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TRAIT PARAMETER ESTIMATION AND IDEOTYPING WITH CANEGRO

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OBJECTIVES

Overall goal: evaluate the suitability of the Canegro model to support sugarcane breeding by predicting the impacts of genetic traits on yield

1. Determine **TP values** for selected genotypes in a pot trial

2. Determine accuracy of simulated **genotypic differences** in **canopy cover, stalk dry mass (SDM)** and **sucrose yield** for selected genotypes grown in irrigated field trials using the TP values estimated from pot trial data

3. Develop a **phenotyping protocol** for estimating TP values

4. Determine **trait impacts** on simulated stalk dry mass for a selected irrigated environment

5. Identify a set of **ideal trait values** for a selected irrigated environment

METHODOLOGY
-TRIAL DESIGN & MEASUREMENTS

- SASRI rainshelter, Mount Edgecombe, KZN

- Fully irrigated & fertilised pot trial (October 2014 – June 2015)

- Complete randomised block design: Five replications of 14 genotypes

- Leaf development (Monthly leaf counts & TVD leaf size on primary stalks)

- Stalk development (Monthly TVD plant height on primary stalks)

- Plant physiology (Stomatal conductance, photosynthesis in 2 experiments)

- Biomass (Stalk dry mass, total dry biomass and sucrose content at harvest)

METHODOLOGY -TP ESTIMATION

Leaf development

Parameter Name	Description	Estimation
PI1 PI2	TT taken between successive green leaves (°Cd)	
LFMAX	Maximum number of fully expanded green leaves per primary stalk	Maximum green leaf number
MXLFAREA	Leaf area of the largest fully expanded leaf (cm^2)	TVD leaf length and width multiplied by shape factor (0.7); Mean of the area of the biggest leaf in each pot
MXLFARNO	Leaf number at which MXLFAREA occurs	Corresponding mean leaf no. of MXLFAREA

METHODOLOGY -TP ESTIMATION

| Leaf development | → | Stalk development |
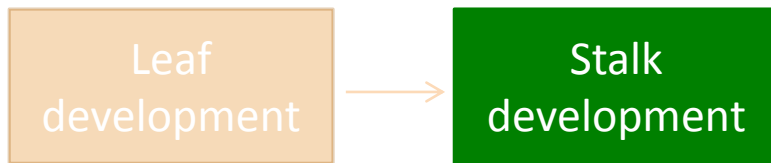
Parameter	Description	Estimation
CHUPIBASE	TT from shoot emergence to the start of stalk elongation (°Cd)	TT taken from shoot emergence to the appearance of leaf no. 10
SERo	Reference stalk elongation rate per unit TT (cm °Cd^{-1})	Gradient of the linear regression of TVD height against TT

METHODOLOGY -TP ESTIMATION

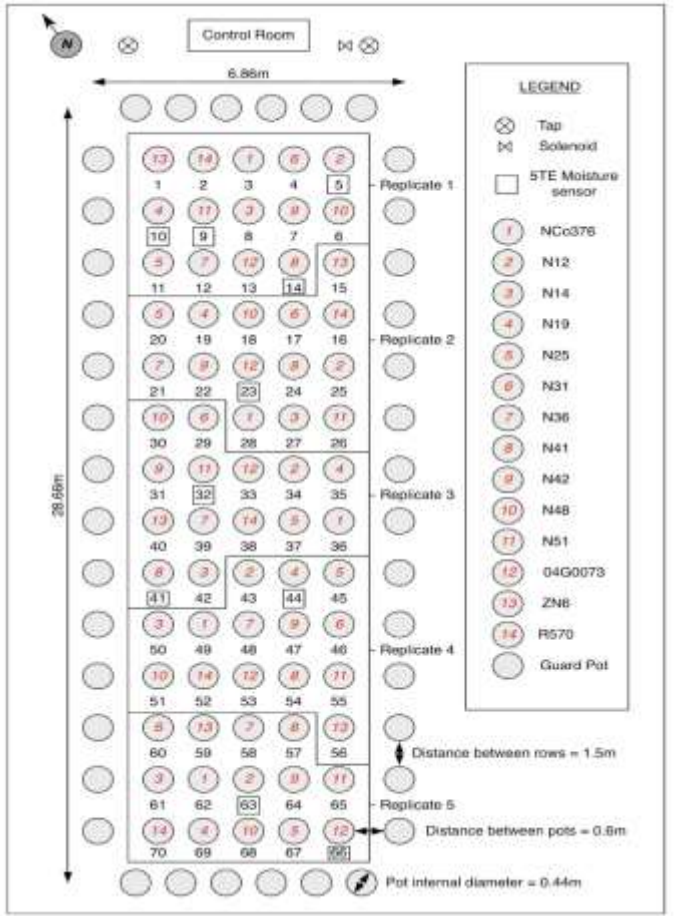| Leaf development	Stalk development	Radiation interception

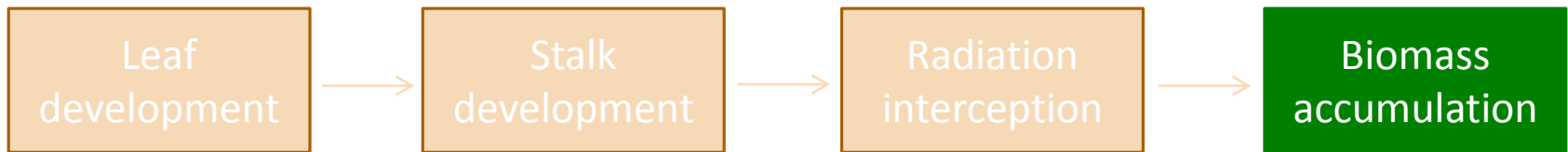| Parameter	Estimation
PARCEmax	Measure stomatal conductance (g_s) and photosynthetic rate (A) with LiCor-6400 portable photosynthesis system and Decagon porometer Hourly measurements from 10am-1pm Two experiments (Five measuring days) Express g_s and A relative to NCo376 ANOVA to combine relative data Multiply normalized A by a field-calibrated value for NCo376 (5.7gMJ^{-1})

METHODOLOGY -TP ESTIMATION

Leaf development	→	Stalk development	→	Radiation interception	→	Biomass accumulation

| Parameter	Definition	Estimation
STKPFMAX	Stalk partitioning coefficient: maximum fraction of aerial dry biomass growth partitioned to stalks ($t\ t^{-1}$)	Normalized stalk dry mass fraction of aerial dry biomass, scaled using a field-calibrated value for NCo376 ($0.7\ t\ t^{-1}$)
SUCA	Sucrose partitioning coefficient: sucrose content in the bottom of a mature stalk ($t\ t^{-1}$)	Normalized sucrose fraction of stalk dry mass, scaled using a field-calibrated value for NCo376 ($0.56\ t\ t^{-1}$)

2. Determine accuracy of simulated genotypic differences in canopy cover, stalk dry mass (SDM) and sucrose yield for selected genotypes grown in irrigated field trials using the TP values estimated from pot trial data

METHODOLOGY -EVALUATION

Parameter	Definition
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})
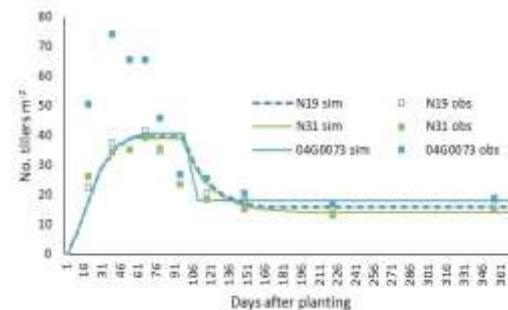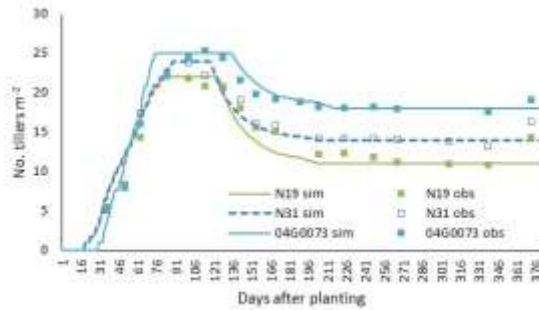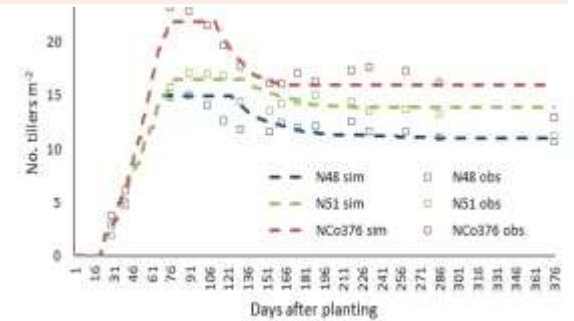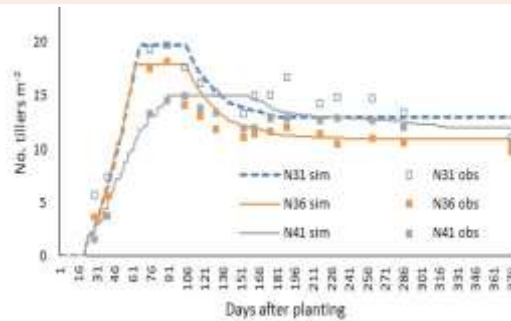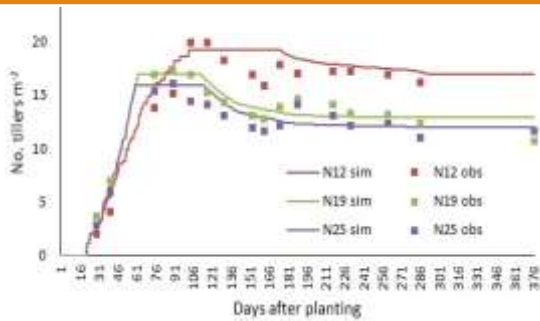
4. Determine trait impacts on simulated stalk dry mass for a selected irrigated environment

Single trait variants: assess the impact of a single trait when all other TP values were identical

Trait parameter	Trait value				
	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)	2.52	3.66	4.79	5.93	7.06
STKPFMAX (t/t)	0.57	0.63	0.69	0.75	0.81
SUCA (t/t[-])	0.50	0.55	0.59	0.64	0.68

Multiple trait variants: assess the combined effects of multiple TPs
- Thirty-two hypothetical genotypes were defined, differing with respect to six TPs
- TP values generated using LP-TAU design in GEM-SA package; Same range of values

Trait impacts: Assess changes in mean (over 30 seasons) SDM
- Max, min, range of mean SDM values; Range % of baseline SDM; Probability distribution
- Path coefficient analysis

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5. Identify a set of ideal trait values for a selected irrigated environment

Ideotyping: Investigate potential SDM gains by simulating a genotype with optimal TP values
- Three most impactful traits (CHUPIBASE, PARCEmax and STKFPMAX)
- Compare mean SDM of ideotype to mean SDM yield of the highest-yielding multiple and single trait variants

Model configuration:
- Simulate crop growth in Pongola
- Two 12 month crops (April and October) from 1980 to 2009 (30 years).
- Well-watered conditions (application of 40mm applied when the soil water content of the top 50 cm reached 60% of field capacity).

- **Significant differences between all TPs** that could be statistically analysed except STKPFMAX

- SERo showed the greatest genetic variation (78%), MXLFAREA (73%), MXLFARNO (63%) and PI2 (52%), PARCEmax (47%), CHUPIBASE (30%), PI1 (24%), LFMAX (23%), STKPFMAX (17%) and SUCA (15%)

- **Significant inter-trait correlations:** PI2 and PARCEmax (r=-0.71**); SUCA and STKPF (0.76**); SERo with STKPFMAX (0.84**), SUCA (0.80**) and CHUPIBASE (0.60*)

- Values of PARCEmax derived from *A* and from g_{sporo} were highly correlated (r=0.79**), as were genotype rankings (r=0.69**)

- PARCEmax values derived from *A* had a better correlation with observed SDM (r=0.66) in Pongola than that of PARCEmax values derived from g_{sporo}* (r=0.30)

RESULTS
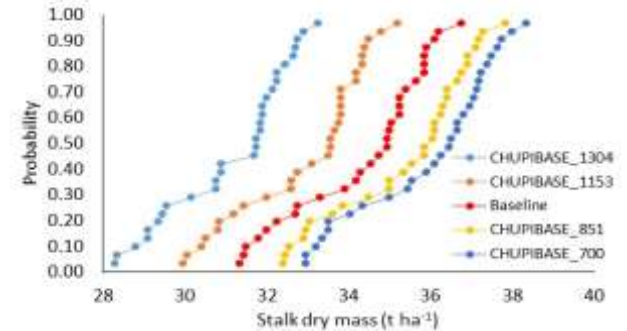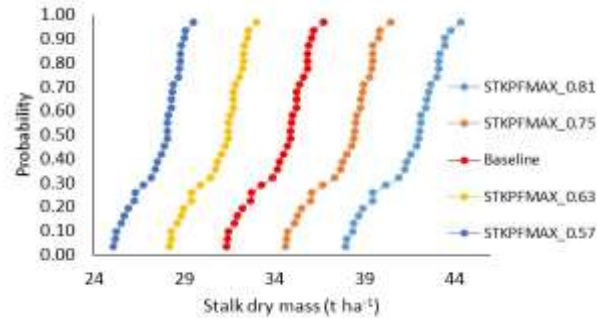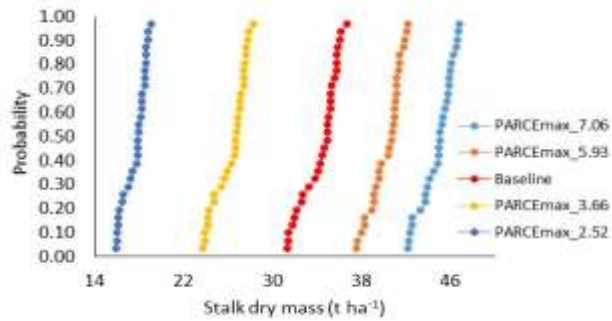# -EVALUATION

- Model showed some potential for simulating genetic differences observed in field trials using TP values estimated from pot trial data
  - Significant differences in observed SDM in Pongola reflected well in simulated SDM rankings (r=0.75*)
  - No significant differences in observed SDM at other sites, and model simulations also showed small differences in SDM
  - Observed genotypic differences in SUCM values and rankings predicted well for Komatipoort_2012

- Model less successful in other respects
  - Unable to predict observed FI_{PC} rankings and values at all sites
  - Over-emphasize PI1, underestimate MXLFAREA
  - Model is unsuitable for exploring trait impacts on canopy yield (empirical; disconnected from biomass growth)
  - Underestimated SDM values for all trials
  - SUCM values and rankings also not predicted well in Pongola and Komatipoort_2011

RESULTS -TRAIT IMPACTS

- Model able to simulate trait impacts on SDM of irrigated crops

- PARCEmax, STKPFMAX and CHUPIBASE were most impactful in single trait variant study
  - Simulated SDM correlated best with PARCEmax (r=0.89**) and STKPFMAX (r=0.41*) in multiple trait variant study;
  - Path coefficient analysis confirmed importance of PARCEmax (0.88) and STKPFMAX (0.40)

- Increasing these TPs increased SDM with no consequences to leaf, tiller & root development

- Ideotype with optimal values (7.06 g/MJ, 0.81 and 700°Cd) increased SDM by 8 and 12 t/ha when compared to the mean SDM values of the highest yielding multiple and single trait variants

FUTURE WORK

- Study identified a possible approach for trait impact studies and ideotyping in future

- Results will be used to:
  - improve Canegro
  - develop procedures for screening populations using HTP, particularly for measuring g_s

- Ph.D. study: "High-throughput phenotyping to assist breeding for drought tolerant sugarcane"
  - Overall aim: develop a HTP protocol for screening early-stage breeding populations for drought tolerance

- Objectives:
  1. Develop a proximal sensing of canopy reflectance procedure for estimating g_c, LAI and g_s
  2. Determine the impacts of traits on yield under well-watered and dry conditions
  3. Determine the heritability of traits
  4. Evaluate the benefit of implementing HTP as a screening procedure in the SASRI breeding program

FUTURE WORK

- Develop correlations between reflectance indices and trait values (Ground-truthing)
  - Pilot trial at SASRI rainshelter
  - Maximum of 2 genotypes will be grown under well-watered and water-deficit conditions

- Measurements will include:
  - Sap flow rate with heat dissipation monitoring
  - Chlorophyll and N content
  - Leaf-level photosynthesis, transpiration, g_s and chlorophyll fluorescence
  - LAI and canopy cover
  - Canopy reflectance with DJI Phantom 4 and Parrot Sequoia in the visual (RGB), near infra-red and thermal bands
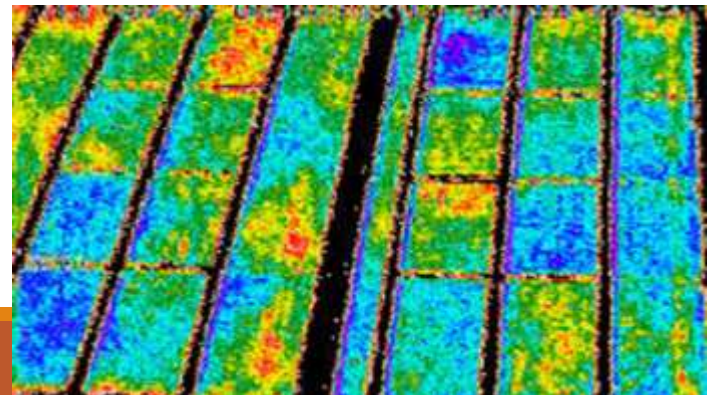
FUTURE WORK

- Estimate trait values for a large number of genotypes with unmanned aerial vehicle (UAV) based HTP
  - refine HTP procedures; genetic variability and impacts of traits on yield
  - Field trial - shallow soil near Komatipoort.

- Measurements will include:
  - Canopy reflectance and emittance; Crop height, yield

- Implement HTP procedure in early-stage plant breeding trial in a dry environment (approximately 35000 genotypes)
  - Genetic variation present in the existing trial and used to elucidate breeding potential of parents for use in subsequent crosses.

ACKNOWLEDGEMENTS

RESULTS -TP VALUES

- **Significant differences between all TPs** that could be statistically analysed except STKPFMAX
- **Significant inter-trait correlations:** PI2 and PARCEmax (r=-0.71**); SUCA and STKPF (0.76**); SERo with STKPFMAX (0.84**), SUCA (0.80**) and CHUPIBASE (0.60*)

Genotype	Leaf development					Stalk development		Photosynthetic efficiency	Biomass partitioning	
	$PI1^a$ (°Cd)	$PI2^a$ (°Cd)	LFMAX	MXLFAREA (cm²)	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

RESULTS -EVALUATION (PONGOLA)

Genotype	FI$_{PC}$ (%)				SDM (t/ha)				SUCM (t/ha)			
	Observed		Simulated		Observed		Simulated		Observed		Simulated	
	Value	Rank	Value	Rank	Value	Rank	Value	Rank	Value	Rank	Value	Rank
PONGOLA 2011												
N12												
N19												
N25												
N31												
N36												
N41												
N48												
N51												
NCo376												
Mean												
Range												
L.S.D.												
RMSE												
r												