The development, validation and implementation of a drought stress index for the evaluation of the drought tolerance potential of South African sugarcane

by

Chandani Sewpersad

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Study Leader: Willem C. Botes

Co-Study Leader: Dr. Riekert van Heerden

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Declaration

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Abstract

In the rainfed areas of the South African sugar industry the unpredictability of rainfall is of major concern for producers. Currently, research into the drought tolerance of South African sugarcane varieties is very limited. Knowledge of varietal drought tolerance potential would allow for more informed decision making when it comes to planting a crop that stays in the ground for between five and fifteen years. The aim of this study was to ascertain the drought tolerance potential of commercial sugarcane varieties using historical field trial data by employing statistical modelling. The first step was to establish a reliable methodology of quantifying the level of drought stress, defined through a drought stress index (DSI), employing the sugarcane growth modelling software Canesim. The second step was to use the selected DSI to evaluate and rate the drought tolerance potential of commercial varieties.

Of the six DSI's calculated, the index comprising a ratio of Canesim simulated rainfed yield (representative of a water stressed environment) to Canesim simulated irrigated yield (representative of a water unstressed environment) was the best at quantifyingthe level of trial drought stress. Using three varieties with previously identified drought potential, two intermediate susceptible (IS) and one intermediate (I) variety, this was the only DSI that was able to quantify all the differences between the varieties.

Using the selected DSI, two different methodologies were used to evaluate varietal drought tolerance potential: General linear regression and Residual maximum likelihood meta-analysis. The regression method proved to be a better method of varietal rating when using historical field data. The two rainfed regions, coastal and midlands were analyzed separately due to the difference in climatic conditions. Using the regression analysis, with N12 as the observed intermediate reference variety, coastal varieties were rated as being susceptible (N16, N19, N39 and NCO376) or intermediate (N27, N29, N33, N36, N41, N45, N47). Rating of the midlands varieties, with both statistical methods, were unsuccessful.

Opsomming

Binne die droëland produksiegebied van die Suid-Afrikaanse suikerindustrie is die wisselvalligheid van reënval ʼn groot bron van kommer vir produsente. Navorsingsresultate aangaande die droogtetoleransie van Suid-Afrikaanse suikerrietvariëteite is baie beperk. Aangesien suikerriet aanplantings vir vyf tot vyftien jaar in produksie mag bly, is kennis aangaande droogtetoleransie noodsaaklik vir ingeligte besluite rondom variëteit keuse. Die doel van hierdie studie was om die droogtetoleransie van kommersiële variëteite met behulp van historiese veldproef resultate en statistiese modellering te bepaal. Die eerste stap was die ontwikkeling van betroubare metodiek wat die graad van droogtestremming kwantifiseer deur middel van droogtestremmingsindekse (DSI's) wat met die suikerriet produksiemodel, Canesim, bereken is. Die tweede stap was om die DSI's te gebruik om geselekteerde kommersiële variëteite vir droogtetoleransie te evalueer en volgens toleransie te rangskik.

Van die ses DSI's wat geëvalueer is, was die indeks wat die verhouding tussen Canesim gesimuleerde droëland opbrengs (verteenwoordigend van ʼn omgewing met droogte) en Canesim gesimuleerde besproeide opbrengs (verteenwoordigend van ʼn omgewing sonder droogte) omskryf het, die mees effektiefste om die graad van droogtestremming te kwantifiseer. Hierdie DSI was vervolgens die enigste wat verskille in droogtetoleransie tussen drie variëteite van bekende droogte toleransie kon kwantifiseer.

Deur gebruik van hierdie DSI is twee verskillende metodes aangewend om die droogtetoleransie van variëteite te evalueer naamlik: Algemene Lineêre Regressie en Residuele Maksimum Aanneemlikheid. Die regressiemetode was die mees effektiefste om variëteite volgens droogtetoleransie, op grond van historiese veldproef resultate, te rangskik. Die twee droëland produksiegebiede, naamlik die kusstrook en Natalse Middellande is afsonderlik geanaliseer as gevolg van klimaatsverskille. Met behulp van die regressiemetode is die kus-variëteite as droogtesensitief of -intermediêr geklassifiseer, met N27, N29, N33, N36, N41, N45 en N47 as droogte-intermediêr en N16, N19, N39 en NCO376 as droogtesensitief. Soortgelyke klassifisering van die variëteite

wat in die Natalse Middellande verbou word was nie met enige van die statistiese metodes suksesvol gewees nie.

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The title page of this thesis contains only my name, which is very deceptive, because there are so many people that have contributed to the creation of this thesis:

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Abbreviations

%	Percentage
°S	Degree South
°Cd	Degree Day
°C	Degree Celsius
AET	Actual Evapotranspiration
AMMI	Additive Main Effects and Multiplicative Interaction
ANOVA	Analysis of Variance
AWC	Available Water Capacity
AWS	Automatic Weather Station
DSI	Drought Stress Index
df	Degrees of Freedom
ERD	Effective Rooting Depth
ET	Evapotranspiration
FC	Field Capacity
FP	Formative Phase
FTSW	Fraction of Transpirable Soil Water
g	Gram
G x E	Genotype x Environment Interaction
GGP	Grand Growth Phase
GGRP	Grand Growth and Ripening Phase
GGE	Genotype Main Effect (G) plus Genotype by Environment (GE) Interaction
GPS	Global Positioning System
ha	Hectares

I	Intermediate Variety
IS	Intermediate Susceptible Variety
ISWC	Initial Soil Water Content
kg	Kilogram
LER	Leaf Extension Rate
LWP	Leaf Water Potential
mm/m	Millimetres per Meter
mm	Millimetre
m	Meter
m_1	Regression Gradient Coefficient
MWS	Manual Weather Station
n	Number of Observations
PCA	Principal Components Analysis
PET	Potential Evapotranspiration
PWP	Permanent Wilting Point
r	Correlation Coefficient
R^2	Percentage Variation Accounted for by Regression Equation
REML	Residual Maximum Likelihood
RUE	Radiation Use Efficiency
S	Susceptible Variety
SASRI	South African Sugarcane Research Institute
SE	Standard Error
SER	Stalk Elongation Rate
SSI	Stress Susceptibility Index

STI	Stress Tolerance Index
SWC	Soil Water Content
sp.	Species
SWSI	Seasonal Water Stress Index (SWSI)
T	Drought Tolerant Variety
TAM	Total Available Moisture
TCH	Tonnes Cane per Hectare
TSH	Tonnes Sucrose per Hectare
TT	thermal time
vs.	Versus
VT1	Primary Variety Trial
VT2	Secondary Variety Trial
YSI	Yield Stability Index
Ψ	Water Potential

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Language and style used in this thesis are in accordance with the requirements of the South African Journal of Plant and Soil. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetitions between chapters have, therefore, been unavoidable.

Chapter 1

Introduction

Chapter 1: Introduction

Sugarcane (*Saccharum* sp.) is the second largest South African field crop by gross value, surpassed only by maize. The South African sugar industry is responsible for generating an average direct income of R8 billion. Approximately 1 million people, more than 2% of the South African population, depend on the industry for a living. There are approxiamtely 29 130 registered sugarcane growers that produce an estimated average of 2.2 million tons of sugar per season. Of this, approximately 0.7 million tons is exported to markets in Africa, Asia and the Middle East. The South African sugar industry therefore makes a very important contribution to the national economy (SASA, 2011/2012).

For any crop to obtain maximum yield, water is essential during its vegetative growth. Sugarcane is a high yielding biomass crop thereby requiring substantial amounts of water to sustain optimal development (Zingaretti *et al*., 2012). Drought is one of the major abiotic stresses that can affect sugarcane productivity worldwide (Venkataramana *et al*., 1986). Drought stress affects the growth and physiological processes in sugarcane which can lead to the yield and quality being significantly affected (Wiedenfeld, 1995; Nyati, 1996; Qing *et al*., 2001). Therefore, the ability of a plant to maintain photosynthesis under conditions of drought stress is an indication of potential drought tolerance (Silva *et al*., 2007).

The level of drought stress experienced by a plant can be evaluated by growth analysis and plant productivity under stressed conditions (Silva *et al*., 2007). Some varieties tolerate stress more effectively than others, but there is a range of different drought tolerance mechanisms that a plant can use under water limiting conditions (Qing *et al*., 2001). Different varieties can show the same phenotype (drought tolerance) due to very different physiological mechanisms. In addition, drought tolerance can also vary according to the age of the plant, water use efficiency and the severity of stress. All these factors make drought tolerance a very complex process to study (Blum, 1996).

Variety choice is an important part of a farmer’s risk-management strategy. This is especially true with sugarcane, as the same crop may be in the ground for five to fifteen years, and annual replanting with different varieties is not a cost effective risk management option (Inman-Bamber, 1994). The effects of climate change have resulted in periods of drought stress becoming more frequent and unpredictable, and this can be a major limiting factor to the growth of sugarcane in rainfed areas (Inman-Bamber *et al*., 2005; Bezuidenhout and Schulze, 2006; Koonjah *et al*., 2006; Silva *et al*., 2008).

The amount of scientific research on drought tolerance of South African sugarcane varieties is very limited. Although there is merit in conducting complex, measurement intensive experiments, the aim of this study was to evaluate the general yield response of commercial varieties to drought stress, using historical field trial data.

The study was divided into two parts. In part one, a pilot study was conducted to establish a methodology to quantify the amount of drought stress experienced by historical field trials, a drought stress index (DSI). This was done by:

- Using different definitions to calculate 6 DSI's;
- Using only varieties with observed* differences in drought tolerance, to evaluate the suitability of the different DSI's (*from anecdotal evidence, as this was the only information available on varietal yield performance when subjected to drought stress); and
- Identifying the DSI that was able to most accurately capture the amount of drought stress experienced by a sugarcane crop.

The second part of the study involved the evaluation of all commercial varieties, with both observed and unknown drought tolerance. This was done by:

- Creating a varietal database for the two rainfed regions in the South African sugarcane industry, coastal and midlands (inland);
- Using the DSI, identified in part one of the study, to quantify the amount of stress experienced during each trial and to evaluate the corresponding varietal yield responses; and
- Rating the different varieties based on their yield response to drought stress.

The results of this study will be useful in classifying the drought tolerance potential of the commercial varieties, and this information can be provided to the sugarcane farmers to facilitate more informed choices in variety selection. In addition, the varieties with differential responses to drought stress can be used for more detailed analysis in future designed experiments.

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Chapter 2

Literature Review

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1. Sugarcane Biology

The sugarcane plant (*Saccharum* sp.) can simplistically be divided into three parts: stalks, leaves and a root system.

1.1 Stalks

The sugarcane plant is made up of a number of unbranched stalks that are tall and cylindrical in shape. The stalk is the most important part of the plant to a sugarcane farmer as it is the site of sucrose storage. Each stalk is made up of nodes and internodes (Figure 2.1).

The nodes are the ring-like structures along the stalk, where the leaves are attached. There is one leaf per node, generally on alternate sides of the stalk. The node is made up of a leaf scar, root band, lateral bud and growth ring. The leaf scar is the remnants of the leaf sheath base that was attached to the node (but has since detached). The root band consists of many root primordia and one lateral bud. Each bud occurs on opposite sides of the stalk. The lateral bud is an embryonic shoot, that is, when the bud germinates a young shoot develops from the growth point of the bud. The size and shape of the buds varies with varieties. The growth ring is a narrow band above the root band. In some varieties a pronounced, shallow, depressed vertical groove extends from the lateral bud, extending into the internode, this is called a bud furrow. Nodes can vary in diameter, colour, configuration and cross-sectional form (Humbert, 1963; Barnes, 1974).

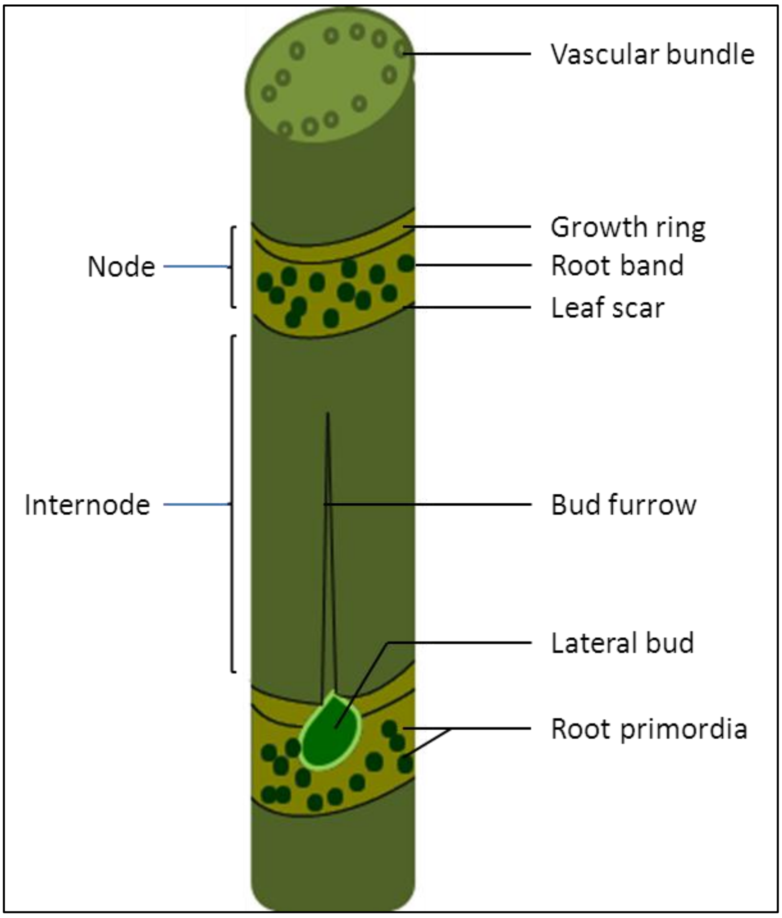

Figure 2.1: Components of a sugarcane stalk (adapted from Humbert (1963)).

The internode is the stem tissue between two nodes. The internodes are covered in a waxy layer and the amount of wax is variety dependent. The length and thickness of the internodes are affected by climatic and cultural conditions. If there is sufficient water available to the plant and the temperature is conducive to growth, then the internodes will be longer because the plant will be growing at a faster rate. However, if the weather conditions are reversed, that is, cool temperatures and limited water availability, the internodes will be shorter (Humbert, 1963; Barnes, 1974). Sucrose is stored in the internodes therefore longer and thicker internodes are preferred by the sugarcane farmer.

The outer portion of the stalk is very hard, consisting of a tough rind. This encloses a soft, fibrous interior, thereby providing protection from damage by external factors, for example, rodents and stem borers. At the upper (younger) end of the stalk the immature internodes are smaller in diameter and shorter, decreasing in size until the stalk growing point (apical meristem) is reached. The stalk growing point is tightly enclosed by the youngest leaf sheaths. As the plant produces sucrose it is stored in the bottom most internodes first. Therefore the topmost part of the stalk contains much less sucrose than the lower part of the fully grown stalk (Hogarth and Allsopp, 2000; Inman-Bamber *et al*., 2002).

1.2 Leaves

Each leaf arises from a node, on alternate sides of a stalk. As the plant develops, the leaves increase in size, up to leaf 14, after which leaf size remains constant. The leaf consists of two principal parts, a lower part (sheath) and an upper part (blade) (Figure 2.2). The sheath is attached to the stalk by a basal ring, completely enclosing the stalk tightly to a height of 7 to 30 cm. The lateral bud is enclosed in the sheath, being protected in its early stages of development (Barnes, 1974). The sheath can be smooth or covered by spiny hairs which may fall off as the leaf matures. In some varieties a purplish tint occurs on the outer surface of the leaf sheath (Humbert, 1963).

At the upper end, the leaf sheath develops into a leaf blade. The junction between the two is a band called the blade joint or collar. At this point, on the inside of the leaf, there is a projection called the ligule. The two wedge-shaped areas called dewlaps are found just above the blade joint. At the margin of the leaf at the collar, a membranous projection called the auricle can be found in some varieties (Barnes, 1974).

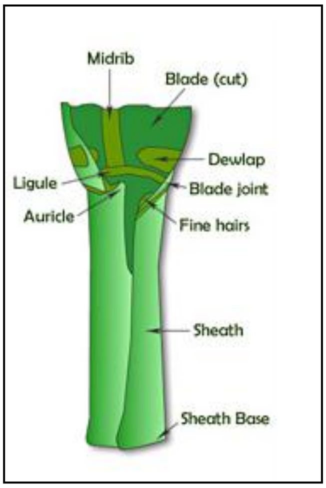

Figure 2.2: Structure of a sugarcane leaf (adapted from http://www.sugarcanecrop.com/growth morphology).

1.3 Roots

The root system of the sugarcane plant is capable of adjusting to its environment. When there is limited water available the plant will extend roots into the deeper layers of the subsoil to extract water. Conversely, when there is excessive soil moisture at the deeper depths, the deeper roots die and the plant develops a much more extensive network of lateral roots (Humbert, 1963; Barnes, 1974).

Sugarcane is planted commercially using pieces of the stalk called setts, where each sett contains at least one node. When the sett is planted a primary shoot develops from the lateral bud and sett roots develop from the root primordia of the root band (Figure 2.3). From the underground lateral buds on the primary shoot, secondary shoots develop. The collection of secondary shoots per sett is called a stool; this will be discussed later on in the chapter. The degree of rooting is variety dependent. These sett roots are thin and branched and provide the young developing plant with nutrients and water. However, this root system limits potential growth rate because the absorbing surfaces of these roots are small so they only last for approximately three months, until the shoot roots take over this function. The shoot roots develop from the root primordia of the lower nodes of the young shoots (Figure 2.3). These roots are thick, white and fleshy. Aside from water and nutrients, they also provide the plant with anchorage (Humbert, 1963).

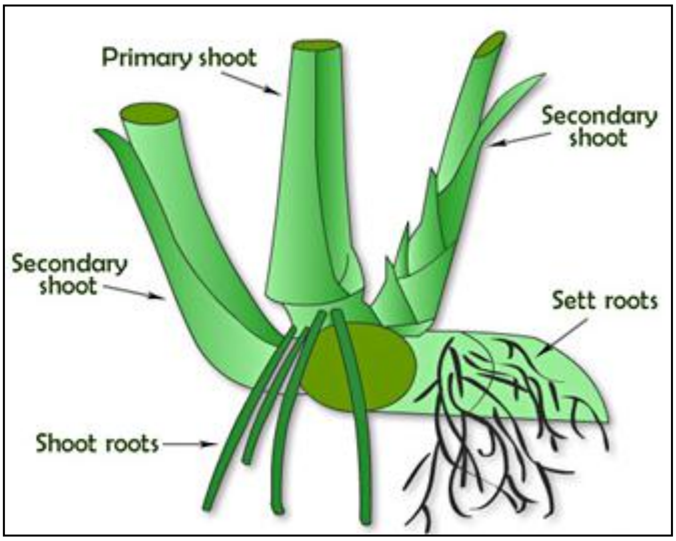

Figure 2.3: A young sugarcane plant showing sett roots and shoot roots (adapted from http://www.sugarcanecrop.com/growth morphology).

The mature root system of established sugarcane plants arises from root bands of shoots, after the initial flush of shoot roots. There are three main types of mature roots: superficial, buttress and rope roots (Figure 2.4).

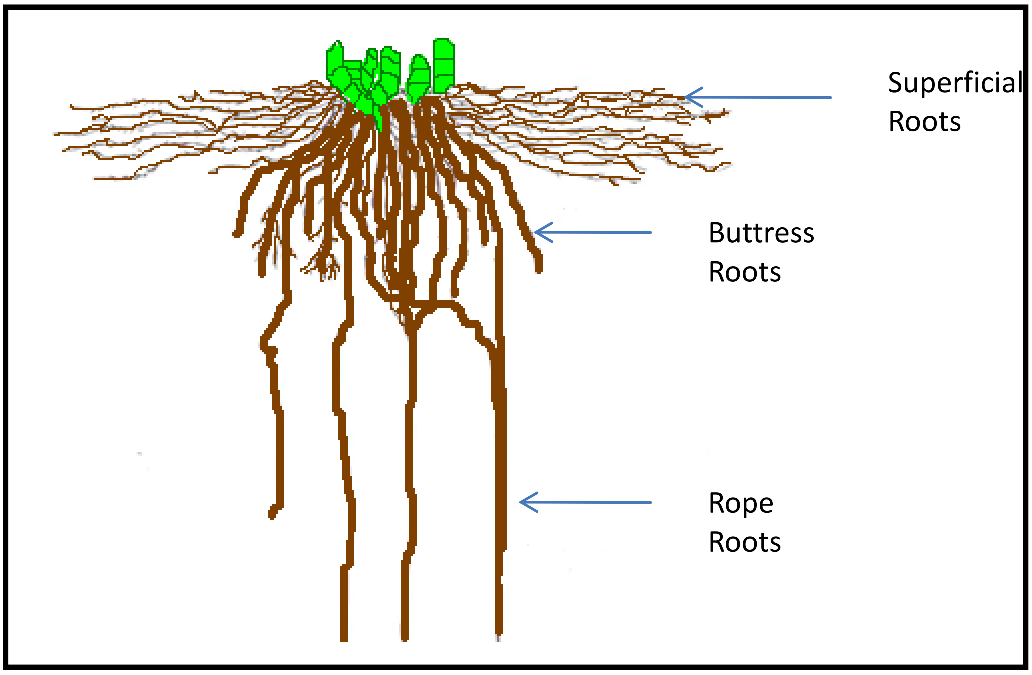

Figure 2.4: The mature root system of a sugarcane plant (adapted from Barnes (1974)).

The root primordia on the nodes higher up on the young shoots give rise to the superficial roots (Figure 2.4). Initially they spread out shallowly but once they have finished extending, they branch vigorously. The main part of these roots is dark and ribbed. When the soil has enough moisture these roots supply the stools with most of the water. However, because these roots are so shallow, they are unable to provide the plant with sufficient moisture under drought conditions. The buttress roots grow downwards, at an angle, thereby providing good anchorage to the plant (Figure 2.4). An added advantage of this root in limited water conditions is that it can penetrate the subsoil, providing the plant with water. The third type of mature root is the rope roots (Figure 1.4). These grow straight down in strands of 15 to 20 individual roots. Like the buttress roots, these roots also provide anchorage and water. They penetrate very deeply into the soil; therefore they are very important in times of drought. The extent, configuration and optimal functioning of the root system are heavily influenced by the physical conditions and the depth of the soil (Humbert, 1963; Barnes, 1974).

2. Sugarcane sucrose formation and storage

The stalk has many roles in a sugarcane plant; it orientates the leaves for maximum radiation interception, translocates water and nutrients from the soil to the leaves, translocates photosynthates from the leaves to the rest of the plant and stores excess photosynthate as sucrose (Barnes, 1974). Most of the sugarcane plant’s daily sucrose production is translocated to the stalk, where it moves towards the base of the plant and the roots with smaller amounts moving towards the apical meristem and immature leaves (Hatch and Glasziou, 1964; Hartt, 1967).

While the plant is still in its active growth phase the photosynthate is predominantly used to increase the mass of the plant body. At this time the number and size of the leaves, stalks and roots are all rapidly increasing. Once the leaves approach full development the rate of photosynthate import (to the leaves) decreases whilst export increases. Maturation (also referred to as ripening) occurs when the plant develops a maximum leaf area, number of stalks and roots that can be maintained under competition for light, water and nutrients.

During ripening the earlier growth processes slow down and are replaced by accelerated accumulation of the photosynthate in the form of sucrose in the internodes. As discussed earlier, sucrose is deposited in the basal internodes first. However, the storage of sucrose does not suddenly begin once the stalk has fully elongated, rather during stalk elongation and for some time post full stalk elongation sucrose is stored in increasing quantities in the stalk (Moore and Maretzki, 1996). Different sugarcane genotypes vary in their ability to store sucrose due to the diversity in net photosynthesis rates and partitioning of the photosynthate (Inman-Bamber *et al*., 2009).

Ultimately, more than half the biomass produced is partitioned into the stalk (Moore and Maretzki, 1996). Of the biomass partitioned to the stalk, 30% is dry matter which is composed of 60% sucrose and 40% fibre (Moore and Maretzki, 1996). The accumulation of sugarcane biomass is dependent on the amount of radiation intercepted by the leaves of the plant and the radiation use efficiency (RUE) of the leaves (Robertson *et al*., 1996). The RUE of a crop is defined as the ratio of biomass accumulated to intercepted radiation (McGlinchey and Inman-Bamber, 1996). The final sugarcane biomass is important because commercial sugarcane yield is based on the fresh weight of millable stalks, which is dependent on the proportion of stalks in the above ground biomass. The sucrose yield is determined by the partitioning of the biomass to sucrose which controls the final yield of sucrose in the millable stalks (Robertson *et al*., 1996).

The rate of photosynthesis depends largely on the prevailing weather conditions. When large amounts of radiation, water and adequate nutrients are available, maximum growth rates can be maintained. However if any of these factors are limiting the growth rate is reduced (Barnes, 1974). Hartt (1967) showed that subjecting sugarcane to drought stress resulted in an 80% reduction in ^{14}C-labeled sucrose transported within 24 hours after stress imposition, however over the long term the quantity (%DM) of sucrose stored increased. This is because the amount of sucrose stored during a crop's cycle depends on the balance between the production and consumption of sucrose. When production exceeds consumption sucrose is stored. In a mildly drought stressed plant for example, the growth of the plant (consumption) is limited therefore allowing a bigger proportion of the sucrose produced to be stored. In irrigated regions some agriculturists manage their sugarcane crop by growing the bulk of the crop under conditions of optimal irrigation followed by a period of "drying off" (irrigation terminated towards the end of the crop cycle) or chemical ripening to encourage sucrose storage (Clements, 1980).

3. The role of soil moisture, soil depth and water movement in the growth of sugarcane

Soil is a valuable resource that supports plant life, and water is an essential part of this system. By understanding the physical properties of a soil, the strengths and weaknesses of the particular soil can be better defined. There are a number of different roles that soil plays in the growth of sugarcane; however, for the purpose of this study the focus of this section will be on the role of soil moisture and soil depth.

3.1 Soil Moisture

Soil texture and structure greatly influence the water holding capacity of a soil.

3.1.1 Soil texture

Soil is made up of soil particles of different shapes and sizes, with coarse sand being the largest particle and clay the smallest (Table 2.1). These particles may exist on their own or in aggregates and are arranged together either tightly or loosely. The spaces between these particles are called the soil pores. The different types of soils originate from the different combinations of soil particle shapes, sizes and arrangements (Marshall and Holmes, 1979).

Table 2.1: Particle size class limits for the South African system of soil classification (Marshall and Holmes, 1979).

Particle size Class	Particle Diameter (mm)
Coarse sand	2 - 0.5
Medium sand	0.5 - 0.2
Fine Sand	0.2 – 0.02
Silt	0.02 – 0.002
Clay	0.002 – 0.0002

The relative proportions of sand, silt and clay differ for different soil types (Humbert, 1963). Different soil types therefore have different textures and can be classified into different textural classes. Figure 2.5 shows an example of soil classification. Consider a soil of 60% sand, 30% clay and 10% silt, projection of any two of these components along their respective axes (indicated by green dotted lines) intersect in the block “sandy clay loam”, which is the textural class of this soil (indicated by blue circle).

The importance of soil textural classes with respect to sugarcane growth is that different textural classes have different secondary properties which are important to plant growth. Some of these properties include water holding capacity, nutrient retention, erosion susceptibility, permeability and mechanical strength. Table 2.2 summarises the differences in some soil properties across different soil textural classes. For example, a clay soil type has the potential to hold more water for the plant as compared to a sandy soil. Therefore if a sugarcane crop was grown on a very sandy soil and subjected to drought conditions, the crop would experience drought stress far quicker than if it was planted on a clay soil type (Silva *et al*., 2007). Approximately 50% of the rainfed sugarcane crop in South Africa is planted on sandy soils (SASRI, 1999), highlighting the vulnerability of these crops to drought events.

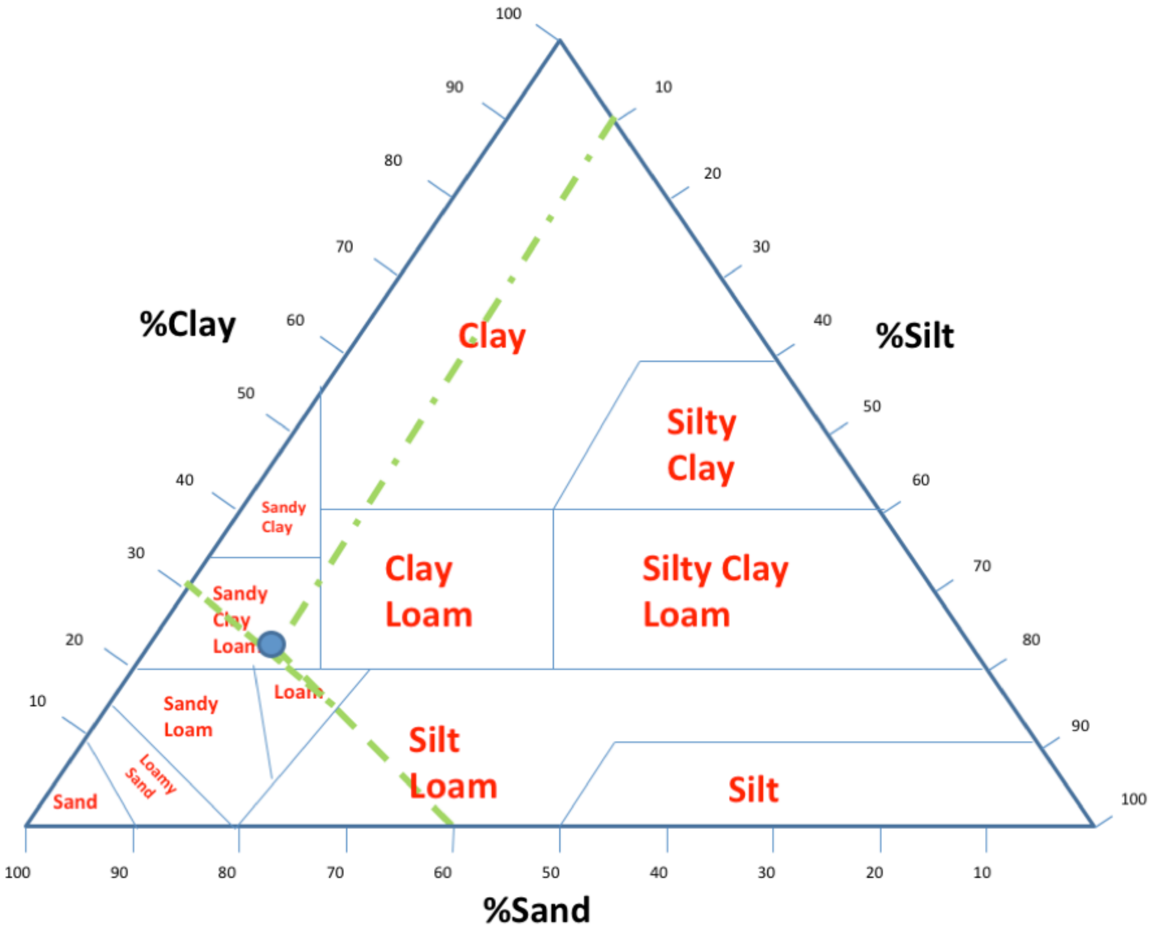

Figure 2.5: Soil textural classification system based on particle size (adapted from Marshall and Holmes (1979)).

Table 2.2: Agricultural significance of soil textural classes (Marshall and Holmes, 1979).

Soil Property	Sand	Loam	Silt Loam	Clay
Internal drainage	Excessive	Good	Fair	Fair – poor
Plant available water	Low	Medium	High	High
Erosion hazard	High	Medium	Low	Low
Run off potential	Low	Low– Medium	Medium-High	High

3.1.2 Soil Water Holding Properties

Water is held in the soil by the capillarity in the pore spaces and by a force of attraction between the soil particles and the water molecules. The soil pores are interconnected thereby allowing the soil to act as a medium for the transport of air and water (Barnes, 1974; Marshall and Holmes, 1979). When it rains (or irrigation occurs) gravity causes the water to move into the soil via soil cracks or fissures. Water continues moving down into the soil until the capillary pressure holding the water in the soil pores exceeds the force of gravity. When this occurs the soil is said to be at its field capacity (FC). FC is the upper limit of water available to the plant, and it is the level at which optimum plant growth occurs (Figure 2.6) (Barnes, 1974). The FC differs across different soil textural classes. Soils with smaller particles (silt and clay) have a larger surface area than those with larger sand particles. A large surface area allows the soil to hold more water, therefore, the FC in sandy soils is much lower than in clay or silt soils (Alway and McDole, 1917; Veihmeier and Hendrickson, 1931; Barnes, 1974).

Water can be removed from the soil by surface evaporation or by plant roots. There is a direct relationship between the amount of water that is removed from the soil and the force with which the water molecules are held within the soil pores; the drier a soil gets the tighter the soil particles hold on to the water molecules (Marshal and Holmes, 1979). On a very hot day, when the surface evaporation rate is very high, plants will wilt because the rate that water is lost by surface evaporation exceeds the rate at which plants can take up water from the soil. The permanent wilting point (PWP) of the soil is reached when the soil holds onto the water so tightly that the plant is unable to take up any water. This is the lower limit of the soil water available to the plant. The available water capacity (AWC) is the amount of water available in the soil that can be removed by the plant. AWC is the difference between upper and lower limits of available soil water, that is, the FC and PWP respectively (Figure 2.6) (Marshal and Holmes, 1979; Van den Berg and Driessen, 2002).

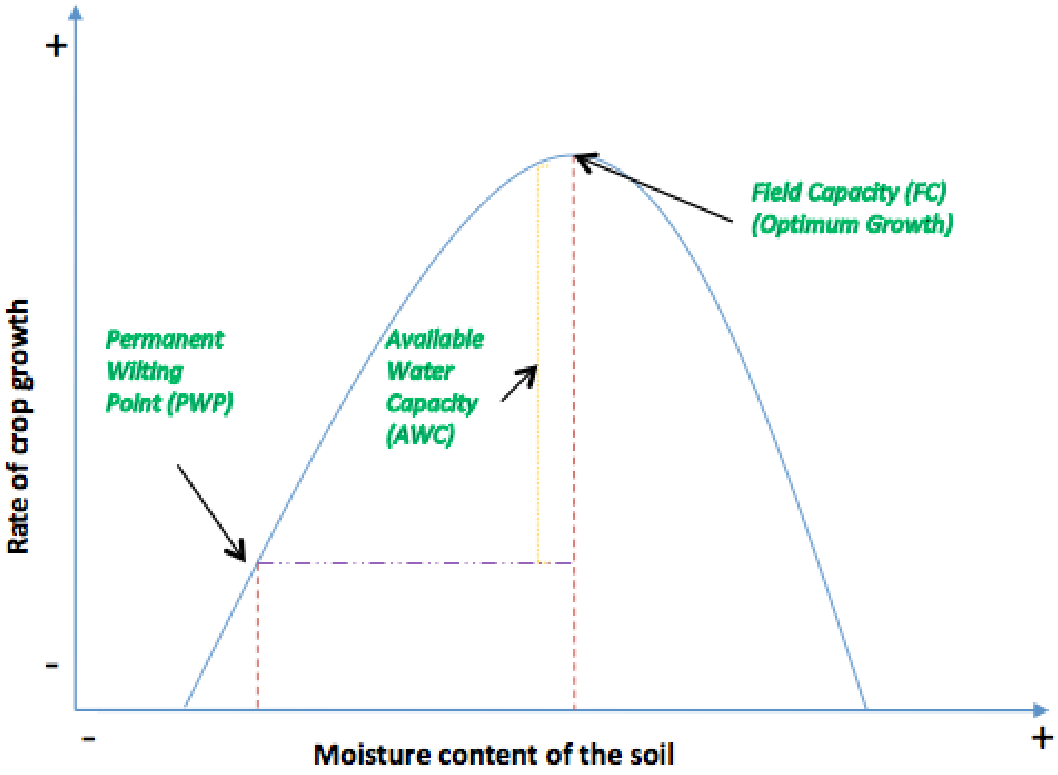

Figure 2.6: The effect of soil moisture on the rate of plant growth (adapted from Barnes (1974)).

AWC depends greatly on the soil texture, as the clay% of a soil increases so does the AWC (Table 2.3) (Barnes, 1974). The growth potential of a crop and its response to drought stress is directly related to the type of soil that it is grown on. The sandier the soil the more affected the crop will be by limited water conditions due to the lower AWC of the field.

Table 2.3: AWC ratings based on clay% ranges (adapted from Barnes, 1974).

Clay Content %	AWC (mm/m)
0-6	<80
7-15	81-100
16-35	101-140
36-55	141-180
>55	>180

3.2 Soil Depth

When evaluating the moisture availability of a soil type, both AWC and soil depth have to be considered simultaneously. The depth of soil is important for plant growth because the deeper the soil, the deeper the plant roots can penetrate for extraction of water and nutrients, and the less likely it will be for the plant to become drought stressed. The effective rooting depth (ERD) of a soil is defined as the soil depth in which 85-90% of the plant roots are found (Humbert, 1963; Barnes, 1974). The ERD is important as it is used in the calculation of the total available moisture (TAM) of a soil. TAM represents the water available to a plant given the depth of the soil (equation 2.1).

Equation 2.1: $\text{TAM (mm)} = \text{AWC (mm/ m)} * \text{ERD (m)}$

For example, if a soil has an AWC of 100 mm/m and an ERD of 0.8m then the TAM of the soil would be 80mm. If the AWC of another soil was also 100mm/m but the ERD was shallow, 0.4m, the TAM of the soil would be only 40mm. Focusing on the AWC in Equation 2.1, if there was a soil that had a very deep ERD (e.g. 2m) this would be of little benefit to the plant if the AWC of that soil was low. A low AWC would result in a low TAM, irrespective of the deep ERD.

The ability of a crop's roots to extract water from the soil depends on the distribution and depth of the roots (Dardanelli *et al.*, 2004). The amount of water received (either via rain or irrigation) will determine if the roots need to penetrate further into the soil profile. If the plant water availability decreases, this forces the roots to penetrate deeper into the soil profile to try to find water. In soils where there is a high clay% in the deeper layers of the soil, the ERD will be lower as roots will be unable to penetrate through this layer. The same occurs for soils where there is a rocky layer in the deeper layers of the soil (Barnes, 1974).

3.3 The movement of water in the soil-plant-atmosphere continuum

Understanding the movement of water between the soil, plant and atmosphere (soil-plant-atmosphere continuum) helps with the understanding of how a plant can become drought stressed. In this continuum, water always moves spontaneously from higher to lower water potentials (Hillel, 1980). When water reaches the soil surface (via either rain or irrigation) it moves into the soil by a process of infiltration (Figure 2.7a).

The water then moves through the soil and to the plant roots by a process called hydraulic flow (Figure 2.7b). When the water leaves the root zone, this is called internal drainage. Water leaves the soil via two processes, the process of transpiration (loss of water vapour from the leaves of the plant) and evaporation (loss of water from the surface of the soil), collectively termed evapotranspiration (ET) (Figure 2.7d,e) (Humbert, 1963; Barnes, 1974; Taylor and Klepper, 1978). The rate of ET depends on the environment that the plant is growing in, for example, the hotter and/or windier an environment, the greater the loss of water from a water saturated plant and or soil into the dry atmosphere. During a drought event, there is less moisture available therefore less infiltration occurs and less water is available to the roots of the plant. However, the movement of water out of the soil via ET still takes place leading to increasingly less water being made available to the roots of the plant. The impact of this water deficiency on the growth of sugarcane is dependent on the growth phase that the crop is in when experiencing the drought event and the duration of the drought stress event (Robertson *et al*., 1999; Cattivelli *et al*., 2008).

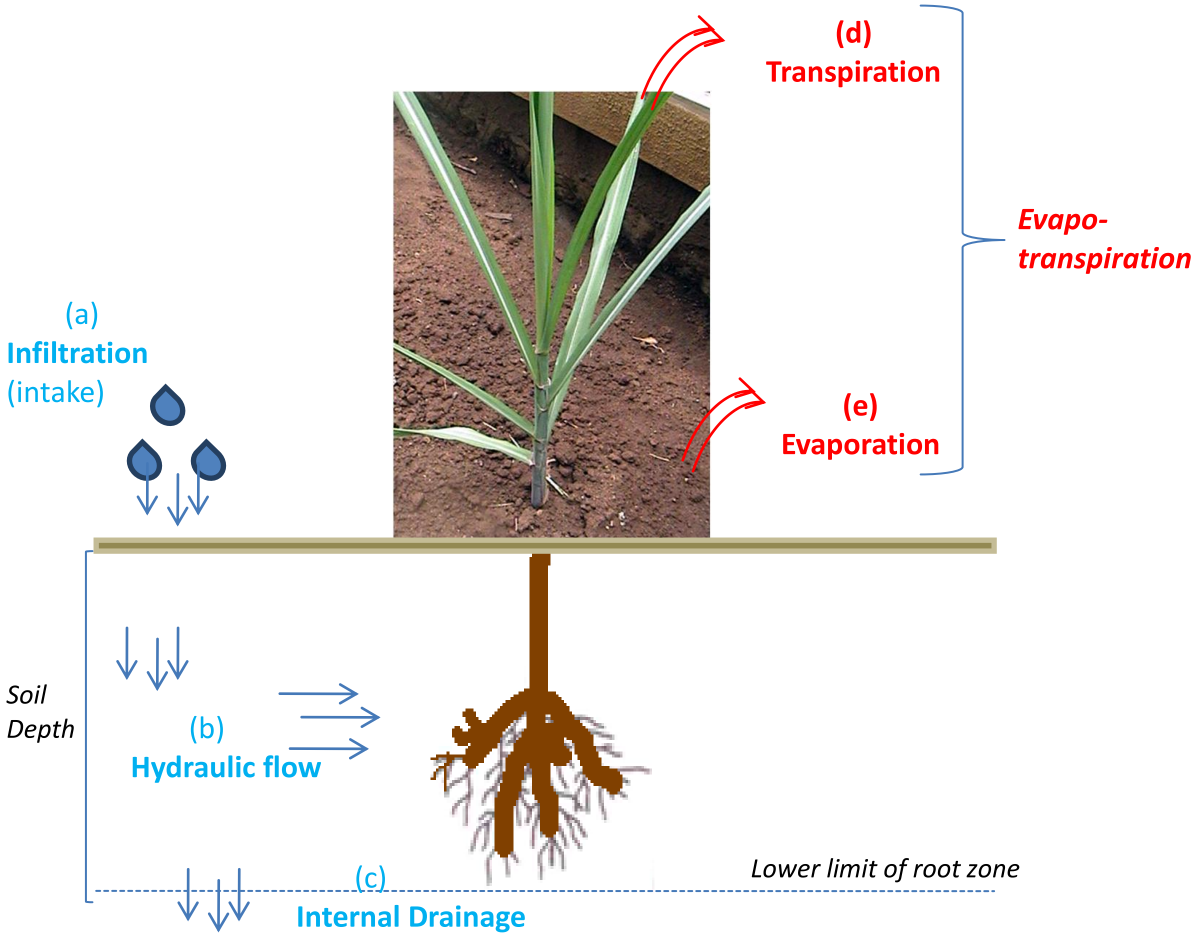

Figure 2.7: The process of water gains and losses in a soil (adapted from Barnes (1974)).

Legend to Figure 2.7:

(a) Water (from rain/irrigation) moves through the soil by a process of infiltration; (b) Movement of water through the soil to the roots by hydraulic flow; (c) Water leaving the root zone by internal drainage; (d) transpiration, process by which water is lost from the leaves of the plant and (e) evaporation, process by which water is lost from the soil surface. (d) and (e) are collectively termed evapotranspiration.

4. The impact of drought stress on sugarcane growth

Before looking specifically at the growth phases it is important to first understand the South African climate.

4.1 The climate of the South African sugarcane industry

South Africa is the southernmost sugarcane industry in the world. It extends between the latitudes 25°S - 31°S. There is 375 590 hectares (ha) under commercial sugar cane production during the 2010/2011 season, 85% of which is rainfed (SASA, 2010/2011, S.I.A.B. Planning and Development Surveys - IA/47/33, 2011). The rainfed area include the coastal area of Kwa-Zulu Natal (highlighted green) and the Midlands region (highlighted orange), each making up 65% and 35% of the total rainfed crop respectively (Figure 2.8). The coastal and midlands areas differ in their climatic conditions, with the midlands being cooler and drier than the coastal region (Table 2.4). Irrigated cane (highlighted blue) makes up 15% of the South African sugar industry, and is located mostly in the north eastern regions of South Africa (Mpumalanga and Pongola) (Figure 2.8) (SASA, 2010/2011).

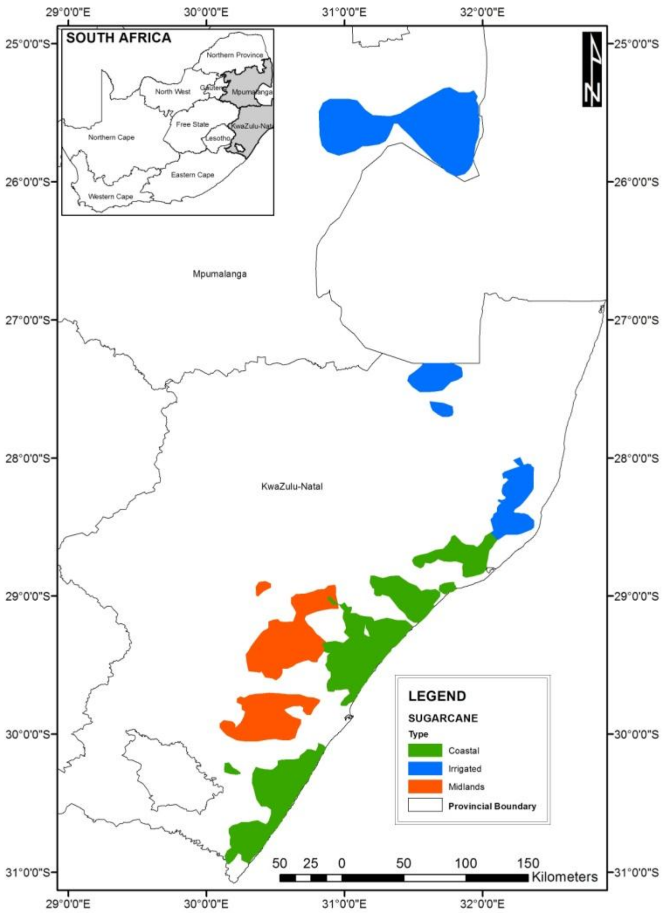

Figure 2.8: A map representing the distribution of the sugarcane industry in South Africa. (GIS department, SASRI, 2011.)

Table 2.4: Annual long term mean climatic conditions at the Midlands, Coastal and the Irrigated regions of the South African sugarcane industry.

Climatic Zone	**Maximum Temperature (°C)**	**Minimum Temperature (°C)**	**Rainfall (mm/year)**	**Sun Hours (hours/day)**
South Coast	24.9	15.1	1032	6.6
North Coast	26.3	15.9	994	6.4
Midlands	24.7	12.3	864	6.5
Pongola **(Irrigated)**	27.3	15.8	898	6.6
Mpumalanga(Irrigated)	29.2	15.6	605	6.7

(Source: SASRI Weather web *http://portal.sasa.org.za*. Date Accessed: 19 June 2011)

The ideal climate for sugarcane growth includes warm temperatures, sufficient rainfall and high solar radiation. In the rainfed sugarcane areas of South Africa, most of the rainfall occurs during the summer months (November – March). A plant is defined as being drought stressed when it does not have access to sufficient water to sustain its growth and/or productivity (Alexander, 1973). Due to the effects of climate change, the periods of drought stress are becoming more frequent and unpredictable. Therefore, in rainfed areas rainfall can be a major limiting factor to the growth of sugarcane (Koonjah *et al.*, 2006; Silva *et al.*, 2008).

4.2. The impact of drought stress on sugarcane growth phases

The growth of sugarcane can be divided into three growth phases; formative, grand growth and ripening phase (Ellis and Lankford, 1990; Tejera *et al.*, 2007). These phases are affected differently by drought stress because there is a change in plant water requirements through the different phases (Zingaretti *et al.*, 2012).

4.2.1 Formative Phase

During the formative phase (FP) germination, tillering and the full development of the leaf canopy occurs (Figure 2.9a). The growth rate in this phase is as fast as the grand growth phase (GGP) (Tejera *et al.,* 2007).

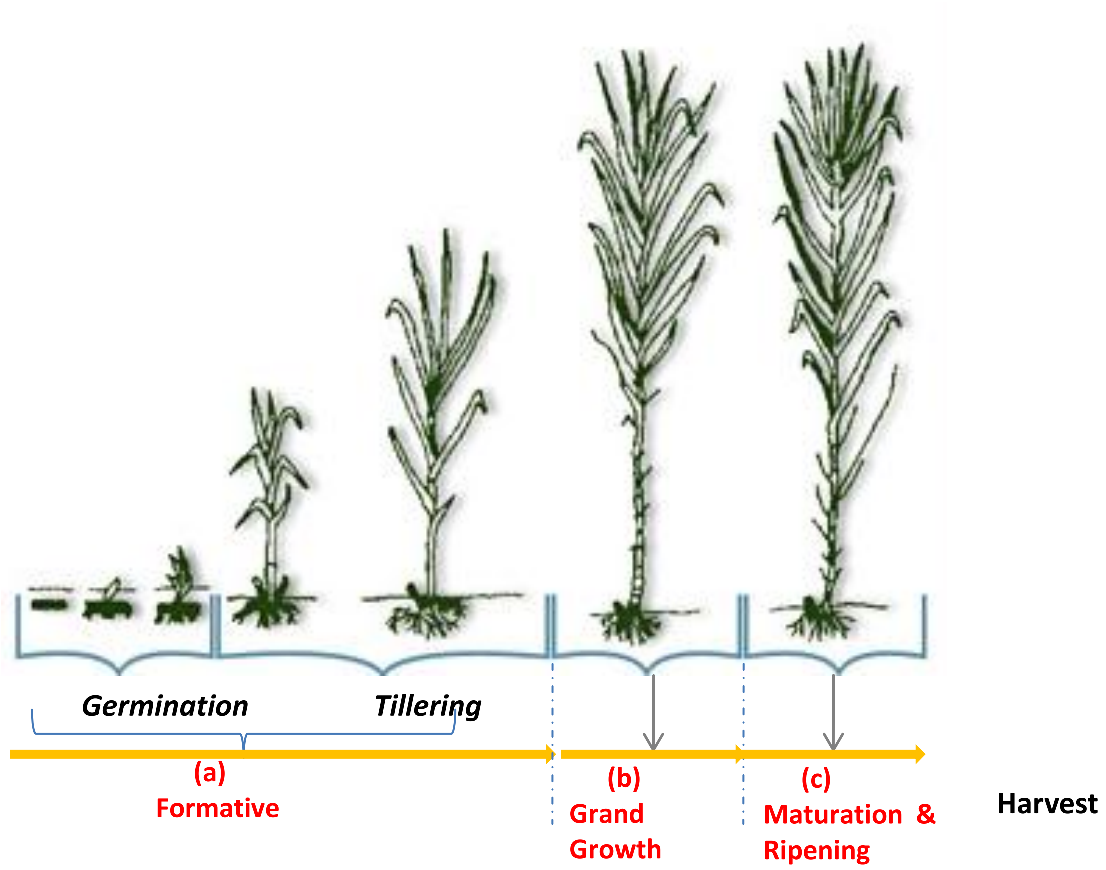

Figure 2.9: The three growth phases of a sugarcane plant.

(adapted from http://www.sugarcanecrops.com/crop_growth_phases/, 2011)

Germination of the setts is only established if there are favourable temperatures and moisture levels (Hogarth *et al.*, 2000). This includes the development of sett roots, growth of the bud into a primary shoot and the formation of shoot roots (Humbert, 1963; Barnes, 1974). The sett only contains enough nutrients and water necessary for the germination of the primary shoot, after which the shoot has to become independent. This is facilitated by producing leaves to allow it to photosynthesize and support its own growth (Barnes, 1974; Hogarth *et al.*, 2000).

Once it has emerged through the soil, the primary shoot grows quickly producing leaves (above the soil) and short internodes (below the surface). The shoot roots support the plant for the rest of the crop cycle. The buds germinate to produce secondary shoots known as tillers (Figure 2.10) (Hogarth *et al.*, 2000). These in turn develop buds at its base and give rise to tertiary shoots. The primary shoot is now independent of the sett. This process is known as stooling or tillering and the structure composed of many secondary shoots is called a stool. The number of secondary shoots determines the number of stalks of cane that makes up a stool. The process of tillering continues until it is limited by factors such as light, space and nutrient availability. Tillering is important as it ultimately determines the productivity of the crop. The more stalks (tillers) that are formed from a stool the higher the productivity of the stool as there will be more volume to store sucrose (when mature). Tillering is very sensitive to environmental conditions (Barnes, 1974; Venkataramana *et al*., 1986; Hogarth *et al*., 2000).

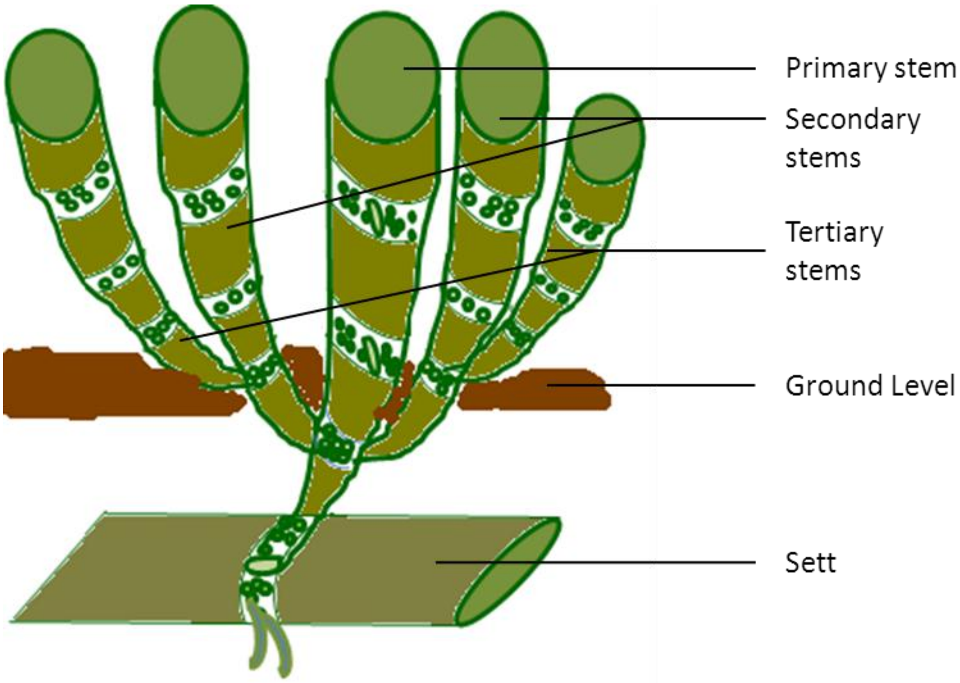

Figure 2.10: The formation of a stool (adapted from Barnes (1974)).

The development of a good leaf canopy is also an important part of the FP and consequently crop growth and final crop yield as it is the leaves which intercept light energy to facilitate the process of photosynthesis (Smit and Singels, 2006). Any light that is not intercepted by the leaf is wasted energy. The canopy also shades out possible growth of weeds. Therefore, the time taken for the leaf canopy to fully form, that is, be able to intercept at least 85% of the incident light should be as short as possible.

The development of the leaf canopy is also sensitive to insufficient water availability. The development of the leaf canopy slows down because there is a decrease in the rate of leaf appearance and an increase in the rate of senescence of the older leaves (Inman-Bamber, 2004; Smit and Singels, 2006). Venkataramana *et al*., (1986) showed that drought stress imposed during the FP can significantly reduce the final cane yield, sucrose content and number of millable cane stalks. In a similar study done by Zhao *et al*. (2010), drought stress imposed during the FP resulted in a reduced number of tillers, green leaf area and number of stalks.

There is a debate in the literature about which phase is the critical water demanding period with respect to sugarcane growth. Singh and Reddy (1980), Naidu and Venkataramana (1987) and Wagih *et al*. (2003) believe that the FP is the most sensitive phase to drought stress. Robertson *et al*., (1999) showed that when drought stress was imposed during the FP, there was a reduction in the above ground biomass, stalk numbers, leaf area and a reduction in tillering; however the crop recovered rapidly when the drought stress was relieved. The final harvest biomass and sucrose yield were not significantly different to the well-watered control. This suggests that if the drought stress is relieved the crop can respond by increasing the rate of tillering and leaf appearance so that the leaf canopy can be re-established. This compensatory growth is the reason for the drought stress imposed during this phase not markedly affecting the final yield. This is supported by work done by Roberts *et al*. (1990), Ellis and Lankford (1990) and Inman-Bamber (1994).

4.2.2 Grand Growth Phase

During the grand growth phase (GGP) the sugarcane plant is growing at a very fast rate with a rapid stalk elongation rate (SER), leaf extension rate (LER) and biomass accumulation (Hogarth *et al*., 2000; Smit and Singels, 2006). This leads to an increase in the demand for water and consequent increase in photosynthetic rate. Therefore, this phase is extremely sensitive to environmental conditions, in particular temperature and soil moisture (Robertson *et al*., 1999). Rapid growth will occur if these conditions are at an optimum and vice versa if they are sub-optimum. Rapid growth will give rise to longer internodes with slow growth producing shorter internodes. At the end of this phase the sugarcane plant is almost fully grown with respect to yield, but the level of sucrose stored is still low as most of the photosynthate has been used to facilitate the growth (Barnes, 1974; Ellis and Lankford, 1990; Hogarth *et al*., 2000).

Robertson *et al*. (1999) showed that when sugarcane was exposed to drought stress during this phase, there was a significant reduction in the biomass and sucrose yield at final harvest when compared to the well watered control. The period of drought stress was of a shorter duration during the GGP compared to the FP, but the impact on final yield was markedly larger showing that the GGP was the more sensitive phase. The numbers of stalks were not significantly reduced but there was a significant reduction in the length of the internodes ($p<0.05$). When the drought stress was relieved, it was not possible for the crop to recover because the loss of stalk length could not be recovered. Silva *et al*. (2008) showed that stalk height, stalk width and stalk number are three attributes of sugarcane that directly affects the final yield of a crop therefore a shorter internode implies less storage space for sucrose, hence a negative impact on the final yield of the crop. Koehler *et al*. (1982) further showed that SER is more sensitive to drought conditions than LER. In addition to being more sensitive to drought stress SER also recovers slower than LER when the stress is relieved (Roberts *et al*., 1990; Batchelor *et al*., 1992; Inman-Bamber, 1995).

Inman-Bamber (1991) also showed that the number of green leaves per stalk was postively correlated with soil water availability ($r=0.85$, $p<0.001$). As mentioned previously, the crop’s ability to maintain the canopy development process is very important for its growth. The ability of the crop to recover from stress imposed during this phase is limited because the crop canopy struggles to re-establish to full cover (Robertson *et al*., 1999). Smit *et al*. (2005) showed that when a crop is stressed in the GGP this results in a decrease in leaf appearance rate and an increase in a leaf senescence rate, consequently decreasing radiation interception.

4.2.3 Ripening Phase

During the ripening (maturation) phase the internode completes its elongation. This occurs while the leaf is still attached. By the time the attached leaf has died, the internode has completed its cycle. The ripening phase happens once the vegetative growth phase is completed, and the product of photosynthesis (sucrose) is deposited in the internodes instead of being used to sustain growth (Barnes, 1974; Hogarth *et al*., 2000).

Sucrose is accumulated in the older internodes first, that is, at the base of the stalk, as these will be the first internodes to reach maturation. The accumulated sucrose can be mobilized to be used to support growth when environmental conditions are not conducive to photosynthesis. As the stalk matures, more internodes accumulate sucrose and the overall sucrose level of the stalk increases (Hartt *et al*., 1963; Hatch and Glasziou, 1964; Inman-Bamber *et al*., 2002).

Natural sugarcane ripening occurs under cool and dry conditions. Stalk elongation is more sensitive to these conditions than photosynthesis, therefore under these conditions photosynthesis continues, but stalk elongation slows down (Hogarth *et al*., 2000). The photosynthate that would have been used to facilitate growth is now directed to sucrose accumulation. The result is an increase in the overall sucrose levels (Hogarth *et al*., 2000). Therefore, drought stress experienced during this phase results in a positive effect in the final sucrose yield (Barnes 1974; Hogarth *et al*., 2000; Inman-Bamber *et al*., 2002). However, sucrose yields only increase if drought stress reduces stalk biomass (tonnes cane per hectare - TCH) by less than 4% (Donaldson and Bezuidenhout, 2000).

In South Africa, the sucrose content in cane is lowest in January – March and at its highest in September – October (Lonsdale and Gosnell, 1976; Sweet and Patel, 1985). Sugarcane under irrigation is “ripened”, in order to increase the final amount of sucrose of the crop (Barnes, 1974). This is done by withholding water prior to harvest, also known as “drying off” (Inman-Bamber and De Jager, 1986; Hogarth *et al*., 2000; Inman-Bamber and Smith, 2005). The application of chemical ripeners also effectively stimulates cane ripening (Barnes, 1974). The sugarcane milling season in South Africa starts from March and ends between October-December. During these periods growers would like to increase sucrose content of the crop to improve milling efficiency. Chemical ripeners allow the growers to effectively manipulate the sucrose content of their crop in a short period of time prior to harvesting.

4.2.4 Ratooning

In South Africa, a sugarcane crop is normally harvested for the first time between 12 – 24 months after planting, depending on the production area. When the sugarcane is harvested there is still a portion of the stalk that is left underground, and it is this portion that gives rise to the subsequent crops known as ratoons (Barnes, 1974). Germination of the underground portion of the stalk is inhibited while the stalk is growing because of apical dominance. This occurs when the apical meristem produces a class of hormones, auxins, in each stalk which suppresses bud development. When the stalk is harvested, the apical meristem is removed and the hormone is not produced hence germination can occur, given favourable temperatures and soil moisture content (Barnes, 1974; Hogarth *et al*., 2000).

Ratooning is very similar to germination except that the primary root system is already present, however this quickly dies once the new growth progresses (Barnes, 1974). Hence the ratoon crop grows much faster than the plant crop. Sugarcane can be repeatedly ratooned for up to 20 years, however stool damage incurred during harvesting and cultivation and/or pest and disease damage is cumulative, and therefore there is a yield decline with successive ratoons. Today, crops are generally ratooned for five to fifteen ratoons (Inman-Bamber, 1994).

Drought stress, if severe, can have a major impact on the ratooning ability of a crop. For example, the drought event in 2010/2011 season in South Africa caused major stool death in the coastal farms with poor soils (Singels *et al*., 2011). This resulted in very poor ratooning and additional replanting that had to be done to replace damaged crops.

4.3 The impact of drought stress on photosynthesis

At the whole plant level drought stress generally leads to a decrease in photosynthesis and growth. Water available to the plant for photosynthesis depends on the amount of water lost to the atmosphere and the amount of water that can be extracted from the soil. During transpiration energy (or potential) gradients are formed along the transpiration pathway, allowing for water to flow from the soil, through the xylem to the leaves for photosynthesis. The energy status of water in a plant is expressed as water potential (Ψ) (Rose, 1966). In practical terms, water in the leaves is usually under tension during transpiration therefore the Ψ in the leaves is more negative than in the roots for example (Zingaretti *et al*., 2012). The pressure required to balance the tension is a measure of the water potential of the leaf water. The Ψ of a plant is a useful indicator of understanding the plant's water status.

Leaf water potential (LWP) decreases, that is, becomes more negative in the morning when transpiration is high. It is increased, closer to zero, in the evenings when transpiration is low. A reduction in the rate of photosynthesis occurs only when the LWP reaches below a certain level (Inman-Bamber and De Jager, 1986). Hsiao (1973) showed that when a sugarcane plant is subjected to drought stress photosynthesis continues long after stalk extension and leaf extension is reduced. In some drought tolerant varieties, when subjected to drought stress their leaves roll up (to reduce surface area) and/or their stomata close to help reduce the amount of water that is lost through transpiration (Yordanov *et al*., 2000).

5. Evaluating a crops yield response to drought stress

Drought affects sugarcane both physiologically, biochemically and morphologically in a complex mechanism which may be further confounded by genotype x environment (G x E) interactions (Singh and Reddy, 1980; Silva *et al*., 2007). One of the major limitations of breeding for varieties that are potentially drought tolerant is that there has not been a single trait identified as being directly related to drought tolerance (Silva *et al*., 2008), rather there is a great deal of interaction between traits (Rizza *et al*., 2004; Smit *et al*., 2005). Another limitation is that there is a large degree of variability in varietal responses to drought (Moore and Maretzki, 1996; Inman-Bamber and Smith*,* 2005) for example, stalk diameter is affected not only by drought stress but also by variety type (Da Silva and Da Costa*,* 2004) and the rate of canopy development (Smit *et al*., 2005). Sugarcane varieties can be classified according to their tolerance to drought stress. A drought susceptible (S) variety would wilt and show reduced cane production early on during the drought event and a tolerant (T) variety would remain turgid and maintain near-optimum growth for longer (Moore and Maretzki, 1996; Silva *et a*l., 2007). The need for researchers to create a quantifiable variable to describe the environment that a crop is exposed to has led to development of drought stress indices (DSI's).

5.1 The use of Drought Stress Indices (DSI's)

Researchers have used a drought stress index (DSI) to quantify the amount of drought stress experienced by a crop. Bakumousky and Bakumousky (1972) calculated a DSI using the yield of the crop under different conditions, rainfed and irrigated. The latter was meant to represent an unstressed condition. The DSI was calculated by expressing the rainfed crop yield as a function of the irrigated (unstressed) yield (equation 2.2a).

Equation 2.2 (a): $DSI = 1-[(Y_i-Y_{ni})/Y_i]$

Bouslama and Schapaugh (1984) calculated a similar type of index in evaluating the response of soyabean varieties to drought stress, called a yield stability index (YSI) (shown in equation 2.2b).

Equation 2.2 (b): $YSI = Y_{ni}/Y_i$
Where:
Y_i = the yield obtained under unstressed conditions (irrigated)
Y_{ni} = the yield obtained under drought stressed conditions

In a similar manner, Fischer and Maurer (1978) also calculated a stress susceptibility index (SSI) for measurement of yield stability in wheat cultivars by expressing the yield of a crop grown under stressed conditions as a function of the yield under unstressed (irrigated) conditions.

Equation 2.2 (c): $SSI = (Y_i - Y_{ni})/(Y_i D)$

Where:

D (stress intensity) = $1-(HY_{ni}/Y_i)$

HY_{ni} = mean yield of all cultivars in drought stressed trials

Hossain *et al.*, (1990) also estimated an index, tolerance, for wheat cultivars, which was calculated as the difference in yields between irrigated and rainfed conditions.

Equation 2.2 (d): $Tolerance = Y_i - Y_{ni}$

Fernandez (1992) proposed a stress tolerance index (STI), which was calculated for each mungbean genotype by multiplying the yield of a genotype grown under rainfed conditions by the yield of the genotype grown under irrigated conditions and dividing the multiplied value by the square of the average mean yield for all genotypes grown under irrigated conditions.

Equation 2.2 (e): $STI = (Y_i * Y_{ni})/(Y_i)^2$

In a study using alfalfa Idso *et al.*, (1981) suggested using a “crop water stress index” (CWSI) derived from the increase in average canopy temperature in relation to that of a well-watered reference plot using infrared thermometry. This index was also used in similar research done on wheat genotypes by Alderfasi and Nielsen (2001) and on broccoli by Erdem *et al.*, (2010).

Equation 2.2 (f): $CWSI= \{[(T_c-T_a) -D_2]/ [D_1-D_2]\} \times 10$

Where:

T_c = average plant canopy temperature (°C)

T_a = air temperature (°C)

$D_2 = 0.41-1.5\times$mean atmospheric vapor pressure deficit

D_1 = is the maximum difference between T_c-T_a

Motzo *et al.*, (2001) calculated a seasonal water stress index (SWSI) based on the soil-plant-atmosphere interaction where stress was quantified as 1-(fraction of transpirable soil water) (FTSW).

Equation 2.2 (g): $SWSI = 1 - FTSW$

Rizza *et al*. (2004) proposed an integrated DSI based on the reduction of plant transpiration relative to the potential transpiration in barley genotypes.

Equation 2.2 (h): $DSI\ (\%) = (1-AET)/PET * 100$
Where:
AET = actual evapotranspiration
PET = potential evapotranspiration

Voltas *et al.* (2005) calculated a DSI using the AWC and ET.

Equation 2.2 (i): $1-(AWC/ET)$

Mohammadi *et al.* (2010) proposed that the YSI was a more useful index to discriminate between drought-tolerance and drought-sensitive wheat genotypes.

5.2. Statistical methods to evaluate a crops response to drought stress

Various statistical methods have been used to relate the yield of a crop to the amount of drought stress it was subjected to. Motzo *et al*. (2001) and Muthuramu *et al*. (2011) used the Additive Main effects and Multiplicative Interaction (AMMI) analysis to evaluate spring triticale and rice yield responses to drought stressed and unstressed environments. The AMMI model is a hybrid model involving both additive and multiplicative components of variance of a two way data structure. The AMMI model separates the additive variance from the multiplicative variance and then applies principal component analysis (PCA) to the interaction portion to extract a new set of coordinate axes which explains the interaction pattern in more detail. However, one of the limitations of AMMI is that it requires a balanced data set (Genstat, v14.1). Rizza *et al*. (2004) used regression analysis to evaluate the yield of different barley varieties over different drought stress levels. The intercept and gradient (m_1) of the regression line was seen as measure of a varieties yield potential and response to drought stress respectively. In a similar manner, Richardson *et al*. 2009 used regression analysis to identify drought tolerant bluegrass varieties. Voltas *et al*. (2004) used a combination of a Genotype main effect (G) plus G x E interaction (GGE) biplot analysis and factorial regression to evaluate wheat varietal yield response to eight different environments. A DSI was calculated for each environment. The advantage of the GGE biplot is that it allows visual evaluation of varietal suitability to different environments. A disadvantage however, is that it requires a balanced data set (Genstat, v14.1). Ndiso *et al*. (2007) and Girdthai *et al*., (2010) used analysis of variance (ANOVA) with post hoc testing to evaluate maize and peanut varietal yield response, respectively, to well-watered and drought stressed experimental conditions. Nouri *et al*., (2011) calculated seven different types of drought tolerance indices, based on the yields of different wheat varieties subjected to both rainfed and irrigated conditions. Using cluster analysis, the varieties were then classified according to their drought tolerance potential: Tolerant, Intermediate or Susceptible.

5.3. Canesim – crop forecasting model

Crop growth modelling began in the 1960's with the aim of increasing insight into crop growth processes by expressing existing knowledge into a series of mathematical equations (Bouman *et al.*, 1996). Crop modelling is a widely used tool in agriculture, being used for research and management decisions.

The need for a crop forecasting model, was highlighted by an in depth survey conducted for the growers and millers of the South African sugarcane industry (Bezuidenhout, 2001). Estimating the expected size of a sugarcane crop is essential to the industry for optimizing milling as well as sugar marketing. The milling season in South Africa is from March to

December. At the beginning of the season, an accurate forecast of the size of the crop expected to arrive at the mill is essential to ensure the profitability and efficiency of the mills (Bezuidenhout and Singels, 2007a).

Canesim is a version of the CERES-MAIZE model (Jones and Kiniry, 1986) that has been modified to simulate the growth and yield of sugarcane over a wide range of climatic and soil conditions in South Africa (Inman-Bamber, 1991). The Canesim model is a daily time step, point-based simulation model predominantly driven by water (Bezuidenhout and Singels, 2007b). The model inputs include soil data (TAM and the amount of water available at the start of the crop), weather data (obtained from the weather stations situated throughout the industry), crop data (start date, harvest date, row spacing, plant/ratoon crop) and irrigation system (if the crop is irrigated). The model accounts for partial canopy conditions and soil water content (SWC) using a single layer soil profile and yield is calculated as a function of transpiration. The model can also simulate irrigated and rainfed crops. For irrigated crops, the crop is "irrigated" when the SWC drops below 50% of the TAM. This threshold was chosen to prevent drought stress (Singels *et al*., 2000).

The weather data used in Canesim comes from a database that collects information from a network of 41 automatic weather stations (AWS) and 8 manual weather stations (MWS) (Singels, 2007). These stations record daily rainfall, solar radiation, relative humidity, wind run, minimum and maximum temperature.

Yield estimates of a crop are calculated by running Canesim model with actual weather data from the start date to the end date of the crop (Singels *et al*., 1999). The yield estimates are often overestimated because the model does not take into account sub-optimal management practices, pests and diseases (Bezuidenhout and Singels, 2007b). Yields of future crops are calculated by using recent weather data (up to the previous day) and data representing a likely future weather scenario (Singels *et al*., 1999).

The daily partitioning of assimilate between the roots and aerial parts is simulated as a non-linear function of total biomass. Partitioning of the stalk dry matter is regulated by the sink capacity and the source to sink ratio. The former is controlled by the current growing conditions, current stalk mass and varietal characteristics. The sucrose accumulation is based on a framework of sucrose distribution within stalks as it is affected by temperature and drought stress (Singels and Bezuidenhout, 2002). The model simulates the interception of radiation by the crop canopy as a direct function of thermal time (Singels and Donaldson, 2000)

Canesim can be used in many different ways: 1.) to assist with agronomic management decisions 2.) yield forecasts (using historical weather data patterns) 3.) identifying factors that may cause yield decline (by estimating yield potential using different scenarios) (Singels *et al*., 1999).

In a Canesim validation study, Bezuidenhout and Singels, (2007b) compared simulated versus actual yields for 22 years (1978-2002). The actual yields for the industry as a whole and for each of the 15 mill areas were obtained from the South African Cane Growers Association. If no weather data was available the nearest weather station was used. One of the tools used to evaluate the model was estimation skill. This is a measure of the quality of an estimate compared to that of a reference estimate, such as the long-term mean, persistence or random guessing (Murphy, 1993). Bezuidenhout and Singels (2007b) showed that the skill for the industry (over the 22 years) was medium, 57.2%. The model was also verified, at mill level, by Gers *et al.* (2001), who reported excellent agreement between simulated and observed regional yields (R^2 = 0.87, p <0.001).

6. List of Equations

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Chapter 3

A pilot study to establish a method of quantifying drought stress in sugarcane

Table of Contents

A pilot study to establish a method of quantifying drought stress in sugarcane

C. Sewpersad

South African Sugarcane Research Institute (SASRI), Private Bag X02, Mount Edgecombe,
4300, South Africa.

Abstract

In the rainfed sugarcane growing regions of South Africa periods of drought stress is a real risk for farmers, with the unpredictability of climate change further compounding this problem. This study employed available historical data to identify sugarcane varieties that are classified, by observation, as intermediate (I, variety N12) and intermediate-susceptible (IS, N19 and NCO376) in their response to drought stress. The variety yields from 66 rainfed trials, in the coastal regions, were extracted from the South African Sugarcane Research Institute's (SASRI's) database, which stretches over 43 years. Canesim, a crop simulating model, was used to generate crop performance data for each individual trial under simulated rainfed and irrigated conditions. Validation of Canesim generated data was achieved by regressing the observed trial data on simulated Canesim yields. This showed that the simulated data were very reliable (R^2=0.85, p<0.001). The Canesim data and the actual trial data were subsequently used to quantify drought stress by means of six different drought stress indices (DSI's): percentage (%) drought stressed days (DSI1); weighted % drought stressed days (DSI2); average Canesim stress (DSI3); weighted average Canesim stress (DSI4); ratio of the observed trial yield over the irrigated Canesim yield (DSI5) and ratio of the rainfed Canesim yield over the irrigated Canesim yield (DSI6). The weightings used were based on the timing of the drought stress event according to the crop's development phase. The varietal yields were regressed on each of the indices. The most sensitive DSI was selected based on its ability to statistically distinguish between varietal yield performances in response to different degrees of drought stress, as quantified by the gradients of the regression lines. DSI 6 was the only index that was able to satisfactorily distinguish between the I and IS sugarcane varieties (R^2=0.75, p<0.001), and was selected for use in subsequent screening of historic data sets available for breeding material and commercially available varieties in rainfed environments (chapter 4).

Keywords: drought stress index, growth phase, Canesim, regression

Introduction

South Africa is host to the southernmost sugarcane industry in the world with 375 590 hectares under commercial sugar cane production during the 2010/2011 season. Depending on the amount of rainfall received, sugarcane farms are either rainfed or irrigated. In South Africa, 85% of the sugarcane industry is rainfed (SASA, 2010/2011, S.I.A.B. Planning and Development Surveys - IA/47/33, 2011). The ideal climate for sugarcane farming is warm temperatures, sufficient rainfall and high solar radiation (Barnes, 1974). Due to the effects of climate change periods of drought stress are becoming more frequent and unpredictable, and this can be a major limiting factor to the growth of sugarcane in rainfed areas (Inman-Bamber and Smith, 2005; Bezuidenhout and Schulze, 2006; Koonjah *et al*., 2006; Silva *et al*., 2008). Bezuidenhout and Schulze (2006) showed that the South African sugarcane industry was subject to prolonged periods of drought stress, commonly referred to as droughts, during 1983, 1992 and 2003, with the most recent drought event occurring in 2010.

A sugarcane plant is defined as being drought stressed when it does not have access to sufficient water to sustain its growth and/or productivity (Alexander, 1973). Sugarcane varieties differ in their response to periods of drought stress, with some being more tolerant than others. A drought susceptible variety would wilt and show reduced cane production early on during the drought event, whereas a more tolerant variety would remain turgid and maintain near-optimum growth for longer (Moore, 1987; Silva *et al*., 2007). This makes variety choice an important part of a farmers risk management strategy, as the same crop will be in the ground for between five to fifteen years (Inman-Bamber, 1994).

The impact of a period of drought stress on the final yield of a sugarcane crop is dependent on the crop’s growth phase, and the length of the drought stress event. Commercially sugarcane is a vegetatively propagated plant, and its growth can be divided into three phases: formative, grand growth, and a ripening phase (Ellis and Lankford, 1990; Tejera *et al*., 2007). The formative phase (FP) involves germination, tillering, and the full development of the leaf canopy. In the grand growth phase (GGP) the cane is growing at a very fast rate with a rapid stalk elongation rate (SER), leaf extension rate (LER), and biomass accumulation rate (Hogarth and Allsopp, 2000). During the ripening phase, the SER decreases, in part, due to environmental and/or management factors. This leads to the accelerated deposition of sucrose in the internodes, and a consequent increase in the whole stalk juice purity (Barnes, 1974; Hogarth and Allsopp, 2000). There is a debate in the literature about which phase is the critical water demanding period with respect to growth translating into final sugarcane yield. Singh and Reddy (1980), Naidu and Venkataramana (1987) and Wagih *et al*. (2003), all reported that the FP was the most sensitive to drought stress. However, Robertson *et al.* (1999) showed that when drought stress was imposed during the FP of a plant crop, the crop recovered rapidly when the drought stress was relieved. This is supported by work done by Roberts *et al.* (1990), Ellis and Lankford (1990), and Inman-Bamber (1994). Robertson *et al.* (1999) further showed that when cane was exposed to stress during the GGP, there was a significant reduction in the biomass and sucrose yield at final harvest. The period of drought stress was of a shorter duration during the GGP compared to the FP, but the impact on final yield was markedly larger, showing that the GGP was the more sensitive to drought stress (Koehler *et al*., 1982; Domaingue, 1996; Ramesh and Mahadevaswamy, 2000; Inman-Bamber, 2004; Silva *et al.*, 2007). However, Wiedenfeld (2000) showed that the effect of drought stress on the final yield of a sugarcane crop was primarily dependent on the degree of drought stress experienced relative to the ET, rather than the growth phase during which the stress occurred.

Drought affects crops physiologically, biochemically and morphologically in a complex mechanism which may be further confounded by genotype by environment (GxE) interactions (Singh and Reddy, 1980; Inman-Bamber *et al*., 2005; Silva *et al*., 2007). One of the major limitations of breeding for sugarcane varieties that are potentially drought tolerant is that there has not been a single trait identified as being directly related to drought tolerance (Silva *et al*., 2008), rather there is a great deal of interaction between traits (Smit *et al*., 2006).

The calculation of a drought stress index (DSI) has proven to be a popular method of quantifying the amount of stress experienced by a crop. Some indices were calculated by comparing a crop's yield response when subjected to drought in relation to well-watered experimental conditions (Bakumousky and Bakumousky, 1972; Bouslama and Schapaugh, 1984; Fischer and Maurer, 1978; Hossain *et al*., 1990; Fernandez, 1992). In a slightly different approach, using alfalfa, Idso *et al*. (1981) used a crop DSI derived from the increase in average canopy temperature in relation to that of a well-watered reference plot using infrared thermometry. This index was also used in similar research done on wheat genotypes by Alderfasi and Nielsen (2001), and on broccoli by Erdem *et al*. (2010). Inman-Bamber and De Jager (1986), Khera and Sandhu (1986), Boroomand-Nasab *et al*. (2005) and Lebourgeois *et al.* (2010) all used the method proposed by Idso *et al.* (1981), to calculate a DSI for sugarcane.

Motzo *et al*. (2001) calculated a DSI based on the soil-plant-atmosphere interaction where stress was quantified as 1-(fraction of transpirable soil water). Rizza *et al.* (2004) proposed an integrated DSI based on the reduction of actual plant transpiration relative to the potential transpiration. Mohammadi *et al.* (2010) proposed that using yield to calculate a DSI was a more useful index to discriminate between drought-tolerant and drought-sensitive wheat genotypes.

A crop growth simulation model (from now on referred to as crop model) is defined as a quantitative scheme for predicting the growth, development and yield of a crop, given a certain set of genetic features (genotype) and environmental variables (Monteith, 1996). Since the late 1960's, crop models have typically been used in agriculture. Crop models are used for many different purposes, one of which is as a decision based tool (Boote *et al*., 1996, Todorovic *et al*., 2009). With climate change being a more prominent factor in the current agricultural world, crop models have been used to assess the impact of changes in climate on the final yield of a crop (Parry *et al*., 2005; Rosenberg, 2010).

A concern around crop models is the amount of error associated with the simulated final crop yield. Palosuo *et al*. (2011) compared the performance of eight different crop simulation models, all of which predicted the yield of winter wheat. These models required input information like daily weather data, soil data, variety type and crop management. The study showed that there was a lot of variability in the different predictions, with three of the models overpredicting yield, three underestimating, and only two of the eight giving reasonable estimates. This shows the uncertainties associated with model predictions –

which is mainly due to inaccurate input data, parameterization and model structure (Palosuo *et al*., 2000), the latter of which is the most difficult to quantify (Chatfield, 1995).

Todorovic *et al*. (2009) compared three different sunflower growth simulation models under different irrigation regimes, as well as rainfed conditions, and they found that the models predicted the actual yield with the index of agreement ranging from 0.80-0.99 across the different models and environmental conditions. The models did underestimate yields when the crop was severely drought stressed, but the estimated yields were not significantly different to the observed yields.

Given the limitations of crop models Van Oijen and Ewert (1999) showed that there is a lot of variability associated with the observed crop yield itself due to sources of variation arising from crop characteristics, the environment and a high sampling error. In addition, crop models generally do not take into account the effect of weeds, diseases or pests on the crop yield.

More recently Ramburan *et al.* (2011) used Canesim to calculate three different DSI's using the following Canesim predicted variables: a) evapotranspiration, b) simulated final yields, and c) actual yield and simulated yields. Canesim is a sugarcane crop model that uses a sugarcane water balance crop model and an on-line weather database to simulate the crop's day-to-day growth status (Singels, 2007). The online weather database contains weather information for the past 80 years. The information is retrieved from the 41 automatic weather stations (AWS) and 8 manual weather stations (MWS) that are located on or near the different farms within each sugarcane growing region in South Africa. These weather stations record daily rainfall, solar radiation, temperature, wind speed and air humidity and the data is downloaded onto the weather database. The crop parameters are then calculated and the model output includes day-to-day values for the sugarcane crop's yield and quality, the crop and soil water status and canopy cover (Bezuidenhout and Singels, 2002; Singels, 2007, Singels *et al*., 2011). The model has an irrigation function, which if selected, allows for the simulation of a trial an irrigated (drought unstressed) trial. If unselected the trial is simulated as a rainfed (susceptible to drought stress) trial.

The accuracy of Canesim has been investigated and validated by Singels *et al.* (1999), Singels and Donaldson (2000), Gers *et al*. (2001) and Singels and Bezuidenhout (2002), revealing that the percentage variation in the observed yields accounted for by the Canesim predicted yields (R^2) was on average greater than 80%, with no significant difference between the observed and predicted mean yields.

The South African Sugarcane Research Institute (SASRI) was established in 1925 and has the main organizational goal of produciing new improved hybrid sugarcane varieties for the different agroclimatic zones (irrigated, coastal short cycle, coastal long cycle, coastal hinterland, midlands) of the sugar industry (Parfitt, 2005). The plant breeding programme conducts varietal evaluation trials within each agroclimatic zone and the trial information is stored in an Oracle database which contains 43 years' (1968-2011) worth of trial and variety data (SASRI Oracle Database (10*g*R2), 2011).

The aim of this pilot study was to make use of the available historical varietal information in the SASRI Oracle database and Canesim to establish a valid methodology for calculating a DSI to accurately quantify the amount of drought stress experienced by sugarcane. The objectives of this study were therefore to:

- Calculate DSI's using different definitions;
- Use varieties, with observed drought tolerance, to evaluate the suitability of the different DSI's;
- Identify the DSI that best describes the amount of drought stress experienced by a sugarcane crop.

Materials and Methods

Variety and trial selection

Varieties were selected based on three criteria, the varieties had to: a.) be a current commercially planted variety in the rainfed coastal regions; b.) have sufficient historical data available in the SASRI Oracle database (n>12) and c.) have an observed yield response to drought stress. Even though anecdotal evidence should never be accepted at face value, this is the only information SASRI has available that reveals varietal yield performance when exposed to drought stress. Following the application of these three criteria the following three varieties were identified: N12 (intermediate (I) response to drought stressed conditions), N19 (intermediate-susceptible response to drought stressed conditions (IS)), and NCO376 (IS) (Table 3.1).

Early work by Inman-Bamber (1982) showed that when N12 was subjected to different levels of drought stress (mild, moderate, severe) it was able to survive these conditions relatively well and recover quickly when the conditions were alleviated. This is supported by observations recorded by SASRI (2006b). Mcintyre and Nuss (1996) showed that N19 responds to drought stress in an IS manner. N19 responded relatively well to drought stress if the conditions were not prolonged and if the soils were of moderate depth. However, if the soils are shallow and clay %< 20% then N19 has a very marked negative yield response to drought stress (SASRI, 2006d). Similarly, NCO376 has been observed to also have an IS response to drought stress (SASRI, 2006a).

Table 3.1: Details of the number of coastal trial data available for N12, N19, and NCO376.

Variety	Year of Release	Observed Rating	No. of Trials
N12	1979	I	38
N19	1986	IS	28
NCO376	1955	IS	57

(Source: SASRI Oracle Database (10gR2). Date Accessed: 14 June 2011.)

The selection criteria for the coastal farms were selected based on: a.) presence of a weather station on the farm; b.) availability of field soil information and c.) trials that contained N12/N19/NCO376. Based on these criteria four coastal trial sites Empangeni, Gingindlovu, Kearsney and Mount Edgecombe were selected (Figure 3.1).

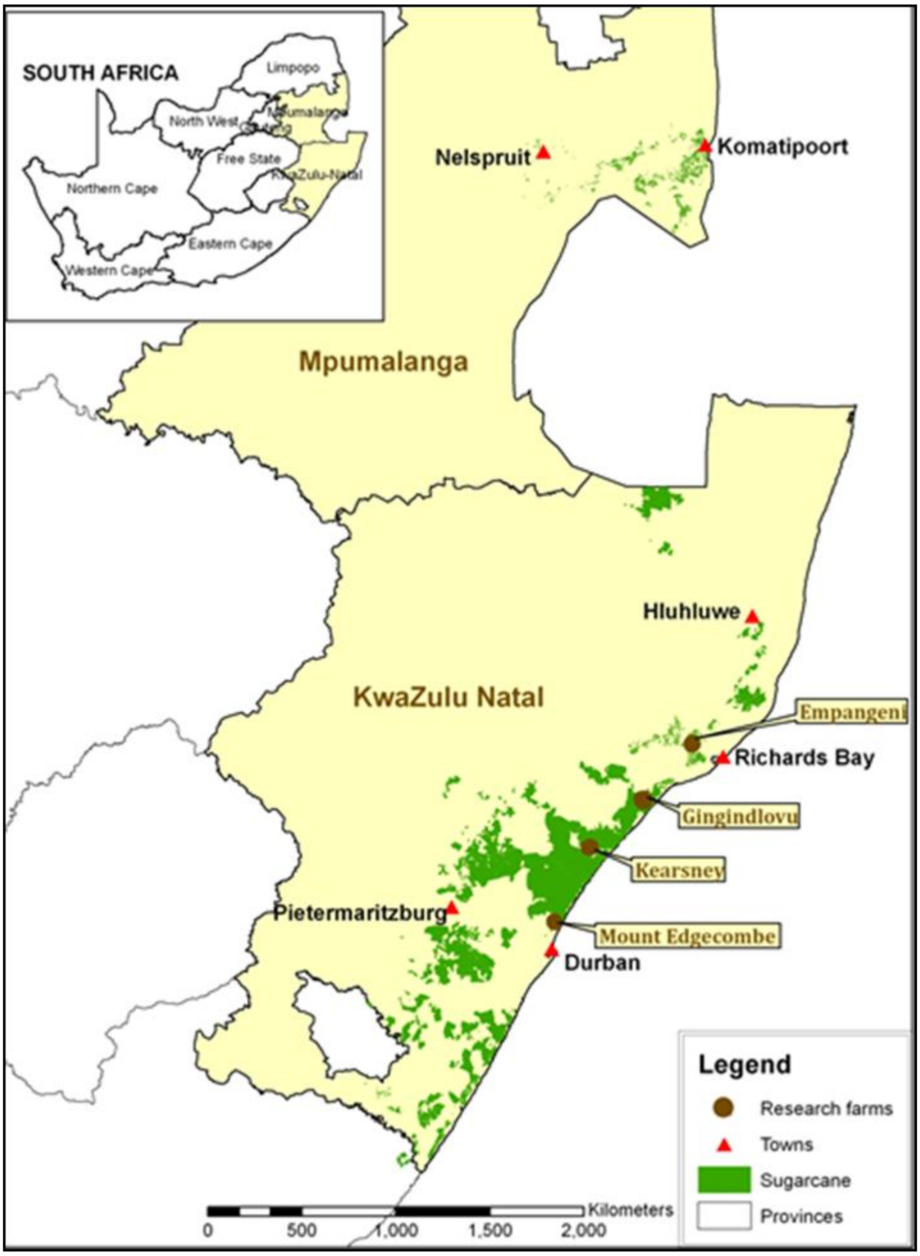


Figure 3.1: Location, within the South African sugar industry, of the four coastal trials selected for this study (GIS department, SASRI, 2011).

Only trials that had information on the ERD and clay% of the fields were considered for this study. Table 3.2 shows the number of trials, for different harvest years (1987-2011), within each of the four farm locations that were selected for this study.

Table 3.2: The number of trials selected from each farm location from 1987-2011. The green, blue and red numbers represent trials that contain 1, 2 or 3 of varieties of interest respectively.

	Mount Edgecombe		**Kearsney**		**Empangeni**		**Gingindlovu**	
GPS Coordinates	Latitude (degrees)	Longitude (degrees)	Latitude (degrees)	Longitude (degrees)	Latitude (degrees)	Longitude (degrees)	Latitude (degrees)	Longitude (degrees)
	29.70	31.03	29.28	31.27	28.80	31.92	29.03	31.60
%Clay	43.8		23.6		53.2		27.7	
1987	**1**							
1988	**3**							
1989	**4**, **1**							
1990	**4**, **1**							
1991	**2**, **1**							
1992	**3**							
1993	**3**							
1994	**3**							
1995	**1**							
1996								
1997	**1**							
1998	**3**							
1999	**3**							
2000	**2**							
2001			**4**					
2002			**4**				**2**	
2003			**2**				**2**	
2004			**1**					
2005							**2**	
2006								
2007							**1**	
2008							**3**	
2009					**1**		2	
2010					**1**,**1**		**1**	
2011					**1**		**2**	

(Source: Oracle Database (10gR2). Date Accessed: 14 June 2011.)

When SASRI variety trials are harvested several parameters are measured whilst others are calculated (from the measured parameters). For example, percentage sucrose and the weight of sugarcane (kg) are measured parameters while tonnes sucrose per hectare (TSH) and tonnes cane per hectare (TCH) are the calculated parameters. All parameters, measured and calculated, are uploaded into the SASRI Oracle database. For the evaluation of varietal performance under drought stressed conditions the calculated yield parameter TCH was used.

Drought stress index (DSI) Methodologies

For the purposes of this study, drought stress is defined as the soil water content (SWC) below which a crop does not grow/yield at its full potential (as it would in an unstressed state). Canesim was used to estimate the yield potential of a crop in an unstressed state.

Running the Canesim model

The Canesim model required certain basic field input data for each trial, including the initial soil water content (ISWC), soil drainage rate, total available moisture (TAM) and cane row spacing. The ISWC is the amount of water in a soil at the time of planting/ratooning the crop. The ISWC of the crop could not be accurately measured as all the trials evaluated were historical trials, therefore the ISWC of the plant crop of a trial was assumed to be 50% TAM of the field (Thompson, 1976). For each of the subsequent ratoon crops, the final SWC of the previous crop was used as the ISWC for the current crop. TAM was estimated using the clay% and the effective rooting depth (ERD) of the soil (Van Antwerpen *et al*., 1994). The soil drainage rate was classified as being slow, medium or fast dependent on the clay%. The cane row spacing for all coastal trials was 1.2m. For each model run, the crop planting date and harvest date were also required.

The Canesim model was run twice (rainfed and irrigated) for each crop of every trial, where the irrigated run represented the full yield potential of an unstressed crop. The irrigated runs for each crop required irrigation information which included the type of irrigation, the irrigation cycle, the soil depletion level (the SWC at which irrigation would have to start) and the amount of water supplied during each irrigation event. Overhead irrigation on a minimum irrigation cycle was used as a standard for all trials. The depletion level was set at 70% TAM, that is, the model provided the crop with water (via irrigation) when the SWC dropped below 70% of TAM. A crop is said to experience drought stress when the SWC drops below 50% of the TAM (Thompson, 1976), therefore by setting the depletion level to 70% TAM ensured that the simulated crop would never experience any drought stress. The amount (mm) of water supplied at each irrigation event was the difference between TAM and 70%TAM.

Estimation of Canesim input variables

The plant crop of a trial planted at Empangeni, TV0105, is shown as an example to illustrate some of the Canesim input and output variables. This crop was planted on 12 March 2005 and harvested on 12 May 2006. The variables TAM, ISWC, irrigation depletion level and the irrigation refill level had to be estimated for each trial. To estimate TAM, the available water content (AWC) of the soil had to first be calculated. This was done by estimating the field capacity (FC) and permanent wilting point (PWP) of the soil (equation 3.1 and equation 3.2), both of which are dependent on clay% (Van Antwerpen *et al.*, 1994).

The FC (%) and PWP (%) were calculated as follows:

Equation 3.1: Y (%) = $\frac{a*(Clay\%)}{b+Clay\%}$

Where Y% = the soil limits (either FC or PWP)%; a = 54.7, for FC and 91.9 for PWP estimation; and b = 24.5, for FC and 135.3 for PWP estimation.

Using the estimated FC and PWP, the AWC (per meter depth of soil) was calculated as follows:

Equation 3.2: AWC (mm/m) = (FC% – PWP%) * $\frac{1000mm/m}{100\%}$

The clay% of the field that TV0105 was planted on was 45.6%. Using equation 3.1 the FC and PWC were estimated to be 35.6% and 23.2% respectively. The AWC was calculated to be 124.2mm/m (equation 3.2).

The ERD of the field for TV0105 was estimated to be 2.0m. This was estimated by SASRI's standard practice. A soil auger was used to dig soil pits at each soil sampling point. The auger has the capacity to dig into 20cm of soil at a time. Each soil pit was sampled to a depth of 2.0m. The depth was only less than 2.0m in cases where the soil profile would not let the auger through, either due to very high clay content or a rocky profile.

The TAM of the field was then estimated using the average ERD and AWC of the field (equation 3.3) as 248.4 mm.

Equation 3.3: TAM (mm) = AWC (mm/m) x ERD (m)

The ISWC, of the plant crop of a trial, assumed to be 50% of the TAM, the ISWC was 124.2mm. This was the ISWC used for the plant crop of this trial, for both the rainfed Canesim runs and the irrigated Canesim runs. The final SWC of the Canesim run was used as the ISWC for the subsequent crops simulation. The parameter TAM was kept constant across all crops for a trial.

Figure 3.2(a) shows how the simulated SWC (blue shaded area) varied according to the amount of daily rainfall received (red bars). For example, on day 344, there was a rainfall event of 123.4mm, and this resulted in a sharp increase in the SWC, which was very low at that time. The drought stress point (green line) was 50% TAM. Figure 3.2 (a) shows that the SWC was below this stress point for most days highlighting that the crop experienced drought stress for most of its life.

For the irrigated Canesim simulations the depletion level of the soil, the SWC at which irrigation occurred, had to be estimated. The depletion level, for the plant crop of TV0105, was calculated (equation 3.4) as 173.9mm. The refill level, maximum SWC to which the crop should be irrigated, was equal to the TAM 248.4mm.

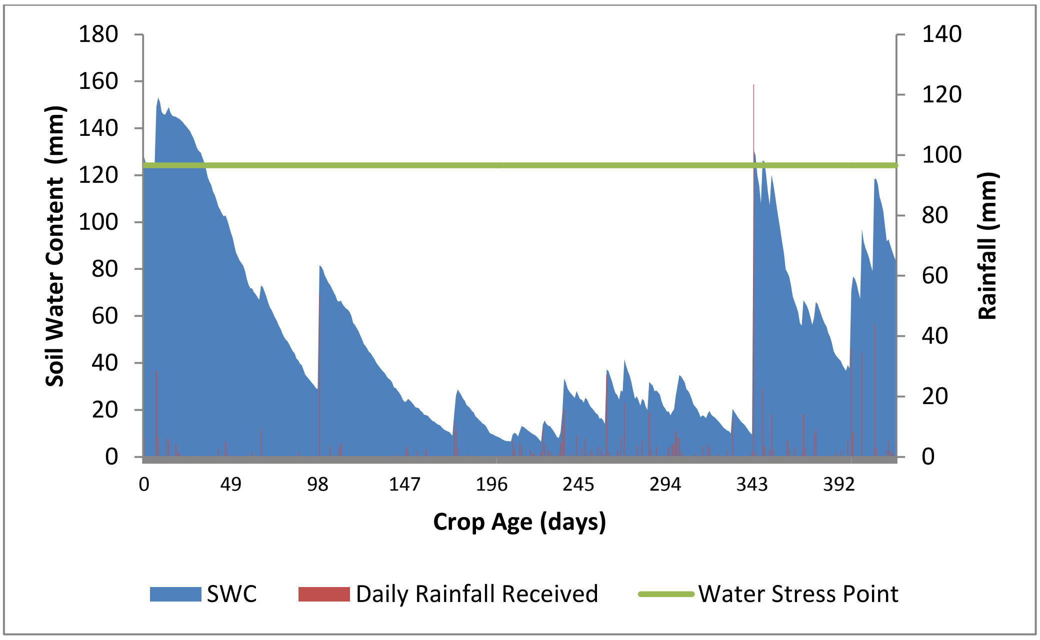
Equation 3.4: Depletion Level (mm) = 70% * (TAM)

The amount of water to be applied during an irrigation event was the difference between the TAM and the Depletion level and was calculated as 74.5mm using the following equation:

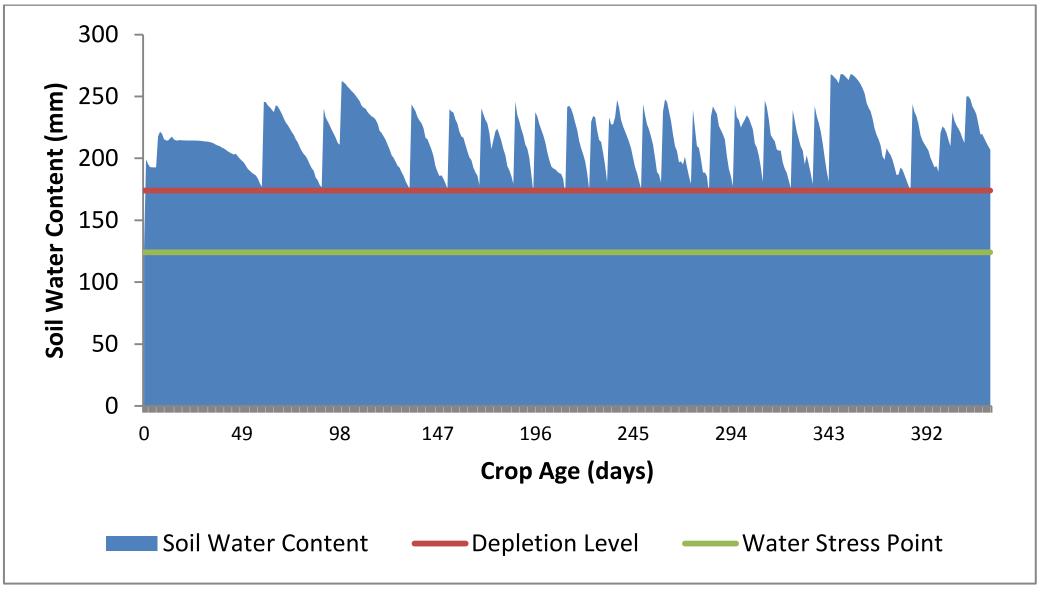
Equation 3.5: Fixed Amount (mm) = Refill Level – Depletion Level

Therefore for the irrigated runs of trial TV0105, the Canesim model would irrigate the crop, with 75.5mm, as soon as the SWC dropped below 173.9mm. Figure 3.2 (b) shows the SWC (blue shaded area) of the irrigated run of the plant crop of trial TV0105. The depletion level is represented by a red line on the graph and the drought stress point by a green line. Whenever the SWC reached the depletion level, the crop was irrigated. The SWC of the irrigated crop never dropped below the drought stress point, so the irrigated run of this crop represented an unstressed crop.

Figure 3.2: The soil water content (SWC) and daily rainfall received, from the Canesim output, of the plant crop of trial TV0105 for the two simulations (a) rainfed and (b) irrigated.



(a) Rainfed



(b) Irrigated

Calculation of the six DSI's

Canesim output parameters were used to calculate DSI's according to six different definitions.

DSI1: Percentage (%) Drought Stressed Days

Drought stress can simplistically be viewed as the number of days where the TAM of the soil is less than 50%, that is, the number of days that the plant does not have sufficient water for optimum growth. If the number of stressed days is expressed as a percentage of the crop age, an index "Percentage drought stressed days" can be calculated as follows:

Equation 3.6: $\%WaterStressedDays = \frac{NumberofDays\ TAM<50\%}{TotalCropAge\ (days)} * 100$

The plant crop of TV0105 was planted on 14 March 2005 and harvested on 12 May 2006; therefore the crop age at harvest was 424 days. The number of days where the SWC was below 50% of TAM, less than 124.2mm, was estimated by examining the daily crop SWC of the Canesim output. The SWC was below 50% of TAM for a total of 386 days. The percentage drought stressed days was calculated as follows 91%. This implies that for the 424 days of the plant crop of TV0105, the crop was drought stressed for 91% of its life, during which optimal growth rates were not possible (Figure 3.2a).

DSI2: Weighted % Drought stressed Days

The objective of this index was to incorporate the relationship between the timing of drought stress, that is, which growth phase the drought stress event/s predominantly occur in and the consequent effect on final cane yield.

Temperature is the main environmental driver of the rate of sugarcane development and in this study temperature effects was measured in terms of thermal time (TT) (Doorenbos and Kassam, 1986). TT, measured in degree days (°Cd), is the summation of cumulative differences between daily mean temperatures and a crop specified base temperature (equation 3.7). The base temperature is defined as the temperature below which there is no growth/development and is estimated to be 10°C for sugarcane (Inman-Bamber, 1994). Thermal time was calculated as follows:

Equation 3.7: TT (°Cd) = $\sum_{i=1}^{N} \frac{Ti\,max + Ti\,min}{2}$ - T_{base}

Where n = total number of crop days and T_{base} = 10°C (Inman-Bamber, 1994).

Singels and Donaldson (2000) showed that stalk elongation in sugarcane starts after 1050°Cd. A TT of 1050°Cd was taken to mark the end of FP and beginning of GGP. The start of the ripening phase is dependent on temperature and a farmer's management practice; therefore there was no unbiased way of separating the GGP and the ripening phase in historical trials. The presence of drought stress in both the GGP and the ripening phase will lead to a decrease in the final cane yield (TCH). Therefore, for the purposes of this study, these two phases were combined. The crop was thus divided into two phases, a FP (FP) and a grand growth and ripening phase (GGRP).

Based on research on the impact (on final crop yield) of drought stress during the different growth phases, a weighting factor was allocated to each of the two phases, namely a weight of 0.3 and 0.7 to the FP and GGRP respectively. The weighted percentage drought stressed days index was calculated by calculating the percentage of stressed days (equation 3.6) within each of the two phases. Each calculated percentage was then multiplied by the phase specific weighting factor and the two values were added.

The weighted DSI was calculated as follows:

Equation 3.8:

$$Weighted\ \%\ WaterStressedDays = 0.3 * (FP\ \%\ WaterStressedDays) + 0.7 * (GGRP\ \%\ WaterStressedDays)$$

Table 3.3 shows the summary of the information used for the calculation of DSI2 for the plant crop of TV0105. The information includes the total number of days that the crop was in each growth phase, the number of drought stressed days within each phase and the calculated percentage drought stressed days (equation 3.6) within each growth phase.

Table 3.3: Summary of the information of the plant crop of the trial TV0105 used for the calculation of DSI2.

Growth Phase Group	Length of Growth Phase (days)	Number of Stressed Days(<50%TAM) in growth phase	% Stressed Days
FP	200	166	83%
GGRP	224	220	98%

Table 3.3 shows that there was more stress experienced during the GGRP, leading to a consequently more heavily weighted DSI compared to DSI1. For the plant crop of TV0105 the negative impact of drought stress on the optimal TCH potential occurred for 93.7% of the crop's life span.

DSI3: Average Canesim Stress

Part of the Canesim daily output was a variable called 'Stress'. This simulated variable was based on the SWC, and it gave an indication of the extent of the drought stress, when present. Canesim stress was calculated by expressing the daily SWC content as a percentage of 50% TAM level of the soil (Raes *et al*., 2009). The range of the variable was between 0 and 100. If the SWC was greater than 50% TAM then the value of the stress variable was always 100, regardless of how much greater than 50% TAM the SWC was. However, if the SWC dropped to below 50% TAM then the stress variable was calculated as a proportion of 50% TAM level. The lower the SWC dropped, below 50% TAM, the closer to 0 the stress variable would approach. Figure 3.3 shows the daily Canesim output, stress (blue line) and SWC (red line) of plant crop of TV0105. The 50% of TAM crop drought stress point is also displayed (green dotted line).

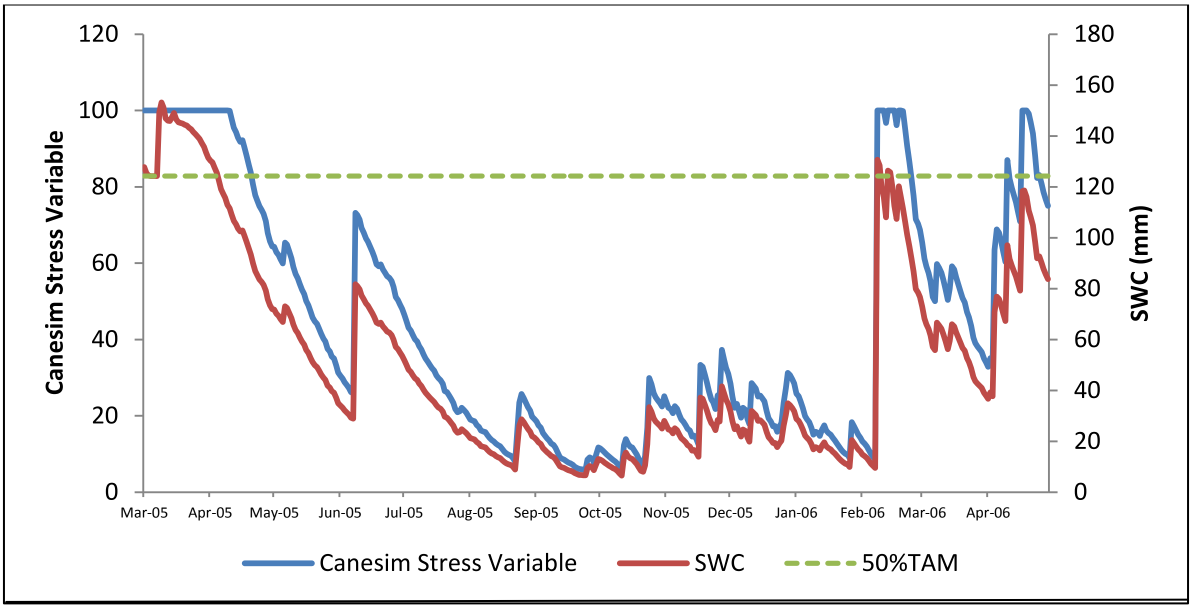


Figure 3.3: The daily values of Canesim variable stress, the SWC and the 50%TAM level for the plant crop of the trial TV0105.

DSI3 was calculated, using equation 3.9, where the daily Canesim stress values were averaged over the crop age as follows:

Equation 3.9: $Average\ Canesim\ Stress\ = \frac{x_1+x_2+x_3+\cdots x_i}{CropAge\ (days)}$

Where x_i = the Canesim simulated stress experienced on the i^{th}day of the crop.

DSI3 of the plant crop TV0105 was calculated to be 44.2. This was an average estimate of the amount of drought stress experienced over the duration of the crop. The stress variable ranged from 100, where there was no drought stress to 5.8, where the soil had almost no soil water available. A value of 100 means that the SWC was at 50% TAM or above, therefore a value of 50 would mean that the average SWC was only 25% of the TAM. By this token, an average value of 44.2 indicates that the plant crop of TV0105 was severely stressed for most of its life, having on average less than 25% TAM as a SWC.

This index differed from DSI1 in that it took into account the extent to which the SWC dropped below the drought stress point.

DSI4: Weighted Average Canesim Stress

A weighted average Canesim stress index was calculated as follows:

Equation 3.10:

$$Weighted\ Average\ Canesim\ Stress = 0.3 * (Average\ FP\ Canesim\ Stress) + 0.7 * (Average\ GGRP\ Canesim\ Stress)$$

Table 3.4 shows the information used in the calculation of DSI4 for the plant crop of TV0105. The information included the total number of days that the crop was in each growth phase and the average Canesim stress experienced within each phase.

Table 3.4: Summary of the information of the plant crop of the trial TV0105 used for the calculation of DSI4.

Growth Phase	Length of Growth Phase (days)	Average Canesim Stress Level
FP	200	52.47
GGRP	224	36.71

The weighted average Canesim stress index was calculated to be 41.4. This value indicates that the SWC for this trial was on average approximately 20.7% of TAM, that is, the crop was severely stressed for most of its life. Most of the stress occurred during GGRP (Table 3.4); therefore the calculated stress value was lower than that for DSI3.

DSI5: Stress Ratio I (observed yield: simulated irrigated yield)

The first stress ratio was calculated by expressing the observed trial yield as a fraction of the final Canesim irrigated yield. If the ratio was equal to 1.0, this would imply that the crop being evaluated experienced no drought stress at all because the simulated yield of the rainfed crop was the same as the yield of the unstressed irrigated crop. If, for example, the ratio equalled 0.5, this would indicate that the rainfed crop was stressed such that it only achieved half its potential yield.

The stress ratio was calculated as follows:

Equation 3.11: $StressRatio\ I = \frac{Observed\ Trial\ Yield}{Final\ Canesim\ Yield_{irrigated}}$

For the plant crop of TV0105 stress ratio I was 0.43, indicating that the crop only achieved 43% of its optimal yield potential.

DSI6: Stress Ratio II (simulated rainfed yield: simulated irrigated yield)

A second stress ratio was calculated by expressing the final Canesim yield of the rainfed crop as a fraction of the final Canesim yield of the irrigated crop. As with DSI 5, if the ratio was equal to 1.0, this would imply that the crop being evaluated experienced no drought stress at all because the simulated yield of the rainfed crop was the same as the yield of the unstressed irrigated crop. The stress ratio was calculated as follows:

Equation 3.12: $Stress\ Ratio\ II = \frac{Final\ Canesim\ Yield_{rainfed}}{Final\ Canesim\ Yield_{irrigated}}$

For the plant crop of TV0105 stress ratio II was calculated as 0.33, indicating that the rainfed crop would have only achieved 33% of its full potential yield.

Doorenbos and Kassam (1986) showed that temperature is one of the main drivers of crop growth, with the other main driver being rainfall (Bezuidenhout and Schulze, 2006). The advantage of DSI6 was that by using the rainfed and irrigated Canesim yields in the simulation, the effects of both temperature and rainfall were accounted for. In addition, the errors associated with the final yields, both rainfed and irrigated, were the same as both were generated using Canesim. This was not the case with DSI5.

Statistical Analysis

Genstat v.14.1 (VSN Intl. Ltd, 2011) was used for all data analyses. Prior to any analyses, the data were first tested for normality and homoscedasticity using the Shapiro-Wilk and Levene's tests respectively. All significance testing were done at the 0.05 level. Transformations were applied as necessary. For graphical representation of data that had to be transformed prior to analysis, the raw data was presented for ease of interpretation. The letters from the *post hoc* Holm-Sidak tests were added to the graph.

The yields were first annualized to account for the variation due to the differences in crop age among all the crops used in this study, with crop ages varying between 346 - 593 days (equation 3.13). Canesim model validation was done by regressing the annualized observed trial mean, which is the average cane yield of all the varieties planted within a trial, on the annualized simulated (rainfed) yields.

Equation 3.13: Annualized Yield (TCH) = $\frac{365}{Crop\ age\ (days)}$ * Actual Yield (TCH)

To validate the observed varietal yield performances of N12, N19 and NCO376, when subjected to different levels of drought stress, the performance was evaluated across different rainfall classes. The annual rainfall for the lifespan of each weather station was downloaded from the SASRI weather web (http://portal.sasa.org.za, 2011). This was done for each of the four weather stations used in the study. The annual rainfall data from each station was sorted from lowest to highest. All the years in the lower third group were classified as 'Below Average', the middle group was classified as 'Average', and the upper third as 'Above Average'. For each weather station the range of rainfall received within each of the three classes was noted. The rainfall for each trial was annualized (equation 3.14) and was classified into one of the three rainfall classes. A residual maximum likelihood (REML) analysis was performed, to establish if there was a significant interaction between variety and rainfall class (y-variate=annualized variety yield; fixed effects=rainfall class*variety; random effects=trial location. This was followed by the *post hoc* multiple comparison Holm-Sidak test.

Equation 3.14: Annualized Rainfall (mm) = $\frac{365}{Crop\ age\ (days)}$ * Actual Rainfall (mm)

To examine the relationship between the unweighted and weighted DSI's a Pearson's correlation matrix was produced.

To investigate how well the DSI's differentiated between the yield performances of the three varieties, the annualized yield of each variety was regressed on each of the 6 DSI's. As stated previously, N12 has an observed intermediate (I) yield response to drought stress where as N19 and NCO376 both have an observed intermediate-susceptible (IS) yield response to drought stress. The ability of a DSI to detect the fine difference between the I and IS yield performance of the three varieties would be indicative of its sensitivity for accurately quantifying the level of drought stress experienced. To evaluate the yield response of a variety to changing levels in drought stress, as quantified by the DSI's, the gradients (m_1) of the regression lines were statistically compared (Rizza *et al.*, 2004).

A general linear regression model was used to compare the gradients of the different varieties. The regression analysis was run twice. The first regression model included N12 as the reference variety, allowing comparisons (of regression parameter estimates) of N12 with N19 and NCO376.

Genstat Regression Model 1:

```
MODEL [DISTRIBUTION=normal; LINK=identity; DISPERSION=*] Annualized Variety Yield
TERMS[FACT=9]Variety*Variety.DSI+Trial Type + FarmLocation + TrialMean + Crop + Harvest
Year + Harvest Season
FIT[PRINT=model,summary,estimates;CONSTANT=estimate;FPROB=yes;TPROB=yes; FACT=9]
Variety*Variety.DSI+FarmLocation +TrialMean + Crop + HarvestYear + HarvestSeason.
```

Using the results of the Student's t-tests, the m_1's of N19 and NCO376 were evaluated. If the m_1's were significantly higher than N12 were classified as being potentially IS varieties, respectively. If the m_1 was not significantly different to N12 the variety would be classified as being a potentially I variety.

The second regression model allowed the separate estimation of regression parameters of individual varieties.

Genstat Regression Model 2:

```
MODEL [DISTRIBUTION=normal; LINK=identity; DISPERSION=*] Annualized Variety Yield
TERMS[FACT=9;FULL=yes]Variety*Variety.DSI+FarmLocation+TrialMean+Crop+Harvest
Year+HarvestSeason
FIT [PRINT=model,summary,estimates; CONSTANT=omit; FPROB=yes;TPROB=yes;FACT=9]
 Variety+Variety.DSI+FarmLocation+TrialMean+ Crop + HarvestYear + HarvestSeason.
```

To validate the chosen DSI, a Student’s t-test was used to determine if there was a statistical significant difference between each of the calculated DSIs among the three different rainfall classes (below average, average, above average).

Results

Canesim Data Validation

The plot of the annualized actual trial yield mean versus the simulated annualized rainfed trial yield mean revealed a few outliers, that is, data points that are distinctly different to the expected average. Further investigation showed that these outliers were due to technical problems with the weather station at the Kearsney farm. This was a result of inaccurate capturing of weather data during 2001-2002, therefore any simulations done during this period were very unreliable. There were 6 outliers subsequently removed from the data set, leaving a total of 60 trials to be analysed. The Shapiro-Wilk test was not significant. Figure 3.4 shows the regression of the observed trial yield mean on the simulated rainfed trial yield mean, excluding the outliers. The Canesim yield accounted for 85% of variation in the observed yields ($p<0.001$).

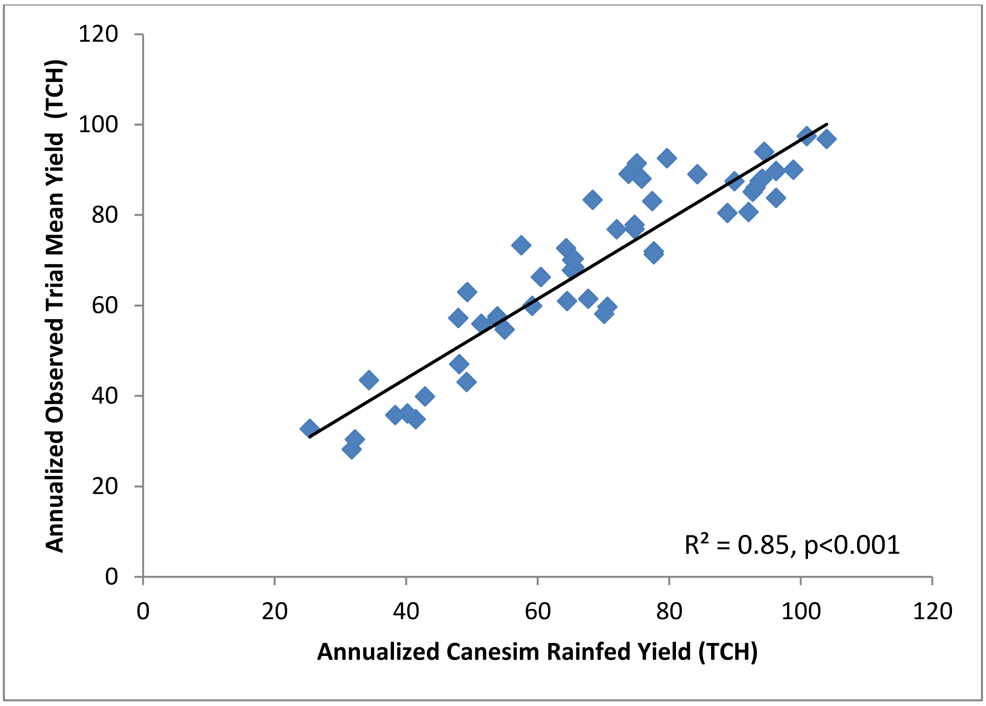


Figure 3.4: Regression of the observed trial yield means versus Canesim rainfed yields.

Validation of observed varietal yield performance to drought stress

The Shapiro-Wilk's test showed that the data was normally distributed. However, the Levene's test showed that the variances of the three varieties were heteroscedastic. The data was log (base 10) transformed. For ease of interpretation, the raw data was presented in Figure 3.5.

REML analysis showed that there was a significant interaction between rainfall class and variety ($F_{4,109}$=4.58, p=0.002). Both N19 and NCO376 produced significantly lower observed yields across each of the rainfall classes (Figure 3.5). For N12, there was only a significant difference between the above average class and the average class (Figure 3.5). This highlighted that there was less of an effect of rainfall on cane yield for the I variety compared with the two IS varieties.

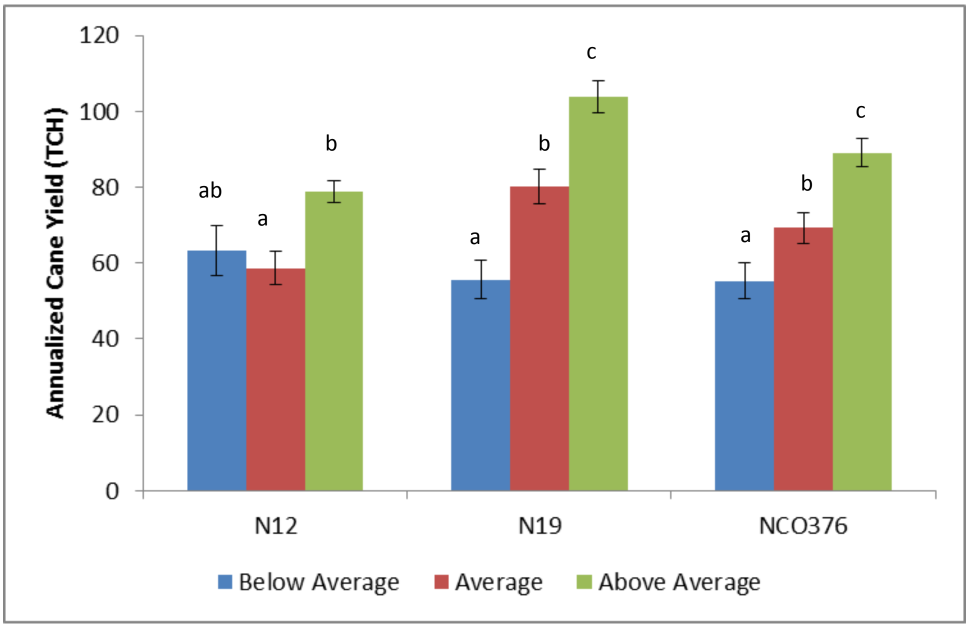


Figure 3.5: The observed yield performance of varieties N12, N19 and NCO376 across the three rainfall classes. The error bar represents the standard error. Classes that do not share the same letters are significantly different to each other ($p<0.05$).

Note: Holm-Sidak ($p<0.05$) tests were done separately for the different rainfall classes within each variety.

Evaluating the DSI's

Figure 3.6 shows the interrelationships between the different indices where the Pearson's correlation coefficients (r) have quantified the nature and strength of each relationship. There were both negative (shown as blue in Figure 3.6) and positive (shown as red in Figure 3.6) r's due to the different types of data used for each of the DSI calculations. For example, DSI1 (and DSI2) range from 0-100% where a value of 100% represents a completely stressed environment (the crop was stressed for every day of its life) whereas a value of 0 represents a completely unstressed crop. DSI3 (and DSI4) were calculated using the Canesim stress variable. As discussed earlier, this variable ranged from 0-100 however, a calculated index value closer to 0 indicates a highly stressed crop and a value of 100 represents a crop that was completely unstressed.

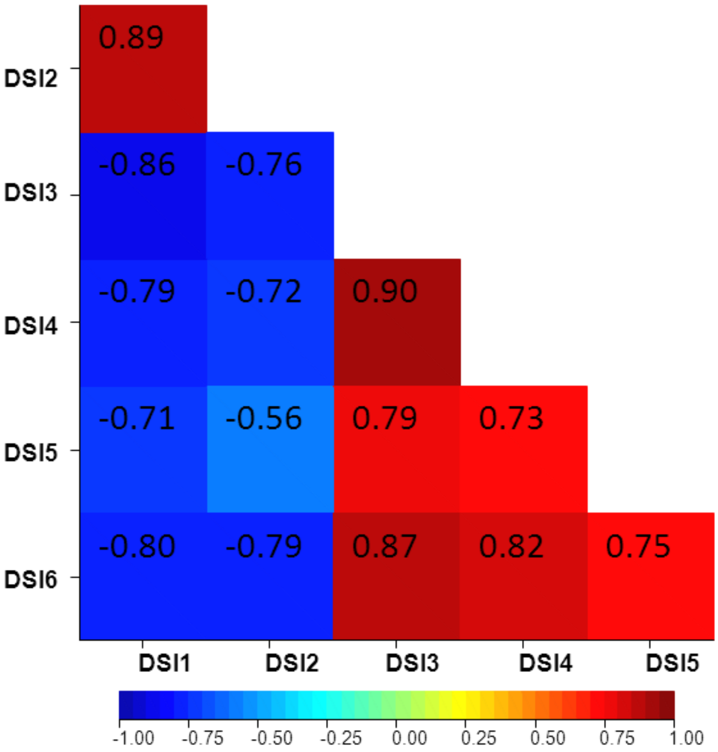
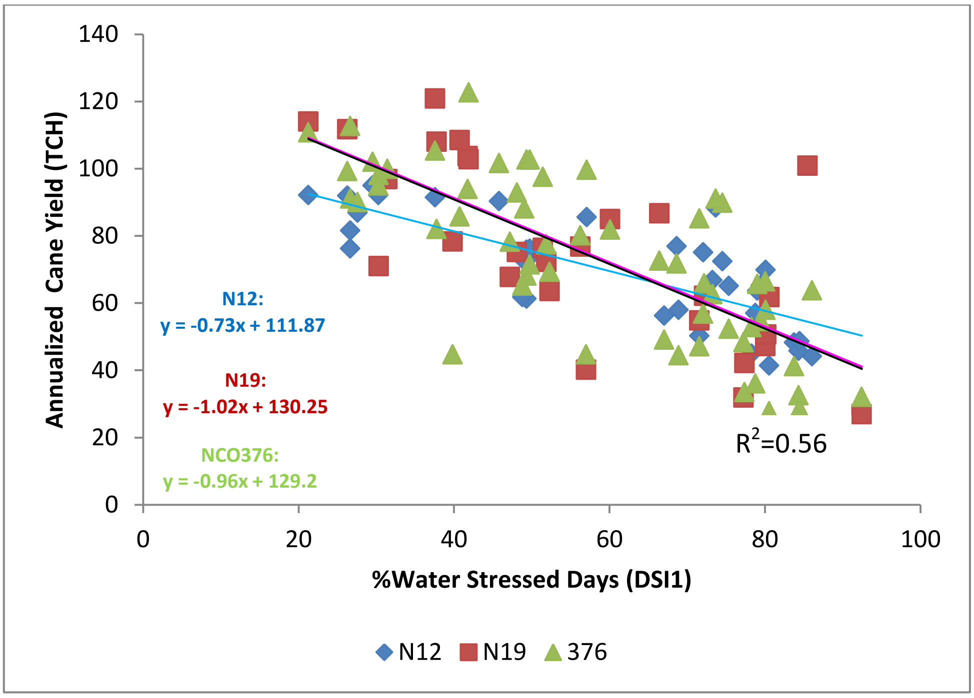


Figure 3.6: A Pearson's correlation matrix graph of the 6 DSI's.

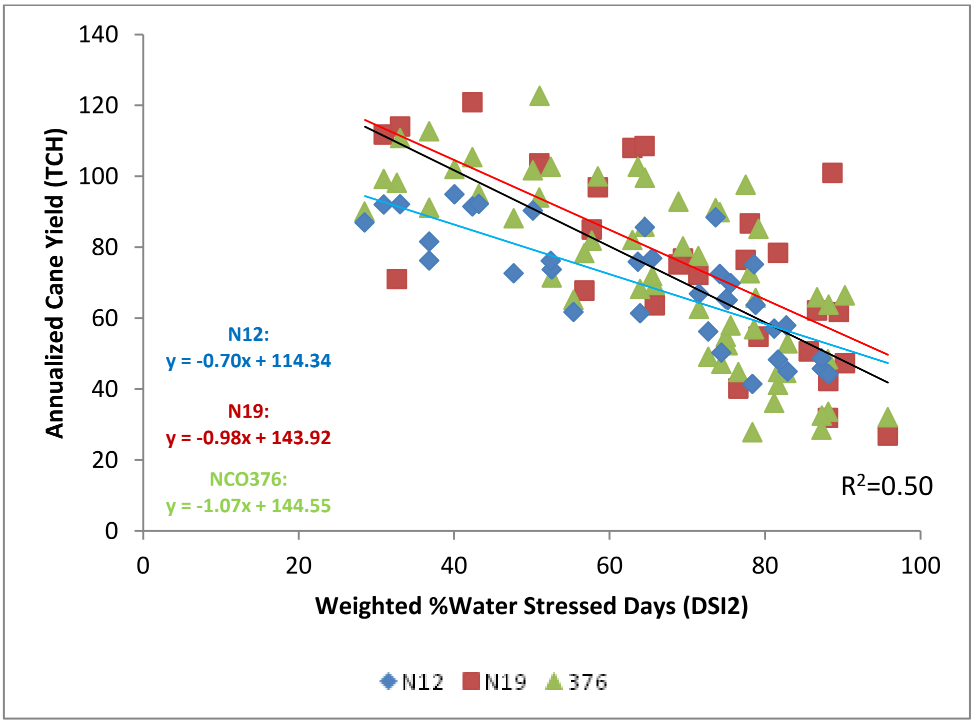
The r's were all significant (p<0.001) and ranged from 0.56-0.90 (Figure 3.6). The unweighted and respective weighted indices (DSI1/2 & DSI3/4) were the most strongly positively correlated (p<0.001) with r=0.89 and r=0.90 respectively, as shown by the red blocks in Figure 3.6.

After removal of the six outliers, there were 35 data points for N12, 28 for N19 and 58 for NCO376. Figure 3.7 shows the regression of the annualized observed yields of the three varieties on each of the six DSI's. The Shapiro-Wilk and Levene tests were not significant. Regression lines were fitted separately for each variety (Genstat Regression Model 2) and the gradients of the each line were tested for differences (Genstat Regression Model 1). The variables (TrialMean, Crop, Harvest Year, Harvest Season) and factor (Farm Location) did not account for significant variation so was not included in the final regression models.

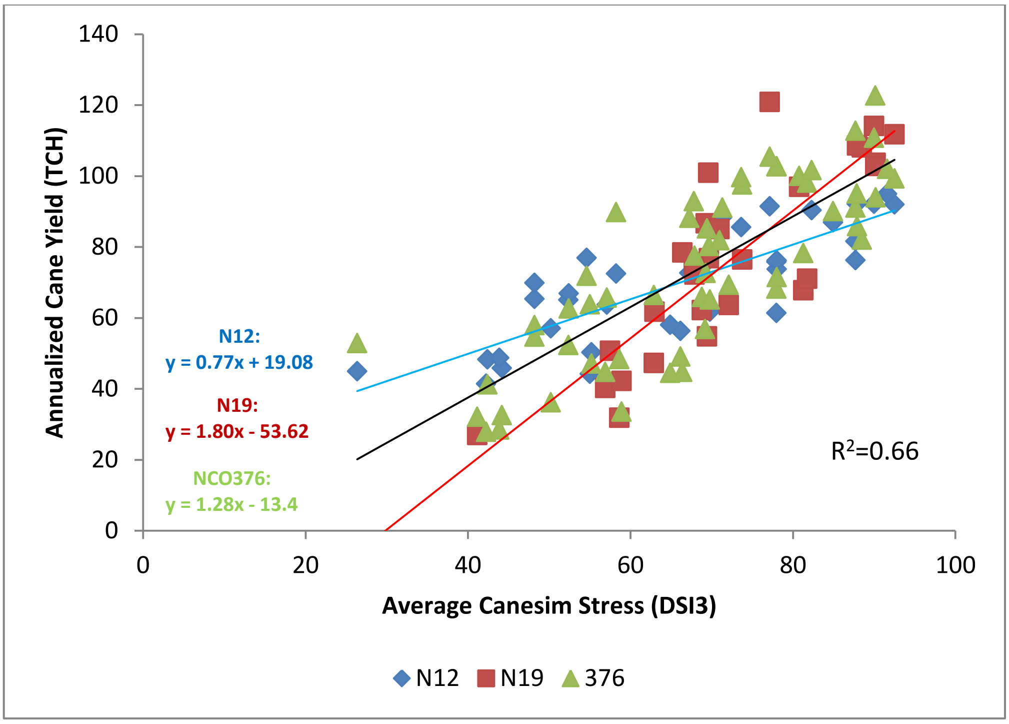
Figure 3.7: Regression lines of the annualised observed cane yield of N12, N19 and NCO376 on each of the 6 DSI's.



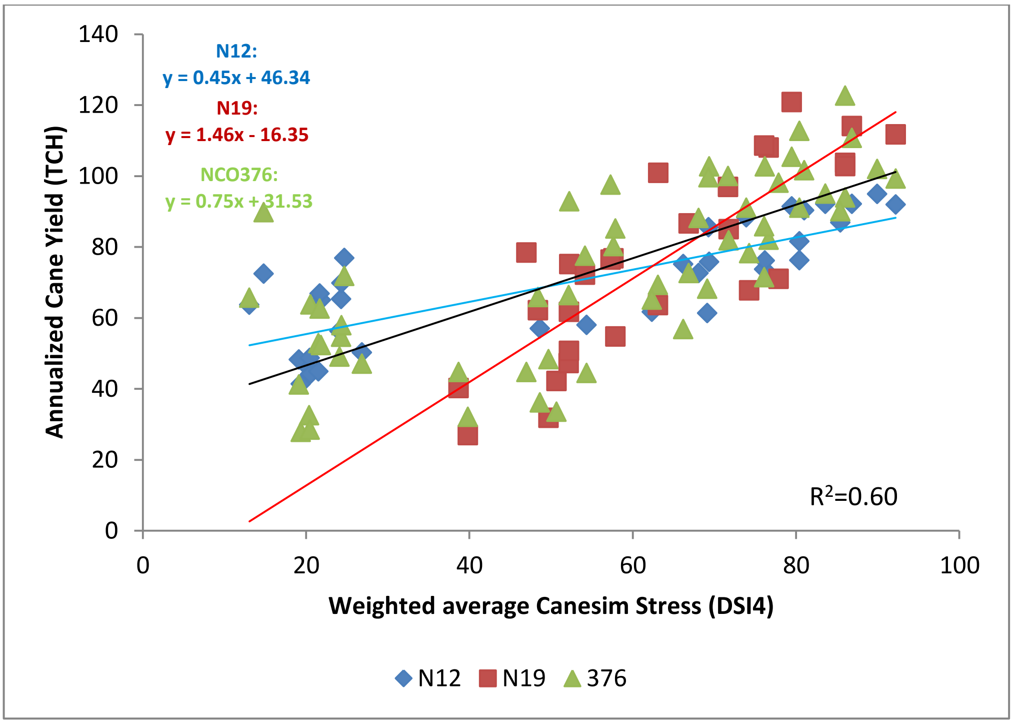
(a) %Water Stressed Days (DSI1)



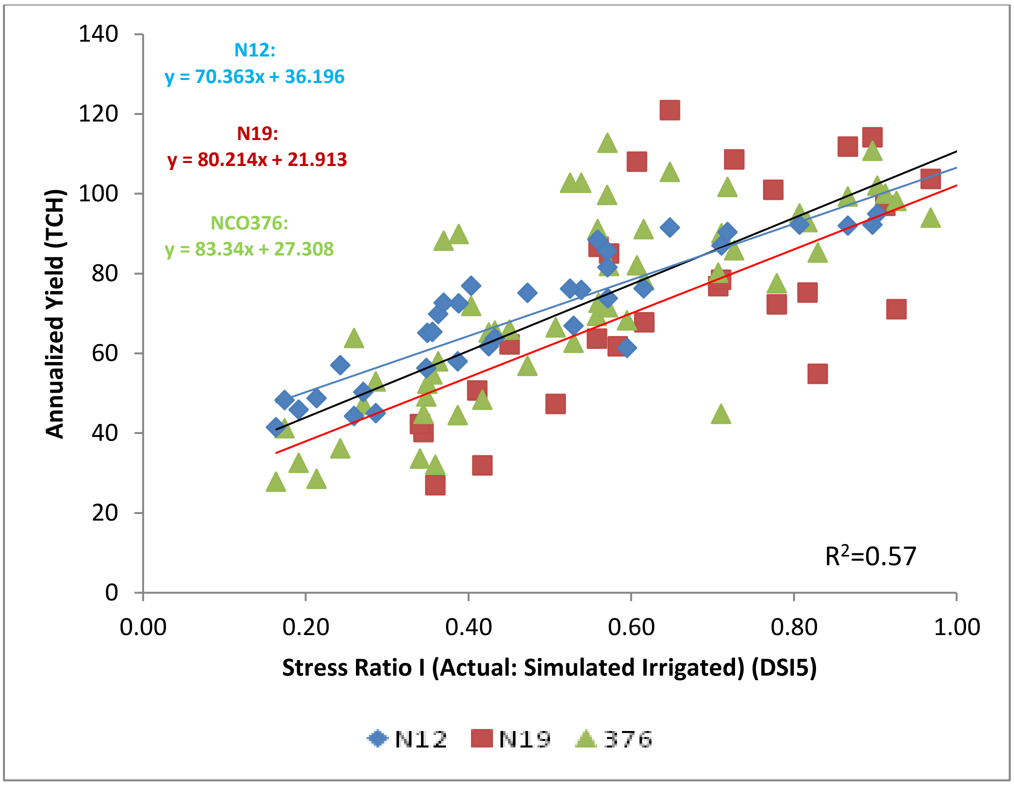
(b) Weighted %Water Stressed Days (DSI2)



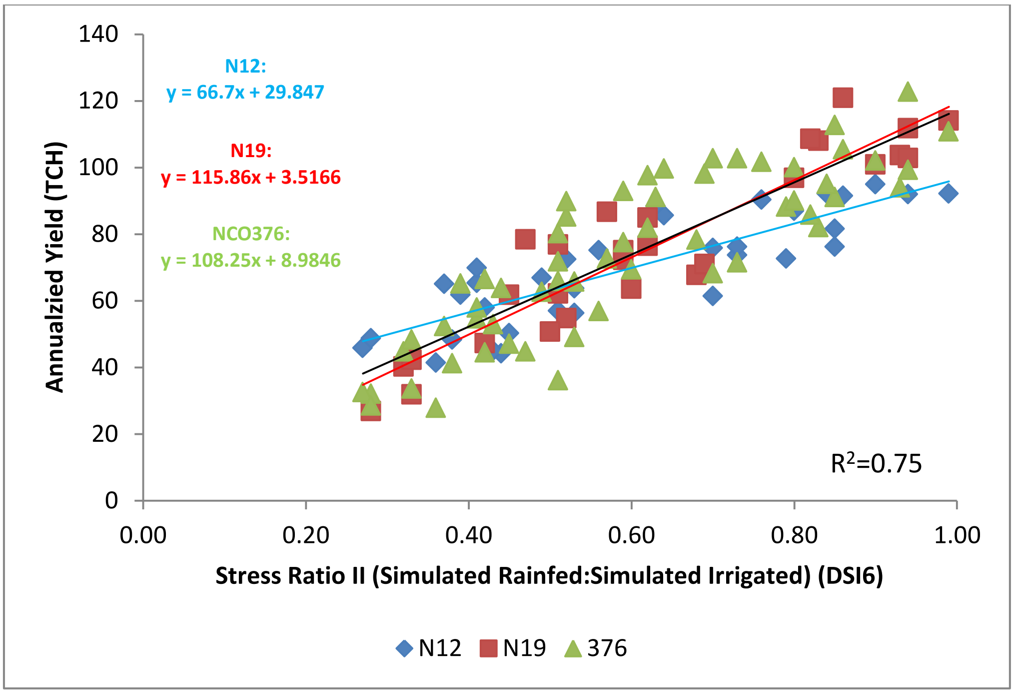
(c) Average Canesim Stress (DSI3)



(d) Weighted average Canesim Stress (DSI4)



(e) Stress Ratio I (Actual: Simulated Irrigated) (DSI5)



(f) Stress Ratio II (Simulated Rainfed:Simulated Irrigated) (DSI6)

Figure 3.7 (a) showed that the gradients (m_1) of N12, N19 and NCO376 regression lines were all negative. This indicated that as the level of drought stress increased there was a consequent decrease in the observed varietal yield. The gradients of N19 and NCO376 were very similar (m_1=-1.02 and -0.96 respectively) and larger than that of N12 (m_1=-0.73), indicating that the yield response of N19 and NCO376 to changes in drought stress similar to each other with each variety losing 1.02TCH and 0.96TCH per 1% increase in stress levels as quantified by DSI1. However, N12 seemed to have a more moderate response to drought stress, compared to N19 and NCO376, losing 0.73TCH per 1% increase in stress levels as quantified by DSI1. The regression analysis, using N12 as the reference level (Genstat regression model 1), could not detect any significant differences between the gradients of N12 compared to N19 or NCO376. Similar results were found with DSI2 (Figure 3.7b).

DSI3 displayed similar results to DSI1 and DSI2 (Figure 3.7c), with N19 and NCO376 having a similar observed yield response to stress whereas N12 was less responsive. However, with DSI3 there was a smaller amount of unexplained error (R^2=0.66) compared with that of DSI1 (R^2=0.56) and DSI2 (R^2=0.50). The Student's t-tests from the first regression analysis showed that there was a significant difference between the gradients of N12 (m_1=0.77) and N19 (m_1=1.80). However, no significant difference could be detected between the gradients of N12 and NCO376 (m_1=1.28). Even though Annualized Yield vs. DSI4 regression had a slightly lower R^2 (R^2=0.60) compared to DSI3, the same results were achieved (m_1: N12=0.45, N19=1.46, NCO376=0.75) (Figure 3.6d).

DSI5, accounted for 57.3% of the variation in observed varietal yield performance. However, the index was unable to detect significant differences between the gradients of N12 (m_1=70.36) compared to N19 (m_1=80.21) or N12 compared to NCO376 (m_1=83.34) (Figure 3.6e)

DSI6 accounted for the most variation (in yield performance) compared to the other five DSI's (R^2=0.75) and was also able to detect significant difference between the gradients of N12 (m_1=66.70) and N19 (m_1=115.86) as well as between N12 and NCO376 (m_1=108.25).

Validation of the chosen DSI

DSI6 was the only DSI that was able to make the distinction between the I variety (N12) and both the IS varieties (N19 and NCO376) and it accounted for the most amount of variation in varietal yield performance. Figure 3.8 shows the average DSI6 for each rainfall class. The Shapiro-Wilk's test showed that the data was normally distributed. The Levene's test showed that the variances across the three rainfall classes were stable. Figure 3.8 shows that as the amount of rainfall decreases, the more severe (lower) are the calculated DSI values. The Student's t-test showed that the calculated index values for the above average rainfall class was significantly higher compared with the average rainfall class ($p<0.001$) and the below average rainfall class ($p<0.001$). In addition, the calculated index values within the average rainfall class was significantly higher compared with the below average class ($p=0.02$).

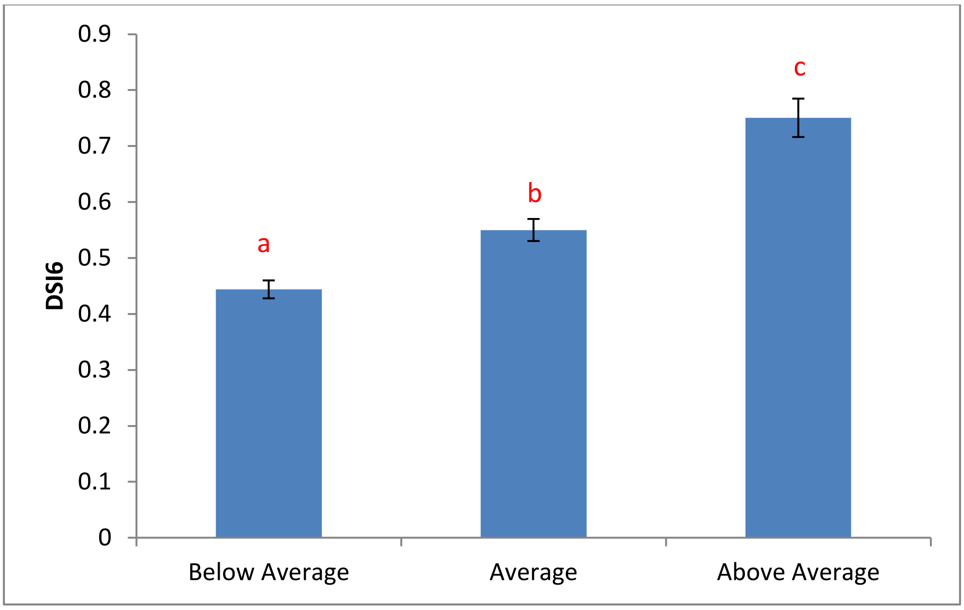


Figure 3.8: Average DSI6 for each of the rainfall classes. The bars represent the standard error. Classes that do not share the same letters are significantly different to each other ($p<0.05$).

Discussion

Validation of the Canesim model highlighted six data points that were considered to be outliers. Further investigation into the data for each of these points revealed erroneous Canesim final yield predictions due to large amounts of missing weather data, within the weather database, during the 2001-2002 period, at the Kearsney farm. Some of the reasons for missing weather data include theft and technical problems with instrumentation which cause parameters to be incorrect or missing and manual records (for an entire month) going missing during mailing (Bezuidenhout and Singels, 2007). When weather data are missing, Canesim uses a data patching algorithm to fill in missing data. However, zero values are substituted for rainfall (Singels *et al.*, 1999). De Lange and Singels (2003) showed that this could lead to serious discrepancies in Canesim yield predictions, especially if the weather station is out of order during a period of significant rainfall. Once these outliers were removed the validity of the data simulations were excellent (R^2=0.85, p<0.001). When Gers *et al*. (2001) validated the Canesim model they obtained similar results (R^2=0.87, p<0.001). Therefore it was assumed that the trimmed Canesim data could be used with confidence for the calculation of the different DSI's.

Research has shown that the timing of the drought stress significantly affects the final cane yield (Robertson *et al.*, 1999; Ramesh and Mahadevaswamy, 2000; Inman-Bamber, 2004; Silva *et al.*, 2008). However, for this study there was a very strong positive correlation (p<0.001) between the unweighted and weighted DSI's showing that weighting the timing of the stress within the FP and GGRP did not prove to provide any significant information on yield response to drought stress. In addition, the regression analysis showed that the unweighted indices accounted for more variation in the varietal yield performance when compared to the (respective) weighted indices. There were 60 trials that were evaluated in this study, with the first crop being planted in March 1986 and the last crop harvested in July 2011. With the 25 year span of crops being evaluated, it was assumed that there was an adequate coverage of the environments (Bezuidenhout and Schulze, 2006). However, the results suggest that the timing of the drought stress event was not a significant element in determining the final yield of the crop.

Different types of information were used to calculate the different DSI's and this had a large impact on how much variation in the observed varietal yield performance the index was able to account for. DSI1 was based on a very basic calculation, where the number of days where the SWC dropped below the drought stress point was counted. The crop days were evaluated according to the number of days where the SWC dropped below 50% TAM. This simple technique of categorising each crop day as being either drought stressed or unstressed was inadequate. This index was unable to adequately quantify the amount of stress in the environment such that it could explain the varietal performances of N12, N19 and NCO376. Since accounting for the timing of the stress did not prove to be useful in this study, DSI2 was also unable to adequately quantify drought stress.

The daily Canesim stress output variable, used to calculate DSI3 (and DSI4), was more sophisticated than the information used to calculate DSI1 and DSI2. The stress output variable quantified the extent to which the SWC dropped below the 50%TAM drought stress point. The stress variable therefore took into account whether the crop was drought stressed or not (similar to DSI1/DSI2), as well as the degree to which the crop was experiencing drought stress.

The calculation of a stress ratio, to quantify the amount of drought stress experienced by a crop, has been extensively used in many different fields of crop research, other than sugarcane. These include crops like wheat, barley, broccoli and alfalfa. However, using this methodology to quantify the amount of drought stress experienced by a historical crop has not been published until recently. Ramburan *et al*. (2011) found that of the three ratios calculated (Canesim rainfed ET:Canesim irrigated ET, Canesim rainfed yield:Canesim irrigated yield, actual trial yield:Canesim irrigated trial yield), the ratio of the observed trial yield to simulated irrigated yield accounted for the most varietal variation in response to changes in environmental differences. These environmental differences include TAM, organic matter, clay% and the soil N mineralization categories. The large error associated with using simulated values to calculate a ratio was the reason Ramburan *et al*. (2011) proposed the observed trial yield to simulated irrigated yield as the best ratio to use to quantify the amount of stress.

The research done in this pilot study showed that the use of the observed trial mean to calculate a stress ratio proved to be a lot less effective compared with using the Canesim rainfed yield. Expressing the actual trial yield as a fraction of the simulated irrigated trial yield makes the assumption that the actual and the simulated (rainfed) yields are similar. It ignores the fact that the actual trial yield is subjected to the natural variation whereas the Canesim simulated yields are only subjected to the error associated with the model. The amount of variation in yield performance when subjected to differing levels of drought stress was the lowest for DSI5 (R^2=0.57), which was approximately 20% less than with DSI6 (R^2=0.75). By expressing the yield of one Canesim yield over another cancels out the error associated with the model and the calculated ratio becomes a relative value of the degree of stress experienced.

DSI6 quantified drought stress in such a way that it accounted for a significant amount of variation in the varietal yield performances over changing levels of drought stress (R^2=0.75, $p<0.001$). This index was able to distinguish between the I and each of the IS varieties. A significant difference between the gradients of N12 (m_1=66.7) and N19 (m_1=115.86) as well as between N12 and NCO376 (m_1=108.25) were found. The ability of this index to detect the fine differences between I and IS varietal performance highlights its superior sensitivity in quantifying degrees of drought stress. Furthermore, there was a significant difference in average DSI6 among the three different rainfall classes, validiting the index's potential of quantifying drought stress.

Conclusion

Of all the DSI's calculated in this study, DSI6, was the only index that was able to make the complete distinction between the I variety and both the IS varieties. This ability to pick up the fine difference between varieties is indicative of its sensitivity and strength as a tool for measuring the amount of drought stress experienced by a trial. The growth of a crop is affected by both temperature and drought stress, and both of these factors are taken into account by the Canesim model. DSI6, was calculated using only the final Canesim yields, therefore one of the strengths of this index is that it takes into account both the drought stress and/or temperature stresses that the crop is exposed to during its development.

Based on this information DSI6 can confidently be used to accurately quantify the amount of stress experienced by a crop in the analysis of all released varieties in Chapter 4.

List of Equations

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Chapter 4

A desktop evaluation of the drought tolerance potential of released sugarcane varieties

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A desktop evaluation of the drought tolerance potential of released sugarcane varieties

C. Sewpersad

South African Sugarcane Research Institute (SASRI), Private Bag X02, Mount Edgecombe,
4300, South Africa.

Abstract

Climate change could result in an increase in frequency and intensity of drought events and is of major concern in the rainfed sugarcane areas of South Africa. In response, the breeding strategy has to now incorporate breeding for drought tolerant varieties. This study used historical yield data, from a SASRI Oracle database, from the rainfed Coastal and Midlands's regions. There were 7 farms (416 trials, 12 varieties) and 6 farms (161 trials, 11 varieties) used for the evaluation of the coastal and midlands regions respectively. Varietal databases containing trial, field, variety information and calculated DSI (from Chapter 3) were created for each region, which were analysed separately using Genstat v14.1. To ensure validity of the calculated DSI's the Canesim model was validated by plotting the actual trial yield against the simulated Canesim rainfed yield. The simulated data were reliable for both regions (Coastal: R^2= 0.72, $p<0.001$; Midlands: R^2= 0.73, $p<0.001$). A correlation analysis between the DSI and trial mean showed that there was a moderate relationship between the two variables for the coastal region ($r=0.54$, $p<0.001$) and a strong relationship for the midlands regions ($r=0.82$, $p<0.001$). A correlation analysis between the distance of a weather station (used in Canesim model) from a farm and the yield deviation (observed yield – Canesim rainfed final yield) showed no relationship between the two variables for either the Coastal ($r=0.08$, $p=0.12$) or Midlands regions ($r=-0.05$, $p=0.59$). To evaluate and rate varietal yield response to drought stress, two different statistical methods were used: 1. General Linear Regression analysis and 2. Residual maximum likelihood (REML) meta-analysis. For the first method, the varietal yield was plotted on the DSI. N12, observed to have an Intermediate (I) yield response to drought stress, was used as a reference variety. The gradients (indicators of yield response to drought stress) of all varieties were compared to that of N12, and varieties were rated accordingly. For the coastal region: N27, N29, N33, N36, N41, N45, N47 were all rated as having an Intermediate (I) response to drought stress and N16, N19, N39 and NCO376 were rated as Susceptible (S) varieties. Due to the smaller gradient differences at the midlands and the 24 month cutting cycle, the regression analysis could not distinguish between the yield response of N12 and the other varieties. For the REML meta-analysis, the DSI's were categorized into four stress environments: ≤0.3, >0.3-0.6, >0.6-0.8, >0.8, representing "environments" of different levels of drought stress. The performance of the different varieties across these environments were evaluated, and rated based on the presence of significant yield differences with an increase in the level of stress experienced. For the coastal regions the ratings were as follows: N12, N27 were rated as being Tolerant (T), N16 and NCO376 were rated as S and the rest, except for N36 and N47, were rated as I. There was insufficient information to evaluate N36 and N47. THE REML meta-analysis was unable to differentiate varietal performance for the midlands data. This was primarily due to a severely unbalanced data set across the different stress environments. The general linear regression method of the rating the yield response of varieties to drought stress is recommended.

Keywords: drought stress index, sugarcane, REML meta-analysis, regression

Introduction

One of the consequences of global warming is the increase in variability of climatic parameters (Arnell and Liu, 2001). This results in an increase in the unpredictability and intensity of drought events which has become a major concern in the rainfed South African sugar producing areas (Schmidt and Purchase, 2002) especially since 85% of the sugarcane industry is rainfed (SASA, 2010/2011, S.I.A.B. Planning and Development Surveys - IA/47/33, 2011).

As part of a drought-adaptation strategy a breeding programme should breed for drought tolerant varieties. However, this is challenging task for conventional breeding as drought affects a number of physiological and morphological traits in plants in a complex manner (Yordanov *et al*., 2000, Silva *et al*., 2008) and there is also a low genetic variance for yield components under drought conditions (Gosal *et al*., 2009). Furthermore, the effect of drought stress on C_4 plants is influenced by the duration and severity of the drought event (Yang *et al*., 1993; Pinheiro and Chaves, 2011) and the age and stage of development at the time of exposure (Chimenti *et al*., 2006).

Research on drought tolerance, involves first identifying drought tolerant and susceptible varieties and subsequently evaluating these varieties, in planned experiments with or without other varieties (of unknown tolerance), for drought related quantifiable traits. Silva *et al.* (2008) showed that there was a positive correlation between productivity (under drought stressed conditions) and cane stalk number, stalk height and stalk weight. Wagih *et al.* (2003) also showed that stalk height and weight were the parameters that could be used for identifying drought tolerant varieties; however there was a high degree of diversity in the 26 sugarcane genotypes evaluated. In another study, Silva *et al.* (2007) identified three physiological traits in cane that could be used to distinguish between drought tolerant and susceptible varieties: chlorophyll fluorescence, leaf chlorophyll content and thermal imaging.

The primary goal of SASRI is to produce sugarcane varieties that yield more sucrose per stalk. SASRI has a five-stage breeding programme that takes between 11-15 years before a variety is released (Parfitt, 2005). This programme is run within each of the five agroclimatic zones of the sugar industry (irrigated, coastal short cycle, coastal long cycle, coastal hinterland, midlands). Only the last two stages of the programme, the primary variety (VT1) and secondary variety trials (VT2), are planted as sugarcane trials. Each trial is replicated three times and evaluated over a plant and two ratoons. These VT1 and VT2's are either planted on SASRI owned research farms, or on farm land leased from sugarcane growers. The latter are called off-station farms. At the end of the five-stage selection process, only one or two varieties are identified as being potentially superior. This variety/ies will be planted on co-operator farms, and further evaluated to confirm its superiority (Parfitt, 2005). If the variety performs well, it is then released to the South African sugarcane industry to be adopted by the sugarcane farmers.

The variety NCO376 was released in 1955 and was one of the first varieties selected for commercial propagation (SASRI, 2006a). Since then many other "N" varieties have been released to the industry (Parfitt, 2005) (Table 4.1). The industry has experienced many drought stressed growing seasons over time, which has allowed observations of varietal yield performance under such conditions. However, it is important to note that these observed ratings are made by individuals and are therefore subjective. Matibiri (1997) demonstrated how the Zimbabwe Sugar Association Experiment Station also took advantage of the drought experienced during the 1995-1996 season, by observing varietal yield response to drought.

There is a variation in variety distribution, between the coastal and midlands region, due to the prevailing conditions within these regions (Table 4.1). They differ with regards to soil type, aspect, altitude, climate and pest and disease levels. The coastal areas have high levels of the pest *Eldana saccharina* Walker and disease *Sporisorium scitamineum* (Syd.), whereas the Midlands areas have high incidence of the disease *sugarcane mosaic virus* as well as *Glomerella tucumanensis* and *Phaeocytostroma sacchari*. The climate in the Midlands is cooler, drier and at a higher altitude compared with the Coastal regions.

Table 4.1: List of varieties (per region), the year of release and the observed growth during drought stress.

Coastal	Midlands	Year of Release	Observed growth during drought stress	Reference
NCO376	NCO376	1955	Poor	Nuss, 2001; Inman-Bamber, 1982; SASRI, 2006a
N12	N12	1979	Moderate to good	Nuss, 2001; Inman-Bamber, 1982; SASRI, 2006b
N16	N16	1982	Poor	SASRI, 2006c
N19		1986	Moderate to Poor	Mcintyre and Nuss, 1996; SASRI, 2006d
N27		1996	Good	SASRI, 2006e
N29		1997	Moderate to Poor	SASRI, 2006f
	N31	1997	Good	SASRI, 2006g
N33		1998	Good	SASRI, 2006h
N36		2000	Moderate	SASRI, 2006i
	N37	2001	Moderate to Poor	SASRI, 2006j
N39		2002	Poor	SASRI, 2006k
N41		2002	Moderate to poor	SASRI, 2006l
N42		2002	*Moderate to good	SASRI, 2006m
	N44	2006	*Moderate to good	SASRI, 2006n
N45		2006	*Moderate	SASRI, 2006o
N47		2007	*Moderate	SASRI, 2006p
	N48	2007	*Moderate to Poor	SASRI, 2006q
	N50	2008	*Good	SASRI, 2006s

*Note: These ratings were based one or more observations by SASRI plant breeders. The ratings have not been recorded in SASRI varietal information sheets because of the insufficient number of observations.

Varieties N31, N37, N44, N48 and N50 are all specifically recommended for the Midlands area. N31 is specifically recommended for the Midlands area where, *E. saccharina*, is not a problem (SASRI, 2006g). N37 is recommended for the high potential humic soils in the Midlands area (SASRI, 2006j). N44, N48 and N50 are recommended for higher altitudes (>600m) and a longer cutting cycle (>20months), therefore suitable for planting in the Midlands area (SASRI, 2006n; SASRI, 2006q; SASRI, 2006s). N50 is also resistant to *sugarcane mosaic virus*.

For the past 43 years, SASRI has stored varietal yield data from all the VT1 and VT2's, in an Oracle database (Oracle Database (10*g*R2), 2011). As a first step, towards a breeding strategy for drought tolerant varieties, the aim of part of the study is to investigate the drought tolerance potential of released varieties, using the historical yield information combined with the DSI, identified in Chapter 3. The objectives of this study were therefore to:

- Create a varietal database for the two rainfed regions, midlands and coastal;
- Use the DSI, identified in Chapter 3, to quantify the amount of stress experienced during a trial and to evaluate the corresponding varietal yield response;
- Rate the different varieties based on their response to drought stress.

Materials and Methods

Variety and Trial Selection

The varietal selection criteria were the same as per Chapter 3 (Table 4.1). Variety performance data, tonnes cane per hectare (TCH), were obtained from two different types of trials, primary variety (VT1) and secondary variety (VT2). There were a total of 416 trials selected from the coastal area and 161 from the midlands area. The trials used varied in different aspects, the trials: i.) were from different farms ii.) were either a plant or ratoon crop iii.) had different average trial means iv.) were planted and harvested in different years and seasons.

Table 4.2 shows the details of the different farms, within each region, that were used in the study. The details include information on whether the farm was a coastal or midlands farm, farm name, whether the farm was an off-station farm or a research farm, the number of trials from the farm that were used in this study, distance of the farm from the closest weather station and the time period for which the weather station was active. Depending on the planting date of the crop being simulated the weather station that was i.) active and ii) closest to the farm was used for Canesim simulations. The coordinates of each farm site was obtained using a Trimble Juno GPS (global positioning system) device. The coordinates of each weather station used was obtained from the SASRI weather web (http://portal.sasa.org.za/weatherweb, 2011). Using the WGS-84 method the distance between the farm and the weather station used was calculated (Table 4.2) (Campbell, 1998).

Table 4.2: Details of the farms used.

Region	Farm Name	Farm type	No. of Trials	*Time Period	*Distance (km)
Coastal	Crookes Brothers	Off station	37	pre 1993	9.92
				post 1993	12.74
Coastal	Musa	Off station	31	pre 2004	7.8
				post 2004	0.25
Coastal	Colin Frost	Off station	29	pre 2004	16.16
				post 2004	8.92
Coastal	Gingindlovu	Research	135	pre 2004	7.04
				post 2004	1.3
Coastal	Empangeni	Research	50	1998-2004	2.84
				post 2004	10.05
Coastal	Kearsney	Research	58	pre 2004	6.83
					14.33
				post 2004	1.2
Coastal	Mount Edgecombe	Research	76	pre 1999	1.59
				post 1999	0.32
Midlands	Anton Woerner	Off station	12	pre 1999	5.44
				post 1999	11.88
Midlands	Fred van Breda	Off station	13	pre 1999	6.17
				post 1999	6.3
Midlands	Conrad Klip (B1)	Off station	21	pre 1999	3.54
				post 1999	8.13
Midlands	Conrad Klip (B2)	Off station	9	pre 1999	5.34
				post 1999	9.65
Midlands	Glenside	Research	44	pre 1999	8.5
				post 1999	11.83
Midlands	Bruynshill	Research	61	pre 1999	7.2
				post 1999	0.77

*Note: Time period refers to the time period for which the weather station was/is active. Distance refers to the distance of the farm from the weather station used.

Quantification of Drought Stress

Through the work of the pilot study (Chapter 3), a drought tolerance index (DSI) of Canesim simulated rainfed yield to Canesim simulated irrigated yield (equation 3.12), was identified as the best method of quantifying drought stress in a desktop study. For each trial evaluated in this study, the Canesim model was run twice, a rainfed and an irrigated run. The calculated DSI was representative of the amount of drought stress experienced by the sugarcane crop for that trial. The values ranged from 0-1.0 with 1.0 representing a crop that experienced no drought stress at all and 0 representing a crop that was completely drought stressed. A DSI value of 0.5 would indicate that the crop was stressed to a point that it was only able to achieve half of its full (unstressed) potential yield.

Creation of a Varietal Database

Two varietal databases were created, one for each region, coastal and midlands. The database was a collation of information on each trial that was used in the study. It included information on the trial name, the varieties that were used from each trial, the crop (plant/ratoon), the field that the trial was planted on, soil information (clay%, Effective Rooting Depth (ERD), Total Available Moisture (TAM)), the rainfall received during the life of the crop, the start and harvest date, crop age, weather station used, distance of weather station from the farm, calculated DSI, Canesim rainfed final yield, Canesim irrigated final yield, observed trial mean yield, variety mean yield, annualized varietal mean yields (equation 3.13).

Statistical Analysis

As with Chapter 3, all data was first tested for normality and homoscedasticity using the Shapiro-Wilk and Levene tests respectively. Where necessary transformations were applied (Genstat v.14.1, 2011). In the case of transformed data, for ease of graphical presentation raw data was used with the statistics from the transformed data anlaysis. All significance testing were done at the 0.05 level. *Post hoc* comparisons were done using the Holm-Sidak test. All statistical analyses were done separately for the coastal and midlands regions.

Validation of the Canesim model

The Canesim model was validated as per methodology in Chapter 3. Outliers were identified and further investigated (see Chapter 3). In addition, a Pearson's correlation coefficient (r) was calculated to evaluate the relationship between the observed trial mean yield and the Canesim rainfed final yield. For each variety, the observed trial mean was regressed on the Canesim rainfed final yield, with all the data as well as with the data excluding outliers.

Evaluation of the trial data used

Box and whisker plots were used to examine the data used from each farm. A REML analysis was used to compare the annualized trial means across the different farms (y variate=annualized trial mean; fixed effects=farm; random effects=crop). A similar REML analysis was done to compare the DSI's across each of the farms. A correlation anlaysis was performed between the variables: annualized trial means and DSI.

To examine the relationship between the observed yield minus the Canesim rainfed yield (from here on referred to as yield deviation) and the distance from a weather station, a Pearson's correlation coefficient was calculated. The distances were grouped into four classes <1km, 1-5km, >5-10km and >10km. A REML analysis was conducted on the absolute yield deviation values to establish significant differences among the different distance classes (y variate=absolute yield deviation; fixed effects=distance class; random effects=crop).

Evaluation of Varietal Yield Response to drought stress

As an initial evaluation of varietal yield response to drought stress, a simple linear regression analysis was performed separately for each of the varieties. The annualized varietal yield (TCH) for each trial was plotted against its calculated DSI.

The next step involved analysing the regional database as a whole, with all the varietal data combined into one dataset. A general linear regression model was used to to compare the gradients (m_1) of the different varieties. The gradient was representative of the varietal yield response to drought stress (Rizza *et al*., 2004). The regression analysis was run twice. The first regression model included N12 as the reference variety, allowing comparisons (of regression parameter estimates) of N12 on all other varieties, using the Student's t-test. Varieties with m_1's that were significantly higher or lower than N12 were classified as being potentially Susceptible (S) or Tolerant (T) varieties, respectively. Varieties with m_1's not significantly different to N12 were classified as being potentially I varieties.

Genstat Regression Model 1:

```
MODEL [DISTRIBUTION=normal; LINK=identity; DISPERSION=*] Annualized Variety Yield
TERMS [FACT=9]Variety*Variety.DSI+ FarmLocation + TrialType + Farm + TrialMean + Crop +
Harvest Year + Harvest Season
FIT[PRINT=model,summary,estimates;CONSTANT=estimate;FPROB=yes;TPROB=yes; FACT=9]
Variety*Variety.DSI+ FarmLocation + TrialType + Farm + TrialMean + Crop + Harvest Year +
Harvest Season.
```

The second regression model allowed the separate estimation of regression parameters of individual varieties.

Genstat Regression Model 2:

```
MODEL [DISTRIBUTION=normal; LINK=identity; DISPERSION=*] Annualized Variety Mean
TERMS[FACT=9;FULL=yes]Variety*Variety.DSI+ FarmLocation + TrialType + Farm + TrialMean
+ Crop + Harvest Year + Harvest Season
FIT [PRINT=model,summary,estimates; CONSTANT=omit; FPROB=yes;TPROB=yes;FACT=9]
 Variety+Variety.DSI+ FarmLocation + TrialType + Farm + TrialMean + Crop + Harvest Year +
Harvest Season.
```

REML meta-analysis was the second type of analysis used to rate varieties. The DSI's were grouped into four drought stress classes: ≤0.3, >0.3-0.6, >0.6-0.8, >0.8. These classes represent a very stressed, stressed, moderately stressed and mildly stressed environment, respectively. A meta-analysis was conducted using REML (y variate=annualized varietal yield; fixed effects=Variety*Drought Stress Class; random effects=Farm Location + Trial Type + Farm + Trial Mean + Crop + Harvest Year + Harvest Season). The variance components of the random model were examined to determine which variables/factors were included in the REML model. A Holm-Sidak *post hoc* test was used to establish if there were significant yield differences across the four stress classes, for each variety. Comparisons were limited to classes of increasing levels of stress. If there were no significant differences among the classes, the variety was rated as a T variety. If there was a significant difference in one of the classes, the variety was rated as an I variety. If there two or more differences between classes, the variety was rated as a S variety.

Results and Discussion

Coastal Data Analysis

Of the 416 trials initially selected for this study only 346 trials were used. Table 4.3 shows (highlighted purple) the details of the number of trials that contained a variety/ies of interest after the removal of the outliers. Trials often contained more than one variety of interest. With NCO376 being released in 1955 (Table 4.1) the most data was available for this variety (n=341). A third of the trial data came from the Gingindlovu farm. For varieties N12 and N19 approximately half of the data on these varieties came from trials at the Gingindlovu farm. Even though N33 was released in 1998 there were only 23 trials available for analysis of this variety. N33 was not a popular variety with farmers because it did not yield as expected in commercial plantings.

Table 4.3: The total number of trials from each coastal farm and the number of trials per variety (purple highlighted text).

	Colin frost	Crookes Bros	Empangeni	Gingindlovu	Kearsney	Mount Edgecombe	Musa
Total number of trials included	**20**	**33**	**44**	**111**	**44**	**66**	**28**
NCO376	19	33	43	110	44	66	26
N12	15	9		34		17	
N16		20			37	16	
N19			23	47		28	9
N27	3	23	42	40	29	3	28
N29	5	13	5	12	31	3	5
N33	2	9		6	2	3	
N36			12	2			14
N39	13	15	3	36	25		2
N41	10	9	24	27	25	2	17
N45	3	6	3	15	5		8
N47	3	5	3	8	7		3

The correlation between the 346 observed trial mean yields and Canesim rainfed final yields was r=0.87 (p<0.001). Figure 4.1 shows the regression between these two variables. The Shapiro-Wilk's test was not significant. The regression analysis showed that 72% of the variation in the observed trial means could be explained by the Canesim rainfed simulated final yields. The Canesim model inputs could therefore be considered to be relatively accurate for the Canesim simulations. As the DSI was calculated using only Canesim output variables, the correlation and regression statistics provide evidence that the DSI's could be used with a high degree of confidence.

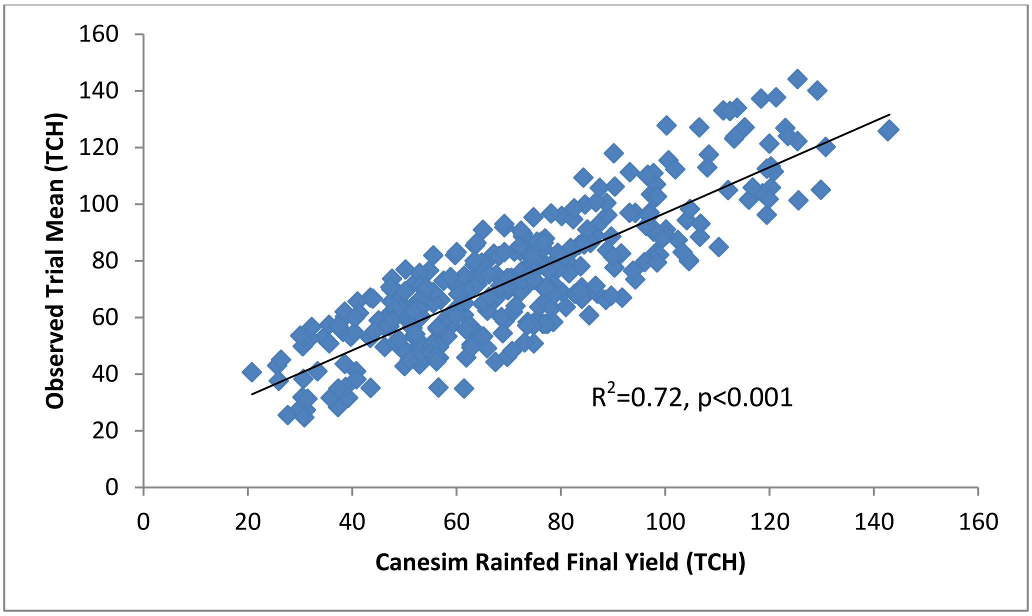

Figure 4.1: Regression of the observed trial mean vs. Canesim rainfed final yield.

Table 4.4 shows the percentage variation accounted for (R^2) between the observed trial mean and the simulated rainfed mean for each variety.

Table 4.4: The R^2 of each varietal regression. The total numbers of trials used are in brackets.

Variety	**R^2**
N12	0.55 (67)
N16	0.62 (63)
N19	0.59 (106)
N27	0.77 (158)
N29	0.77 (71)
N33	0.83(22)
N36	0.45 (28)
N39	0.74 (90)
N41	0.75 (109)
N45	0.74 (38)
N47	0.56 (24)
NCO376	0.72 (321)

Figure 4.2 shows the box and whisker plots of the annualized trial means among the different coastal farms. The Shapiro-Wilk and Levene tests were significant. A square root transformation was applied to the data. The REML analysis showed that there were significant differences between the annualized trial means among the coastal farms ($F_{6,337}$ = 38.21, p<0.001).

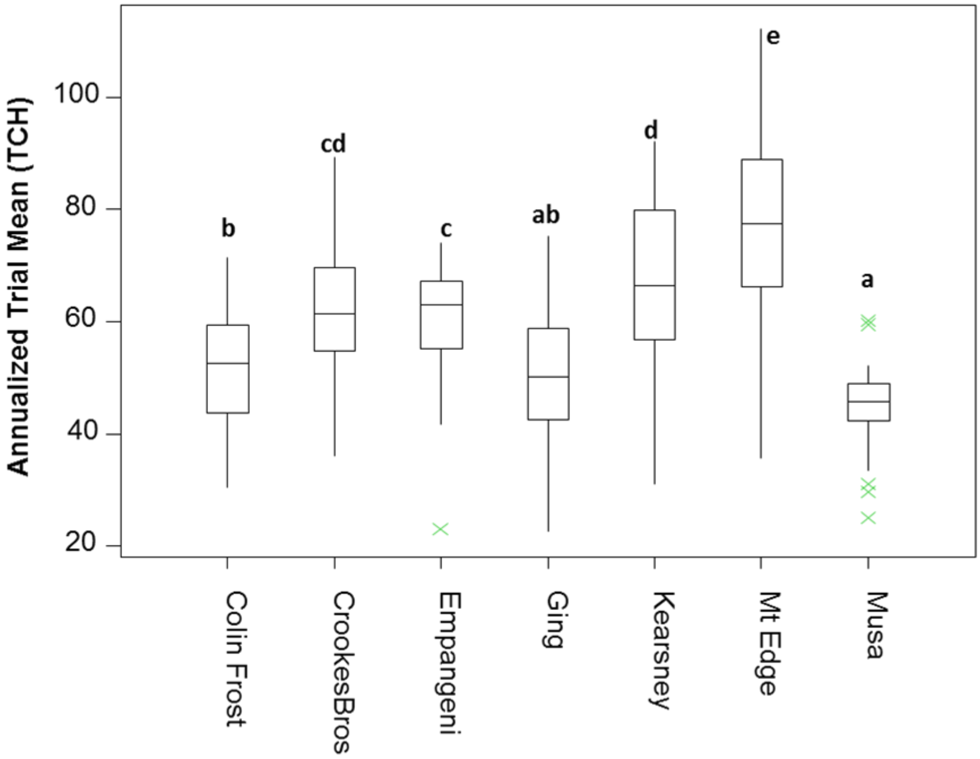

Figure 4.2: Box and whisker plot of the annualized trial mean (TCH) for each coastal farm. Farms that share the same letter are not significantly different to each other (p<0.05)[1].

The Mount Edgecombe farm had a significantly higher trial mean compared with all other farms. This farm had a very high clay% (>40%). Some of the trials at this farm had annualized means of greater than 100TCH. Musa (clay%=28.0%) had significantly lower trial means compared to all farms except Gingindlovu (clay%=39.6%). The latter was also not significantly different to Colin Frost (clay=24.5%). There were a few outliers (as shown by the green crosses in Figure 4.2). The weather data for these outliers were accurate; therefore these trials were not excluded from the analysis. Further investigation showed that a trial at Empangeni had an annualized mean of 22.4TCH and a DSI of 0.21. This

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[1] Raw means are presented in the box and whisker plots, however the a's and b's come from the Holm-Sidak *post hoc* test on square root transformed data.

indicated that the trial was severely drought stressed, yielding only approximately 21% of its full yield potential. In contrast the DSI of three low yielding trials at Musa were >0.65 indicating that the trials were not severely drought stressed. The range of annualized trial means at Gingindlovu was 20.2TCH-90.7TCH. Most of the trials with annualized means <40TCH were severely drought stressed.

Figure 4.3 shows the box and whisker plots of DSI for the different farms. The Shapiro-Wilk and Levene tests for the DSI were significant. A square root transformation was applied to the data. The REML analysis showed that there were significant differences in the amount of drought stress experienced among the farms in the coastal regions ($F_{6,339} = 56.62$, $p<0.001$).

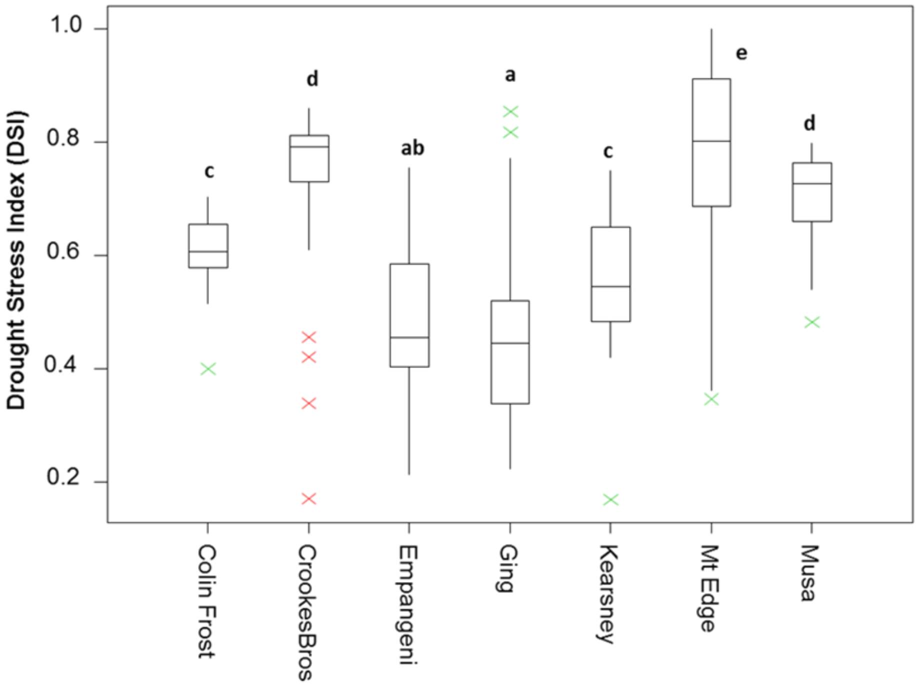

Figure 4.3: Box and whisker plot of the DSI for each coastal farm. Farms that share the same letter are not significantly different to each other ($p<0.05$)[2].

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[2] Raw means are presented in the box and whisker plots, however the a's and b's come from the Holm-Sidak *post hoc* test on square root transformed data.

The farms Mount Edgecombe experienced significantly less stress compared to all farms. Most of the trials at Crookes Bros experienced moderate to little drought stress, however there were a few trials that experienced more severe drought stress (as shown by the red crosses in Figure 4.3). For Mount Edgecombe, the low stress levels at this site were reflected in the high yields achieved at the farm (Figure 4.2 and 4.3). Despite having lower drought stress levels the yields at Crookes Bros were not as high as would be expected (Figure 4.2 and 4.3). Musa had significantly lower yields compared with five of the six other farms, even though it was one of the farms with little drought stress (Figure 4.2) and had a very high clay content (>40%). Gingindlovu and Empangeni experienced the most drought stress (Figure 4.3). Despite Empangeni having similar drought stress levels as Gingindlovu, the trials at this farm had significantly higher yields compared to Gingindlovu (Figure 4.2). There was a moderate correlation between the level of drought stress experienced and the annualized yields for the coastal farms ($r=0.54$, $p<0.001$). Even though some trials experienced a higher degree of drought stress, they did not necessarily have lower yields. This could be attributed to the types of varieties planted within the trial, as well as to the type of soils that the trials were planted on. A sandier soil is a lot more susceptible to drought stress compared to a soil with a higher clay%.

Figure 4.4 shows the box and whisker plots of the absolute yield deviations of each of the four distance from weather station classes. The Shapiro-Wilk and Levene tests were not significant. The Pearson correlation coefficient showed that there was no significant relationship between the two variables (actual distance from weather station and annualized trial yield) ($r=0.08$, $p=0.12$). REML analysis also showed that there was no significant difference in absolute yield deviation between the different weather classes ($F_{3,342}=2.62$, $p=0.052$). It can therefore be concluded that there was no significant relationship between the yield deviation and distance from the weather stations. This is an important finding as 69 and 84 trials had to use weather stations that were >10km and >5-10km away from the farms, respectively.

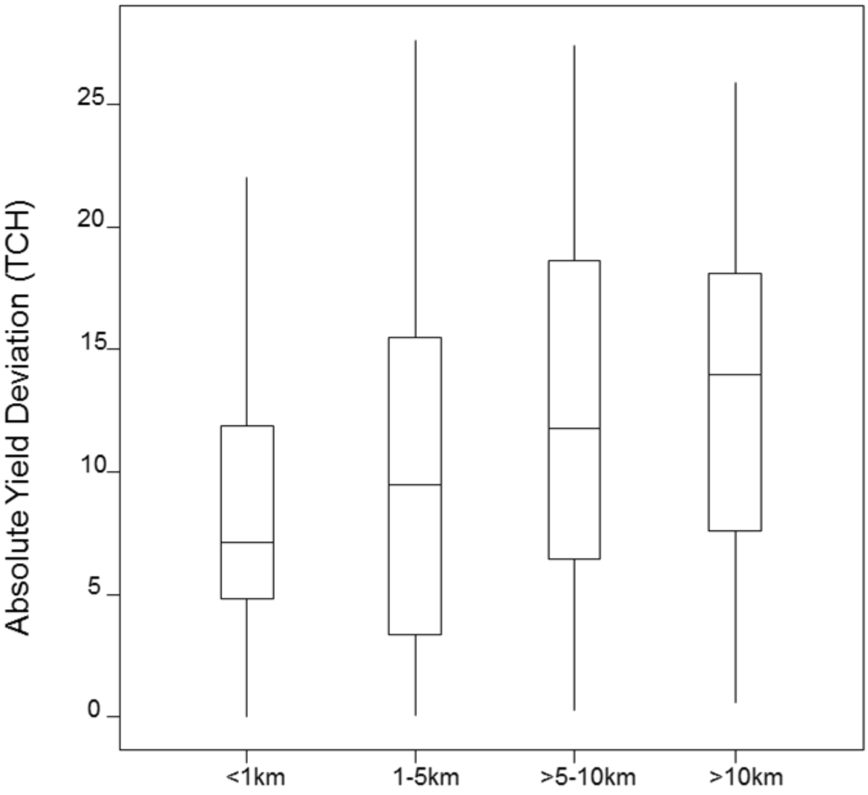

Figure 4.4: Box and whisker plot of the absolute yield deviation for each distance class.

Table 4.6 shows the results of the first general linear regression analysis (annualized varietal yield on DSI, with variety as the group factor). N12 was the reference variety. The Shapiro-Wilk and Levene tests were not significant. The t-probability, in the table, refers to the comparison of each variety m_1 with the m_1 of N12. Only the factor farm location and the variable trial mean accounted for significant variation, 17.0% and 16.8% of the variation respectively, hence these two were included in the final regression model. A Genstat default alphabetical reference level (reference level = Colin Frost) was allowed for the factor farm location, as comparison of this factor was not the aim of this analysis.

Table 4.5 shows the results of the second regression model, with individual parameter estimations for each variety.

Table 4.5: General Linear Regression output parameters showing individual parameter estimates (Ho: m_1=0).

	Parameter	estimate	SE	t(1070)	t pr.
	Variety N12	20.79	3.65	5.70	<.001
	Variety N16	-5.42	5.98	-0.91	0.365
	Variety N19	9.10	3.12	2.92	0.004
	Variety N27	22.20	3.13	7.09	<.001
	Variety N29	11.18	4.13	2.71	0.007
	Variety N33	19.03	6.90	2.76	0.006
	Variety N36	17.76	8.12	2.19	0.029
	Variety N39	6.77	4.18	1.62	0.106
	Variety N41	16.65	4.10	4.06	<.001
	Variety N45	19.23	6.04	3.18	0.002
	Variety N47	27.81	7.45	3.73	<.001
	Variety NCO376	9.35	2.37	3.95	<.001
m_1	DSI.Variety N12	3.59	5.90	0.61	0.54
	DSI.Variety N16	49.53	8.89	5.57	<.001
	DSI.Variety N19	28.22	4.92	5.74	<.001
	DSI.Variety N27	6.69	4.96	1.35	0.18
	DSI.Variety N29	18.98	6.92	2.74	0.006
	DSI.Variety N33	6.40	11.3	0.56	0.57
	DSI.Variety N36	19.50	12.50	1.56	0.12
	DSI.Variety N39	30.63	6.50	4.71	<.001
	DSI.Variety N41	12.26	6.46	1.90	0.058
	DSI.Variety N45	16.49	9.65	1.71	0.088
	DSI.Variety N47	-10.20	12.70	-0.80	0.42
	DSI.Variety NCO376	29.46	3.57	8.25	<.001
	Farm Location Colin frost	-4.48	1.73	-2.59	0.01
	Farm Location CrookesBros	-0.70	1.54	-0.45	0.65
	Farm Location Empangeni	12.99	1.47	8.81	<.001
	Farm Location Ging	3.78	1.45	2.61	0.009
	Farm Location Kearsney	3.27	1.60	2.04	0.041
	Farm Location Mt Edge	14.64	1.56	9.41	<.001
	Farm Location Musa	1.94	1.00	1.94	0.052
	Trial Mean	0.42	0.018	23.66	<.001

Table 4.6: General Linear Regression output parameters showing the comparison of the parameters of 11 varieties with the parameters of the reference variety, N12[3].

Parameter	estimate	SED	t(1070)	t pr.*
Constant	16.31	3.58	4.56	<.001
DSI	3.59	5.90	0.61	0.54
Variety N16	-26.21	6.68	-3.92	<.001
Variety N19	-11.69	4.21	-2.77	0.006
Variety N27	1.41	4.23	0.33	0.74
Variety N29	-9.61	5.10	-1.88	0.06
Variety N33	-1.76	7.48	-0.24	0.81
Variety N36	-3.03	8.53	-0.35	0.72
Variety N39	-14.02	5.17	-2.71	0.007
Variety N41	-4.14	4.92	-0.84	0.40
Variety N45	-1.56	6.62	-0.24	0.81
Variety N47	7.02	8.01	0.88	0.38
Variety NCO376	-11.44	3.70	-3.09	0.002
DSI.Variety N16	45.9	10.1	4.55	<.001
DSI.Variety N19	24.64	7.01	3.51	<.001
DSI.Variety N27	3.1	7.18	0.43	0.67
DSI.Variety N29	15.39	8.58	1.79	0.073
DSI.Variety N33	2.8	12.30	0.23	0.82
DSI.Variety N36	15.9	13.70	1.17	0.24
DSI.Variety N39	27.04	8.53	3.17	0.002
DSI.Variety N41	8.68	8.34	1.04	0.30
DSI.Variety N45	12.9	11.00	1.17	0.24
DSI.Variety N47	-13.8	13.80	-1	0.32
DSI.Variety NCO376	25.88	6.11	4.24	<.001
FarmLocation CrookesBros	3.78	1.50	2.52	0.012
Farm Location Empangeni	17.46	1.47	11.89	<.001
Farm Location Ging	8.25	1.34	6.16	<.001
Farm Location Kearsney	7.74	1.42	5.45	<.001
Farm Location Mt Edge	19.12	1.59	12.05	<.001
Farm Location Musa	4.48	1.73	2.59	0.01
Trial Mean	0.418	0.018	23.66	<.001

Figure 4.5 shows the fitted model of the combined regression analysis. N12, which has been observed to be an I variety, displayed a more T response, with a m_1 of 3.6TCH/0.1DSI. This indicates that with a 10% increase or decrease in the level of drought stress

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[3] Standard Error of Difference (SED), DSI.Variety refers to the difference in gradient (m_1) of a variety to N12 , the t-probability, column 5 in Table 4.10, refers to the comparison of each varietal parameter with that of N12.

experienced by N12, there would be a corresponding yield decrease or increase of 3.6TCH, respectively. The t-probabilities from Table 4.6, shows that there were only two groupings of varieties: N16, N19, N39 and NCO376 all had significantly higher m_1's compared to N12; meaning that they had a larger yield response to drought stress when compared to N12. These varieties were classified as S varieties. All the other varieties had m_1's that were not significantly different to N12, were classified as I varieties. Variety N47 showed a negative response to changes in drought conditions, with a m_1 of -10.2TCH/0.1DSI. When N47 experienced a 10% increase or decrease in the level of drought, there was a corresponding yield increase or decrease of 10.2TCH, respectively. N47 has been shown to perform better in poorer soils and yield worse (than other varieties) under good conditions (SASRI, 2006p). The R^2 for the N47 regression alone was 36.7% indicating that a straight line does not fit the data well. This could be due to the fact that the variety was exposed to very little drought stress (the highest DSI for the N47 data set was 0.78). Variety N16 had the largest yield response to drought with a m_1 of 49.5TCH/0.1DSI, indicating that with a 10% decrease or increase in the amount of drought, there will be corresponding 49.5TCH yield increase or decrease, respectively.

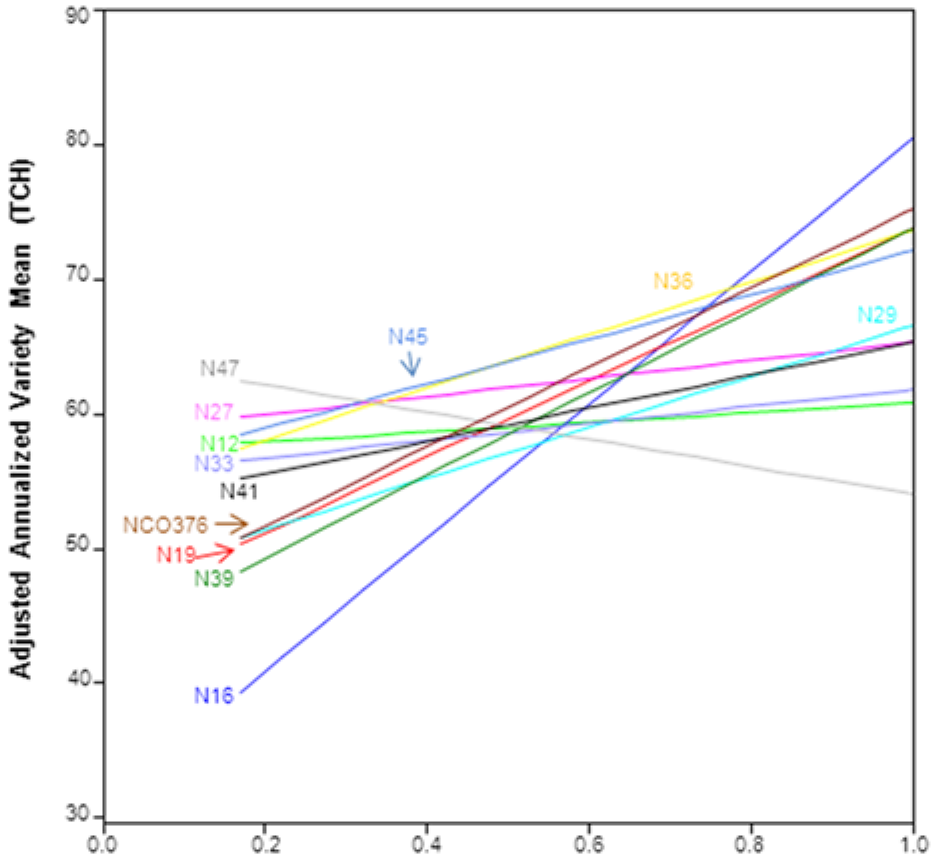

Figure 4.5: The fitted model of the general linear regression analysis of coastal varieties.

The Shapiro-Wilk and Levene tests for the REML meta-analysis were not significant. REML meta-analysis showed that only the trial type (off station/research) did not account for any variation in the data, so this term was removed from the model. There were highly significant interaction effect, Variety.Drought stress class ($F_{30,1050}$=2.26, p<0.001). Figure 4.6 illustrates the significant interaction between Variety and the Drought tolerance classes. The Holm Sidak *post hoc* test showed that the varieties N12 and N27 had a T response to drought. N16 and NCO376 had a S response to drought. N19, N29, N33, N39, N41, N45 all had an I response to drought. N19, N39, N41 and N45 had no significant yield difference among the lower three classes, but there was a significant increase in yield when the drought tolerance index is >0.8. With varieties N29 and N33, when the DSI was ≤0.3 the varietal yields were significantly lower than all the other classes. However, there was no significant difference among the yields of the classes >0.3-0.6, >0.6-0.8, >0.8. It is important to note that N29 had 8 data points for the ≤0.3 class whereas N33 had only 2 data points.

Variety N47 showed no significant difference across the three drought stress classes, there was no information available for this variety within the >0.8 drought tolerance class. Therefore, this variety could be rated as either an I variety or a T variety. More information was needed to classify N36 according to this method, because there was no information available for the >0.8 class and there was only one data point for the ≤0.3 class.

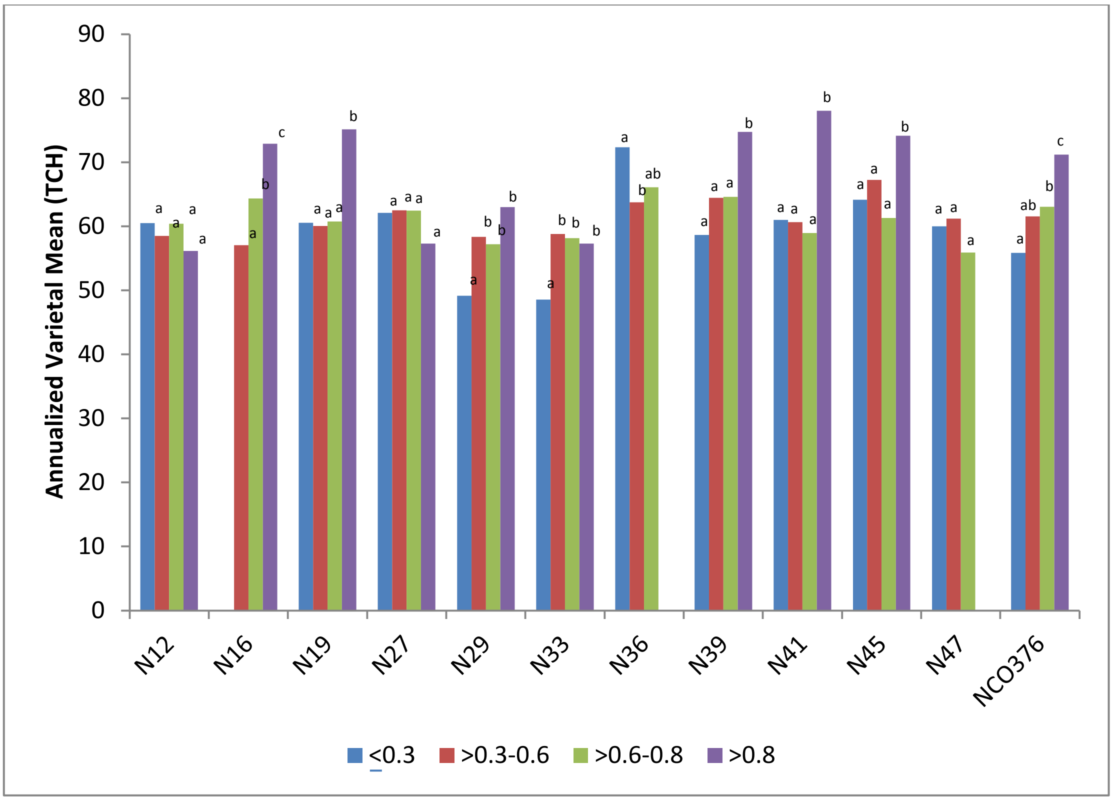

Figure 4.6: Annualized varietal mean for each of the drought stress classes (from REML meta-analysis). The comparisons are limited to only within each variety. Classes that do not share the same letters are significantly different to each other ($p<0.05$).

Table 4.7 shows the comparisons of the varietal drought stress ratings derived from two different methodologies, regression analysis and REML meta-analysis. N16 and NCO376 were both classified as S varieties with both methods. N29, N33, N41, N45 were also classified as I varieties with both methods. N12 was classified as a tolerant variety with the REML analysis but was assumed to be an I variety with the regression analysis (based on anecdotal evidence). N19 and N39 were rated as S with the regression analysis and I with the REML analysis. N27 was rated as an I variety with the regression analysis and a T variety with REML analysis.

Table 4.7: Summary of the drought stress ratings using regression analysis and REML meta-analysis.

Variety	Regression Rating	REML meta-analysis Rating
N12	I (reference rating)	T
N16	S	S
N19	S	I
N27	I	T
N29	I	I
N33	I	I
N36	I	*
N39	S	I
N41	I	I
N45	I	I
N47	I	*
NCO376	S	S

*Note: More information is needed to rate this variety with REML meta-analysis.

Midlands Data Analysis

Table 4.8 (blue text) outlines the number of trials, from each midlands farm after the removal of trials due to inaccurate weather data. Of the 161 trials initially selected for this study, after the removal of the outliers, only 113 trials were used, with Conrad Klip B2 having more than half of the initial number of the trials discarded. Table 4.8 (purple highlighted text) also shows the details of the number of trials (from each farm) that contained a variety/ies of interest, after the removal of the outliers. At least half of the trials for each variety, except N44, came from the Bruynshill farm.

Table 4.8: The total number of selected trials from each midlands farm (blue) and the number of trials per variety (in purple highlighted text).

	Anton Woerner	**Bruynshill**	**Fred van Breda**	**Glenside**	**Conrad Klip B1**	**Conrad Klip B2**
Total number of trials included	10	45	20	8	13	17
NCO376	6	40	8	16	20	8
N12	9	42	11	17	10	5
N16	4	40	1	7	16	8
N31	10	21	8	14	11	2
N37	3	25	1	9	13	7
N44	6	6	5	1	2	4
N48	1	6	1	5	3	0
N50	2	6	2	0	2	0

Table 4.9 shows the percentage variation accounted for (R^2), between the observed trial mean and the simulated rainfed mean, for the simple regression of each variety.

Table 4.9: The R^2 of the observed trial mean vs. Canesim rainfed final yield, excluding the outliers. The total numbers of trials used are in brackets.

Variety	**R^2**
N12	0.70 (94)
N16	0.68 (76)
N31	0.72(66)
N37	0.67 (58)
N44	0.79 (24)
N48	0.78 (16)
N50	0.77 (12)
NCO376	0.50 (98)

Figure 4.7 shows the relationship between the observed trial mean yields and Canesim rainfed final yields. The Shapiro-Wilk's tests were not significant. 73.3% of the variation in observed trial means could be explained by the simulated rainfed final yields ($p<0.001$). As with the coastal data, this shows that the model inputs that were used for the Canesim trial simulations significantly and closely approximated the true trial conditions. As the DSI's were calculated using only Canesim values, these DSI's could be trusted to accurately represent the level of drought stress experienced by a trial.

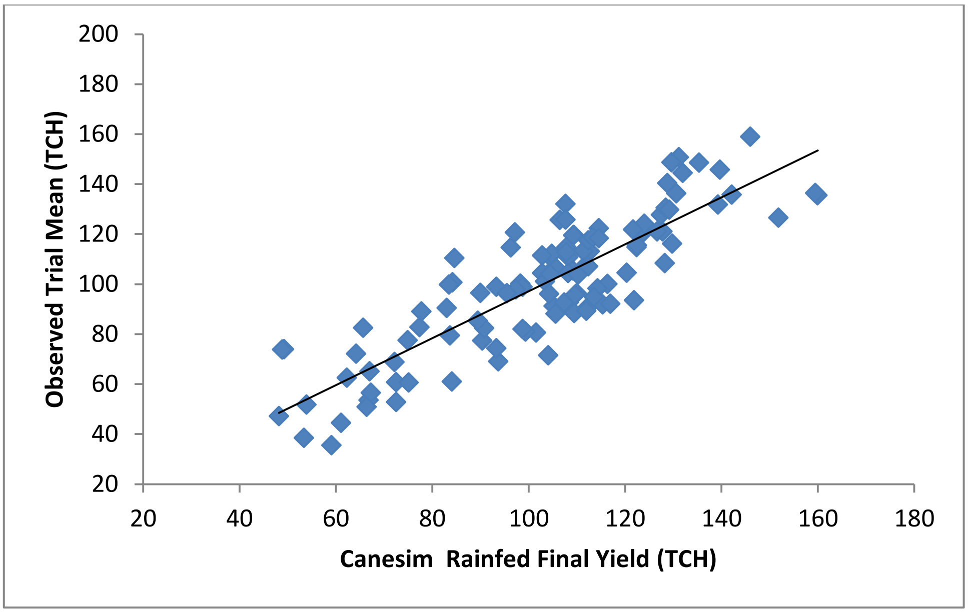

Figure 4.7: Regression of the observed trial mean vs. Canesim rainfed final yield for all midlands trials.

Figure 4.8 shows the average annualized trial yield among the midlands farms. The Shapiro-Wilk and Levene tests were not significant. The REML analysis showed that there were significant differences among the farms ($F_{5,107} = 20.94$, $p<0.001$) (Figure 4.8).

Anton Woerner was the lowest yielding midlands farm, being significantly lower than all other farms except for Fred van Breda (Figure 4.8). A contributing factor could have been the low clay% at this site. Anton Woerner had a very low clay% of 10% while Fred van Breda had a clay% of 19%. Conrad Klip (B2) (clay%=50%) and Bruynshill (clay%=35%) farms had significantly higher yields than all farms, except Conrad Klip (B1) (clay%=21%). Glenside (clay%=20%) had a similar clay% to Conrad Klip B1, and the yields at this farm were not significantly different to that at Conrad Klip B1.

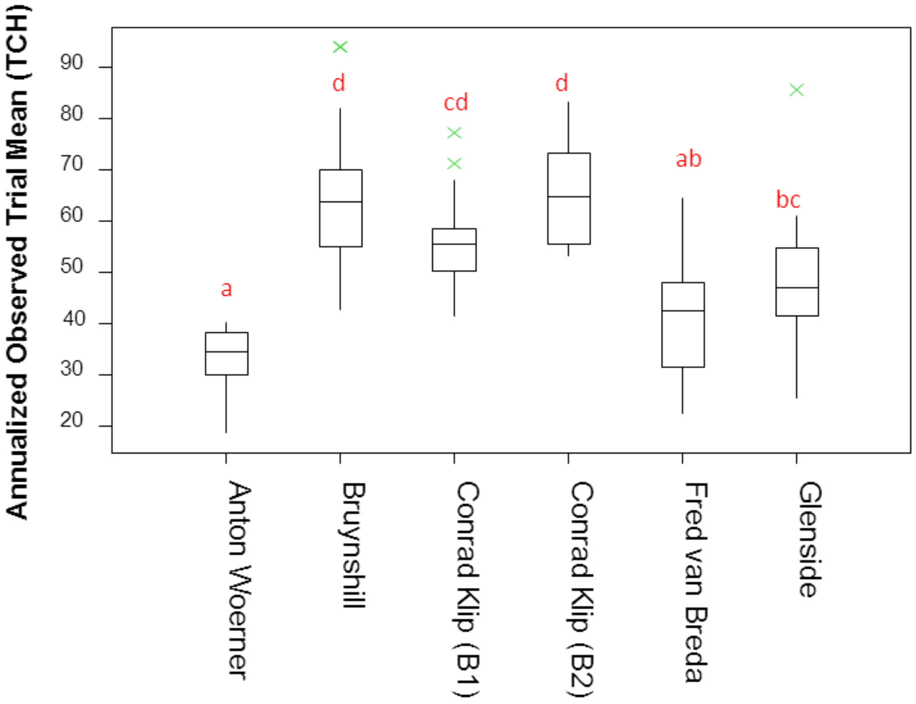

Figure 4.8: Box and whisker plot of the annualized trial mean (TCH) for each midlands farm. Farms that share the same letter are not significantly different to each other (p<0.05).

Figure 4.9 shows that the box plots of the level of drought stress experienced across the different farms. The assumptions of normality and homoscedasticity were not violated. The REML analysis showed that there were significant differences in the average DSI's among the midlands farms ($F_{5,107} = 16.68$, p<0.001) (Figure 4.9).

Anton Woerner had a significantly smaller DSI compared with all other farms, except for Fred van Breda. The low clay% content at Anton Woerner could be a reason for the extreme level of drought stress experienced at this farm. The high levels of stress at Fred van Breda and Anton Woerner was reflected in the low yields obtained at these farms (Figure 4.8). The trials that had the lowest levels of drought stress, of all midlands farms, were Bruynshill, Conrad Klip (B1) and Conrad Klip (B2). The clay% at these farms was higher than at the other farms. These farms had, on average, a DSI of 0.6, indicating that the yield potential at these farms was only 60% of its full potential. The average annualized trial yield and the DSI among the different farms were significantly positively correlated (r=0.82, p<0.001) (Figure

4.8 and Figure 4.9). Unlike the coastal farms, at the midlands farms, when the DSI was high, this indicated a low level of drought stress and led to a consequently higher average annualized yield.

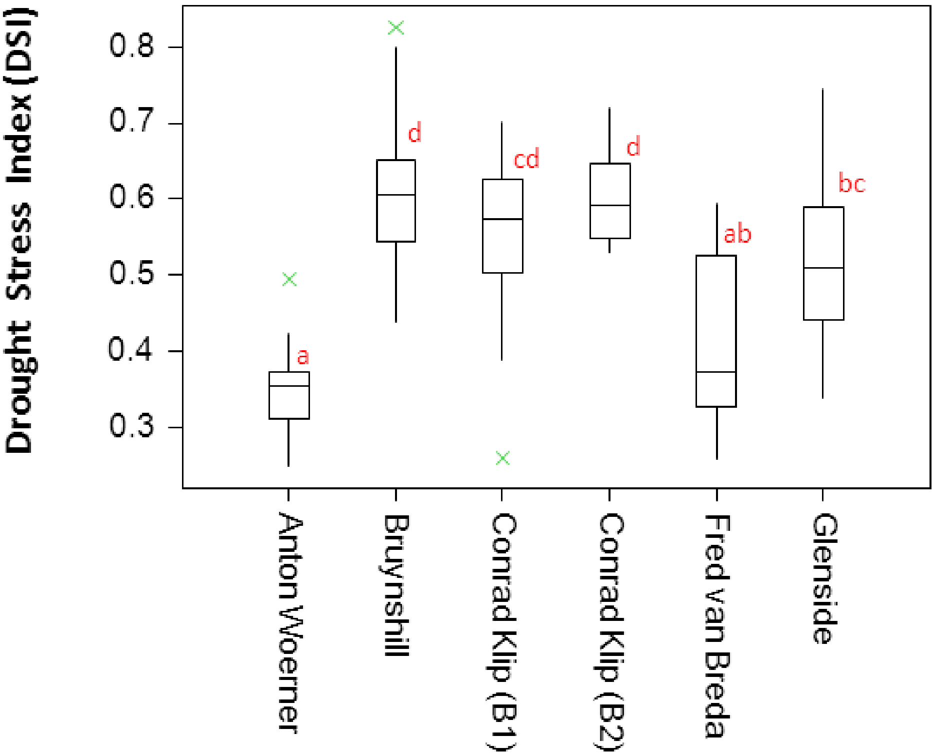

Figure 4.9: Box and whisker plot of the DSI for each midlands farm. Farms that share the same letter are not significantly different to each other ($p<0.05$).

The correlation coefficient between the variables: distance from the weather station and yield deviation, showed that there was no linear relationship between the two ($r=-0.05$, $p=0.59$). Figure 4.10 shows spread of the absolute yield deviation for each of the four weather distance classes. The REML analysis found no significant differences among the distance classes ($F_{3,109}=0.29$, $p=0.83$). As 74% of all midlands trials used weather stations that were at least >5km away from the farm site, a non-significant effect of distance on yield deviation was important.

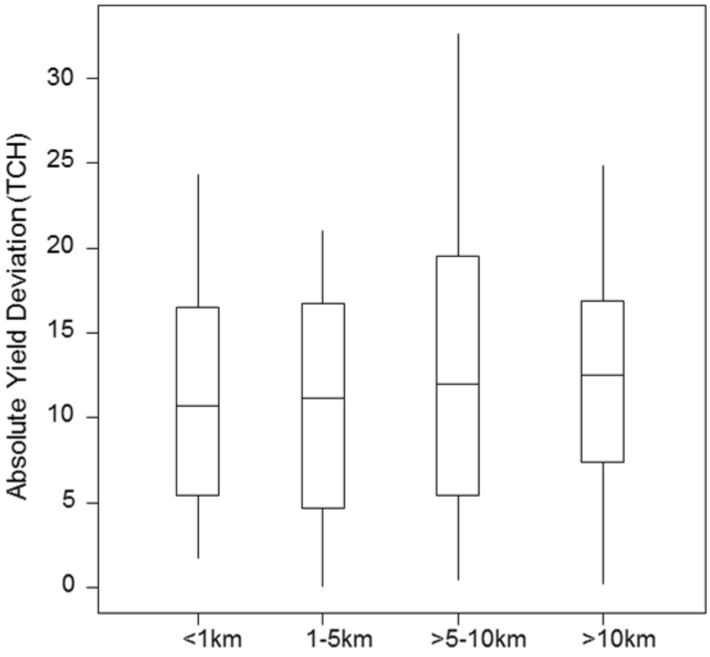

Figure 4.10: Box and whisker plot of the absolute yield deviation for each distance class for midlands trials.

Table 4.11 shows the results of the first combined regression analysis, with N12 as the reference variety. The assumptions of normality and homoscedasticity were not violated. With the midlands data, only the variable trial mean accounted for significant variation (20.9%), hence this variable was included in the final regression model.

Table 4.10 shows the results of the second combined regression analysis, with individual regression parameters for each variety.

Table 4.10: General Linear Regression output parameters showing individual parameter estimates (Ho: $m_1=0$).

	Parameter	**estimate**	**SE**	**t(427)**	**t pr.**
	Variety N12	-1.20	2.88	-0.42	0.677
	Variety N16	-1.97	3.99	-0.49	0.622
	Variety N31	10.44	3.44	3.04	0.003
	Variety N37	-10.27	4.54	-2.26	0.024
	Variety N44	12.84	4.4	2.92	0.004
	Variety N48	-0.61	6.47	-0.09	0.925
	Variety N50	0.18	9.15	0.02	0.984
	Variety NCO376	4.40	3.19	1.38	0.168
m_1	DSI.Variety N12	14.42	6.24	2.31	0.021
	DSI.Variety N16	22.64	7.7	2.94	0.003
	DSI.Variety N31	17.95	7.26	2.47	0.014
	DSI.Variety N37	29.36	8.54	3.44	<.001
	DSI.Variety N44	12.61	9.03	1.4	0.163
	DSI.Variety N48	29.90	11.9	2.52	0.012
	DSI.Variety N50	35.60	16.2	2.21	0.028
	DSI.Variety NCO376	2.04	6.3	0.32	0.746
	Trial Mean	0.46	0.020	22.65	<.001

Table 4.11: General Linear Regression output parameters for the comparison of the parameters of 7 varieties against the parameters of the reference variety, N12.

Parameter	**estimate**	**SED**	**t(427)**	**t pr.**[4]
Constant	-1.2	2.88	-0.42	0.68
Stress_Ratio	14.42	6.24	2.31	0.021
Variety N16	-0.77	4.91	-0.16	0.88
Variety N31	11.64	4.48	2.6	0.01
Variety N37	-9.07	5.37	-1.69	0.092
Variety N44	14.04	5.24	2.68	0.008
Variety N48	0.59	7.09	0.08	0.93
Variety N50	1.38	9.59	0.14	0.89
Variety NCO376	5.6	4.27	1.31	0.19
DSI.Variety N16	8.22	8.65	0.95	0.34
DSI.Variety N31	3.53	8.30	0.43	0.67
DSI.Variety N37	14.94	9.37	1.59	0.11
DSI.Variety N44	-1.82	9.98	-0.18	0.86
DSI.Variety N48	15.5	12.40	1.25	0.21
DSI.Variety N50	21.2	16.70	1.27	0.20
DSI.Variety NCO376	-12.38	7.64	-1.62	0.11
Trial Mean	0.4616	0.020	22.65	<.001

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[4] The t-probability, column 5 in the table, refers to the comparison of each varietal parameter with that of N12, DSI.Variety, in Table 4.11, refers to the difference in gradient (m_1) between a variety and N12.

The combined regression analysis was unable to distinguish between the m_1 of N12 and the rest of the varieties, even though the size of the varietal m_1's varied (2.04–35.6TCH/0.1DSI) (Figure 4.10). The levels of variation of the m_1's were similar to that of the coastal region, however, the limiting factor may have been that the gradient differences (between a variety and N12) was larger (3.1-45.9) compared with midlands (1.82-21.1). Another contributing factor is that a midlands crop is grown over 24 months, so a drought event would have to persist for a considerable length of time in order to cover the entire growing period. Normally drought events occur and are then relieved, allowing the plant to recover. Therefore, with the long growing season, the midlands varieties are more buffered against intermittent stresses like drought. This therefore makes it difficult to quantify the effect of a drought event on final varietal yield.

Looking at the m_1's in Table 4.10 NCO376 seemed to have had the lowest response to drought stress with a m_1 of 2.04 TCH/0.1DSI. This showed that NCO376 had a more tolerant response to drought stress. This was unexpected, as NCO376 has been observed as having a more susceptible response to drought stress (Table 4.1). N31 and N44 had m_1's similar to N12, suggesting that these varieties showed a more intermediate response to drought stress. N37 and N48 had very similar yield responses to drought stress (Table 4.10), and the m_1's suggest that these two varieties had a more susceptible response to drought stress. N50 had the largest yield response to drought stress (m_1=35.6TCH/0.1DSI), suggesting that it also had a susceptible response to drought. N50, however, has been observed to have a tolerant response to drought stress (Table 4.1). Even though there were only 12 data points available for analysis, the amount of stress experienced ranged from DSI 0.34-0.80, so there was a representative range of drought stressed environments. Pair-wise Student's t-tests was used to tentatively classify midlands varieties.

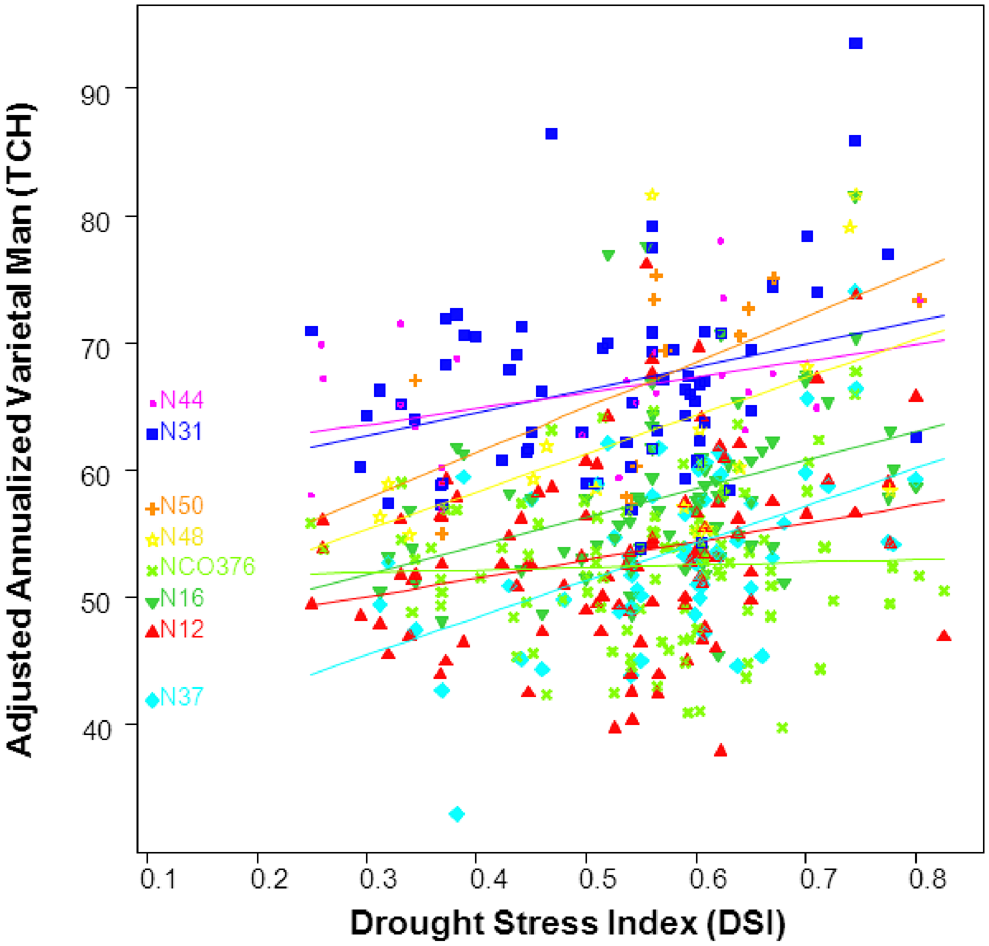

Figure 4.11: The fitted model of the general linear regression analysis of midlands varieties.

For the REML meta-analysis, the Shapiro-Wilk test showed that the data was normally distributed, however, the Levene's test showed that the variance among the different varieties were not stable. A square root transformation improved the result. For the REML meta-analysis, the drought tolerance class, >0.8, was excluded from the analysis as there were only two trials within this class. For the midlands, there were only four trials were the DSI was ≤0.3. Of the 113 trials, 65% trials were classified as 0.3-0.6, with 31% being in the 0.6-0.8 class. For this analysis, Farm area was the only factor that accounted for variation in the data set. There was no significant interaction ($F_{10,416}$=0.52, p=0.97), indicating that there was no significant difference in the way different varieties responded to varying levels of drought stress. There was a significant increase in yield from one drought stress class to the next ($F_{2,18}$=25.31, p<0.001). There were also a highly significant Variety main effect ($F_{7,370}$=25.07, p<0.001). Figure 4.12 shows raw means of the eight varieties being evaluated, information was only completely available for three of drought stress classes in four varieties. N12, N31, N44, NCO376 showed a higher mean yield for the ≤0.3 class, compared with the 0.3-0.6 class. It should be noted that there were only 4, 2, 3 and 2 data points available for each variety, respectively, for the ≤0.3 class. Therefore more data needs to be available for the ≤0.3 class to properly evaluate the varietal performance.

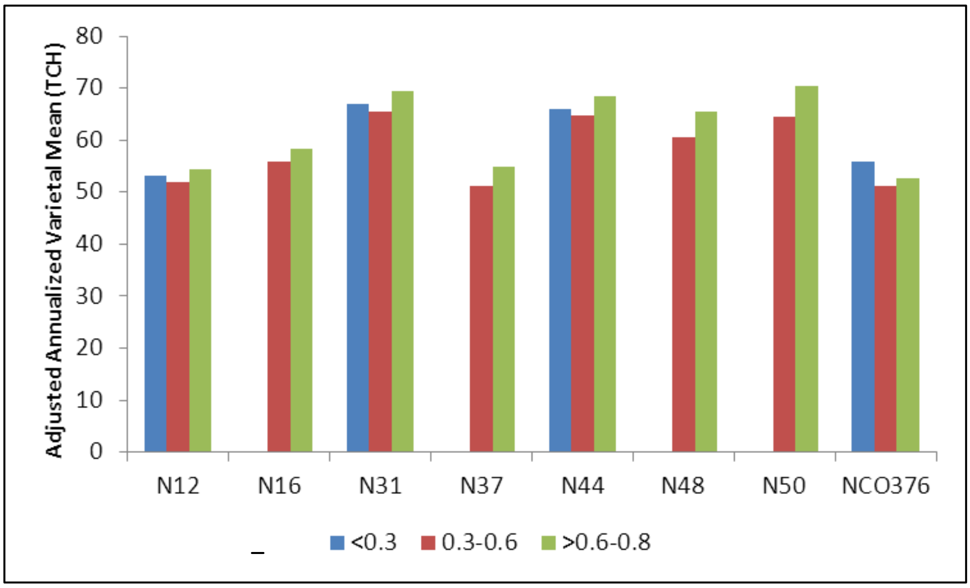

Figure 4.12: Annualized varietal means (midlands) for each of the drought tolerance classes (from REML meta-analysis).

Table 4.12: Tentative drought tolerance ratings for midlands varieties.

Variety	m_1	Tentative Drought Tolerance Rating[5]
N12	14.42	I
N16	22.64	S
N31	17.95	S
N37	29.36	S
N44	12.61	T
N48	29.9	S
N50	35.6	S
NCO376	2.04	T

The tentative ratings of the midlands varieties correspond to some degree to the observations in the field. N12 is observed to have an intermediate to good response, and statistically it has shown a similar response to drought stress. N16, N37 and N48 which have been observed to have a poor response to drought stress (Table 4.1), all had a m_1 that corresponded with the performance of a susceptible variety (Table 4.10). N31 had a more susceptible response to drought stress, even though this variety has been observed to have a more tolerant response to stress. N44 has been observed to have a tolerant response to drought stress. NCO376 has been observed to have a poor response to drought stress, however it has been tentatively rated as tolerant (Table 4.1).

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[5] Based on the m_1's of the coastal varieties (Table 4.6).

Conclusion

Valid Canesim simulations gave confidence that the calculated DSI's closely approximated the level of drought stress experienced by a trial. Within the coastal region, varieties were rated as either intermediate or susceptible to drought stress. N12, N27, N29, N33, N36, N41, N45 and N47 were all rated as having an intermediate response to drought stress. Although N12, N27 and N33 showed a more tolerant yield response to drought stress, this was not picked up with the regression analysis. However, the REML analysis rated N12 and N27 as having a tolerant response to drought stress. N16, N19, N39 and NCO376 were all rated as having a susceptible response to drought stress. These ratings are closely aligned with the observations that have been made on these varieties over time. Even though the REML meta-analysis offered more separation between varieties, the limitation to using this method is the requirement of sufficient data, for each water stress class for each variety, to confidently rate varietal response to stress. Rating of the midlands varieties, with either method, was not possible. This could be attributed to smaller differences in gradients. The inability to distinguish between varieties was compounded by longer cutting cycle for a midlands crop (24 months). Given the limitations of a desktop study, a regression analysis is recommended as a first step towards rating sugarcane varieties.

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Chapter 5

General discussion and conclusion

General discussion and conclusion

Research has shown that the impact of drought stress on the growth of the sugarcane is affected by the growth phase within which the stress event occurs. However, in this study, taking into account the timing of the stress event in the calculation of the DSI did not account for any additional variation in varietal yield response to drought stress. This held true when applied to the simple index of %drought stressed days (DSI2) as well as to the Canesim stress variable (DSI4). Of the 60 trials analysed, in the pilot study, 67% of the trials were planted on fields that had a high clay% (>40%), with the rest of the trials being planted on fields with a medium clay% (+/-25%). Fields with higher clay% take a longer time to respond to periods of drought stress because they have a higher TAM. This may therefore have been a confounding factor, contributing to the inability of account for the impact of stress within the different phases. Another potential factor may have been the grouping of the GGP and the ripening phase. Even though the impact of drought stress has a negative effect on TCH within each of these phases, the impact during the GGP is much larger.

Attempting to quantify the amount of drought stress experienced by a historical trial is very challenging, especially as trials in the study dated back to the 1980's. Field soil properties, clay% and ERD, had to be estimated for the older trials. Due to erosion and land preparation these factors could have changed to some extent over time, with this change potentially contributing to the amount of unexplained variation in the relationship between the DSI's and varietal yield data. The six indices, in the pilot study, accounted for 50-75% of the variation in varietal yield data. DSI6, captured the most variation in varietal yield data (R^2=75%). The other five indices accounted for 50-66% of variation, significantly lower than DSI6. In the calculation of DSI6 only Canesim simulated yields (rainfed:irrigated) were used, whereas with DSI5 the actual yield to the irrigated simulated yields were used. The errors associated with the actual yield (e.g. natural variation, pests, diseases, management factors) are different to that of the Canesim simulated yields (model error). DSI5 accounted for approximately 20% less variation in the varietal yield compared with DSI6. A further advantage of DSI6 using only Canesim generated yields was that the Canesim model took into account the extent to which the crop is drought stressed, that is, how much the SWC dropped below the 50% TAM threshold. The Canesim model also accounted for the effect of temperature, which is a very important driver of crop growth.

The use of the Canesim model came with its own limitations. This included the model not being able to account for the effect of pests and diseases or the effect of farm management practises on the growth of sugarcane. The inconsistencies in some weather stations to record daily weather data, proved to be another limitation. This was especially true for the Midlands farms.

For the analysis of the released varieties, the coastal and midlands varietal database comprised of 346 and 113 trials respectively. Of the 346 coastal trials, 40% of the trials were planted on soils with high clay% (>40%) whereas there were only 7% of midlands trials with high clay%. Given the soil composition alone, it would be expected that it would be easier to detect differences in drought tolerance at Midlands, however, the ability of DSI6 to distinguish differences in the drought tolerance of the different varieties proved to be more limited in the midlands region compared with the coastal (Table 5).

Some of the reasons for the inability of the DSI to quantify varietal differences at the midlands could include the fact that the gradient differences (between a variety and N12) was smaller at midlands (1.82-21.1) compared with coastal region (3.1-45.9). The midlands trials were also exposed to different environmental conditions compared to the coastal trials, with the weather being colder and drier in the midlands. It is for this reason that the crop cutting cycle in the midlands is a 24 month cycle. With a longer cutting cycle a drought event would have to persist for a considerable length of time in order to cover the entire growing period. The long growing period therefore acts a buffer against intermittent stress. Farmers and researchers have observed that a drought event may for example occur in the midlands for four months and then when good conditions return the crop is able to recover. This compared to a coastal crop harvested at 12 months, if a drought persists for four months, this represents a considerable portion of the crops total life. It therefore does not have the time to recover compared to a 24 month crop which can capatilize when good condtions return.

The two methods of analysis both had their strengths and weaknesses. A strength of the REML analysis was that when there was sufficient data for all stress classes it was able to pick up finer differences between varieties compared to the regression analysis. For the Coastal region, I and S varieties were easily identified, using regression analysis. However, it was unable to detect T varieties. The REML analysis identified N12 and N27 as T varieties. A weakness of REML analysis was that it would be unable to accurately rate varieties if the data (for the stress classes) were severely unbalanced. The REML analysis also involved dividing the DSI into classes, to create different stress "environments". This division could lead to a loss of information and precision in the analysis. The strength of the regression analysis was that with the gradient, it was able to quantify rate of change in yield over varying levels of drought stress. The unequal spread of data points across the different levels of DSI was not as much of a disadvantage for the regression anlaysis compared with the REML analysis.

This study was meant to be the first step in investigating the drought tolerance potential of released varieties in South Africa. This study used anecdotal evidence of varietal yield performance when subjected to drought stress. In general, anecdotal evidence should never be accepted at face value because it is based purely on observation and the response of varieties during drought may be affected by a range of other environmental factors. However, anecdotal evidence is the closest possible information that SASRI has to the truth therefore it was the only comparative information available. This is the reason why the crude ratings were used as validation. One of the limitations of this study is that there was no scientific validation of the drought ratings derived for the different released varieties (Table 5). However, when SASRI does embark into a scientific drought tolerance project, using the information from this study, SASRI will be able to validate these ratings.

It is recommended that before a trial is planted, soil sampling be conducted on the field. This soil data should be added to the current SASRI varietal database. This can give scientists more insight into the crop yield performance. The management of the weather stations also needs to be improved, with flags within the system to detect any outliers immediately, so that these can be investigated. Also, additional notes on anything that occurred during the life of the crop needs to be noted and uploaded into the database. For example, diseases, pests, hail or poor management practices. This will improve the understanding of crop yield performance and the validity of future ratings of varieties. It is recommended that regression analysis be used for rating of varieties. If at such time there is enough varietal information, within each of the drought stress classes, the REML analysis can be revisited. It is also recommended that the results of this study be workshopped with the sugarcane farmers in South Africa, to provide them with more varietal information and to get feedback on their experience.

Table 5: Summary of the drought tolerance ratings for the coastal and midlands released varieties.

	Coastal Variety Rating	**Midlands (only) Variety Rating**[6]
N12	I	I
N16	S	S
N19	S	
N27	I	
N29	I	
N31		S
N33	I	
N36	I	
N37		S
N39	S	
N41	I	
N44		T
N45	I	
N47	I	
N48		S
N50		S
NCO376	S	T

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[6] These ratings are tentative based on individual Student’s t-tests.