:00 in experiment 1 and from 8:00-9:00 in experiment 2), then decreased to about 200 µmol mol^{-1} at 14:00 in experiment 1, and 15:00 in experiment 2, declining further thereafter.

A and g_s are known to be sensitive to VPD, SRAD and temperature (Jackson *et al*. 2016). *A* and g_s should be measured when RH is between 50 and 90% (i.e. VPD is low to moderate) with little variation over time, and when SRAD is high, to ensure that stomata are wide open (N Taylor, 2015, pers. comm.[1]). Based on the information presented, the most suitable period for measuring *A* and g_s seems to be from 10:00 to 13:00.

[1] Dr N Taylor, Department of Plant Production and Soil Science, University of Pretoria


(a)
Temp (°C)
SRAD (W m-2), VPD (Pa), Ecref (µm h-1)
Hourly interval
8-9
9-10
10-11
11-12
12-13
13-14
14-15
15-16
16-17
Temp
SRAD
Ecref
VPD
(b)
gs (mmol m-2 s-1)
A (µmol m-2 s-1), E (mmol m-2 s-1)
Hourly interval
gsLiCor
gsPoro
A
E

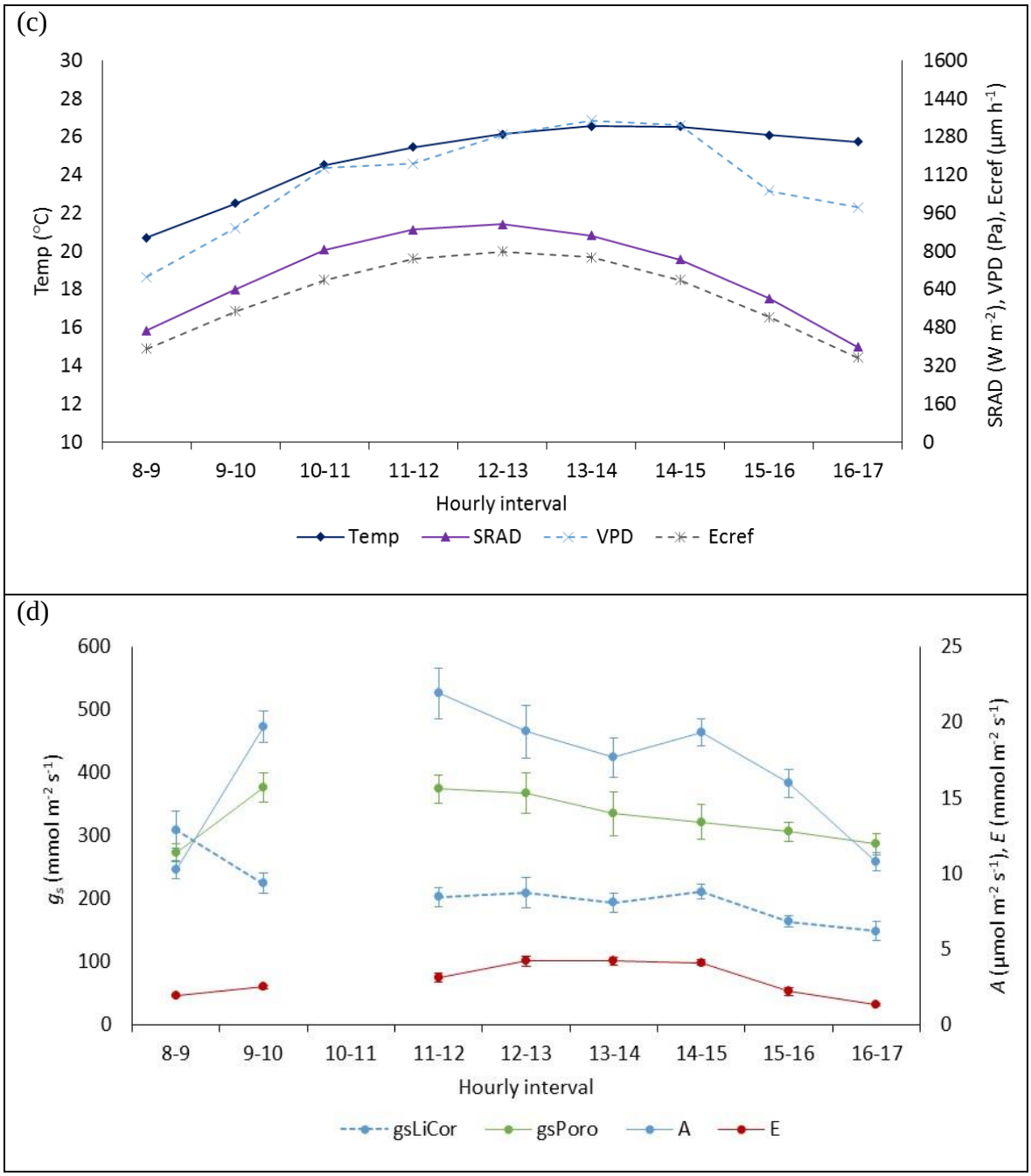


Figure 4.1. Hourly mean weather and plant variables measured in diurnal experiments 1 (a, b) and 2 (c, d). Weather variables include temperature (Temp), solar radiation (SRAD), vapour pressure deficit (VPD) and sugarcane reference evapotranspiration rate (Ecref). Plant variables include stomatal conductance using the LiCor-6400 (g_{sLicor}) and Decagon porometer (g_{sPoro}), photosynthetic rate (A) and transpiration (E). Vertical bars indicate standard error of the mean value.

Phenotyping experiments

Analysis of variance indicated that hourly datasets of normalized A (A*) could be combined in experiments 1 and 2 (Table 4.4), and that there were significant differences between genotypes. In experiment 1, A* ranged from 68% (ZN6) to 101% (04G0073) (Table 4.5), with the value for ZN6 being significantly lower than those for all other genotypes except N12, N19, N36 and N51. The value for 04G0073 was not significantly different to those of other genotypes except ZN6, N12 and N19. In experiment 2, A* ranged from 50% (N12) to 100% (NCo376), with the value for N12 being statistically lower than that for all other genotypes except ZN6. The value for NCo376 was statistically higher than that of most of the other genotypes. For the combined dataset, NCo376 was found to be the highest-ranking genotype (100%) and N12 the lowest (60%) (Table 4.5).

Analysis of variance indicated that hourly datasets of normalized g_{sLiCor} (g_{sLiCor}*) could be combined in experiment 1 (Table 4.4), with values ranging from 56% (N12) to 102% (N41) (Table 4.5). The lowest rankings genotypes N12 and ZN6 did not differ significantly, while the value for NCo376 was significantly higher than those of all other genotypes except 04G0073, N42, N25, N14 and N48. In experiment 2, g_{sLiCor}* values differed significantly between hours for all genotypes except for R570 (Table 4.6) and therefore could not be combined. Due to the variability observed, these results were not analysed further.

Analysis of variance indicated that hourly datasets of normalized g_{sPoro} (g_{sPoro}*) could also be combined in both experiments (Table 4.4), and significant genotype differences were found. g_{sPoro}* values ranged from 59% (N12) to 107% (04G0073) in experiment 1, and from 50% (N12) to 112% (04G0073) in experiment 2 (Table 4.5). In both experiments, the value for N12 was not significantly different from genotypes ZN6, N51 and N25, while the value for 04G0073 was significantly higher than that of all genotypes except NCo376, N42 and N41.

Table 4.4. Analysis of variance for normalized photosynthetic rate (A*) and stomatal conductance using the LiCor-6400 (g_{sLiCor}*) and Decagon porometer (g_{sPoro}*) for two experiments, and for combined data where statistical criteria allowed it. Sources of variation include genotype (G), measurement hour (H) and experiment (E), as well as the interactions between them. Degrees of freedom (d.f.), probability of the F-statistic (F.pr.) and mean squares (m.s.) are shown.

Source of variation	A*			g_{sLiCor}*			g_{sPoro}*		
	d.f.	F.pr.	m.s.	d.f.	F.pr.	m.s.	d.f.	F.pr.	m.s.
Phenotyping experiment 1									
G	13	<0.001	3175.8	13	<0.001	5102.6	13	<0.001	6483.6
H	2	0.570	244.1	2	0.002	6002.8	2	<0.001	4206.0
G x H	26	0.524	416.6	26	0.719	806.4	26	0.520	490.0
Residual	371	-	434.2	371	-	981.7	369	-	509.2
Phenotyping experiment 2									
G	13	<0.001	5402.9	13	<0.001	7202.1	13	<0.001	9659.6
H	2	<0.001	9250.3	2	<0.001	32111.1	2	<0.001	6444.3
G x H	26	0.095	364.4	26	0.008	960.1	26	0.237	588.1
Residual	368	-	260.4	368	-	521.9	367	-	492.6
Combined data									
E	1	0.211	12630	-	-	-	1	0.877	122.2
G	13	<0.001	8156.8	-	-	-	13	<0.001	15426.2
G x E	13	0.232	469.6	-	-	-	13	0.094	819.1
Residual	795	-	372.6	-	-	-	792	-	528.4

Table 4.5. Mean normalized values of photosynthetic rate (*A**, %) and stomatal conductance measured with the LiCor-6400 (g_{sLiCor}*, %) and Decagon porometer (g_{sPoro}*, %) for two experiments, and for combined data where statistical criteria allowed it. Standard error of the mean value is indicated in brackets. Values with common superscripted letters do not differ significantly at p=0.001. The coefficient of variance (CV, %) is also shown.

	Genotypes														CV%
Plant Variables	ZN6	N12	N19	N36	N51	N31	N41	R570	N48	N14	N25	N42	NCo376	04G0073	
Phenotyping experiment 1															
A*	67.5 ^{d} (2.38)	70.8 ^{cd} (4.10)	77.6 ^{bcd} (3.72)	83.9 ^{abcd} (3.60)	84.9 ^{abcd} (4.77)	87.6 ^{abc} (4.81)	83.9 ^{ab} (4.86)	90.1 ^{ab} (3.38)	90.7 ^{ab} (4.25)	94.7 ^{ab} (5.31)	96.5 ^{a} (4.72)	98.1 ^{a} (4.71)	100 ^{a}	100.7 ^{a} (4.94)	23.6
g_{sLiCor}*	77.9 ^{abcd} (6.24)	56.3 ^{d} (3.52)	70.3 ^{bcd} (6.44)	78.8 ^{abcd} (5.20)	82.4 ^{abcd} (7.79)	69.1 ^{cd} (4.60)	102.3 ^{a} (8.94)	78.2 ^{abcd} (4.72)	88.2 ^{abc} (7.27)	83.1 ^{abcd} (6.90)	93.0 ^{abc} (5.19)	97.1 ^{ab} (5.02)	100 ^{a}	93.9 ^{abc} (6.57)	37.7
g_{sPoro}*	63.5 ^{fg} (4.35)	58.7 ^{g} (4.14)	80.3 ^{cdef} (5.24)	77.7 ^{defg} (5.00)	70.4 ^{efg} (4.52)	73.7 ^{defg} (3.74)	104.1 ^{ab} (5.61)	71.1 ^{efg} (4.02)	86.3 ^{bcde} (4.78)	78.1 ^{defg} (3.99)	77.5 ^{defg} (4.23)	93.2 ^{abcd} (4.42)	100 ^{abc}	106.6 ^{a} (5.73)	28.2
Phenotyping experiment 2															
A_N*	60.5 ^{fg} (1.90)	49.8 ^{g} (2.26)	69.2 ^{ef} (3.02)	86.8 ^{abcd} (5.60)	72.1 ^{def} (3.52)	75.8 ^{cdef} (3.35)	83.4 ^{bcde} (2.83)	83.6 ^{bcde} (2.80)	84.6 ^{abcd} (2.96)	87.3 ^{abcd} (3.81)	89.2 ^{abc} (5.56)	91.4 ^{ab} (3.82)	100 ^{a}	90.3 ^{abc} (3.85)	20.1
g_{sPoro}*	56.6 ^{f} (3.76)	49.7 ^{f} (3.47)	85.1 ^{bcd} (3.62)	90.9 ^{bc} (4.39)	61.6 ^{fe} (4.06)	76.8 ^{cde} (4.23)	98.6 ^{ab} (4.94)	80.5 ^{bcde} (3.72)	89.3 ^{bc} (4.82)	84.5 ^{bcd} (3.99)	68.4 ^{def} (5.70)	97.9 ^{ab} (5.11)	100 ^{ab}	111.9 ^{a} (5.49)	27.0
Combined data															
A_N*	64.0 ^{fg} (1.58)	60.2 ^{g} (2.67)	73.4 ^{ef} (2.44)	84.8 ^{bcde} (3.18)	78.4 ^{de} (3.02)	81.7 ^{cde} (3.01)	86.4 ^{bcd} (2.82)	86.8 ^{bcd} (2.22)	87.7 ^{bcd} (2.60)	90.9 ^{abc} (3.28)	92.8 ^{abc} (3.65)	94.7 ^{ab} (3.04)	100 ^{a}	95.5 ^{ab} (3.18)	22.9
g_{sPoro}*	60.0 ^{gh} (2.89)	54.2 ^{h} (2.74)	82.7 ^{cde} (3.17)	83.8 ^{cde} (3.52)	65.9 ^{fgh} (3.06)	75.3 ^{def} (2.81)	101.3 ^{ab} (3.72)	75.8 ^{def} (2.79)	87.8 ^{bcd} (3.37)	81.3 ^{de} (2.83)	72.9 ^{efg} (3.57)	95.5 ^{abc} (3.37)	100 ^{ab}	109.3 ^{a} (3.95)	28.1

There was a good correlation between A^* and g_{sPoro}^* genotype values (r=0.62 and 0.78 for experiments 1 and 2, respectively) and rankings (r=0.60 and 0.71 for experiments 1 and 2, respectively). The combined datasets showed highly significant correlations between A^* and g_{sPoro}^* values (r=0.79**), and rankings (r=0.69**).

In conclusion, A^* and g_{sPoro}^* values and rankings were found to be closely correlated. The porometer confers an advantage over the LiCor-6400 as it requires less training and skill to operate, is relatively inexpensive, and measurements can be conducted rapidly.

Table 4.6. Normalized values of stomatal conductance (%) measured with the LiCor-6400 in phenotyping experiment 2, for different genotypes measured in three hourly intervals.

Genotype	Hour		
	10:00-11:00	11:00-12:00	12:00-13:00
N42	133.2	88.4	80.2
04G0073	129.2	93.6	68.1
N25	120.9	96.9	70.2
N48	110.4	80.4	77.8
N14	106.1	82.1	63.3
N36	100.2	100.9	75.3
NCo376	100.0	100.0	100.0
N41	99.0	78.8	71.3
N51	91.0	61.8	52.0
R570	86.3	78.5	67.8
N31	85.4	70.9	66.1
N19	76.5	72.4	57.0
ZN6	65.9	67.6	54.5
N12	61.1	49.9	37.4
L.S.D.[a] = 11.7			

[a] L.S.D. – Least significant difference at p=0.05.

Biomass components

The stalk fraction of above-ground dry biomass ranged from 0.485 (N14) to 0.584 (N41), with a mean value of 0.552. Stalk fractions did not differ significantly between genotypes (Table 4.7).

Stalk component fractions differed significantly between genotypes (Table 4.7). The mean fibre fraction of stalks was 0.465, with an overall range of 12% of the mean. Sucrose fraction of stalks ranged from 0.403 (N14) to 0.473 (N19). The sucrose content of the lowest ranking genotype N14 was not significantly different to that of N25, N31 and N51, while the value of the highest ranking genotype N19 was not significantly different to that N41, N48 and R570. The non-sucrose fraction of stalks ranged from 0.038 (R570) to 0.057 (N14 and N25).

The meristem and leaf sheath fractions of above-ground biomass did not differ significantly between genotypes. Meristem fractions ranged from 0.053 (R570) to 0.086 (N14), and leaf sheath fraction ranged from 0.062 (ZN6) to 0.085 (N12), with a mean value of approximately 0.06 for both fractions. Senesced leaf fraction of above-ground biomass differed significantly between genotypes, ranging from 0.086 (N12) to 0.148 (ZN6). Green leaf fraction of above-ground biomass also differed significantly between genotypes, with a mean value of 0.188 and a range of 45% of the mean. N41 had the lowest leaf fraction (0.14) and did not differ significantly from that of N48 and ZN6. N14 had the highest leaf fraction (0.224), although this did not differ significantly from that of most other genotypes.

There were no significant differences between genotypes in aerial dry biomass or SDM. Aerial biomass ranged from 2.93 (N14) to 3.83 kg pot^{-1} (N19), with a mean value of 3.35 kg pot^{-1} and a range of 29% of the mean. N14 and N19 were also the lowest and highest ranking genotypes in terms of SDM, with values of 1.43 and 2.19 kg pot^{-1}, respectively. Average SDM was 1.87 kg pot^{-1}, with a range that was 41% of the mean.

Genotypes differed significantly with regards to leaf area, with a mean value of 5.12 m^2 pot^{-1}. N41 had the lowest leaf area (3.75 m^2 pot^{-1}), which did not differ significantly from that of NCo376 and ZN6, while N12 had the highest (6.97 m^2 pot^{-1}), which did not differ significantly from that of N25 and R570.

Table 4.7. Fractions of stalk and biomass components, above-ground dry biomass, stalk dry mass and leaf area for different genotypes measured at harvest. Standard error of the mean value is indicated in brackets. Values with common superscripted letters do not differ significantly at p=0.05.

	Genotypes										
Biomass fractions	NCo376	N12	N14	N19	N25	N31	N41	N48	N51	R 570	ZN6
Stalk	0.560^{a} (0.028)	0.528^{a} (0.018)	0.485^{a} (0.015)	0.567^{a} (0.023)	0.575^{a} (0.031)	0.528^{a} (0.022)	0.584^{a} (0.038)	0.577^{a} (0.037)	0.547^{a} (0.017)	0.561^{a} (0.021)	0.564^{a} (0.035)
Fibre	0.464^{bc} (0.004)	0.475^{b} (0.004)	0.478^{ab} (0.006)	0.442^{d} (0.004)	0.438^{d} (0.011)	0.493^{a} (0.006)	0.452^{cd} (0.004)	0.453^{cd} (0.010)	0.479^{ab} (0.002)	0.475^{b} (0.004)	0.463^{bc} (0.006)
Sucrose	0.430^{bcd} (0.008)	0.436^{bcd} (0.009)	0.403^{e} (0.005)	0.473^{a} (0.010)	0.428^{bcde} (0.005)	0.423^{cde} (0.018)	0.467^{a} (0.008)	0.469^{a} (0.013)	0.411^{de} (0.003)	0.455^{ab} (0.006)	0.440^{bc} (0.004)
Non-sucrose	0.052^{abc} (0.002)	0.045^{cd} (0.004)	0.057^{a} (0.002)	0.043^{d} (0.003)	0.057^{a} (0.002)	0.044^{cd} (0.006)	0.041^{d} (0.003)	0.040^{d} (0.003)	0.055^{ab} (0.001)	0.038^{d} (0.002)	0.047^{bcd} (0.003)
Meristem	0.062^{a} (0.010)	0.079^{a} (0.007)	0.086^{a} (0.006)	0.067^{a} (0.009)	0.056^{a} (0.006)	0.065^{a} (0.005)	0.060^{a} (0.015)	0.062^{a} (0.008)	0.074^{a} (0.007)	0.053^{a} (0.006)	0.060^{a} (0.009)
Senesced leaf	0.118^{c} (0.007)	0.086^{d} (0.003)	0.125^{abc} (0.006)	0.106^{cd} (0.007)	0.111^{cd} (0.015)	0.147^{ab} (0.006)	0.150^{d} (0.010)	0.116^{c} (0.008)	0.103^{cd} (0.009)	0.122^{bc} (0.013)	0.148^{ab} (0.014)
Leaf sheath	0.081^{a} (0.008)	0.085^{a} (0.004)	0.081^{a} (0.008)	0.067^{a} (0.006)	0.068^{a} (0.007)	0.075^{a} (0.007)	0.066^{a} (0.009)	0.072^{a} (0.008)	0.078^{a} (0.011)	0.068^{a} (0.006)	0.062^{a} (0.008)
Green leaf	0.180^{b} (0.011)	0.221^{a} (0.010)	0.224^{a} (0.006)	0.192^{ab} (0.011)	0.190^{ab} (0.015)	0.185^{b} (0.010)	0.140^{c} (0.012)	0.174^{bc} (0.020)	0.197^{ab} (0.009)	0.195^{ab} (0.015)	0.166^{bc} (0.009)
Above-ground dry biomass (kg pot^{-1})	2.99^{a} (0.23)	3.55^{a} (0.14)	2.93^{a} (0.24)	3.83^{a} (0.25)	3.29^{a} (0.31)	3.22^{a} (0.24)	3.46^{a} (0.25)	3.67^{a} (0.26)	3.09^{a} (0.12)	3.68^{a} (0.23)	3.15^{a} (0.13)
Stalk dry mass (kg pot^{-1})	1.70^{a} (0.22)	1.88^{a} (0.12)	1.43^{a} (0.14)	2.19^{a} (0.20)	1.91^{a} (0.24)	1.70^{a} (0.15)	2.05^{a} (0.24)	2.14^{a} (0.25)	1.69^{a} (0.08)	2.08^{a} (0.19)	1.79^{a} (0.19)
Leaf area (m^2 pot^{-1})	4.01^{cd} (0.06)	6.97^{a} (0.62)	5.60^{b} (0.43)	5.21^{bc} (0.27)	5.72^{ab} (0.59)	5.10^{bc} (0.84)	3.75^{d} (0.23)	5.10^{bc} (0.57)	5.21^{bc} (0.27)	5.69^{ab} (0.19)	4.00^{cd} (0.22)

4.1.2 Trait parameter values

This section presents (1) an overview of the TP values estimated from data collected in the pot trial, as well as correlations between traits; and (2) an analysis of the phenotyping and estimation procedures for selected TPs to develop the phenotyping protocol.

Overview

Significant genotypic differences were found for all TPs that could be statistically analysed, with the exception of STKPFMAX (Table 4.8). SERo showed the greatest genetic variation (the range of values was 78% of the mean), followed by MXLFAREA, MXLFARNO and PI2. PARCEmax also showed high genetic variation with a range that was 47% of the mean, followed by CHUPIBASE with 30%. PI1, LFMAX and STKPFMAX showed less variation. SUCA showed the least genetic variation with a range below 20% of the mean.

The range of values for PI1 and PI2 found in this study are mostly supported by Inman-Bamber (1994) and Donaldson *et al*. (2003). The PI2 values for flowering genotypes N42 and 04G0073 were much lower than the lowest PI2 value gleaned from the literature (103 °Cd, Donaldson *et al*. 2003). The LFMAX values were closer to those reported by Sexton *et al*. (2015), while Marin *et al.* (2011) and Castro-Nava *et al*. (2016) reported generally lower values. The range of MXLFAREA and MXLFARNO values were mostly within the ranges reported by Inman-Bamber (1994), Donaldson *et al*. (2003), and Castro-Nava *et al*. (2016).

The range of CHUPIBASE values used in the trait impact study included the CHUPIBASE value of 1050 °Cd reported by Marin *et al.* (2011). Sexton *et al*. (2015) reported CHUPIBASE values ranging from 1200 to 1900 °Cd which were higher than those used in this study.

The range of estimated PARCEmax values was within the range reported by Sexton *et al*. (2015).

The range of STKPFMAX values mostly agree with values reported by Marin *et al.* (2011) and Sexton *et al*. (2015), while SUCA values were also in agreement with those reported by Marin *et al.* (2011) and Sexton *et al*. (2015).

Table 4.8. Trait parameter values for different genotypes estimated in a pot trial. Parameter descriptions are given in Table 2.1. Overall mean, and range as a percentage of the mean (%Range), are also shown. Values with common superscripted letters do not differ significantly at p=0.05.

Genotype	Leaf development					Stalk development		Photosynthetic efficiency	Biomass partitioning	
	PI1[a] (°Cd)	PI2[a] (°Cd)	LFMAX	MXLFAREA (cm^2)	MXLFARNO	CHUPIBASE[b] (°Cd)	SERo[a] (mm $°Cd^{-1}$)	PARCEmax (g MJ^{-1})	STKPFMAX[c] (t t^{-1})	SUCA[c] (t t^{-1})
NCo376	101^{bc}	114^{ab}	14^{a}	256^{h}	19^{ef}	930	1.19^{cd}	5.7^{a}	0.70^{a}	0.58^{bcd}
N12	107^{ab}	143^{a}	13^{a}	370^{de}	22^{d}	1020	1.18^{d}	3.43^{fg}	0.66^{a}	0.59^{bcd}
N14	101^{bc}	114^{ab}	14^{a}	326^{efg}	25^{c}	947	0.92^{f}	5.19^{abc}	0.61^{a}	0.54^{e}
N19	100^{bc}	117^{ab}	13^{a}	391^{cd}	20^{def}	942	1.23^{cd}	4.18^{ef}	0.71^{a}	0.63^{a}
N25	104^{b}	103^{b}	13^{ab}	355^{def}	33^{a}	987	1.07^{e}	5.29^{abc}	0.71^{a}	0.58^{bcde}
N31	111^{ab}	129^{ab}	11^{b}	329^{efg}	30^{b}	1028	1.10^{de}	4.66^{bcde}	0.66^{a}	0.57^{cde}
N36	114^{a}	101^{b}	14^{a}	459^{b}	18^{f}	1140	1.49^{b}	4.83^{de}	-	-
N41	116^{a}	112^{ab}	14^{a}	300^{fgh}	21^{de}	1038	1.36^{bc}	4.92^{bcd}	0.73^{a}	0.63^{a}
N42	102^{bc}	87^{b}	14^{a}	396^{cd}	29^{b}	1042	1.22^{cd}	5.40^{ab}	-	-
N48	108^{ab}	114^{ab}	13^{ab}	435^{bc}	19^{ef}	1053	1.32^{c}	5.00^{bcd}	0.73^{a}	0.63^{a}
N51	104^{ab}	116^{ab}	13^{ab}	339^{defg}	29^{b}	952	1.20^{cd}	4.47^{cde}	0.69^{a}	0.55^{de}
04G0073	112^{ab}	85^{b}	14^{a}	286^{gh}	26^{c}	1119	1.90^{a}	5.44^{ab}	-	-
ZN6	106^{ab}	119^{ab}	14^{a}	448^{bc}	18^{f}	987	1.25^{cd}	3.65^{g}	0.70^{a}	0.59^{bc}
R570	91^{c}	109^{ab}	13^{ab}	526^{a}	32^{a}	838	1.25^{cd}	4.95^{bcd}	0.70^{a}	0.61^{ab}
Mean	105	112	13	373	24	1002	1.26	4.79	0.69	0.59
Range	25	58	3	270	15	302	0.98	2.27	0.12	0.09
%Range	24	52	23	73	63	30	78	47	17	15
L.S.D.[d]	-	-	1.94	58.40	2.20	-	-	0.39	0.096	0.035

[a] Significant genotypic differences in PI1, PI2 and SERo were assessed with Student's t-test.

[b] Values of CHUPIBASE were derived from PI1 and could not be statistically analysed.

[c] Values of parameters STKPFMAX and SUCA values could not be determined for genotypes N36, N42 and 04G0073 due to flowering.

[d] L.S.D. – Least significant difference at p=0.05.

Table 4.9 gives the correlations between TPs. CHUPIBASE was significantly correlated to PI1, presumably because CHUPIBASE was calculated as function of leaf appearance rate, which is driven by PI1. PARCEmax was negatively correlated with PI2, which suggests that genotypes with a faster leaf development rate after leaf 14 have higher PARCEmax values. SERo was positively correlated with STKPFMAX, SUCA and CHUPIBASE. These correlations could not be explored as stalk elongation has no downstream effects on biomass growth and partitioning in the Canegro model. SUCA was positively correlated to STKPFMAX.

Table 4.9. Correlation coefficients for the relationships between trait parameters. Parameter descriptions are given in Table 2.1. Statistical significance of correlations was tested at p=0.05 (*) and p=0.01 (**).

	PI2	MXLFAREA	MXLFARNO	LFMAX	CHUPIBASE	SERo	PARCEmax	STKPFMAX	SUCA
PI1	0.00	-0.35	-0.38	0.03	0.86**	0.46	-0.09	0.15	0.13
PI2		0.00	-0.22	-0.52	-0.31	-0.52	-0.71**	-0.41	-0.10
MXLFAREA			0.00	-0.06	-0.16	-0.03	-0.34	0.22	0.39
MXLFARNO				-0.43	-0.30	-0.22	0.30	-0.23	-0.41
LFMAX					0.16	0.33	0.25	0.12	0.07
CHUPIBASE						0.60*	0.06	0.13	0.16
SERo							0.16	0.84**	0.80**
PARCEmax								0.10	-0.12
STKPFMAX									0.76**

Leaf parameters

Genotype N41 had the highest PI1 value determined by method 1, which was approximately 10% higher than the mean PI1 value of 105 °Cd (Table 4.10). R570 had the lowest PI1 value (approximately 27% lower than that of N41 and 14% lower than the mean), which was significantly different from values for all other genotypes except N42, NCo376, N14 and N19. N12 had the highest PI2 value, which was 28% higher than the mean, and 68% higher than that of 04G0073, which had the lowest PI2 value (31% below the mean). With method 1, the goodness of fit for PI1 and PI2 was highly significant for all genotypes. The PI2 values estimated for the flowering genotypes N36, 04G0073 and N42 were all lower than PI1, against the expected trend (Bonnett 1998). This suggests that the 5th and 6th set of

measurements that had to be removed due to flowering, may be essential for accurate estimation of PI2.

The PI1 values estimated with method 2 were all higher than those estimated with method 1, and differed by more than one standard error (Table 4.10). The PI2 values in method 2 were within one standard error of the values predicted with method 1, and the goodness of fit was significant for all non-flowering genotypes. In addition, there was an excellent correlation (r = 0.97) between the genotype rankings of PI2 between method 1 and method 2.

These results suggest that three measurements of leaf number are required prior to the appearance of leaf no. 14 for accurate estimation of PI1. Three bi-monthly leaf number measurements after leaf 14 and before the onset of flowering are required to reliably predict PI2.

LFMAX ranged from 11 (N31) to 14 leaves (several genotypes), with a mean value of 13 leaves (Table 4.8). Results suggest that it would be possible to estimate LFMAX values reliably by recording green leaf number shortly after transplanting, and again after TT of 720 ^{o}Cd (base 10°C) has elapsed. This recommendation is based on the reasoning that LFMAX values are unlikely to be less than 10 or more than 16 based on the mean and range of values found. Given that speedlings had about 8 leaves shortly after transplanting (Table 4.1), another 8 leaves would require a minimum of 720 ^{o}Cd for genotypes with a rapid leaf development rate (based on the lowest PI1 value of 91 °Cd, Table 4.8). Assuming a mean daily temperature of 25°C, this amounts to approximately 36 days (or about one month) between the two measurements. Thereafter, no further measurements of green leaf number are needed.

Table 4.10. Estimated phyllochron intervals (PI1 and PI2) for different genotypes using monthly (method 1) and bi-monthly (method 2) datasets. Standard error of the estimate is indicated in brackets. Values with common superscripted letters do not differ significantly at p=0.05. The number of observations (n) is indicated. The correlation (r) between the values and ranks calculated with method 1, and those calculated with method 2, is shown. The correlations and goodness of fit (R^2) are indicated as significant (*, $p<0.05$) or highly significant (**, $p<0.01$).

Genotype	Method 1								Method 2						
	PI1 (°Cd)	Rank	R^2	n	PI2 (°Cd)	Rank	R^2	n	PI1 (°Cd)	Rank	n	PI2 (°Cd)	Rank	R^2	n
N41	115.69 ^{a} (3.48)	1	0.99**	3	112.09 ^{ab} (13.66)	9	0.94**	6	122.74	1	2	101.68 ^{de} (11.76)	10	0.98*	3
N36	113.72 ^{a} (1.42)	2	0.99**	3	101.21 ^{b} (10.16)	12	0.98**	4	116.45	3	2	102.29	9	-	2
04G0073	111.83 ^{ab} (3.75)	3	0.99**	3	85.27 ^{b} (4.96)	14	0.99**	4	119.51	2	2	81.31	14	-	2
N31	110.94 ^{ab} (2.76)	4	0.99**	3	128.95 ^{ab} (11.45)	2	0.97**	6	116.45	4	2	119.41 ^{b} (5.55)	2	0.99*	3
N48	108.22 ^{ab} (3.98)	5	0.99**	3	113.97 ^{ab} (9.88)	6	0.97**	6	116.45	5	2	105.35^{cde} (5.14)	6	0.99*	3
N12	107.39 ^{ab} (3.05)	6	0.99**	3	143.26 ^{a} (10.99)	1	0.97**	6	113.54	6	2	132.57 ^{a} (0.22)	1	0.99*	3
ZN6	106.10 ^{ab} (3.62)	7	0.99**	3	118.66 ^{ab} (11.07)	3	0.96**	6	113.54	7	2	111.12 ^{bc} (13.38)	3	0.98*	3
N51	104.01 ^{ab} (3.29)	8	0.99**	3	116.27 ^{ab} (12.34)	5	0.96**	6	110.77	8	2	106.77 ^{cd} (7.95)	5	0.99*	3
N25	103.65 ^{b} (1.03)	9	0.99**	3	102.53 ^{b} (13.12)	11	0.94**	6	105.62	12	2	92.07 ^{e} (17.39)	12	0.97*	3
N42	101.65 ^{bc} (4.34)	10	0.99**	3	87.24 ^{b} (12.67)	13	0.96**	4	110.77	9	2	88.08	13	-	2
NCo376	101.34 ^{bc} (2.16)	11	0.99**	3	113.95 ^{ab} (11.09)	7	0.96**	6	105.62	13	2	105.35^{cde} (5.14)	7	0.99*	3
N14	100.94 ^{bc} (3.50)	12	0.99**	3	113.57 ^{ab} (9.85)	8	0.97**	6	108.13	10	2	105.25 ^{de} (0.22)	8	0.99*	3
N19	99.78 ^{bc} (3.99)	13	0.99**	3	116.97 ^{ab} (11.89)	4	0.96**	6	108.13	11	2	110.24 ^{c} (4.31)	4	0.99*	3
R570	91.27 ^{c} (0.24)	14	0.99**	3	109.35 ^{ab} (14.57)	10	0.93**	6	90.83	14	2	97.10 ^{de} (13.37)	11	0.98*	3
r	-	-	-	-	-	-	-	-	0.94**	0.95**	-	0.97**	0.97**	-	-

MXLFAREA ranged from 256 (NCo376) to 526 cm^2 (R570), with a mean value of 373 cm^2. MXLFARNO ranged from 18 (N36) to 32 (R570), with a mean value of 24 (Table 4.8). Results suggest that it would be possible to estimate values of MXLFAREA and MXLFARNO by recording leaf size and number of all green fully expanded leaves shortly after transplanting, and at TT intervals of about 900°Cd thereafter. This is the minimum TT required to develop 10 green leaves (the likely lowest LFMAX value) for genotypes with rapid leaf development (91 °Cd, Table 4.8). Measurements should continue up to leaf number 39 (likely maximum MXLFARNO), or until leaf size remains constant or declines with increasing leaf number. This amounts to measurements of leaf size and number every 60 days for typical conditions at Mount Edgecombe.

Reference stalk elongation rate

SERo values ranged from 0.92 (N14) to 1.90 mm $°Cd^{-1}$ (04G0073) in the monthly dataset (Table 4.11), with the values for N14 and 04G0073 being significantly lower and higher respectively, than values for all other genotypes. The goodness of fit was highly significant for all genotypes in method 1.

Genotype 04G0073 had a significantly higher SERo value than all other genotypes in method 2, while N14 had the lowest value (Table 4.11). SERo values estimated with method 2 were well correlated with that of method 1, and all of the method 2 values were within one standard error of method 1 values, except for the flowering genotype N36. There was an excellent correlation between the genotype rankings of SERo values estimated with method 1 and 2 ($r = 0.98$).

N41 had the highest SERo value using method 3, which differed significantly from that for all other genotypes except N48 and ZN6. The value for N14 was significantly lower than that of all other genotypes and was 26% lower than that of N41. Although the SERo values and rankings derived from method 1 and 3 were significantly correlated, 9 out of 14 of the values differed by more than one standard error.

Method 4 only yielded one value that was within one standard error of those predicted with method 1. In addition, the correlation between the rankings found in method 1 and 4 was much poorer than reported in methods 2 and 3.

Results suggest that it should be possible to reliably predict SERo values from bi-monthly measurements of stalk height, in conjunction with daily temperatures.

Maximum radiation conversion efficiency

The PARCEmax values derived from measurements of A^* ranged from 3.43 (N12) to 5.70 g MJ^{-1} (NCo376), while those derived from measurements of g_{sporo}^* ranged from 3.09 (N12) to 6.23 g MJ^{-1} (04G0073) (Table 4.12). Values of PARCEmax derived from A^* and from g_{sporo}^* were highly correlated, as were genotype rankings. A preliminary assessment showed that PARCEmax values derived from A^* had a better correlation with observed SDM (r=0.66) in Pongola (Table 4.13) than that of PARCEmax values derived from g_{sporo}^* (r=0.30). PARCEmax values derived from A^* were therefore used in the TP evaluation process.

Table 4.11. Reference stalk elongation rate (SERo) values for different genotypes using monthly (method 1) and bi-monthly (method 2) datasets. Datasets with stalk heights measured three times (method 3) and two times (method 4) are also shown. Standard error of the estimate is indicated in brackets. Values with common superscripted letters do not differ significantly at p=0.05. The number of observations (n) is indicated. The correlation (r) between SER_0 values and ranks calculated with method 1, and those calculated with methods 2, 3 and 4, is shown. The correlations and goodness of fit (R^2) are indicated as significant (*, p<0.05) or highly significant (**, p<0.01).

	Method 1				Method 2				Method 3				Method 4		
Genotype	SERo (mm °Cd^{-1})	Rank	R^2	n	SERo (mm °Cd^{-1})	Rank	R^2	n	SERo (mm °Cd^{-1})	Rank	R^2	n	SERo (mm °Cd^{-1})	Rank	n
04G0073	1.90^{a} (0.07)	1	0.98**	6	1.85^{a} (0.06)	1	1.00**	3	1.82	1	-	2	1.82	1	2
N36	1.49^{b} (0.03)	2	1.00**	6	1.45^{b} (0.12)	2	0.99*	3	1.49	2	-	2	1.49	4	2
N41	1.36^{bc} (0.04)	3	0.99**	8	1.40^{b} (0.08)	3	0.99**	4	1.30^{a} (0.11)	3	0.99*	3	1.51	2	2
N48	1.32^{c} (0.07)	4	0.98**	8	1.32^{b} (0.04)	4	1.00**	4	1.26^{a} (0.06)	4	1.00**	3	1.38	8	2
ZN6	1.25^{cd} (0.03)	5	1.00**	8	1.28^{bc} (0.11)	5	0.99**	4	1.20^{b} (0.15)	5	0.99*	3	1.51	3	2
R570	1.25^{cd} (0.06)	6	0.98**	8	1.25^{bc} (0.09)	6	0.99**	4	1.17^{b} (0.12)	7	0.99*	3	1.43	7	2
N19	1.23^{cd} (0.06)	7	0.99**	8	1.25^{bc} (0.10)	7	0.99**	4	1.18^{ab} (0.13)	6	0.99*	3	1.44	6	2
N42	1.22^{cd} (0.07)	8	0.99**	6	1.20^{bc} (0.06)	10	1.00**	3	1.11	10	-	2	1.11	13	2
N51	1.20^{cd} (0.05)	9	0.99**	8	1.20^{bc} (0.07)	9	0.99**	4	1.10^{b} (0.06)	11	1.00**	3	1.29	10	2
NCo376	1.19^{cd} (0.04)	10	0.99**	8	1.22^{bc} (0.10)	8	0.99**	4	1.14^{bc} (0.16)	9	0.99*	3	1.45	5	2
N12	1.18^{d} (0.05)	11	0.99**	8	1.17^{bc} (0.04)	11	1.00**	4	1.15^{b} (0.04)	8	1.00**	3	1.24	11	2
N31	1.10^{de} (0.06)	12	1.00**	8	1.11^{bc} (0.09)	12	0.99**	4	1.07^{bc} (0.13)	12	0.99*	3	1.35	9	2
N25	1.07^{e} (0.08)	13	0.98**	8	1.09^{c} (0.06)	13	0.99**	4	1.07^{c} (0.05)	13	1.00**	3	1.16	12	2
N14	0.92^{f} (0.07)	14	0.98**	8	0.91^{c} (0.09)	14	0.98*	4	0.96^{d} (0.01)	14	1.00**	3	0.98	14	2
r	-	-	-	-	0.99**	0.98**	-	-	0.98**	0.96**	-	-	0.85**	0.81**	-

Table 4.12. Values of maximum radiation conversion efficiency (PARCEmax) estimated from normalized measurements of stomatal conductance (g_{sporo}*) and photosynthetic rate (*A**) for different genotypes. Values with common superscripted letters do not differ significantly at p=0.05. Overall mean is shown. The correlation (r) between the values and ranks of PARCEmax (*A**) and PARCEmax (g_{sporo}*) are indicated as highly significant (**, p<0.01).

Genotype	PARCEmax (*A**) (g MJ^{-1})		PARCEmax (g_{sporo}*) (g MJ^{-1})	
	Value	Rank	Value	Rank
NCo376	5.7	1	5.7	3
04G0073	5.44^{ab}	2	6.23^{a}	1
N42	5.40^{ab}	3	5.45^{abc}	4
N25	5.29^{abc}	4	4.16^{efg}	11
N14	5.19^{abc}	5	4.63^{de}	8
N48	5.00^{bcd}	6	5.00^{bcd}	5
R570	4.95^{bcd}	7	4.32^{def}	9
N41	4.92^{bcd}	8	5.78^{ab}	2
N36	4.83^{de}	9	4.78^{cde}	6
N31	4.66^{bcde}	10	4.29^{def}	10
N51	4.47^{cde}	11	3.76^{fgh}	12
N19	4.18^{ef}	12	4.71^{cde}	7
ZN6	3.65^{fg}	13	3.42^{gh}	13
N12	3.43^{g}	14	3.09^{h}	14
Mean	4.79	-	4.67	-
r	-	-	0.78**	0.69**
L.S.D.[a]	0.39	-	0.47	-

[a] L.S.D. – Least significant difference at p=0.05

4.1.3 Phenotyping protocol

This study proposes the following phenotyping protocol for estimating TP values. Effective use of resources and practicality were the main considerations.

Cane setts should be pre-germinated to ensure uniform shoot emergence. Speedlings should then be transplanted into pots containing a synthetic soil medium (described in section 3.2.2), and grown under well-watered, stress-free conditions.

The following measurements are recommended for estimating TP values:

(1) Three measurements of fully expanded leaf number prior to the appearance of leaf number 14 for estimating PI1, followed by three bi-monthly measurements for accurate estimation of PI2;
(2) Measurements of green leaf number shortly after transplanting, and again after 720 °Cd (base 10), for accurate estimation of LFMAX;
(3) Measurements of leaf size and number of all green fully expanded leaves shortly after transplanting, and at TT intervals of about 900°Cd (base 10) thereafter. This should continue up to leaf number 39, or until leaf size remains constant or declines with increasing leaf number, for accurate estimation of MXLFAREA and MXLFARNO.
(4) Bi-monthly measurements of stalk height to estimate SERo;
(5) Measurements of g_s for gaseous exchange on well-lit TVD leaves with a leaf porometer between 10:00 and 13:00 to estimate PARCEmax values. All genotypes within a replicate should be measured within one hour.
(6) Measurements of biomass fractions at harvest to estimate biomass partitioning fractions (STKPFMAX and SUCA).

TPs should be estimated from phenotypic measurements conducted before the onset of flowering. It should be noted that PARCEmax, STKPFMAX and SUCA values cannot be directly estimated from phenotypic measurements, and require normalization relative to that of a reference genotype.

The phenotyping procedure recommended here could be used in the development of high-throughput technologies to assist sugarcane plant breeding. For example, canopy-level conductance (g_c) can be estimated through proximal sensing of surface reflectance and by

thermal imagery using high-throughput technologies (Munns *et al*. 2010; Yang *et al*. 2013). This would require the development of transfer functions between g_c measured manually (comprising measurements of g_s and green leaf area index) and g_c measured with proximal sensing. Basnayake *et al*. (2015) reported that leaf-level g_s showed a generally positive association with cane yield, although the strength of the relationship varied widely (r=0.29 to 0.94). The study further reported that canopy-level conductance (g_c) generally showed larger positive correlations with observed yield, and concluded that g_c could potentially be used as a screening measurement in the early stages of the sugarcane plant breeding programme.

4.1.4 Evaluation

Pongola_2011

FI_{PC} values and rankings were not accurately simulated as evidenced by poor correlations for values and rankings. For example, observed FI_{PC} differed significantly between genotypes, ranging from 39% (N12) to 75% (N31) (Table 4.13). The model simulated a much smaller difference in FI_{PC} values between N12 and N31 (64 vs. 65%). Simulated FI_{PC} showed a highly significant negative correlation with PI1 (r = -0.85), which was not reflected in the observed values (Table 4.14).

Simulated FI_{PC} is governed by tiller and leaf development, and their associated TPs. Although N12 (lowest observed FI_{PC} value) had fewer tillers than N31 (highest observed FI_{PC}) after emergence, new tillers continued to appear for a longer period which resulted in a higher maximum population compared to N31 (Table 3.3 and Figure 3.2). The PI1 values (most relevant for canopy development) estimated in the pot trial for N12 and N31 did not differ significantly (Table 4.8), and N12 had a MXLFAREA value that was 12% larger than N31. Although the PI1 values calculated from the Pongola field trial data (Ngobese 2015) were higher than those estimated in the pot trial (162 vs. 107°Cd for N12, and 145 vs. 110°Cd for N31), the respective genotypic differences were small in both cases, and presumably had negligible impact on canopy development. However, the MXLFAREA value observed in the field trial for N12 was 25% lower than that of N31, contradicting the trend observed in the pot trial. It is noteworthy that using these TP values estimated from field data did not result in more accurate predictions of FI_{PC} (39 and 49% vs 64 and 65% for N12 and N31, respectively). This information suggests that the Canegro model overemphasises the

influence of PI1 on FI_{PC}, and underestimates the influence of MXLFAREA. Furthermore, MXLFAREA values and rankings determined from the data sets differed greatly, which suggests that a strong environmental influence is at play, as was found by Singels *et al.* (2005). The suitability of MXLFAREA as a genetic trait parameter to represent the effect of leaf size on canopy development therefore needs rethinking.

Observed SDM values differed significantly between genotypes, ranging from 37 (N12) to 46 t ha^{-1} (NCo376) (Table 4.13). Observed SDM values showed a significant correlation with simulated values ($r = 0.67$) but were underestimated by 17% on average. SDM rankings were predicted well ($r = 0.75$). Observed SDM values showed a positive correlation with PARCEmax values, while simulated SDM correlated significantly with PARCEmax, PI2 and STKPFMAX (Table 4.14). This suggests that PARCEmax is an important determinant of stalk yield, although its influence was overestimated in the model.

Observed SUCM values differed significantly between genotypes, ranging from 12.2 (N31) to 19.5 t ha^{-1} (N25) (Table 4.13). Simulated SUCM values and rankings did not show significant correlations with observed SUCM values and rankings. Observed SUCM values correlated significantly with LFMAX, STKPFMAX and PI2, while simulated values correlated best with PARCEmax, PI2 and STKPFMAX (Table 4.14).

Table 4.13. Observed and simulated values of fractional interception at partial canopy cover (FI_{PC}), stalk dry mass (SDM) and sucrose yield (SUCM) for selected genotypes grown in Pongola. Standard error of the observed genotype mean value is indicated in brackets. Values with common superscripted letters do not differ significantly at p=0.05. The overall mean and range of values, and the root mean square error (RMSE, t ha^{-1}) of simulated values are shown. The correlation (r) between observed and simulated values and rankings is shown and statistical significance at p<0.05 is indicated by *.

	FI_{PC} (%)				**SDM (t ha^{-1})**				**SUCM (t ha^{-1})**			
	Observed		**Simulated**		**Observed**		**Simulated**		**Observed**		**Simulated**	
Genotype	**Value**	**Rank**	**Value**	**Rank**	**Value**	**Rank**	**Value**	**Rank**	**Value**	**Rank**	**Value**	**Rank**
N31	74.7[a] (1.76)	1	65.3	5	39.3[bc] (1.51)	8	32.0	7	12.2[b] (2.25)	9	14.2	7
N25	69.9[ab] (2.16)	2	68.3	3	42.1[abc] (1.53)	3	38.8	2	19.5[a] (1.40)	1	18.4	4
NCo376	69.7[ab] (1.99)	3	74.6	1	46.4[a] (1.35)	1	40.7	1	18.6[a] (2.51)	5	19.5	1
N48	67.7[b] (1.86)	4	62.0	8	40.9[abc] (2.38)	4	38.0	3	18.9[a] (2.84)	3	19.1	2
N36	64.2[bc] (2.05)	5	63.8	6	39.7[abc] (0.70)	5	30.2	8	19.5[a] (1.71)	2	14.2	8
N19	58.2[cd] (1.78)	6	72.0	2	39.5[abc] (0.74)	6	32.5	6	17.5[a] (1.40)	7	15.6	5
N51	58.1[cd] (3.49)	7	65.3	4	45.6[ab] (4.22)	2	33.3	5	18.7[a] (4.18)	4	14.6	6
N41	53.8[d] (2.66)	8	53.3	9	39.5[abc] (1.88)	7	37.4	4	18.6[a] (0.83)	6	18.6	3
N12	38.9[e] (2.36)	9	63.5	7	36.5[c] (4.09)	9	23.7	9	15.8[ab] (4.45)	8	9.8	9
Mean	61.7	-	65.3	-	41.1	-	34.1	-	17.7	-	16.0	-
Range	35.8	-	21.3	-	9.9	-	17.0	-	7.3	-	9.7	-
L.S.D.[a]	6.48	-	-	-	6.92	-	-	-	3.87	-	-	-
RMSE	-	-	10.52	-	-	-	7.92	-	-	-	3.17	-
r	-	-	0.35	0.44	-	-	0.67*	0.75*	-	-	0.51	0.30

[a] L.S.D. – Least significant difference at p=0.05 for observed values.

Table 4.14. Correlation coefficient for the relationships between trait parameters estimated in a pot trial and simulated and observed fractional interception at partial canopy cover (FI_{PC}), stalk dry mass (SDM) and sucrose yield (SUCM) for selected genotypes grown in Pongola. Parameter descriptions are given in Table 2.1. Statistical significance of correlations was tested at p=0.05 (*) and p=0.01 (**).

	FI_{PC}		**SDM**		**SUCM**	
	Observed	**Simulated**	**Observed**	**Simulated**	**Observed**	**Simulated**
PI1	-0.07	-0.85**	-0.48	0.03	-0.12	0.10
PI2	-0.53	-0.04	-0.44	-0.87**	-0.75*	-0.87**
LFMAX	-0.29	-0.09	0.25	0.44	0.83**	0.49
MXLFAREA	-0.07	-0.15	-0.46	-0.13	0.20	-0.02
MXLFARNO	0.27	0.07	0.17	-0.11	-0.29	-0.25
CHUPIBASE	0.00	-0.60	-0.55	0.03	0.02	0.12
SERo	-0.20	-0.53	-0.20	0.28	0.48	0.39
PARCEmax	0.78*	0.19	0.66	0.95**	0.43	0.90**
STKPFMAX	0.19	-0.23	0.11	0.71*	0.78*	0.82*
SUCA	-0.19	-0.33	-0.48	0.14	0.24	0.32

Komatipoort_2011

There were no significant genotypic differences in observed FI_{PC} or SDM (Table 4.15). The model simulated a much larger range in FI_{PC} than was observed. The simulated range of SDM was smaller than the observed range, which was not significant. There were significant differences in observed SUCM values, which ranged from 5.4 (04G0073) to 14.2 t ha^{-1} (N19). The model underestimated SUCM values for N31 and N19 by 27% on average, but predicted SUCM value for 04G0073 accurately. SUCM rankings were not simulated well.

Komatipoort_2012

Observed FI_{PC} differed significantly between genotypes, with 04G0073 having a significantly higher FI_{PC} value than N31 and N19 (Table 4.16). The model was not able to accurately simulate the observed genotypic differences in FI_{PC}. Observed SDM did not differ significantly between genotypes, while the simulated range in SDM was smaller than the observed range. Observed SUCM values differed significantly between genotypes, ranging from 5.8 (04G0073) to 19.4 t ha^{-1} (N19). The model was able to predict the observed differences in SUCM values and rankings well.

Table 4.15. Observed and simulated values of fractional interception at partial canopy cover (FI_{PC}), stalk dry mass (SDM) and sucrose yield (SUCM) for selected genotypes grown in Komatipoort. Standard error of the observed genotype mean value is indicated in brackets. Values with common superscripted letters do not differ significantly at p=0.05. The overall mean and range of values, and the root mean square error (RMSE, t ha^{-1}) of simulated values are shown. The correlation (r) between observed and simulated values and rankings is shown (all statistically insignificant at p=0.05).

	FI_{PC} (%)				**SDM (t ha^{-1})**				**SUCM (t ha^{-1})**			
	Observed		**Simulated**		**Observed**		**Simulated**		**Observed**		**Simulated**	
Genotype	**Value**	**Rank**	**Value**	**Rank**	**Value**	**Rank**	**Value**	**Rank**	**Value**	**Rank**	**Value**	**Rank**
N31	56.4[a] (11.4)	1	42.6	2	30.3[a] (2.4)	3	21.6	1	11.4[b] (0.4)	2	9.4	1
N19	52.4[a] (10.8)	2	48.2	1	31.6[a] (3.4)	2	20.2	3	14.2[a] (2.1)	1	9.1	2
04G0073	52.1[a] (11.6)	3	35.4	3	32.7[a] (1.7)	1	20.8	2	5.4[c] (0.2)	3	5.4	3
Mean	53.6	-	42.1	-	31.5	-	20.9	-	10.3	-	8.0	-
Range	4.3	-	12.8	-	2.4	-	1.4	-	8.8	-	4.0	-
L.S.D.[a]	23.5	-	-	-	7.3	-	-	-	2.1	-	-	-
RMSE	-	-	12.7	-	-	-	10.8	-	-	-	3.2	-
r	-	-	0.13	0.5	-	-	-0.61	-0.5	-	-	0.93	0.5

[a] L.S.D. – Least significant difference at p=0.05 for observed value

Table 4.16. Observed and simulated values of fractional interception at partial canopy cover (FI_{PC}), stalk dry mass (SDM) and sucrose yield (SUCM) for selected genotypes grown in Komatipoort. Standard error of the observed genotype mean is indicated in brackets. Values with common superscripted letters do not differ significantly at p=0.05. The overall mean and range of values, and the root mean square error (RMSE, t ha^{-1}) of simulated values are shown. The correlation (r) between observed and simulated values and rankings is shown (all statistically insignificant at p=0.05).

	FI_{PC} (%)				**SDM (t ha^{-1})**				**SUCM (t ha^{-1})**			
	Observed		**Simulated**		**Observed**		**Simulated**		**Observed**		**Simulated**	
Genotype	**Value**	**Rank**	**Value**	**Rank**	**Value**	**Rank**	**Value**	**Rank**	**Value**	**Rank**	**Value**	**Rank**
04G0073	68.7[b] (4.5)	1	36.5	3	44.4[a] (2.8)	3	30.2	3	5.8[a] (0.4)	3	8	3
N31	46.0[a] (4.0)	2	36.9	2	51.4[a] (2.2)	1	31.5	1	16.6[b] (0.8)	2	14.4	2
N19	40.1[a] (3.5)	3	41.8	1	49.1[a] (1.4)	2	31.5	2	19.4[c] (0.6)	1	15.4	1
Mean	51.6	-	38.4	-	48.3	-	31.1	-	13.9	-	12.6	-
Range	28.6	-	5.3	-	7.0	-	1.3	-	13.6	-	7.4	-
L.S.D.[a]	11.4	-	-	-	18.7	-	-	-	1.8	-	-	-
RMSE	-	-	19.3	-	-	-	17.4	-	-	-	2.9	-
r	-	-	-0.71	-1	-	-	0.95	1	-	-	0.99	1

[a] L.S.D. – Least significant difference at p=0.05 for observed value

Mount Edgecombe_2011

Observed SDM and SUCM did not differ significantly between the two genotypes (Table 4.17). The simulated ranges of SDM and SUCM values were smaller than the observed ranges, which were not statistically significant. The model underestimated SUCM values, but predicted the ranking correctly.

Table 4.17. Observed and simulated values of stalk dry mass (SDM) and sucrose yield (SUCM) for selected genotypes grown in Mount Edgecombe. Standard error of the observed genotype mean is indicated in brackets. Values with common superscripted letters do not differ significantly at p=0.05. The overall mean and range of values are shown.

	SDM (t ha^{-1})				**SUCM (t ha^{-1})**			
	Observed		**Simulated**		**Observed**		**Simulated**	
Genotype	**Value**	**Rank**	**Value**	**Rank**	**Value**	**Rank**	**Value**	**Rank**
04G0073	18.7[a] (1.2)	1	11.1	2	4.5[a] (0.2)	2	2.6	2
N19	17.1[a] (1.4)	2	11.3	1	6.1[a] (0.5)	1	3.8	1
Mean	17.9	-	11.2	-	5.3	-	3.2	-
Range	1.6	-	0.2	-	1.6	-	1.2	-
L.S.D.[a]	5.1	-	-	-	1.5	-	-	-

[a] L.S.D. – Least significant difference at p=0.05 for observed value

The model showed some promise in simulating genetic differences observed in field trials using TP values estimated in the pot trial. For example, the model was able to predict rankings of SDM well in Pongola. The model simulated very small differences in SDM between for the Komatipoort and Mount Edgecombe trials, which agrees with the non-significant differences in observed SDM. The observed genotypic differences in SUCM values and rankings were also predicted well for Komatipoort_2012.

In other ways, the model was unsuccessful in predicting genetic differences in crop growth and yield. The observed genotypic differences in FI_{PC} were simulated poorly for the Pongola trial, and were consistently under-estimated for the Komatipoort trials. The model also consistently underestimated SDM values for all trials. This may have been caused by the poor simulations of canopy cover, and/or of incorrect simulation of environmental impacts on crop growth and development. Significant genotypic differences in SUCM values and rankings were also not predicted well in Pongola and Komatipoort_2011.

The hypothesis of "it is possible to accurately simulate genetic differences in crop growth and yield observed in field trials using TP values estimated in a pot trial" was therefore proven to be partially true for SDM only. Datasets for testing the hypothesis require an adequate number of genotypes with significant genotypic differences in variables related to crop growth and development. Only the Pongola dataset remotely fulfilled these requirements.

4.2 Trait impact study

4.2.1 Single trait variants

The PI1 variants produced mean SDM values declining from 35 to 33 t ha^{-1} as PI1 increased, with the range being 3.5% of the baseline mean (Figure 4.2). The optimal PI1 value with the highest simulated SDM was 93 °Cd.

CHUPIBASE variants showed mean SDM values that declined from 36 to 31 t ha^{-1} as CHUPIBASE increased. The range was 14% of the baseline mean (Figure 4.2). The decline in SDM increased with each increment in CHUPIBASE (Figure 4.3). The "optimal" CHUPIBASE value was 700 °Cd which consistently outperformed the other variants (Figure 4.3).

PI2 variants produced mean SDM values from 33 to 34 t ha^{-1} with a range of 4% of the baseline mean (Figure 4.2). There was an increase in SDM with the first increment in PI2 from 54°Cd to 83°Cd, with very small decreases in SDM with subsequent increments (Figure 4.3). The optimal value for PI2 was 112 °Cd.

PARCEmax variants produced the largest range in mean SDM values from 18 to 45 t ha^{-1} (79% of the baseline mean) (Figure 4.2). SDM increases diminished gradually as PARCEmax increased. The highest PARCEmax value (7.06 g MJ^{-1}) produced a 31% increase in SDM and consistently outperformed the other PARCEmax variants (Figure 4.3).

STKPFMAX variants produced mean SDM values that ranged from 28 to 41 t ha^{-1}, (40% of the baseline mean) (Figure 4.2). The response of SDM to increases in STKPFMAX was linear over the range tested, as can be expected. The highest STKPFMAX value (0.81) increased SDM by approximately 20% relative to the baseline value and consistently outperformed the other PARCEmax variants (Figure 4.3).

SUCA variants produced mean SDM values ranging from 34 to 35 t ha^{-1}. This TP appeared to affect SDM the least of all the parameters, as the range of SDM values was 1% of the baseline mean (Figure 4.2). The optimal value was found to be 0.68.

In summary, trait parameters PARCEmax, STKPFMAX and CHUPIBASE showed the largest impacts on simulated SDM. These results therefore suggest that maximum SDM can be achieved when optimal values for these TPs are combined. This may be possible in reality since no significant correlations were found between these traits in this study.

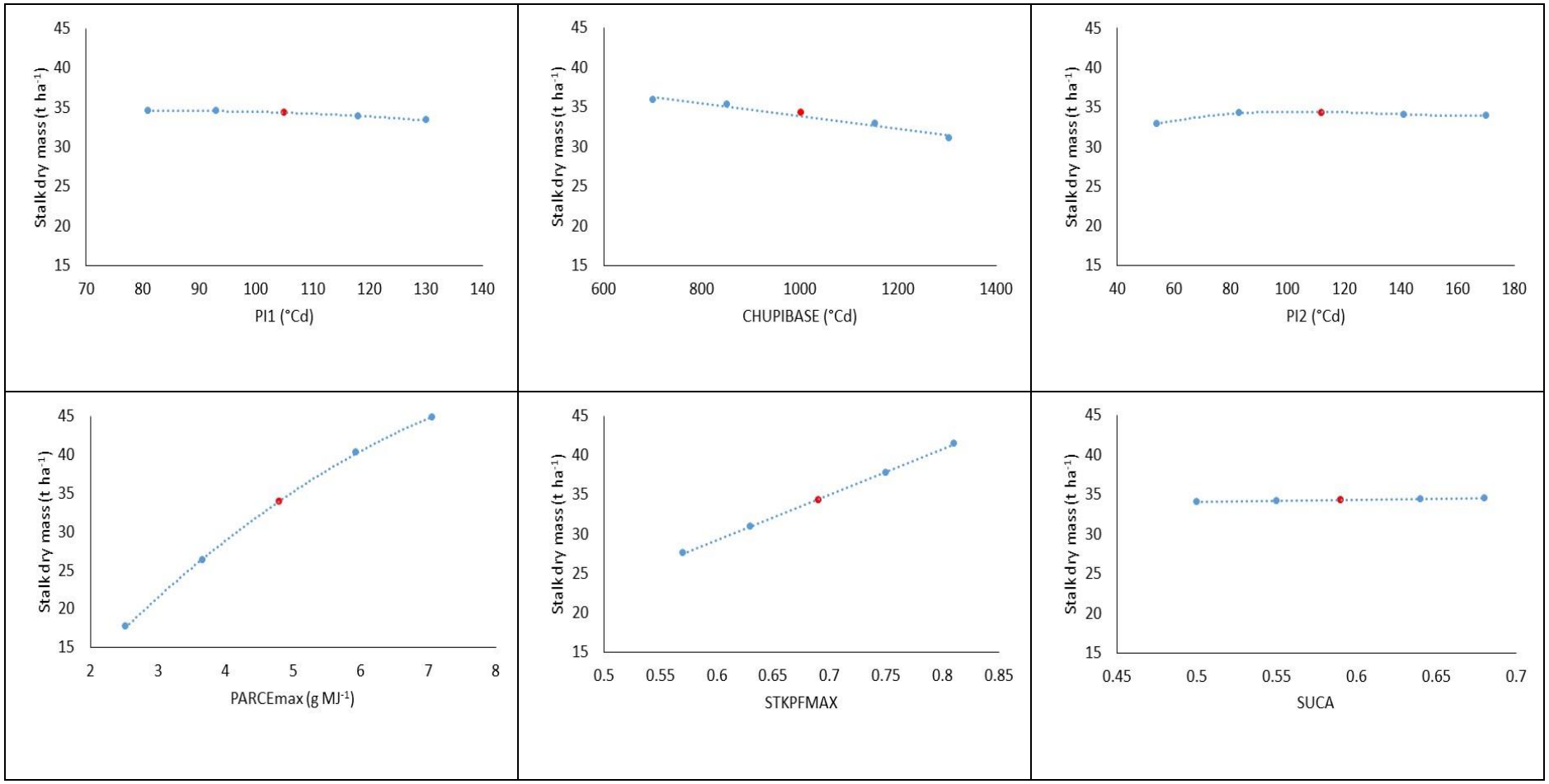


Figure 4.2. Mean stalk dry mass simulated for different single trait variants. Parameter descriptions are given in Table 2.1. The baseline value is indicated in red.

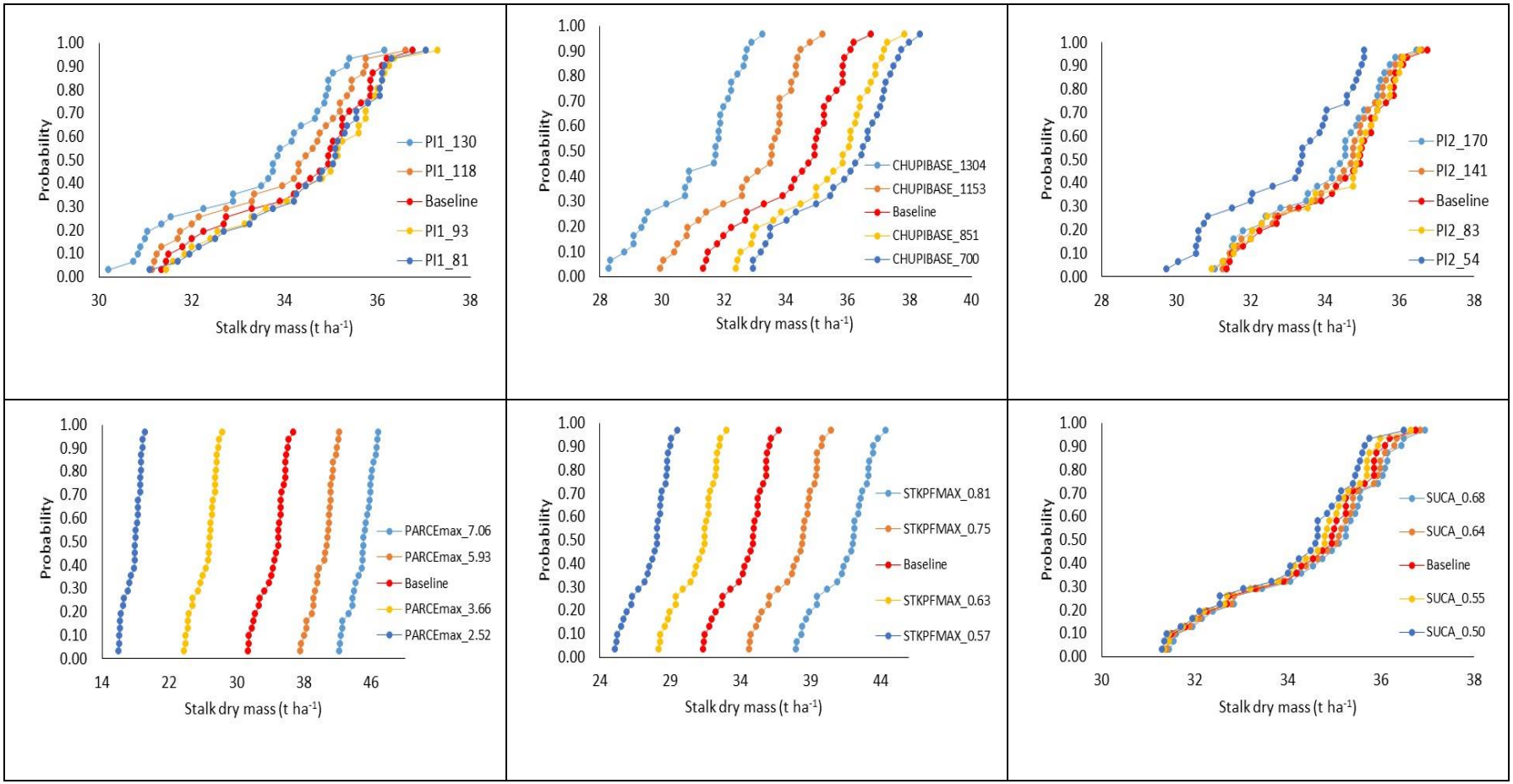


Figure 4.3. Cumulative distribution frequency of simulated stalk dry mass for hypothetical genotypes that differ only with respect to one trait at a time, for Pongola. Parameter descriptions are given in Table 2.1. The baseline value is indicated in red. Trait parameter values used in the simulations are also shown.

4.2.2 Multiple trait variants

The path coefficient analysis showed that the only TPs that had significant correlations with simulated SDM were PARCEmax (r=0.89**) and STKPFMAX (r=0.41*). PARCEmax and STKPFMAX were also the only traits which had significant direct effects of 0.88 and 0.40, respectively. In both cases the direct effects dominated these correlations (no indirect effects found). All other correlations and indirect effects were insignificant.

These results are comparable with that of Sexton *et al*. (2015), where maximum radiation conversion efficiency was found to be the most influential parameter out of 10 traits in APSIM-Sugarcane, having the highest effect on stalk dry mass and sucrose yield in 2 of 3 sites that were investigated.

4.2.3 Ideotyping

A hypothetical ideotype with optimal PARCEmax, STKPFMAX and CHUPIBASE values of 7.06 gMJ^{-1}, 0.81 and 700°Cd respectively, produced SDM values ranging from 53 to 59 t ha^{-1}, with a mean SDM of 57 t ha^{-1}. In this example, the simulation results suggest that combining optimal values for these three traits in a single genotype could result in SDM increases of 8 and 12 t ha^{-1}, when compared to the mean SDM values of the highest yielding multiple and single trait variants, respectively. These results suggest that PARCEmax, STKPFMAX and CHUPIBASE could be candidate traits for screening in early plant breeding stages.

The results of the trait impact study suggest that Canegro has the potential for identifying key traits that are most impactful on yield and to identify ideal values for these for a given environment. This information could be used to develop crop ideotypes for target environments and thus guide breeding efforts. However, further work is required to address model weaknesses that have been identified in this study.

5. CONCLUDING DISCUSSION

Canegro (v4.5_C2.0, Singels *et al*. 2016a) trait parameter (TP) values were estimated for 14 genotypes grown in a pot trial conducted at Mount Edgecombe. Values of TPs governing leaf and stalk development were determined directly from monthly measurements of leaf number and size, and of stalk height. TPs that were more challenging to estimate, such as those governing photosynthetic efficiency and biomass partitioning, were estimated from measurements of leaf gas exchange and biomass fractions, respectively, and normalized with respect to the reference genotype NCo376.

Reference stalk elongation rate (SERo) showed the greatest genetic variation (range of 78% of the mean), followed by maximum leaf area (MXLFAREA, 73%), the leaf number at which MXLFAREA occurs (MXLFARNO, 63%) and phyllochron interval 2 (PI2, 52%). Maximum PAR conversion efficiency (PARCEmax) also showed significant variation with a range of 47% of the mean. Phyllochron interval 1 (PI1, 24%), maximum number of green leaves (LFMAX, 23%) and maximum sucrose content (SUCA, 15%) showed less variation, although this was still significant. A range of 17% for the maximum stalk partitioning fraction (STKPFMAX, 17%) was not statistically significant. The range for thermal time required to the start of stalk elongation (CHUPIBASE) was 30%, although this could not be tested for significance.

Significant trait correlations were found between (1) CHUPIBASE and PI1, as could be expected; (2) PARCEmax with PI2, suggesting that genotypes with a faster leaf development rate after leaf 14 had higher PARCEmax values; (3) STKPFMAX and SUCA with SERo, suggesting that genotypes with faster stalk elongation partitioned more biomass to stalks as well as to stalk sucrose; (4) SERo and CHUPIBASE, suggesting that genotypes that commence stalk elongation late, have more rapid stalk elongation later on. These trait correlations need to be confirmed with larger data sets from more experiments.

The model showed some potential for simulating genetic differences observed in field trials using TP values estimated from pot trial data. For example, significant differences in SDM observed in an irrigated field trial in Pongola were reflected well in the simulations of SDM rankings. There were no significant genotype differences in observed SDM in the other field trials, and model simulations also showed small differences in SDM. The model also

simulated SUCM values and rankings well for the Komatipoort_2012 trial. The model was unable to predict canopy development rankings and values observed in field trials. The model seemed to over-emphasize the influence of PI1, and under-estimate the influence of MXLFAREA, on the development of canopy cover. A concern was that the accuracy of simulated canopy cover for the Pongola field trial did not improve when using leaf TP values estimated from data from this trial. In hindsight the Pongola dataset was the only one that was suitable for testing the hypothesis, as it included an adequate number of genotypes with significant genotypic differences in crop growth and development variables. The first hypothesis of "it is possible to accurately simulate genetic differences in crop growth and yield observed in field trials using trait parameter values estimated in a pot trial" was therefore proven to be partially true, with the model being able to predict genotype rankings for SDM observed in the Pongola field trial.

The study provided guidelines for effective phenotyping procedures for estimating TP values. Measurements should be conducted before the onset of flowering. Three measurements of fully expanded leaf number prior to the appearance of leaf number 14 are required for accurate estimation of PI1, followed by three bi-monthly measurements to estimate PI2. One measurement of green leaf number shortly after transplanting, and again after 720 °Cd, is recommended for accurate estimation of LFMAX. Measurements of leaf size and number of all fully expanded green leaves should be recorded shortly after transplanting, and at TT intervals of about 900°Cd thereafter, and up to leaf 39 (if required) for accurate estimation of MXLFAREA and MXLFARNO. SERo could be estimated from measurements of stalk height once every two months. The study recommends measuring stomatal conductance with the leaf porometer between 10:00 and 13:00 to estimate PARCEmax values, and normalization should be performed on replicate data measured within an hour. Values of STKPFMAX and SUCA could be estimated from biomass fractions at harvest, and before the onset of flowering where possible, and then normalized relative to that of a reference genotype.

The trait impact study showed that parameter PARCEmax had the strongest impact on SDM under irrigated conditions, followed by STKPFMAX and CHUPIBASE. Simulated SDM ranged by 79, 40 and 14%, respectively, in response to single TP variation based on observations from the pot trial. The path coefficient analysis of simulated SDM responses to

multiple TP changes also showed that PARCEmax and STKPFMAX were strong determinants of SDM. A hypothetical genotype with optimal values of PARCEmax, STKPFMAX and CHUPIBASE produced a simulated SDM that was 12 t ha^{-1} higher than that of the highest yielding single variant genotype, and 8 t ha^{-1} higher than that of the highest yielding multiple variant genotype.

Results suggest that the Canegro model was not suitable for exploring trait impacts on canopy development. For example, it underestimated genetic variation in FI_{PC} observed in field trials, and it underestimated the influence of MXLFAREA on the canopy development. However, results also show that the Canegro model was able to simulate trait impacts on SDM of irrigated crops, although the influence of PARCEmax was overemphasised. The trait impact study further showed that increasing PARCEmax, CHUPIBASE and STKPFMAX resulted in increased simulated partitioning of biomass to stalks and thus higher SDM, with no consequences to simulated leaf, tiller and root development. This is unlikely to be the case in reality. This weakness limits the model's suitability for trait modelling, as discussed by Jones *et al*. (2011). For these reasons, the second hypothesis of "the Canegro model can be used to identify a set of ideal trait parameter values for a target environment" could not be accepted. The simulation capability of the Canegro model should be improved by linking the simulation of plant growth and developmental processes with the mass balance.

In summary the key findings of this study were:

(1) TP values were determined for a number of genotypes which can be used in future trait modelling studies;
(2) Inter-relationships between some of the TPs were identified. This should be taken into account when generating parameter values for trait impact studies;
(3) Effective phenotyping procedures were formulated for generating data required for TP estimation. In particular the proposed protocol for measuring g_s could be used to develop high-throughput phenotyping technologies in future;
(4) Three traits were identified that were most impactful on SDM under irrigated conditions, namely maximum radiation conversion efficiency, the stalk partitioning fraction, and the thermal time period to start of stalk elongation. Simulations suggest that combining the optimal values for these traits into one genotype could increase

SDM under irrigation beyond the highest yields of genotypes with single optimal values.

(5) The Canegro model in its present form is not suitable for exploring trait impacts on canopy development, a key process in sugarcane yield formation. The canopy development algorithm is too empirical, it is disconnected from the biomass growth and partitioning algorithm and is therefore unable to simulate interactions between these processes.

(6) The knowledge generated in this study will be useful for improving the suitability of the Canegro model for supporting sugarcane breeding, and for developing procedures for screening sugarcane populations for desirable traits.

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APPENDIX A

Pilot pot trial

A pilot pot trial was conducted to optimize trial management and measurement procedures.

Methodology

Trial design

The trial was established at the SASRI traysite, Mount Edgecombe as a randomised design consisting of five genotypes replicated five times (Figure A. 1).

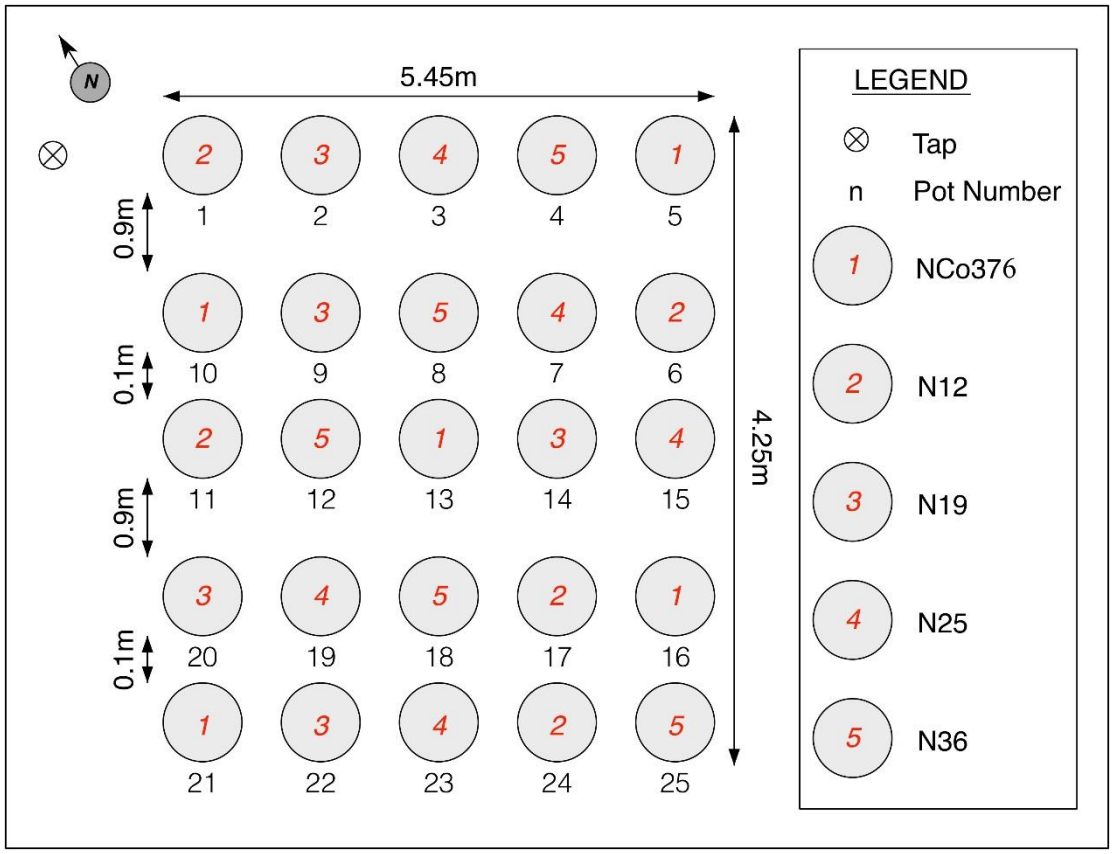


Figure A. 1. Layout of the pilot pot trial.

Trial operations

Single-budded setts were pre-treated with a cold water dip of Benomyl® (Arysta LifeScience, South Africa) at 0.5g l^{-1} for 10 minutes with gentle agitation.

The trial was planted on 10th October 2014, where four setts were planted into each large white bin (80*l* capacity) with coarse gravel stones (5cm depth) in the base to facilitate

drainage, filled with a synthetic medium of river sand, sugarcane filter press and vermiculite (4:2:1). The trial was fertilised according to SASRI recommendations (330 kg ha^{-1} N; 200 kg ha^{-1} P; 720 kg ha^{-1} K). Harvesting was carried out on 30 June 2014.

Measurements

Weather data

Hourly average solar radiation (SRAD, Wm^{-2}), temperature (°C), relative humidity (RH, %), rainfall (mm) and wind speed (m s^{-1}) were recorded from samples taken at ten second intervals using an automatic weather station located at the SASRI rainshelter approximately 150m from the trial site.

Thermal time (TT, °Cd) was calculated as follows:

$$TT = \sum_{d=i}^{n} Max\left(0, \left(\frac{Tmax+Tmin}{2} - T_b\right)\right) \quad \text{(Equation A.1)}$$

where Tmax and Tmin are the daily maximum and minimum temperatures respectively, and Tb is the base temperature, taken as 10°C and 16°C for leaf and stalk development (Singels *et al*. 2016a), respectively.

Crop water status

Irrigation was applied manually with dripper lines. Volumetric soil water content (VWC, m^3 m^{-3}) was measured using two 5TE soil moisture sensors (Decagon Devices, Washington, USA) installed at a depth of 15-20cm in pot numbers 12 (N36) and 16 (NCo376). Sensor output (dielectric permittivity logged at 30-minute intervals with a CR1000 data logger, Campbell Scientific, Inc.) were converted to VWC values with the Topp equation (Topp *et al*. 1980). Field capacity (FC, m^3 m^{-3}) and permanent wilting point (PWP, m^3 m^{-3}) of the synthetic medium were determined by pressure plate technique. Values of VWC were then adjusted by a sensor-specific conversion factor so that the sensor estimated PWP value corresponded with the laboratory-determined PWP value. Stress point (SP, m^3 m^{-3}) was taken as 50% of the difference between FC and PWP.

Leaf and stalk development

One primary shoot in each pot was tagged for weekly leaf measurements. The number of fully expanded leaves were recorded from shortly after planting (October 2013) to harvest (June 2014).

TVD leaf length and maximum width were measured using a tape measure, and a leaf shape coefficient of 0.7 used for the estimation of TVD leaf area (Sinclair *et al*. 2004).

Stalk height was measured weekly on tagged primary shoots from the base of the stalk up to collar of the TVD leaf.

Biomass components at harvest

Destructive sampling of biomass components was carried out on 30th June 2014. In each pot, stalks were cut at the base and total above-ground fresh biomass recorded. Each pot sample was divided into dead leaves (trash), millable stalk, leaf sheath, meristem (tops), green leaf and flower components where appropriate and weighed prior to sub-sampling of each component. Sub-samples were weighed for fresh mass, before being dried in an oven at 80°C to constant mass, and weighed once again for dry weight. These data were used to estimate dry matter content (%), from which the dry mass of biomass components and total above-ground biomass could be inferred. Analyses of stalk composition (fibre, sucrose and non-sucrose contents) were conducted by the SASRI mill-room. A pooled sample of 12 stalks across replicate pots per genotype was shredded in a blender, filtered, and juice samples assessed with a polarimeter and refractometer to determine pol and brix% (Schoonees-Muir *et al*. 2009) respectively. The percentage of stalk material that consisted of fibre, sucrose and non-sucrose (fresh mass basis) was determined according to established methods. Stalk dry mass was calculated as the product of stalk fresh mass and stalk dry matter content, while sucrose content on a dry mass basis was calculated from sucrose content fresh mass basis and stalk dry matter content.

Green leaf area per pot was then calculated by multiplying specific leaf area (SLA, $m^2\,kg^{-1}$) with green leaf fresh mass per pot. SLA was determined for three sub-samples per pot consisting of ten green leaves each, that was weighed and the leaf area recorded using the LI-3000C scanning head coupled to the LI-3050C desktop accessory (LiCor, Lincoln, NE).

Data analysis

Leaf parameters

Variation in PSWITCH introduces unwanted variation in PI1 and PI2 (Table 2.1) due to their interdependency, and thus a constant value of 14 (Inman-Bamber 1994) was assumed for all genotypes. The feasibility of reducing sampling frequency was evaluated by comparing PI1 and PI2 estimates from weekly measurements to fortnightly and monthly measurements where every second data point was removed to generate the reduced datasets. The sampling frequency was determined by evaluating the gradient of the limited experimental dataset, which was required to be within one standard error of the gradient of the full data set.

Reference stalk elongation rate

The feasibility of reducing sampling frequency was evaluated by comparing SERo (Table 2.1) estimates from weekly measurements to monthly measurements. The sampling frequency was determined by evaluating the gradient of the limited experimental dataset, which was required to be within one standard error of the gradient of the full data set.

Results

Weather data

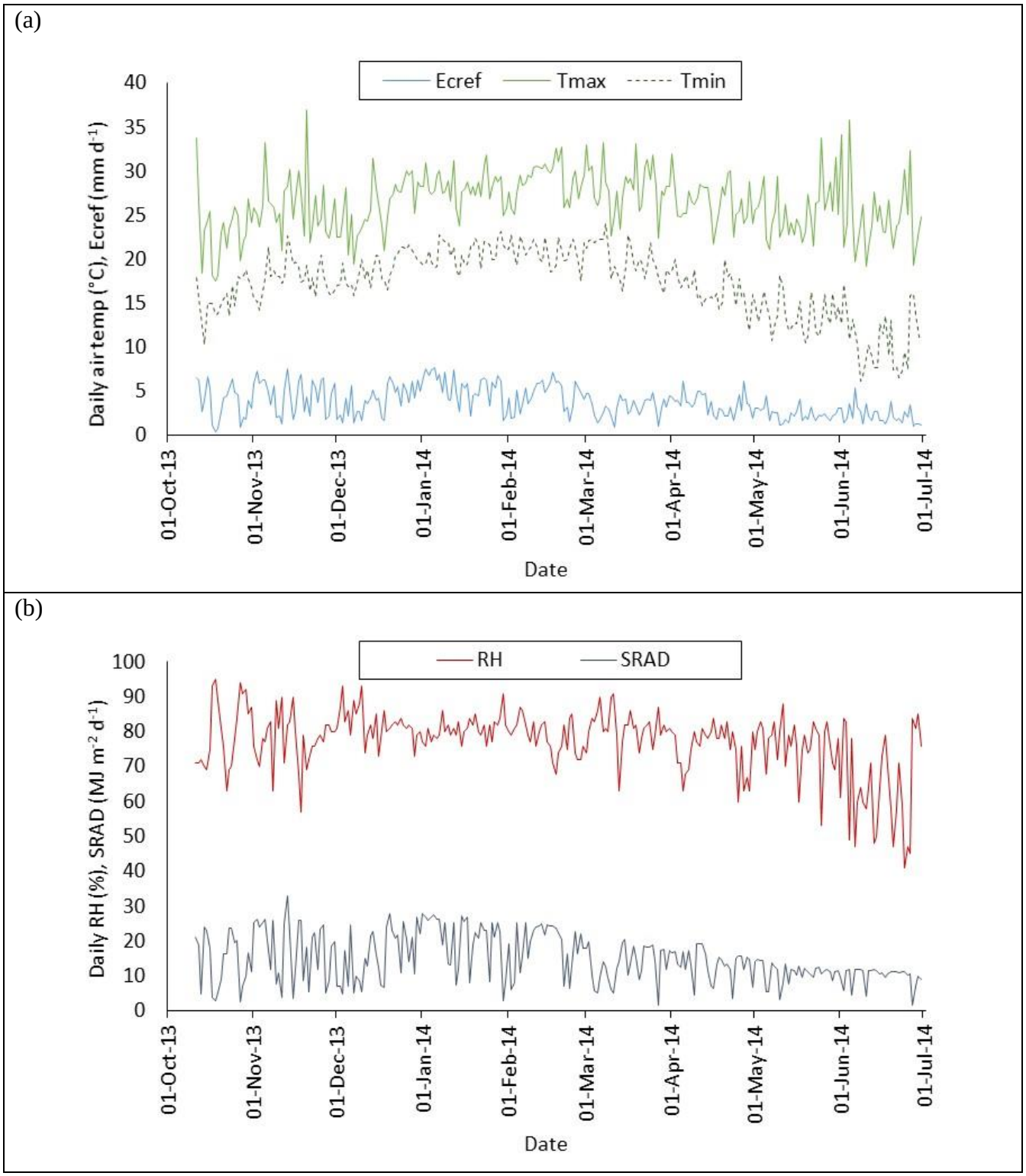


Figure A. 2. Daily maximum (Tmax) and minimum (Tmin) temperature, reference sugarcane evapotranspiration rate (Ecref), relative humidity (RH) and solar radiation (SRAD) at the SASRI traysite for the duration of the experiment.

Crop water status

The moisture content recorded in the trial did not show evidence of water stress, as it remained mostly above the assumed stress point (Figure A. 3). However, signs of severe water stress (leaf yellowing and curling) was observed during the period from December 2013 to January 2014, when irrigation was inadequate. Measuring soil water content in two pots at one depth was therefore inadequate to accurately monitor soil water status. In addition, relatively large irrigation amounts were mostly applied once a day, causing some drainage of the pots. This method of irrigation was therefore deemed insufficient for keeping the crop unstressed.

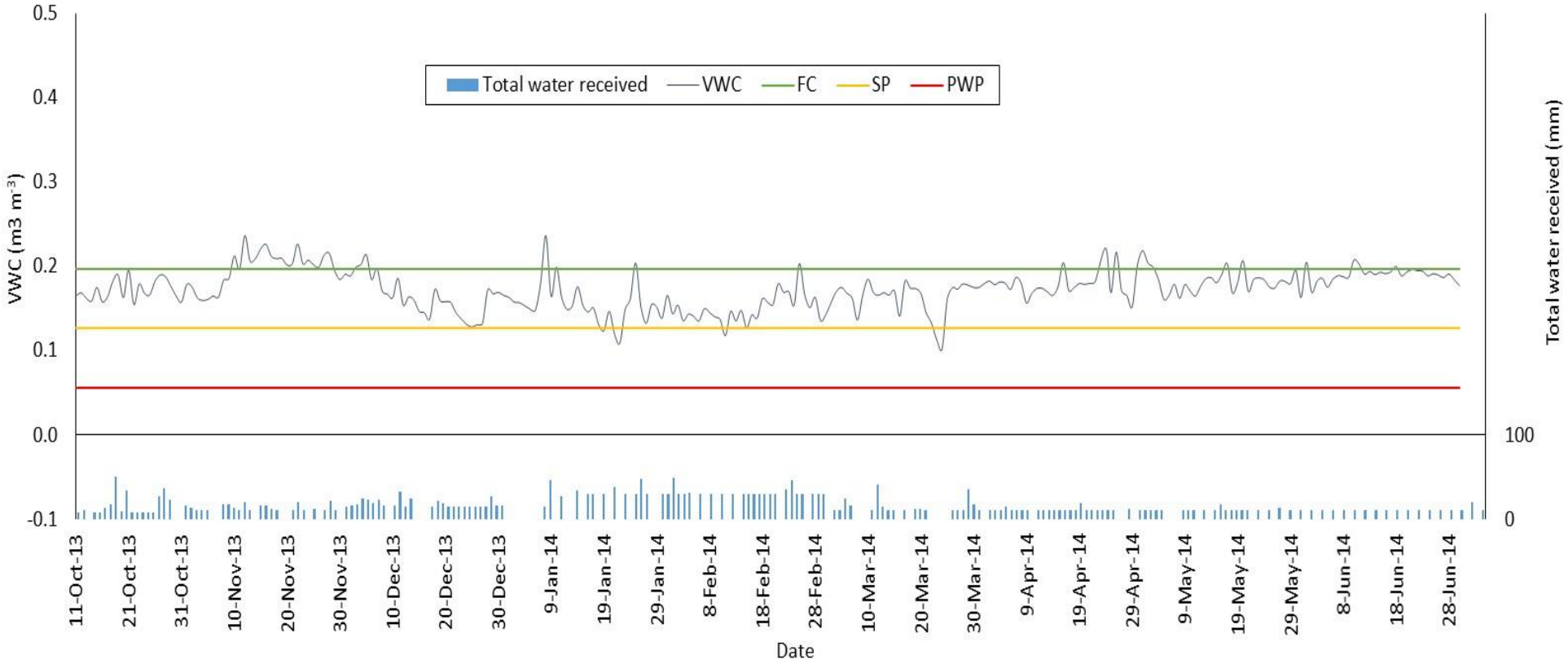


Figure A. 3. Mean volumetric water content (VWC) measured at soil depths of 15-20cm for two sugarcane genotypes (N36 and NCo376). Field capacity (FC), stress point (SP, taken as 50% of the difference between FC and PWP) and permanent wilting point (PWP) of the soil medium are shown. Water received (irrigation plus rainfall, mm), is shown as bars. Daily VWC was taken as the mean value of the two sensors at 12:00am.

Preliminary trait parameter values

Leaf parameters

The PI1 and PI2 values estimated with method 1 differed significantly between genotypes in both cases, with N12 having significantly higher values than all other genotypes at 89°Cd and 130°Cd, respectively (Table A. 1). On the other hand, N25 had significantly lower values compared to the other genotypes at 76°Cd and 96°Cd, respectively. The goodness of fit was found to be highly significant in all cases using method 1.

The PI1 and PI2 values estimated with method 2 were all within one standard error of the respective values estimated with method 1 (Table A. 1). The goodness of fit was found to be highly significant in all cases, and there were highly significant correlations between the PI1 and PI2 values and rankings estimated with methods 1 and 2.

The PI1 values estimated with method 3 were all within one standard error of the values estimated with method 1, except for genotypes N12 and N25 (Table A. 1). The PI2 values were also within one standard error of the values estimated with method 1 with the exception of N19.

Reliable estimates of PI1 and PI2 could therefore be reliably estimated for the majority of genotypes from monthly measurements of leaf number.

Table A. 1. Estimation of phyllochron intervals 1 and 2 (PI and PI2) for five sugarcane genotypes using weekly (method 1), fortnightly (method 2) and monthly (method 3) datasets. Standard error of the estimate is indicated in brackets. Values with common superscripted letters do not differ significantly at p=0.05. The number of observations (n) is indicated. The correlation (r) between the PI2 and PI2 values and ranks calculated with method 1, and those calculated with methods 2 and 3, are shown. The correlations and goodness of fit (R^2) are indicated as significant (*, p<0.05) or highly significant (**, p<0.01).

Genotype	Method 1								Method 2								Method 3							
	PI1 (°Cd)	Rank	R^2	n	PI2 (°Cd)	Rank	R^2	n	PI1 (°Cd)	Rank	R^2	n	PI2 (°Cd)	Rank	R^2	n	PI1 (°Cd)	Rank	R^2	n	PI2 (°Cd)	Rank	R^2	n
N12	88.89^{a} (1.57)	1	0.99**	14	129.80^{a} (2.54)	1	0.99**	19	88.34^{a} (2.35)	1	0.99**	8	130.75^{a} (3.29)	1	0.99**	10	87.27^{a} (5.86)	1	0.99**	4	127.71^{a} (3.94)	1	0.99**	6
N19	79.15^{b} (1.38)	2	0.99**	13	108.12^{b} (2.38)	2	0.99**	20	80.13^{b} (1.86)	2	0.99**	7	107.35^{b} (3.84)	2	0.99**	9	79.23^{a} (2.63)	2	0.99**	4	104.88^{b} (5.74)	3	0.99**	6
N36	78.76^{b} (1.17)	3	0.99**	13	96.12^{c} (1.45)	4	0.99**	20	78.82^{b} (1.98)	3	0.99**	7	95.20^{c} (1.87)	4	0.99**	9	78.49^{a} (2.66)	3	0.99**	4	96.55^{c} (3.94)	4	0.99**	6
NCo376	76.91^{bc} (1.23)	4	0.99**	13	106.96^{b} (2.30)	3	0.99**	20	76.47^{b} (1.97)	4	0.99**	7	105.57^{b} (3.52)	3	0.99**	9	75.94^{a} (3.51)	5	0.99**	4	107.17^{b} (6.27)	2	0.99**	6
N25	75.48^{c} (1.27)	5	0.99**	13	96.06^{c} (2.06)	5	0.99**	20	76.36^{b} (1.82)	5	0.99**	7	94.50^{c} (3.06)	5	0.99**	9	77.45^{a} (1.66)	4	0.99**	4	95.55^{c} (5.31)	5	0.99**	6
r	-	-	-	-	-	-	-	-	0.99**	1**	-	-	0.99**	1**	-	-	0.98**	0.9*	-	-	0.99**	0.9*	-	-

Reference stalk elongation rate

The SERo values estimated with method 1 differed significantly between genotypes and ranged from 1.07 (N12) to 1.43 mm $°Cd^{-1}$ (N36), with N36 being significantly higher than N12 (Table A. 2). The goodness of fit was found to be highly significant for all genotypes.

The SERo values estimated with method 2 differed significantly between genotypes, and were all within one standard error of the values estimated with method 1 (Table A. 2). The goodness of fit was found to be highly significant for all genotypes, as were the correlations between method 1 and method 2 SERo values and rankings.

The SERo values estimated with method 3 differed significantly between genotypes, and were found to be within one standard error of the values estimated with method 1 for all genotypes except N36 (Table A. 2). The goodness of fit was found to be highly significant for all genotypes, and there were highly significant correlations between the SERo values and rankings estimated with methods 1 and 3.

These data suggest that reliable estimates of SERo values can be derived from monthly measurements of stalk height.

Table A. 2. Reference stalk elongation rate (SERo) values for five sugarcane genotypes using weekly (method 1), fortnightly (method 2) and monthly (method 3) datasets. Standard error of the estimate indicated in brackets. Values with common superscripted letters do not differ significantly at p=0.05. The number of observations (n) is indicated. The correlation between the SERo values and ranks calculated with method 1, and those calculated with methods 2 and 3 are shown. The correlations and goodness of fit (R^2) are indicated as significant (*, $p<0.05$) or highly significant (**, $p<0.01$).

Genotype	Method 1				Method 2				Method 3			
	SERo (mm °Cd^{-1})	Rank	R^2	n	SERo (mm °Cd^{-1})	Rank	R^2	n	SERo (mm °Cd^{-1})	Rank	R^2	n
N36	1.43^{a} (0.03)	1	0.98**	35	1.44^{a} (0.05)	1	0.98**	18	1.37^{a} (0.06)	1	0.99**	9
N19	1.28^{a} (0.02)	2	0.99**	35	1.27^{a} (0.03)	2	0.99**	18	1.27^{a} (0.06)	2	0.99**	9
NCo376	1.22^{a} (0.03)	3	0.99**	35	1.22^{a} (0.04)	3	0.99**	18	1.19^{ab} (0.06)	3	0.98**	9
N25	1.11^{ab} (0.02)	4	0.99**	35	1.11^{ab} (0.02)	4	0.99**	18	1.10^{ab} (0.04)	4	0.99**	9
N12	1.07^{b} (0.03)	5	0.98**	35	1.06^{b} (0.04)	5	0.98**	18	1.05^{b} (0.06)	5	0.98**	9
r	-	-	-	-	1**	1**	-	-	0.99**	1**	-	-

Biomass components at harvest

Table A. 3. Fractions of biomass components, cane yield, above-ground dry biomass and leaf area for five sugarcane genotypes measured at harvest. Standard error of the mean value is indicated in brackets.

Biomass fractions	N12	N19	N25	N36	NCo376
Stalk	0.535 (0.014)	0.586 (0.008)	0.499 (0.025)	0.554 (0.004)	0.569 (0.006)
Fibre	0.469	0.453	0.439	0.444	0.480
Sucrose	0.471	0.496	0.481	0.505	0.453
Non-sucrose	0.060	0.051	0.079	0.051	0.068
Meristem	0.004 (0.001)	0.002 (0.000)	0.003 (0.000)	0.005 (0.001)	0.005 (0.002)
Senesced leaf	0.248 (0.015)	0.260 (0.006)	0.339 (0.019)	0.319 (0.010)	0.266 (0.011)
Leaf sheath	0.081 (0.004)	0.058 (0.001)	0.058 (0.003)	0.054 (0.003)	0.067 (0.007)
Green leaf	0.131 (0.005)	0.095 (0.006)	0.102 (0.006)	0.048 (0.003)	0.080 (0.009)
Above-ground dry biomass (kg pot^{-1})	3.74 (0.100)	3.95 (0.164)	3.07 (0.088)	3.74 (0.262)	2.99 (0.284)
Cane yield (kg pot^{-1})	2.00 (0.038)	2.32 (0.128)	1.53 (0.090)	2.08 (0.159)	1.70 (0.161)
Leaf area (m^2 pot^{-1})	5.40 (0.220)	3.40 (0.234)	3.68 (0.265)	1.74 (0.119)	2.20 (0.115)

Recommendations

Several instances of poor germination or germination failure were observed in the first pot trial. In order to ensure uniformity of plants, the study recommends pre-germination of setts prior to planting in the second pot trial.

The soil water content data collected suggested that monitoring soil moisture content in few pots at one depth was insufficient for detecting crop water stress. In addition, irrigating once daily were shown to cause drainage at times. It is therefore recommended that soil water content be monitored in a number of pots with genotypes with diverse canopy development characteristics at two depths (15-20cm and 35-40cm), and that smaller irrigation events be applied several times a day.

The phenotypic data suggest that leaf and stalk parameters can be accurately estimated from monthly measurements, which is a more practical approach for experiments with numerous treatments.

APPENDIX B

Phenotyping pot trial

Weather data

The weather conditions for the duration of the trial are shown in Figure B. 1. Daily maximum (Tmax) and minimum (Tmin) temperatures ranged from 19 to 41°C and from 10 to 23°C, respectively, with a mean daily temperature of 22°C. Sugarcane reference evapotranspiration rate (Ecref) ranged from 0.8 to 9.0 mm d^{-1}, with a daily mean of 3.5 mm d^{-1}. Relative humidity ranged from 32 to 92%, with a daily mean of 76%. Daily solar radiation (SRAD) ranged from 3 to 29 MJ m^{-2} d^{-1}, with a daily mean of 15 MJ m^{-2} d^{-1}.

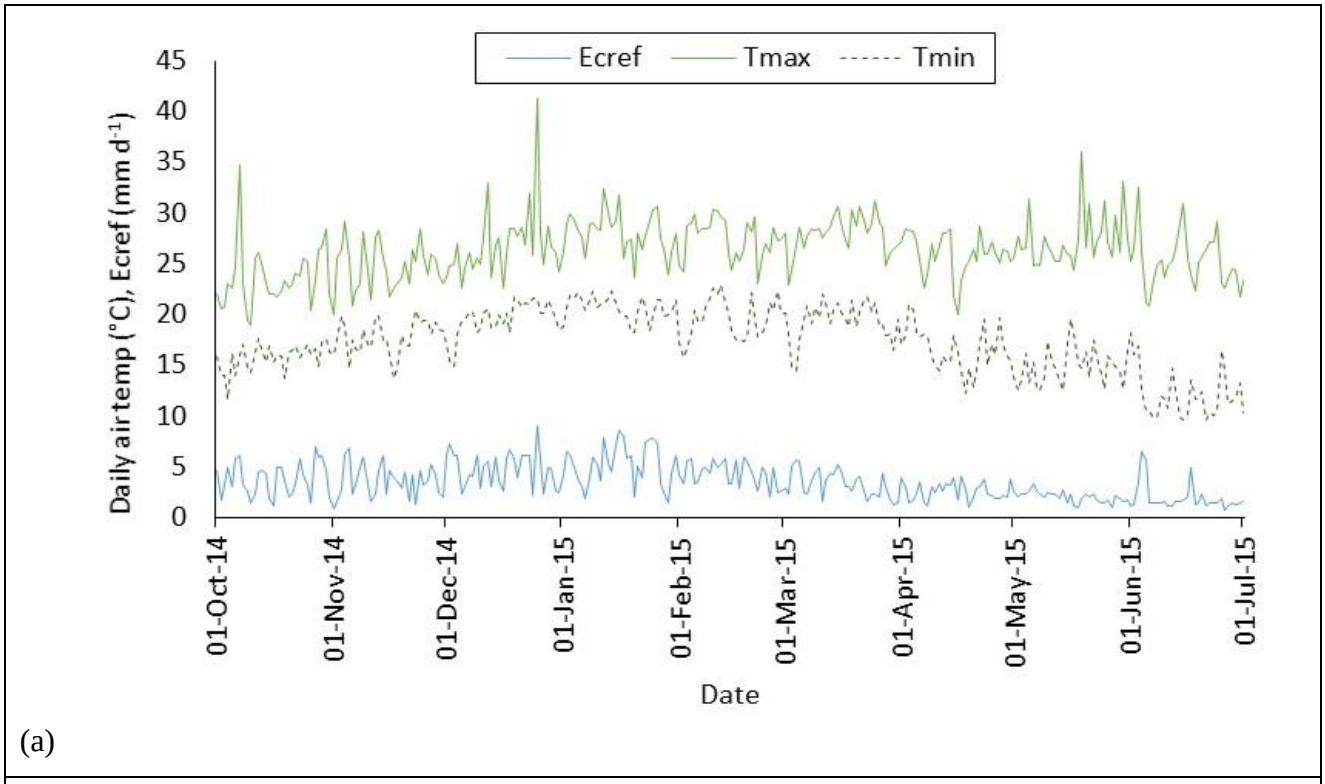
Crop water status

Genotype N12 experienced optimal soil water status for the duration of the trial (Figure B.2). 04G0073 extracted water more aggressively, causing soil water content to drop slightly below stress point during January, March and May. This was largely due to the unforeseen unavailability of water supply at times. Overall, both genotypes experienced well-watered conditions for the majority of the trial.

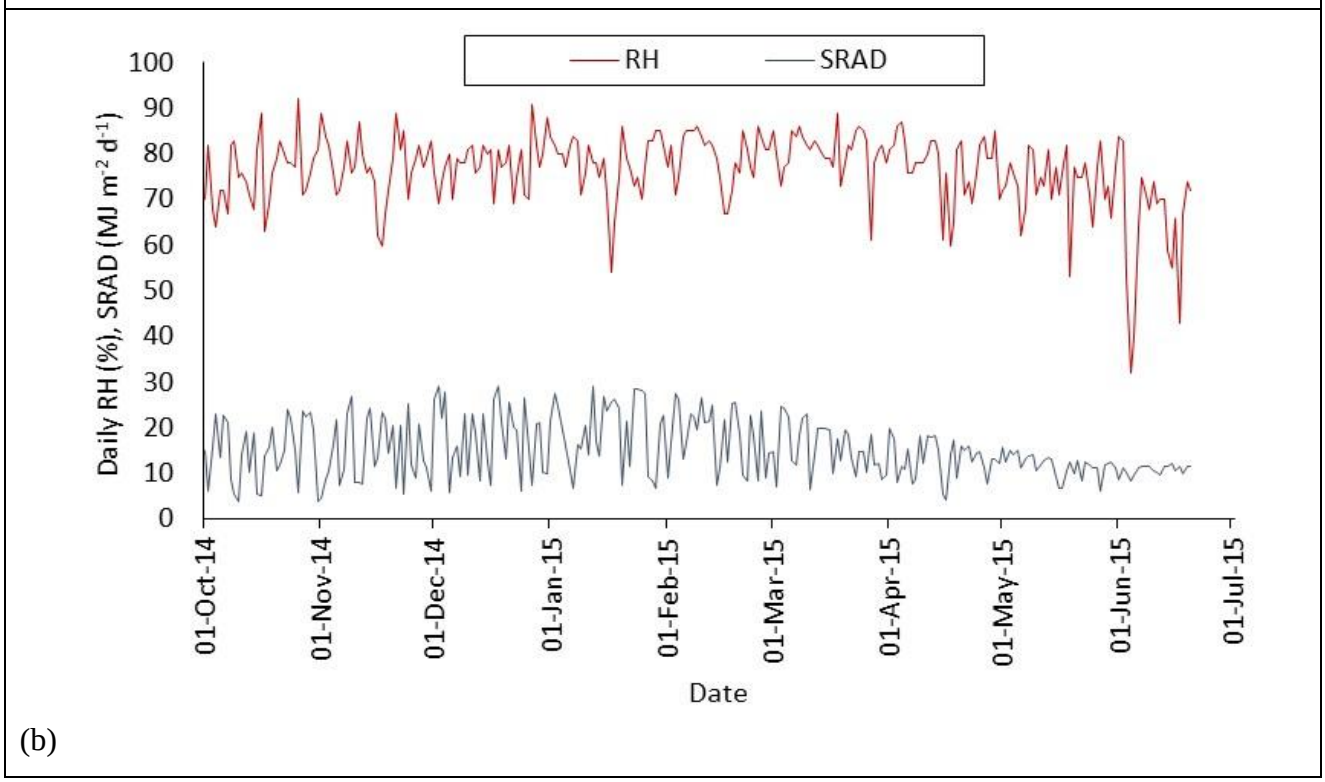
Photosynthetic rate and stomatal conductance

Diurnal experiments

Two experiments were conducted to better understand diurnal variation in A and g_s and to refine measurement protocols. Results are shown in Table B.1.



(a)



(b)

Figure B. 1. Daily maximum (Tmax) and minimum (Tmin) temperature, reference sugarcane evapotranspiration rate (Ecref), relative humidity (RH) and solar radiation (SRAD) recorded at the SASRI rainshelter at Mount Edgecombe.

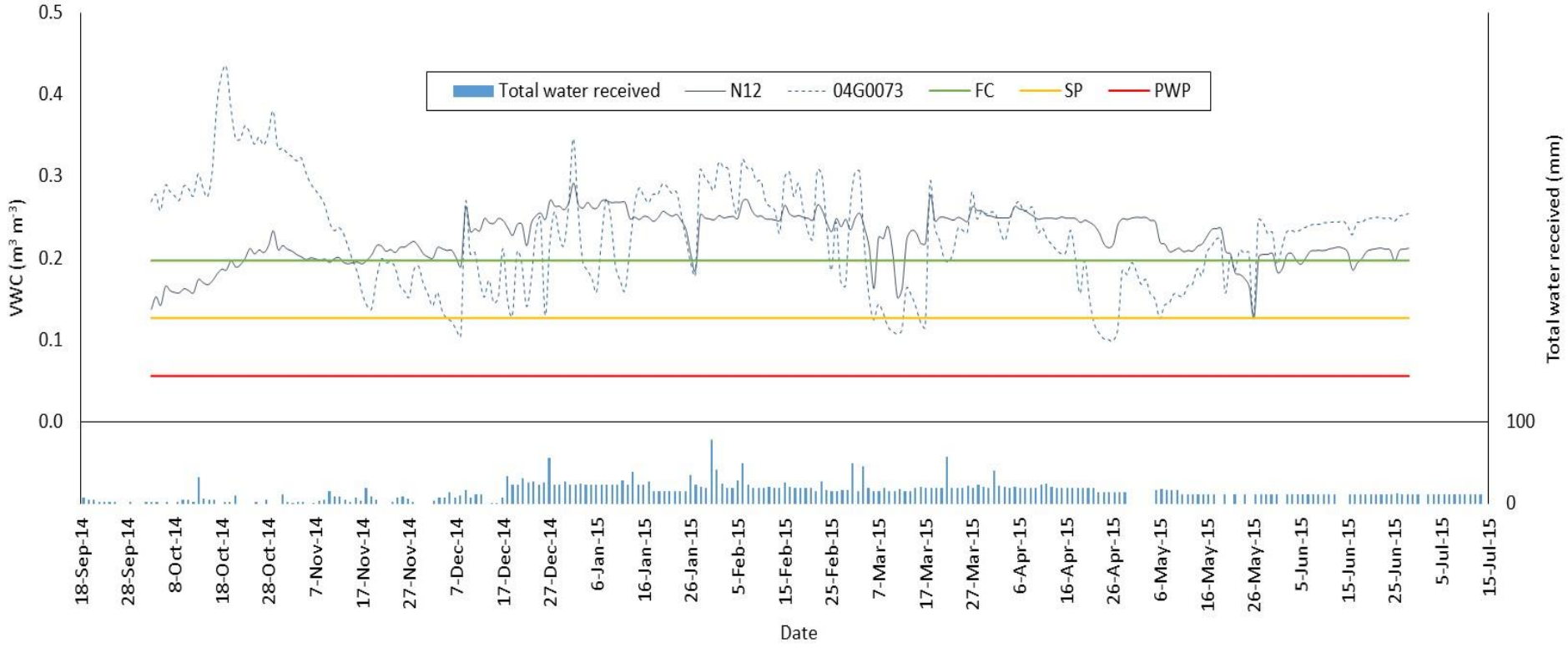


Figure B. 2. Mean volumetric water content (VWC) measured for two contrasting sugarcane genotypes (N12 and 04G0073). Field capacity (FC), permanent wilting point (PWP) and stress point (SP, taken as 50% of the difference between FC and PWP) of the soil medium are shown. Water received (irrigation plus rainfall, mm) is shown as bars. Daily VWC for a given pot was taken as the mean value of the two sensors at soil depths of 15-20cm and 35-40cm at 12:00am.

Table B. 1. Hourly mean photosynthetic rate (*A*), stomatal conductance measured with the LiCor-6400 (g_{sLicor}) and Decagon porometer (g_{sPoro}) and transpiration (*E*), measured in two experiments. Standard error of the mean value is given in brackets. Hourly means of weather data, namely solar radiation (SRAD), temperature (Temp), relative humidity (RH), vapour pressure deficit (VPD) and sugarcane reference evapotranspiration rate (Ecref) are also shown.

	Diurnal experiment 1									Diurnal experiment 2								
	Plant variables				Weather variables					Plant variables				Weather variables				
Hour	*A* (µmol m^{-2} s^{-1})	g_{sLiCor} (mmol m^{-2} s^{-1})	g_{sPoro} (mmol m^{-2} s^{-1})	*E* (mmol m^{-2} s^{-1})	SRAD (W m^{-2})	Temp (°C)	RH (%)	VPD (Pa)	Ecref (µm h^{-1})	*A* (µmol m^{-2} s^{-1})	g_{sLiCor} (mmol m^{-2} s^{-1})	g_{sPoro} (mmol m^{-2} s^{-1})	*E* (mmol m^{-2} s^{-1})	SRAD (W m^{-2})	Temp (°C)	RH (%)	VPD (Pa)	Ecref (µm h^{-1})
8-9	20.2 (1.12)	260.0 (16.1)	340.9 (32.1)	2.1 (0.08)	412.1	22.2	66.4	902	221	10.3 (0.59)	309.3 (29.1)	273.5 (14.3)	1.9 (0.08)	413.8	20.3	74.7	606	390
9-10	22.7 (0.96)	249.5 (13.8)	367.3 (26.1)	2.4 (0.10)	613.8	23.9	60.4	1177	455	19.7 (1.05)	224.8 (15.3)	376.8 (22.9)	2.5 (0.13)	602.6	22.0	68.2	845	547
10-11	23.8 (0.98)	201.5 (12.1)	444.3 (29.2)	2.2 (0.08)	773.5	25.0	59.4	1287	602	-	-	-	-	772.5	24.0	63.4	1092	679
11-12	-	-	-	-	876.5	25.0	60.0	1264	713	20.6 (1.67)	202.6 (15.4)	374.0 (21.6)	3.1 (0.28)	873.0	25.3	64.5	1144	769
12-13	25.1 (1.19)	217.4 (14.6)	374.2 (25.1)	2.9 (0.13)	918.0	25.4	60.3	1286	779	19.4 (1.74)	209.1 (23.9)	368.4 (32.1)	4.2 (0.31)	911.0	25.9	62.8	1244	799
13-14	23.5 (1.18)	213.9 (17.2)	369.5 (33.2)	2.6 (0.13)	891.0	25.5	63.0	1203	777	17.7 (1.33)	193.7 (15.0)	334.7 (34.3)	4.2 (0.29)	880.5	26.4	61.0	1345	774
14-15	20.7 (1.28)	169.9 (14.1)	312.2 (18.8)	2.4 (0.21)	815.5	25.6	63.0	1218	744	19.3 (0.88)	211.3 (11.2)	322.1 (27.2)	4.1 (0.18)	794.0	26.5	61.1	1351	679
15-16	18.4 (1.81)	159.5 (14.5)	335.6 (17.0)	2.1 (0.21)	667.3	25.8	65.5	1146	665	16.0 (0.91)	164.0 (9.2)	306.2 (15.0)	2.2 (0.28)	645.2	26.1	67.7	1088	524
16-17	15.2 (1.21)	112.5 (8.4)	268.9 (18.8)	1.6 (0.10)	492.7	25.9	65.8	1145	547	10.8 (0.59)	148.8 (14.6)	287.1 (17.1)	1.3 (0.08)	451.8	25.9	69.8	1011	354