ESTIMATION OF WATER USE EFFICIENCY OF SOYBEAN (*GLYCINE MAX*) FOR BIODIESEL PRODUCTION IN KWAZULU-NATAL

Kyle Trent Cameron Reddy

Submitted in fulfilment of the academic requirements of
Master of Science in Hydrology

Centre for Water Resources Research

School of Agricultural, Earth and Environmental Sciences

College of Agriculture, Engineering and Science

University of KwaZulu-Natal

Pietermaritzburg

Supervisor: Mr R.P Kunz

ABSTRACT

The production of biofuels from crops is an alternative approach to that of fossil fuels, which is expected to increase in order to ensure both cleaner energy and energy security. Knowledge of water use and yield of biofuel crops under different crop management practices and rainfed conditions at a smallholder scale is scarce in South Africa. Therefore, the main aim of this study was to estimate the crop water use and yield of soybean (*Glycine max* L.) as well as the crop's response to inoculation. A field study was conducted at Swayimane in KwaZulu-Natal (South Africa) to estimate the seasonal water use, seed yield, water use efficiency (WUE_C) and biodiesel yield of two genetically modified soybean varieties (CAPG3 and LS6161R). The trial was grown under rainfed conditions with optimum fertilization (100%) and two inoculation levels (0 and 100%). Seasonal crop water use (m^3 ha^{-1}) was derived from actual crop evapotranspiration (ET_C in mm) that was estimated using the soil water balance method. Final biomass production and seed yield were measured at harvest, while biodiesel yield was determined post-harvest using measured seed oil content. The inoculated LS6161R variety consumed 4810 m^3 ha^{-1} of water and produced 4.59 t ha^{-1} of seed, from which a WUE_C of 0.95 kg m^{-3} was calculated. For the CAPG3 variety, comparable figures of 5083 m^3 ha^{-1}, 4.35 t ha^{-1} and 0.86 kg m^{-3} were obtained for water use, yield and WUE_C, respectively. Both varieties produced similar theoretical biodiesel yields of 845-850 L ha^{-1}, based on a seed oil content of 17.9-18.9%. The non-inoculated treatment produced lower seed yields and WUE_C. However, there were no statistically significant differences between varieties and inoculation treatments for measured crop water use and yield. Observations of phenological growth stages were used to partially calibrate the AquaCrop model. The model was then used to simulate crop water use, yield and WUE_C, which was then compared to observations. Simulated values of WUE_C correlated poorly with observed data for both varieties and inoculation treatments. In conclusion, LS6161R is more water use efficient than CAPG3 and thus, may be better suited for biodiesel production under rained conditions for both smallholder and commercial farming systems. CAPG3 produced a higher proportion of biomass instead of seed yield and thus, is less suited for biodiesel production. With the implementation of good crop management practices, the yield gap between smallholder and commercial farmers can be reduced as is evident in this study. Finally, a full calibration of AquaCrop under optimum (i.e. irrigated) growing conditions is recommended for both soybean varieties.

DECLARATION-PLAGIARISM

I, Kyle Trent Cameron Reddy declare that

(i) The research reported in this dissertation, except where otherwise indicated, is my original work.

(ii) This dissertation has not been submitted for any degree or examination at any other university.

(iii) This dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

(iv) This dissertation does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

(a) their words have been re-written, but the general information attributed to them has been referenced;

(b) Where their exact words have been used, their writing has been placed inside quotation marks, and referenced.

(v) This dissertation does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the Dissertation and in the References sections.

Signed: ………………………………….

Kyle Trent Cameron Reddy

Supervisor: ………………………………...

Mr Richard Kunz

ACKNOWLEDGEMENTS

All praise is due to the Almighty for guiding and blessing me throughout my studies and for allowing me to accomplish things that I had always believed were out of my reach.

I would like to thank the Water Research Commission of South Africa (WRC) for funding this research project.

I would like to specially acknowledge and thank my supervisor (Mr Richard Kunz) and co-supervisor (Professor Tafadzwanashe Mabhaudhi), for their continuous support and understanding throughout the year, for making sure that my concerns were always addressed and for lending me their time in the midst of their busy schedules.

I am grateful to Thinaysha Govender and Keanu Singh, for assisting me with field and laboratory work in the midst of their busy schedules.

I would like to thank UKZN staff members, namely Ian Doidge, Vivek Naiken and Vimbayi Chimonyo for assisting me with the technical and crop science aspect of my study.

I would also like to thank my parents for being understanding and providing me with guidance, motivation and allowing me to empower myself through education. I would like to thank my brother for supporting and motivating me in every way so that I was able to obtain my degree.

And lastly, I would like to thank both academic and administrative staff in the Hydrology programme for making both my undergraduate and postgraduate experiences memorable.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ARC	Agricultural Research Council
AWS	Automatic weather station
B5	5% biodiesel blend
BIS	Biofuel industrial strategy
BNF	Biological nitrogen fixation
C	Carbon
CC	Canopy cover (%)
CC_O	Initial canopy cover (%)
CC_X	Maximum canopy cover (%)
CCI	Chlorophyll content index
CD	Calendar days (days)
CDC	Canopy decline coefficient (% day^{-1} or % GDD^{-1})
CGC	Canopy growth coefficient (% day^{-1} or % GDD^{-1})
CN	Curve number
CO_2	Carbon dioxide
CV	Coefficient of variation (%)
D	Willmott's index of agreement
DAFF	Department of Agriculture, Forestry and Fisheries
DAS	Days after sowing
DME	Department of Minerals and Energy (former)
DIFN	Diffuse non-intercepted radiation
DoE	Department of Energy
DWS	Department of Water and Sanitation
ET	Evapotranspiration (mm)
ET_C	Crop water use (mm day^{-1})
ET_O	Reference crop evapotranspiration (mm day^{-1})
E_S or E	Soil water evaporation (mm)
E2	2% bioethanol blend
FAO	Food and Agriculture Organization of the United Nations

FAO56	Food and Agriculture Organization, Paper No. 56
FC	Field capacity
GDD	Growing degree days (day ºC)
HI	Harvest index (%)
IFA	International Fertilizer Industry Association
K	Potassium
K_C	Crop coefficient
K_{SAT}	Saturated hydraulic conductivity
LAI	Leaf area index
LN	Leaf number
LSD	Least significant difference
N	Nitrogen
NRMSE	Normalised root mean square error
P	Phosphorus
P	Precipitation (mm)
PAW	Plant available water
PHT	Plant height (cm)
PWC	Profile water content
R	Pearson correlation coefficient
R^2	Pearson correlation coefficient squared
R	Surface runoff (mm)
RMSE	Root mean square error
RSA	Republic of South Africa
S	soil water storage (mm)
ΔS	Change in soil moisture (mm)
SA	South Africa
SAT	Saturation
SC	Stomatal conductance
SPAW	Soil-Plant-Air-Water
SWB	Soil water balance
T_C	Crop transpiration (mm)
T_n	Minimum air temperature (ºC)
T_x	Maximum air temperature (ºC)

UKZN	University of KwaZulu-Natal
UK	United Kingdom
USA	United States of America
VWC	Volumetric water content
WMO	World Meteorological organization
WUE_B	Water use efficiency of biodiesel production (kg m^{-3})
WUE_C	Crop water use efficiency (kg m^{-3})
Z_{eff}	effective rooting depth (m)
Z_{min}	minimum rooting depth (m)
Z_{max}	maximum rooting depth (m)

1. INTRODUCTION

Globally, the demand for energy has risen in recent decades due to various drivers (e.g. population growth) However, alternative energy sources that are deemed both cleaner and more environmentally friendly, are required to meet the increasing demand (Brent, 2014). The production of biofuels from crops is one solution, which has received much attention worldwide. Internationally, biofuel production is expected to reduce the use of fossil-based fuels and improve energy security (DME, 2007). Liquid biofuel products consist of bioethanol and biodiesel, both of which are produced from a variety of crops (i.e. feedstocks). Bioethanol is made from sugar- and starch-producing crops, while biodiesel is produced from vegetable seed oil (Jewitt *et al*., 2009).

Biofuel production in South Africa is also expected to improve economic development and create employment, especially at a smallholder farm scale (DME, 2007). The objective of the Biofuel Industrial Strategy (BIS) of 2007 was to blend biofuels with the national liquid fuel supply within a five-year period (DME, 2007). To date, this strategy has failed to stimulate biofuel production, as it is currently economically unviable, due mainly to the lack of required subsidies and incentives (DoE, 2014). The BIS recommended various feedstocks (e.g. sugarcane, sugarbeet, soybean, canola and sunflower) for biofuel production. However, through the Biofuel Regulatory Framework of 2014, soybean and grain sorghum were selected as reference feedstocks for bioethanol and biodiesel production, respectively (DoE, 2014). The selection of these two crops was based on social, economic and environmental risks and benefits when compared to the other recommended crops (Greiler, 2007).

The issue of food security has resulted in staple crops such as maize being excluded as a potential biofuel feedstock (DME, 2007). Feedstocks that are irrigated in South Africa have implications on available water resources (DWS, 2016). Irrigated agriculture accounts for the majority (~62%) of freshwater withdrawals in South Africa (FAO, 2016). Therefore, biofuel feedstocks should be produced under rainfed conditions to reduce competition for irrigated water required by food crops (DWS, 2016). Feedstock production also requires vast areas of arable land and thus, national biofuel policies propose that underutilized land in the former homelands should be used and not commercial farmland (DoE, 2014; DWS, 2016).

Biofuel manufacturers are encouraged to obtain approximately 10-30% of required feedstock from smallholder farmers (DoE, 2014). Smallholder farmers refers to previously disadvantaged subsistence farmers as well as emerging farmers. However, smallholder farmers mainly produce soybean for subsistence under rainfed conditions across different agroclimatic regions in the country (DAFF, 2010a).

Since South Africa is mostly a semi-arid country, average soybean yields are often low due to erratic climatic and other stress-related factors (e.g. soil fertility, weeds, pests and diseases), as well as improper crop management activities (i.e. no inoculation of soybean seed prior to planting, incorrect concentration and application of fertilizers and poor weed management) during the growing season (DAFF, 2010a). These factors may also have adverse implications on rainfed agriculture as water availability for plant growth is limited and unreliable. Therefore, with low water availability, efficient water use in agriculture is essential to maximize agricultural productivity (Gheewala *et al*., 2011).

In the agricultural sector, the concept of crop water use efficiency (WUE_C) is often used to highlight the relationship between crop growth (i.e. crop yield) and the amount of water used (i.e. crop water use) (Gheewala *et al*., 2011). Therefore, WUE_C is an important indicator in the agriculture sector, especially in a water scarce country like South Africa, where agricultural expansion is deemed important for alleviating poverty and improving economic growth (Janda *et al*., 2012). It is therefore important to improve knowledge of WUE_C in rainfed agricultural systems where water availability can be unreliable or limited. The benefits of improved WUE_C will help ensure that available water resources are utilized in a productive manner, thus contributing to economic growth, poverty alleviation and sustainable biofuel production.

For smallholder farmers, the average crop yields produced under rainfed conditions are generally low when compared to commercial farmers, which thus results in low WUE_C. Therefore, in order to improve WUE_C at a smallholder farm scale, especially for biofuel production, knowledge of crop water use, yield and WUE_C within a smallholder environment is necessary to evaluate how efficiently biofuel feedstocks utilize water. Although some work has been done on soybean in South Africa with respect to WUE_C (e.g. Kunz *et al*., 2015a; Lembede, 2017; Mbangiwa *et al*., 2019; Masanganise, 2019), there are unresolved questions pertaining to the effects of different crop management practices (e.g. inoculation) on soybean WUE_C grown at the smallholder farm scale. Therefore, there is a need for soybean crop WUE_C data at this scale that can be used to develop agricultural production guidelines to facilitate the incorporation of smallholder farmers within the biofuel value chain. This study seeks to address such questions, focussing on soybean as the selected feedstock.

1.1 Aims and Objectives of Study

The main aim of this study was to quantify the WUE_C of soybean grown under rainfed conditions at the smallholder farm scale. In addition, the effect of inoculation on seed yield and oil content was also assessed. This research is needed to develop production guidelines and best management practices regarding the cultivation of soybean under rainfed conditions in rural communities. The specific objectives that were addressed through field-based research are as follows:

- To assess the effects of inoculation on soybean water use and seed yield.
- To obtain field-based measurements using appropriate instruments and techniques to estimate crop water use and yield over the growing season.
- To determine the water use efficiency of soybean under rainfed conditions in a smallholder farming environment.
- To derive monthly water use coefficients of soybean under rainfed conditions.
- To perform a partial calibration of the AquaCrop model using datasets collected from the field.
- To evaluate the performance of the AquaCrop model by comparing simulations of crop water use, yield and WUE_C to observations.

1.2 Structure of Dissertation

This dissertation begins with a review of relevant literature related to biofuel production and policies (**Chapter 2**). The chapter includes an assessment of water use and yield of two feedstocks, *viz*. soybean and grain sorghum. **Chapter 3** focuses on crop water use efficiency as well as the factors that affect it. A summary indicating why soybean was selected in this study is also provided. In **Chapter 4**, a review is given of different techniques used to measure crop water use and yield. The chapter ends with a discussion on the crop yield model used in this study (see C**hapter 4.4**). Thereafter, the methodology adopted in this study is explained and justified in **Chapter 5.** This section details the experimental site, the experimental design, the planting material used, the agronomic practices applied, data acquisition from both field and laboratory work and lastly, the linking of field observations to modelling. The results are presented and discussed in **Chapter 6**. Based on the main findings of this study, the conclusions drawn and recommendations made are presented in **Chapter 7**.

2. BIOFUEL PRODUCTION AND RELATED POLICY

In this chapter, the definitions of biofuels are given as well as a review of biofuel-related policies. The role of smallholder farmers within the biofuel supply chain is also considered as well as a brief overview of water use and yield of two feedstocks.

2.1 Definition of Biofuels

There are several definitions of the term biofuels in the literature. The most common definition, according to Watson *et al.* (2008) and Blanchard *et al*. (2011), is liquid, solid and gaseous fuel produced from biomass. Liquid fuels are produced from biomass in the form of bioethanol and biodiesel (Kunz *et al*., 2015a). In this study, the focus is on liquid biofuels produced for the transport sector, in particular biodiesel produced from soybean feedstock.

2.2 Biofuel Policies in South Africa

The development of a viable biofuels sector in South Africa requires support from local government, as well as coherent policies and frameworks that consider the economic, environmental and social impacts (Knothe, 2010). In this subsection, biofuel-related policies are discussed, followed by a summary highlighting key aspects that influenced this study.

2.2.1 Biofuel industrial strategy (2007)

The main objective of the biofuel industrial strategy (BIS) was to achieve a 2% blending target of biofuels with fossil-based fuels (i.e. diesel and petrol) within a five-year period (DME, 2007). The biofuel industrial strategy was primarily driven by the need to address issues related to poverty and slow economic development in rural communities (DME, 2007). However, this short-term target was not achieved, due mainly to a lack of financial support and incentives from government (DoE, 2014). Therefore, there is a need for clear economic incentives and subsidies, as well as effective policy regulations support and stimulate the biofuel industry (DME, 2007). These issues were addressed by the biofuel regulatory framework, as discussed in **Section 2.2.3.**

2.2.2 Mandatory blending (2012)

In August 2012, the Department of Energy (DoE) published regulations regarding the mandatory blending of biofuels in the Government Gazette (DoE, 2012). The mandatory biodiesel blending rate is B5 (i.e. 5% biodiesel v/v), which equates to a minimum blending of ~465 million litres of biodiesel. Two of the four proposed biodiesel plants will produce enough biofuel to satisfy the B5 blending rate, provided both plants operate at full capacity (DoE, 2014). Soybean is the preferred feedstock and an annual supply of ~2.48 million tons (from ~1.46 million ha) is required for the B5 blending (Kunz *et al.*, 2015c).

The mandatory bioethanol blending rate is E2 (i.e. 2% bioethanol v/v), which equates to a minimum blending of ~240 million litres of bioethanol (DoE, 2012). Two of the four proposed bioethanol plants will produce sufficient bioethanol to satisfy the E2 blending, assuming both plants operate at full capacity (DoE, 2014). Grain sorghum is the preferred feedstock and an annual supply of ~576000 tons (from ~217000 ha) is required for the E2 blending (Kunz *et al*., 2015c).

2.2.3 Biofuel regulatory framework (2014)

The biofuel regulatory framework (BRF) largely addressed the major shortcomings of the BIS by introducing economic incentives for biofuel production. The BRF also provides a platform for stakeholder involvement, including smallholder communities (DoE, 2014). The biofuel regulatory framework provided an improved implementation plan via mandatory blending of biofuels (with petrol and diesel), licensing of biofuel manufacturers and pricing strategies that guarantee a return on investment. The biofuel regulatory framework identified two reference feedstocks that require the least subsidies and financial support, *viz*. soybean and grain sorghum for biodiesel and bioethanol production, respectively (DoE, 2014). However, further research pertaining to crop WUE_C of these two crops at a smallholder farm scale is needed for this framework to be successfully implemented.

2.2.4 Water use in biofuel production (2016)

According to the Department of Water and Sanitation (DWS), South Africa is classified as a water scarce country, with around 60% of the country's freshwater resources being utilized for irrigation of commercial crops (DWS, 2016). When compared to rainfed crop production, several authors (e.g. Hughes *et al.,* 2007; DWS, 2016) noted that large-scale use of irrigation negatively affects both ground and surface water resources (DWS, 2016). Other studies (e.g. Uhlenbrook, 2007; Jewitt *et al*., 2009) have highlighted that minimal information exists on water use of biofuel crops. Thus, DWS (2016) recommends the production of biofuel feedstocks under rainfed conditions as opposed to irrigated conditions. However, more research is required to address water use of feedstocks grown by smallholder farming communities under rainfed conditions.

2.3 The Role of Smallholder Farmers

The biofuel related policies reviewed above emphasise the role of the smallholder farmer in South Africa's biofuel industry. In particular, the BRF noted that biofuel manufacturers would only be eligible for government-funded incentives if they meet the following criteria:

- A minimum of 10% of biofuel feedstock should be sourced from emerging, smallholder and previously disadvantaged farmers within four years of plant manufacturing operations (DoE, 2014).
- Manufacturing plants must be owned and controlled by a minimum 25% of previously disadvantaged farmers (DoE, 2014).

2.4 Feedstock Review

Numerous authors have assessed the suitability of various feedstocks for biofuel production in South Africa (e.g. Jewitt *et al*., 2009; Khomo, 2014; Kunz *et al.*, 2015b; Lembede, 2017) and thus, is not repeated here. The two reference (i.e. key) feedstocks (i.e. grain sorghum and soybean) highlighted in the biofuel regulatory framework (DoE, 2014) are briefly reviewed next, with emphasis on what is known about their typical yields, particularly in the smallholder farming environment.

2.4.1 Grain sorghum

Grain sorghum (*Sorghum bicolor* L. Moench), is a C4 grass crop that belongs to the *Poaceae* family and is native to Africa (EL Bassam, 2010). Sorghum is typically planted in 0.91 m, 1.5 m or 2.3 m rows. In-row spacing that varies from 0.01 to 0.15 m is suggested to achieve a desired population ranging from 28895 to 444444 plants ha^{-1} (du Plessis, 2008). Grain sorghum can be grown for human consumption (i.e. sorghum meal and sorghum rice) and is produced at a smallholder scale. In addition, sorghum is suitable to drier conditions as it is more tolerant than other cereal crops, making it an ideal crop to be produced by smallholder farmers under rainfed conditions. drought tolerant than other crops, e.g. maize (DAFF, 2010b). Therefore, it is highly suitable for smallholder farmers to produce. In the Limpopo province, smallholder farmers produce 20000 tons of sorghum on 25342 ha of land (average yield of 0.8 t ha^{-1}) and mainly consume what they grow (DAFF, 2010b). In comparison, commercially grown sorghum varies around 60000 ha per annum. Commercial farmers in the Free State produce approximately 300000 tons on 150000 ha of land, which is about 2 t ha^{-1} (DAFF, 2010b). According to Statistics SA (2007), the national average yield is 2.1 t ha^{-1} (dryland) and 2.6 t ha^{-1} (irrigated). However, only approximately 5% of the total area planted to sorghum is irrigated.

The biofuels regulatory framework (DoE, 2014) highlighted grain sorghum as the reference feedstock to represent the production of bioethanol from starch. Kotze (2012) reported that PANNAR supplies three grain sorghum hybrids suitable for bioethanol production, *viz*:

- PAN8816, a tannin-free sorghum currently used by 85% of the market for malting and milling,
- PAN8909, a new hard-seed hybrid suitable for milling and bioethanol, and
- PAN8906, which is similar to PAN8909 and thus, also suited for bioethanol.

PAN8625 is a tannin sorghum used mainly for malting and is not suitable for bioethanol production.

2.4.2 Soybean

Soybean *(Glycine max*) is a leguminous annual C3 plant belonging to the *Fabaceae* or *Leguminosae* family, which is native to China and is classed as an oilseed crop (Schulze and Maharaj, 2007; El Bassam, 2010). According to DAFF (2010a), an inter-row spacing of 0.40 to 0.90 m and intra-row spacing of 0.05 to 0.15 m is recommended for soybean in order to achieve a planting density of 250000 to 400000 plants ha^{-1}. Annual production of commercial soybean ranges from 400000 to 500000 tons on average and occurs mainly in Mpumalanga (DAFF, 2010a). According to Statistics SA (2007), 15% of the total area under soybean is irrigated.

Crop yields from commercial farms are typically higher than that from smallholder farms due to several factors, e.g. crop variety grown, use of irrigation, planting density and other management activities such as seed inoculation, use of fertilizers, control weeding, application of insecticides, herbicides and fungicides (e.g. to prevent soybean rust) (El Bassam, 2010; Khomo, 2014). The low yields are also attributed to the lack of willingness of smallholder farmers to improve soybean growth, due to a lack of knowledge pertaining to the crop and the agronomic practices specific to soybean (Khomo, 2014). Therefore, soybean is not commonly produced by smallholder farmers as compared to grain sorghum (Khomo, 2014). The average yields in South Africa range from 1.5 t ha^{-1} and 2.4 t ha^{-1} for dryland and irrigated conditions, respectively. However, for smallholder farmers, the average rainfed yield is 1.6 to 1.7 t ha^{-1} (Schulze and Maharaj, 2007).

2.5 Summary

From the biofuel policies and frameworks reviewed above, soybean and grain sorghum have been selected as reference feedstocks for biodiesel and bioethanol production respectively, as they require the least subsidies and financial support. Current policies strongly support the production of biofuel feedstocks under rainfed conditions as well as the inclusion of smallholder farmers in the biofuel supply chain. The inclusion of smallholder farmers should improve both job creation and economic development, but more importantly, boost agricultural productivity in rural areas.

However, soybean is not commonly grown on smallholder farms due to a lack of the following: 1) the farmer’s willingness to grow this crop; 2) knowledge on how to grow the crop; and 3) access to resources required to grow the crop. Hence, less is known about expected yields and water use of soybean at this farming scale, as well as the recommended management practices to improve yields, e.g. inoculation and fertilization (Letete and von Blottnitz, 2012; Maonga *et al.*, 2015). The next section reviews the available knowledge on water use and yield of these two reference feedstocks grown under rainfed conditions.

3. FACTORS AFFECTING WATER USE EFFICIENCY

In this chapter, water use efficiency is defined, along with factors that affect this metric.

3.1 Definition of WUE_C

The most common definition of WUE_C (kg m^{-3}) is the ratio of utilisable portion of biomass used for biofuel production, relative to the amount of water consumed by the biomass (Mengistu *et al*., 2014; Kunz *et al*., 2015a):

$$\boldsymbol{WUE_C = \frac{Y_C}{ETc}} \qquad \textbf{Equation 1}$$

where Y_C is the total yield (stem, tuber, grain or seed yield) in (kg ha^{-1}) and ET_C is the actual evapotranspiration in (m^3 ha^{-1}). The utilisable portion of the biomass contains the sugar, starch or vegetable oil, which can be used for biofuel production. It is important to state the type of yield (i.e. wet/fresh or dry mass) being used in **Equation 1** (Kunz *et al.*, 2015a).

Feedstock water use is ET_C accumulated over the growing season and is defined as the sum of transpired water, soil water evaporation as well as canopy and litter interception (Kunz *et al*., 2015a). These four processes are difficult to measure separately and thus, ET_C is either measured directly or indirectly using empirical methods (Voloudakis *et al*., 2015). ET_C is generally calculated in mm, which is then converted to m^3 via multiplication by a factor of 10.

3.2 Crop WUE_C

A review of the literature was undertaken to determine WUE_C values of both grain sorghum and soybean grown in South Africa. This information is presented next.

3.2.1 Grain sorghum

The yield, water use and WUE_C of grain sorghum from the literature is given in **Table 3.1**. Kunz *et al*. (2015a) measured the water use and yield of grain sorghum over two seasons at two different sites (Ukulinga and Hatfield). Hadebe (2015) also considered the water use of different sorghum genotypes at two study sites. These studies highlight the large range in water use efficiencies estimated for grain sorghum in South Africa.

Table 3.1: Yield, water use and water use efficiency of grain sorghum obtained from the available literature

Source	**Study site**	**Seed yield**	**Water use (ET_C)**	**Water use efficiency (WUE_C)**
		t ha^{-1}	**m^3 ha^{-1}**	**kg m^{-3}**
Kunz *et al.* (2015a)	Ukulinga Hatfield	2.10 to 5.70	4360 to 5020	0.41 to 1.02
Hadebe (2015)	Ukulinga Mbumbulu	1.90 to 4.82	2580 to 3730	1.16 to 2.67

3.2.2 Soybean

Minimal information exists on WUE_C (see **Subsection 3.1** for definition) of soybean produced in South Africa, especially in smallholder farming environments. The yield, water use and WUE_C of soybean obtained from a few studies is given in **Table 3.2.** It is worth noting that Baynesfield and Swayimane represent a commercial and smallholder farming environment, respectively. It is clear from **Table 3.2** that the WUE_C metric is strongly influenced by yield, which differs between the two sites, considering crop ET_C was similar for both sites. The yield and water use of soybean were measured on a commercial pig farm at Baynesfield during the 2012/13 season as part of a WRC-funded project (K5/2066) by Mengistu *et al.* (2014). From this work, Kunz *et al.* (2015a) published a water use value of 469 mm measured over the cropping season using the surface renewal technique. Based on eddy covariance measurements, Mbangiwa *et al.* (2019) then published a lower water use value of 347 mm, due to poor data quality caused by occasional system failures and heavy rainfall events. Kunz *et al.* (2015a) also recommended that a second season of water use and yield measurements should be undertaken at Baynesfield, which was completed in 2017/18 by Masanganise (2019) using an improved surface renewal method. Preliminary results from this study are also included in the **Table 3.2**.

Table 3.2: Yield, water use and water use efficiency of soybean produced in KwaZulu-Natal that was obtained from the available literature

Source	Study site	Seed yield	Water use (ET_C)	Water use efficiency (WUE_C)
		$t\ ha^{-1}$	$m^3\ ha^{-1}$	$kg\ m^{-3}$
Masanganise (2019)	Baynesfield	5.14	3810	1.35
Kunz *et al*. (2015a)		5.28	4690	1.12
Mbangiwa *et al*. (2019)		5.28	3474	1.52
Lembede (2017)	Swayimane	1.61	4895	0.33

No information on WUE_C of soybean at a smallholder farm scale was found in the available literature, apart from a study done by Lembede (2017), who considered the effects of mulch and fertilizer application on water use and yield of soybean at Swayimane in South Africa. Although soil fertilization is the most common technique for improving soil fertility, it may be difficult for smallholder farmers to apply as it is expensive and not easily accessible in rural environments. However, Lembede (2017) noted that the soybean variety was not inoculated prior to planting at Swayimane, which may have affected the outcome of the WUE_C results (i.e. obtained lower yields). Lembede (2017) thus concluded that "soybean inoculation is essential" and that "the nodule number should be counted in the growing season".

3.3 Management Practices

Crop yields are known to influence WUE_C more so than accumulated ET_C. Crop yields are influenced by *inter alia*, the choice of cultivar selected for planting. Genetically modified varieties of soybean can produce high seed yields when grown in drier climatic conditions (PANNAR, 2006; DAFF, 2010a; FAO, 2015). Hence, improvement in crop WUE_C is achievable by ensuring correct cultivar selection at the smallholder farm scale (DAFF, 2010a; FAO, 2015).

More importantly, crop yields are strongly influenced by the implementation of good crop management practices (i.e. inoculation, fertilization, weeding, application of herbicides, insecticides and fungicides) throughout the growing season. The adoption of best management practices would ensure optimum crop development and thus, maximize yield and WUE_C (Greiler, 2007; Donburg *et al*., 2010). These management practices are discussed next in more detail.

3.3.1 Fertilization

3.3.1.1 Nitrogen

Nitrogen (N) is an element that is mostly absorbed by plants from the soil (DAFF, 2010a). Nitrogen promotes leaf area growth and is a vital component of chlorophyll (IFA, 1992; Olivar *et al*., 2014). It is also a vital constituent of essential cellular and protein components such as amino acids and nucleic acids (Olivar *et al*., 2014). Plants growing in semi-arid environments require water for integrating growth and metabolic activity at the cellular level and therefore, N acts as osmotic agent, retaining water in their vacuoles (Olivar *et al*., 2014).

Plants take up nitrogen in inorganic forms such as ammonium (NH_4^+) and nitrates (NO_3^-) which are constituents of fertilizers but are also found organically in soil (Islam *et al*., 2018). Although legume plants have a special adaptation that enables them to absorb atmospheric nitrogen (N_2) and use it for growth through a process known as biological nitrogen fixation or BNF (Nieuwenhuis and Nieuwelink, 2002; Khonje, 2016; Leggett *et al*., 2017). Therefore, nitrogen application in the form of fertilizers is not needed for soybean crops. Excess N will cause increased vegetative growth, but not influence yield (Nieuwenhuis and Nieuwelink, 2002; Mutegi and Zingore, 2014). Instead, soybean should be inoculated with a compatible nitrogen-fixing bacterium before planting (PANNAR, 2006). This will result in the plant fixing its own nitrogen to meet its requirements (see **Section 3.3.2**). However, if there is deficient soil available N to meet the legume's (e.g. soybean) requirement, the plant will remobilize N from other parts of the plant (e.g. leaves) to the pods. This will then diminish the photosynthetic capacity of the leaves and thus, reduce the yield potential (Salvagiotti *et al*., 2008).

3.3.1.2 Phosphorus

Phosphorus (P) is an important nutrient that is required in larger quantities by legumes. Similar to N, P is often deficient in many soils in southern Africa. Even in fertile soils, there is generally not sufficient levels of P (Gyaneshwar *et al*., 2002). However, large amounts for P applied to soils as fertilizers can be mobilized through reactions with precipitation and highly reactive elements, i.e. aluminium (Al^+) and iron (Fe^{3+}) in acidic soils and calcium (Ca^{2+}) in calcareous or normal soils. This results in high levels of P being leached from the soil profile (Gyaneshwar *et al*., 2002). Phosphorus deficiency is reported to cause deep green, blue and/or purple coloured leaves, which results in poor photosynthesis due to a lack of chlorophyll and thus, plants become stunted and yield potential is reduced (Malik *et al.*, 2006). In addition, P deficiency can cause poor regulation of transpiration (Fageria *et al*., 2001).

3.3.1.3 Potassium

Potassium (K) is the most abundant nutrient in plants and contributes to about 10% of the dry plant mass (Leigh and Jones, 1984). It is an important macro nutrient for metabolic growth (i.e. cell functioning and stress adaption), regulation of opening and closing of stomata for gaseous exchange of CO_2 and water vapour, as well as facilitating transport of nutrients within the plant (Tiwani *et al.*, 2001). However, potassium deficiency is not as common as P deficiency (Fageria *et al*., 2001). The common K deficiency signs are yellow scorching or chlorosis along the leaf margins (Fageria *et al*., 2001). Deficiency result in reduction in both vertical and lateral roots and reduced growth of aerial parts (Mokoena, 2013). Deficiencies are caused by leaching of K due to poorly structured soils and high plant uptake from previous crops (Mokoena, 2013). Potassium is reported to not only increase yields but also increase uptake of other nutrients such as Ca, Mg, N and P required for crop growth (Mokoena, 2013). It is also responsible for increasing oil content, seed yield and protein (Tiwani *et al.*, 2001; Mokoena, 2013).

3.3.2 Inoculation

Nitrogen (N) is an important macro-nutrient that is responsible for improved biomass development and yield potential in most crops (Nieuwenhuis and Nieuwelink, 2002; Salvagiotti *et al*., 2008; Lamptey *et al.*, 2014). However, in many natural ecosystems, nitrogen losses are significant due to crop uptake, soil erosion, leaching and denitrification processes (Khonje, 2016; Rurangwa *et al.*, 2018). For smallholder farmers, poor crop management practices such as not adopting inter-cropping and tillage are the main causes of significant losses of soil nitrogen (Swanepoel *et al*., 2010). The most common treatment used universally to improve soil fertility is fertilization, where a combination of nutrients is applied in solution or granular form (Mokoena, 2013; Khonje, 2016). However, chemical fertilizer treatments are often expensive, and suppliers are located far away. Hence, fertilizers are therefore not readily available and/or affordable by smallholder farmers, which results in farmers applying insufficient amounts of fertilizers (DAFF, 2010a and Mokoena, 2013). However, an inexpensive and environmentally friendly alternative to nitrogen fertilization is seed inoculation.

As soybean is not native to southern Africa, the soils in this region have low native compatible bacteria populations (PANNAR, 2006 and Salvagiotti *et al*., 2008; Swanepoel *et al*., 2010; Rurangwa *et al*., 2018). The low bacteria populations result in poor nodulation (i.e. poor fixation of atmospheric N_2), which has consequential effects on the development and yield of legumes (Lamptey *et al*., 2014; Rurangwa *et al.*, 2018). The solution is to inoculate (or coat) soybean seed with a compatible bacterial strain prior to planting (DAFF, 2010a, Mokoena, 2013; Khonje, 2016; Siyeni, 2016).

It is important that legumes are inoculated with the correct strain of bacteria (i.e. *Bradyrhizobium japonicum*) in order to maximize BNF capacity (Benizri *et al*., 2010; Maphosa, 2015). These compatible bacteria are called rhizobia and induce the root hairs of legumes to form effective nodules, which produces soil nitrogen (Mutegi and Zingore, 2014). Studies done by Singh (2005), and Schulz *et al*. (2005) showed that inoculation of seeds with a compatible rhizobium bacteria increased plant height, pod numbers, nodules and biomass than compared to non-inoculated seeds. An increase of approximately 40% in crop yields has been observed in soybean seed inoculated with *B. japonicum*, compared to non-inoculated seed (Schulz *et al.*, 2005). According to Javaid and Mahmood (2010), inoculation of soybean has the potential of increasing crop yield, nitrogen yield and residual N levels. Soybean inoculation can also benefit the rotation with non-leguminous crops as there would be a higher concentration of nitrogen in the soil from the previous leguminous crop, therefore reducing the cost of applying nitrogen fertilizer to the soil. Inoculation can ensure a sustainable increase in agricultural productivity, without causing adverse effects on the environment (Hardarson *et al.*,1987; Salvagiotti *et al*., 2008; Mutegi and Zingore, 2014).

3.3.3 Inoculation and fertilization

Being resource poor, most smallholder farmers in sub-Saharan Africa (SSA) typically apply negligible amounts of mineral fertilizers which are often render inoculation treatments ineffective and thus reducing the quantity and quality of seed yields (Njeru *et al*., 2013). The application of other essential nutrients such as P and K for soybean are often overlooked as farmers believe that soybean production does not require fertilizer input (Mokoena, 2013). The application of P and K fertilizer is a major problem amongst farmers in South Africa due to 1) incorrect rates of P and K application, and 2) lack of knowledge on soil properties that affect these nutrients (Mokoena, 2013). However, for inoculation treatments to be successful there are other factors that are needed to ensure nitrogen fixation is efficient, such as high soil fertility (Njeru *et al*., 2013). Even with the best yielding varieties, soils in SSA cannot support optimal soybean yields without soil fertility amendment (Njeru *et al*., 2013).

It is noted that inoculated soybean may not always result in higher yields as adverse soil conditions may render this treatment ineffective (Mutegi and Zingore, 2014). Adverse soil conditions related to its moisture content, pH, temperature and fertility may result in the inoculation treatment being ineffective. Low soil fertility due to a lack of macro nutrients such as phosphorus (P) and potassium (K) can cause a reduction in BNF activity, thus affecting the symbiotic relationship between the legume and rhizobia (Salvagiotti *et al*., 2008; Lamptey *et al*., 2014; Rurangwa *et al*., 2018). According to studies done by Mokoena (2013) and Rurangwa *et al*. (2018), high concentrations of P and K in the soil are needed for BNF to be effective. The nutrients (i.e. P and K) are responsible for supporting nitrogen fixation by increasing the quantity and quality of nodules and thus, improving crop development and yield as shown in **Figure 3.1.** As a result, N_2 fixation is highly sensitive to P and K deficiency due to reduction in nodule mass (Sinclair and Vadez, 2002). These nutrients also affect the seed quality and thus oil produced from these seeds that is used for biodiesel production (Sparks, 2010). Therefore, it highlights the importance of applying P and K fertilizers in conjunction with seed inoculation (Mokoena, 2013; Mutegi and Zingore, 2014; Rurangwa *et al*., 2018).

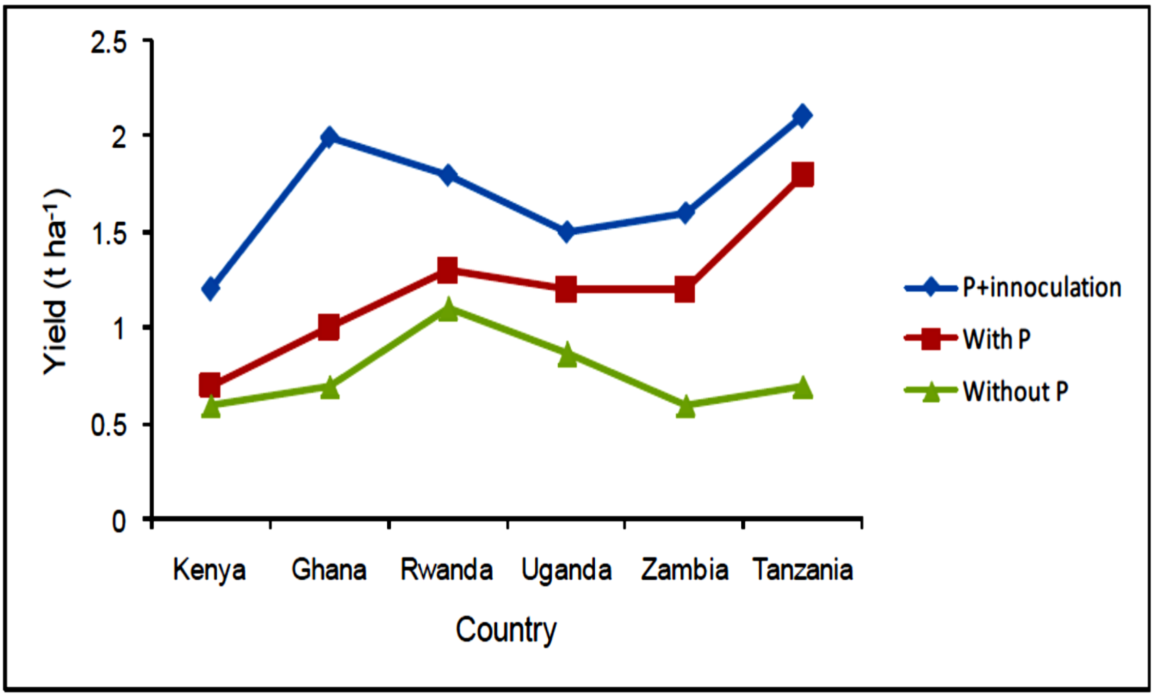


Figure 3.1: Average soybean yield with and without P fertilization and inoculation over three seasons from 2010 to 2012 (Mutegi and Zingore, 2014)

3.3.4 Mulching

Soil water evaporation is liquid water that is converted to water vapour (i.e. vapourization) and is removed from the evaporating surface, i.e. soil surface (Allen *et al.*, 1998). Soil evaporation affects the available soil moisture as well as soil temperature, therefore affecting the microclimate of partially vegetated crops (Kustas and Agam, 2014). As a result, soil water evaporation can affect transpiration indirectly. In sparsely covered crops, soil water evaporation can affect the relative fraction of rainfall to runoff, which in turn influences available soil water content. Crops can become water stressed, which results in decreased crop development and yield (Kustas and Agam, 2014). Similar to transpiration, soil water evaporation cannot be measured easily due to the complexity of the process. The factors that highly influence soil water evaporation include:

- plant and inter-row spacing, i.e. the smaller the plant and row spacing, the greater the canopy closure, which reduces the exposure of the soil to climatic conditions, decreasing soil water evaporation);

- mulching i.e. mulches (such as straw, hay and plastic) act as a blanket and cover the soil surface, thus reducing soil water evaporation (Ren *et al*., 2016). However, according to Lembede (2017), these materials containing a high C:N ratio were shown to decompose, which resulted in reduced soil nitrogen availability. Mulching can also influence soil temperatures by reducing surface temperatures, which may affect crop growth and thus, reduce yields. It is also shown that mulching can retard seed germination and early development of the plant (Hillel, 1998).

3.3.5 Soil water availability

One of the major effects that hinders agricultural production under rainfed conditions is low soil water availability (Molden *et al.*, 2010). Soil water availability is influenced by several factors, especially soil texture (Molden *et al*., 2010). Soil texture can be defined as the relative portion of particles of various sizes in the soil (Islam *et al.*, 2018). The percentage of each particle size (i.e. sand, silt and clay) makes up the soil texture. Soil textures generally differs with soil depth and thus, distinctive soil textures can often be seen with increasing soil depth. Sandy (or lighter) textured soils are poorly structured, thus allowing water to infiltrate much quicker over a period. Clay (or heavier) textured soils have a lower infiltration rate and high-water holding capacity (Mutegi and Zingore, 2014).

Sandy soils with low organic matter are not well suited for soybean production. However, soybean tolerates heavier (i.e. clayey) soils better than other crops (Nieuwenhuis and Nieuwelink, 2002; PANNAR, 2006; DAFF, 2010a). With heavier textured soils, water is retained in the soil matrix, which allows more water to be available for plant uptake. This reduces crop water stress during critical crop development stages such as flowering, which does not impede crop growth and yield (Karuku *et al*., 2012).

Estimation of soil water retention parameters help provide insight into soil water content patterns and to determine when a crop is water stressed during a growing season (Saxton and Rawls 2006; Steduto *et al.,* 2012). These parameters include saturation (SAT), field capacity (FC), permanent wilting point (PWP), plant available water (PAW), saturated hydraulic conductivity (K_{SAT}) and soil bulk density. Soil water retention characteristics are strongly influenced by soil texture and thus, affects crop water availability (Lorentz *et al*., 2001; Saxton and Rawls, 2006) as shown in **Table 3.3**.

Table 3.3: Estimated soil water characteristics for texture classes (Saxton and Rawls, 2006)

Texture Class	**Sand**	**Clay**	**Wilting point**	**Field capacity**	**Saturation**	**Plant available**	**Saturated conductivity**	**Soil bulk density**
	%		**% V**				**mm h^{-1}**	**g cm^{-3}**
Sand	88	5	5	10	46	5	108.1	1.43
Loamy Sand	80	5	5	12	46	7	96.7	1.43
Sandy Loam	65	10	8	18	45	10	50.3	1.46
Loamy Sand	40	20	14	28	46	14	15.5	1.43
Silty Loam	20	15	11	31	48	20	16.1	1.38
Silty	10	5	6	30	48	25	22.0	1.38
Sandy Clay Loam	60	25	17	27	43	10	11.3	1.50
Clay Loam	30	35	22	36	48	14	4.3	1.39
Silty Clay Loam	10	35	22	38	51	17	5.7	1.30
Silty Clay Loam	10	45	27	41	52	14	3.7	1.26
Sandy Clay Loam	50	40	25	36	44	11	1.4	1.47
Clay	25	50	30	42	50	12	1.1	1.33

Permanent wilting point (PWP) can be defined as the amount of soil water held tightly by the soil matrix, which prevents plant roots from extracting soil water, leading to senescence of the plant (Steduto *et al.*, 2012). PWP is reached when plants extract soil water at a suction force of -1500 kPa. FC can be defined as the amount of soil water retained by the soil matrix once soil water equilibrates after drainage over time (Steduto *et al.*, 2012). At FC, plants can extract soil water at a suction force of -33 kPa. PAW is defined as the difference between FC and PWP. SAT is defined as the amount of water that saturates all soil pores within the soil matrix. A saturated soil will not allow more water to infiltrate and will result in surface runoff. K_{SAT} represents the flow of water when the soil is saturated, which is subjected to a hydraulic gradient. K_{SAT} typically decreases with increasing soil depth due to the presence of less organic matter and increasing clay content (Karuku *et al*., 2012). Soil dry bulk density is defined as the mass of dry soil (mass of solids) per unit volume of soil (White, 2003). A low bulk density means less compaction and a favourable soil structure for root penetration, which is ideal for soybean (Karuku *et al*., 2012). Typical bulk density ranges for fine textured soils are 1.0 to 1.6 g cm^{-3} as seen in **Table 3.3**.

Soil water availability is also strongly influenced by soil water evaporation, which can lead to declining soil water content that can affect crucial crop development stages such as flowering and pod formation (DAFF, 2010a). This decline can cause water stress in crops, which inhibits crop growth and yield potential. There are several crop management practices that can reduce soil water evaporation, of which mulching is a highly effective (see **Section 3.3.4**). Alternatively, a more appropriate method used to reduce soil water evaporation is by increasing the planting density and reduce intra-plant and inter row spacing. As the crop develops, there will be higher canopy closure, which will help shade the soil surface reducing soil water evaporation, thus increasing soil water content in the root zone for plant uptake. The benefit of this approach is that it prevents N or other nutrient losses through leaching, which helps improve crop development and yield (Ritchie and Basso, 2008). Canopy closure also results in less surface runoff, which in turn reduces soil erosion (Ritchie and Basso, 2008).

3.4 Summary

This study aims to assess the crop water use and yield of soybean grown under rainfed and smallholder farming conditions. When compared to sorghum, a review of the literature highlighted a lack of knowledge pertaining to 1) water use, yield and WUE_C of soybean at a smallholder farm scale, and 2) the effects of plant management practices that influence WUE_C (i.e. seed inoculation and fertilization). Furthermore, more land is required for soybean cultivation destined for biodiesel production when compared to the land area required for sorghum (and bioethanol production). Therefore, the environmental impacts of soybean cultivation are likely to exceed those of sorghum production. Based on this, soybean was selected for study and not sorghum. Seed inoculation is a cheaper alternative to fertilization, which can also maximize the effectiveness of other management practices (e.g. mulching or fertilizing). Thus, additional research is required to assess the effects of soybean inoculation on water use and yield at a smallholder farming scale. This knowledge is needed to facilitate the development of agronomic guidelines for feedstock production.

4. ESTIMATION OF CROP WATER USE, GROWTH AND YIELD

In this subsection, various techniques used to estimate crop water use and yield are discussed. The objective is to identify suitable methods that may be used to assess the water use and yield of soybean grown at the smallholder farming scale. A crop model capable of estimating WUE_C is also discussed.

4.1 Crop Water Use

4.1.1 Reference evapotranspiration

Reference evapotranspiration (ET_O) is an important variable for calculating water use coefficients over a crop's growth cycle (Allen *et al.*, 1998). ET_O is defined as the potential evaporation rate from a reference surface, which is generally a hypothetical grass surface of uniform height (0.12 m), which is actively growing (albedo of 0.23) under optimum conditions, i.e. well-watered and well maintained (Allen *et al*., 1998). The most common technique recommended by Chimonyo *et al*. (2016) and Roby *et al*. (2017) for estimating ET_O (in mm) is the modified Penman-Monteith equation:

$$ET_O = \frac{0.408\Delta(R_n - G) + \gamma \frac{900}{T + 273} u_2(e_s - e_a)}{\Delta + \gamma(1 + 0.34\, u_2)}$$ **Equation 2**

where R_n is the net radiation in (MJ m^{-2} day^{-1}), T is the air temperature in (°C), u_2 is the wind speed in (m s^{-1}), G is the soil heat flux density in (MJ m^{-2} day^{-1}), e_s is the saturated vapor pressure (kPa), e_a is the actual vapour pressure in (kPa), Δ is the slope of saturated vapour pressure curve in (kPa $°C^{-1}$) and γ is the psychrometric constant in (kPa $°C^{-1}$). These parameters can be determined from sensors located on an automatic weather station. ET_O can be determined using software utilities developed by the Food Agricultural Organization (FAO) such as the ET_O Calculator, which is based on the Penman-Monteith equation shown in **Equation 2** (WMO, 2008). Once ET_O has been determined together with crop ET_C (see subsections that follow), water use coefficients can be derived for the growing period.

4.1.2 Actual evapotranspiration

The three processes that constitute ET_C (i.e. crop transpiration, soil water evaporation and interception loss) cannot be measured individually due to the complexity of these processes. Therefore, ET_C is measured as a combined value and is an important variable in calculating both WUE_C and crop coefficients for different crop types. The common techniques used to estimate ET_C as recommended by WMO (2008), Kunz *et al*. (2015a), Chimonyo *et al.* (2016) and Mbangiwa *et al*. (2019), include conventional micrometeorological techniques such as surface renewal, eddy covariance, surface layer scintillometry and the use of remote sensing. However, these methods are relatively expensive and require adequate fetch to accurately determine crop water use and thus, are not well suited to experiments conducted at the smallholder farming level.

ET_C can also be estimated using empirical methods such as the soil water balance (SWB) method, where ET_C is obtained indirectly using **Equation 3** and therefore, is better suited for small-scale studies of crop water use. The SWB equation for estimating ET_C mm is as follows:

$$\boldsymbol{ET_C = P + I + U - R - D \pm \Delta S} \qquad \textbf{Equation 3}$$

where ET_C is the actual evaporation, P is precipitation, I is irrigation, U is capillary rise, R is surface runoff, D is drainage and ΔS is the change in soil water content. In order to estimate ET_C, other variables in the equation need to be estimated or measured as a depth of water in mm. Precipitation can be measured using a rain gauge and irrigation is ignored for rainfed crop production. Furthermore, capillary rise is considered negligible if the ground water table is deep. Surface runoff can be measured using runoff plots. The most difficult parameter in the equation to estimate is deep drainage beyond the root zone. However, if soil moisture content is monitored at various depths within the profile:

- drainage can be estimated using soil moisture sensors installed at different soil depths or simulated using a crop model (Zeleke and Wade, 2012), and
- the change in soil moisture storage (ΔS) can also be determined.

4.1.3 Soil water content

Soil water measurements are important for monitoring water availability and abstraction within the rhizosphere (Stevens, 2007). The amount of soil water found within the root zone over a growing season can be estimated 1) directly by measuring soil water content, or 2) indirectly by measuring soil water potential or matric potential (Lembede, 2017). Common techniques used to estimate soil moisture as recommended by Kunz *et al.* (2015a), Lembede (2017) and Chimonyo *et al.* (2016) include PR2/6 profile probes, Watermark sensors, gravimetric sampling and CS650 probes. These techniques are discussed next in more detail.

4.1.3.1 Watermark sensors

Watermark sensors (Irrometer, Riverside CA, USA) are commonly used to measure the electrical resistance that changes with the presence of water in soil (Stevens, 2007). Although Watermarks are cheap and easy to obtain, they require calibration with other reliable and more expensive sensors. Watermarks needs to be placed at appropriate depths in the rooting zone in order to monitor profile water content more accurately throughout the growing season (Irmak *et al.*, 2016). However, they are sensitive to soil temperature fluctuations, which can cause inaccuracies and thus, the need for adjustment using measured soil temperatures is also important (Chard, 2002). Furthermore, Watermarks are also susceptibility to inaccuracies that are caused by soil disturbance during installation (Chard, 2002).

4.1.3.2 PR2/6 profile probe

The PR2/6 profile probe (Dynamx Inc, Texas, USA) measures soil moisture content at multiples depth within a soil profile (Delta-T, 2016). The probe is made up of a sealed rod with electrical sensors at different intervals its length. Once placed in the soil profile, an electromagnetic field is generated, which is influenced by the presence of water (Delta-T, 2016). The method is quick to use, is considered accurate and samples a relatively large volume of soil (Delta-T, 2016). However, the sealed rod can get easily damaged, which affects the accuracy of measurements.

4.1.3.3 Gravimetric sampling

For the gravimetric method, soil samples are obtained at various depths using a soil auger. Soil samples are initially weighed to determine wet mass, then oven dried at constant temperature (105˚C) for 24 hours and then re-weighed to calculate the soil's gravimetric water content (Rahaman *et al*., 2014). However, soil bulk density is needed to convert gravimetric water content to volumetric water content. Soil bulk densities need to be determined in the same area and depths where the gravimetric samples were taken. This labour-intensive method does not require calibration and provides accurate results if done correctly (Rahaman *et al*., 2014).

4.1.3.4 CS650 soil moisture probes

Although expensive, CS650 soil moisture probes (Campbell Scientific, Utah, USA) measure volumetric water content, electrical conductivity, dielectric permittivity and temperature of soils or other porous media (Campbell Scientific, 2012). The CS650 sensors are made up of 30 cm long steel rods that create a large radius for soil moisture and temperature monitoring. CS650s do not require calibration with other sensors as it measures soil water content directly, which is one of the advantages of using the sensor. The outputs derived from the CS650 (i.e. soil temperature) can be used to calibrate other instruments/sensors such as the Watermarks, which does not measure soil water content directly.

4.2 Crop Growth and Yield

This subsection discusses various techniques for estimating feedstock growth and yield. The objective is to identify suitable techniques for estimating the growth and yield of soybean produced at the smallholder farming scale.

4.2.1 Crop growth

Destructive sampling can be used to determine biomass accumulation over a crop's growing period (Kunz *et al*., 2015a; Hadebe *et al*., 2017). This technique involves the periodic removal of above-ground foliage to measure both fresh and oven-dry biomass yields (i.e. leaf, stem, head and/or tuber weight). Dry yields are obtained by oven drying fresh plant biomass (with roots removed) at a temperature ranging from 65 to 75ºC for a period of 48 hours (Kunz *et al*., 2015a).

4.2.2 Final crop yield

There are different methods of harvesting and obtaining final seed yield. Final crop yields are commonly determined by harvesting entire plant rows of an experiment in order to get an accurate estimation of crop yields (Mokoena, 2013). However, this method is labour intensive and therefore, studies often utilize the quadrant approach, especially if experimental sites are large, i.e. commercial farms (Fermont and Benson, 2011). Harvested crops are generally air dried in a glasshouse and then weighed to determine final biomass (i.e. stem and pods with seeds). Air dried pods are shattered to obtain the seeds and then weighed to determine seed yield.

4.2.3 Harvest index

Final seed yield and biomass measurements can be used to calculate the harvest index (*HI*), which is defined as the ratio of dry seed yield (Y in t ha^{-1}) relative to the total biomass (B in t ha^{-1}) as shown in **Equation 4** below.

$$\boldsymbol{HI = \frac{Y}{B} \times 100}$$ **Equation 4**

4.3 Biodiesel Yield

According to Kunz *et al*. (2015a), biodiesel yields can be determined using **Equation 5**. In this equation the seed oil content (%) is determined using a hexane extraction process described by Meyer *et al*. (2008). This process results in oil content extracted from soybean seeds in a laboratory. Seed quality highly influences the amount of oil extracted, i.e. high-quality seeds produce high oil content (PANNAR, 2006, Mokoena, 2013).

$$\boldsymbol{Y_B = Y_C \times O_C \times 10 \times 0.95/0.92}$$ **Equation 5**

where Y_B is the biodiesel yield (L ha^{-1}), Y_C is the dry seed yield (t ha^{-1}) and O_C is the seed oil content (%). The factor of 10 accommodates the units of yield and seed oil content. The above theoretical biodiesel yield equation is based on the following assumptions, *viz*.:

- all bio-oil can be extracted from the seed,
- the conversion efficiency is 95% (Nolte, 2007), and
- a typical oil density for soybean of 0.92 kg L^{-1} (Atabani *et al*., 2013).

Water use efficiency of biodiesel production (WUE_B in L ha^{-1}) is defined by Kunz *et al*. (2015a) as the ratio of biodiesel yield (Y_B in L ha^{-1}; see **Equation 5**) relative to the crop water use (ET_C in m^3 ha^{-1}), as shown in **Equation 6**. WUE_B is strongly influenced by crop yield, i.e. low crop yield results in low WUE_B (Kunz *et al*., 2015a).

$$\boldsymbol{WUE_B = \frac{Y_B}{ETc}} \qquad \textbf{Equation 6}$$

4.4 Modelling of Crop Water Use and Yield

In this subsection, a brief description on the AquaCrop model (Raes *et al*., 2009) is given, with regard to its ability to simulate crop water use and yield. Thereafter, the application of AquaCrop in both international and local soybean case studies is discussed.

4.4.1 AquaCrop model

Due to the complexity of the terrestrial environment, simulation models are often used to help simplify complex processes and to provide valuable predictions of important variables (Schulze, 1995). Estimation of crop water use and yield are no exceptions, as they can be difficult and expensive to measure in different conditions due to the influence of the climate, soil and crop management practices (Kunz *et al.*, 2015a). Therefore, it becomes necessary to simulate or predict crop water use and yield across a wide range of growing conditions and management practices (Farahani *et al*., 2009; Kunz *et al*., 2015a).

Most crop simulation models are deterministic in design, meaning that a specific set of input parameters should produce a unique output (Gary *et al*., 1998). According to Steduto (2006), all mechanistic crop models are based on one or more growth engines (i.e. water-, solar- and/or carbon-driven), which simulate the production of biomass and yield. AquaCrop is a daily crop simulation model developed by the Food and Agricultural Organisation or FAO (Raes *et al.*, 2009). AquaCrop is based on a water-driven growth engine (Steduto *et al.*, 2012), which estimates biomass production from crop transpiration as shown in **Figure 4.1.** Hence, AquaCrop can simulate crop yield responses to water availability under different crop management and environmental conditions (Steduto *et al.*, 2012). The model is considered a robust model that is user friendly and does not require many input parameters, most of which can be determined from field measurements (Heng *et al.*, 2009). However, AquaCrop has been reported to perform well under moderate water stress conditions as compared to severe stress conditions (Heng *et al.*, 2009; Todorovic *et al.*, 2009; Battisti *et al.*, 2017).

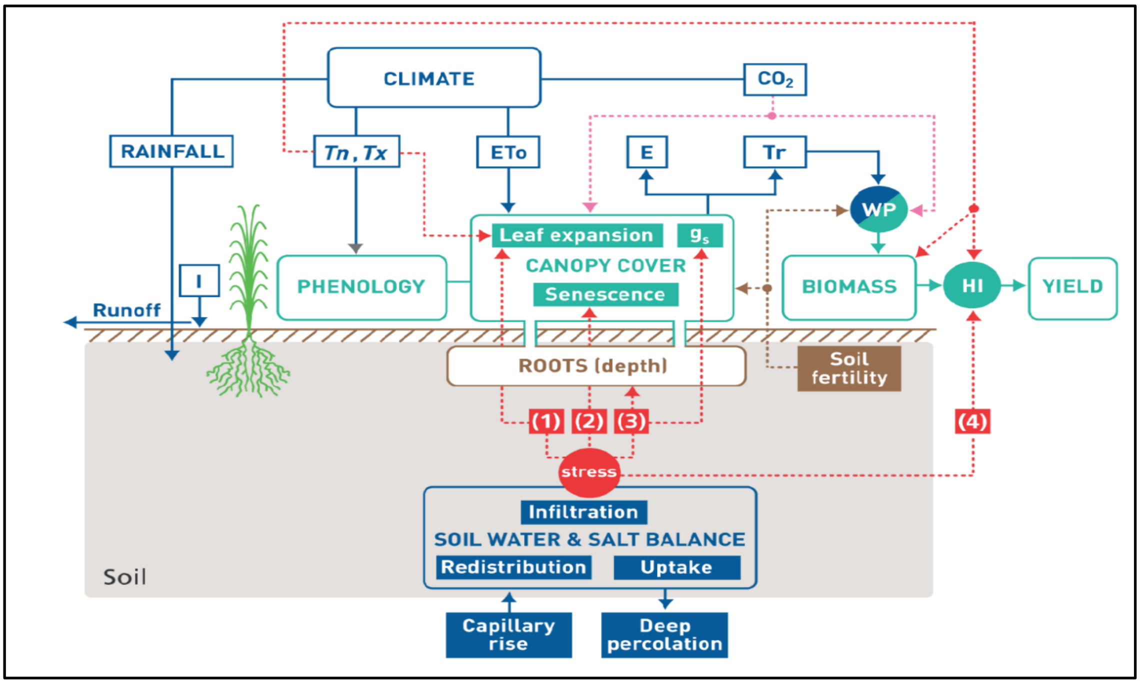


Figure 4.1: Diagram of the AquaCrop water-driven growth engine (Steduto *et al.*, 2012)

The model does not account for interception loss, which can be an issue for 1) certain crops that have a high leaf area index, and 2) where plant and inter-row spacings are small resulting in full canopy closure (Steduto *et al.*, 2012). The model has also been criticised for its simplistic field management options, since AquaCrop does not account for inoculation or the type and proportion of nutrients being used (Heng *et al.*, 2009). The model simulates crop water use (ET_C) using the soil water balance method. The model generates surface runoff, soil water content, capillary rise and deep percolation for the growing period specified within the model (Saab *et al.*, 2015).

4.4.2 Model application

A review of available literature was undertaken to determine where AquaCrop had been applied to simulate the water use and yield of soybean. Of all the studies found, the main five are presented next as case studies.

4.4.2.1 Case study 1: China (2008-11)

AquaCrop was applied in the North China Plain to estimate crop water use and yield of soybean by Paredes *et al.* (2015). The main outcomes of this study were as follows:

- A partial calibration of the canopy cover (CC) curve, as compared to the use of default parameters, did improve model simulations with respect to 1) available soil water content and soil water evaporation, as well as 2) biomass and yield estimations.
- However, the model does not simulate available soil water content over the crop season very well, due to the abandonment of the FAO dual crop coefficient (K_C) approach within the model.

According to Paredes *et al*. (2015), AquaCrop partitions crop evapotranspiration (ET_C mm) into crop transpiration (T_C mm) and soil water evaporation (E_S mm). Using this approach, the models simulates daily crop water use via a soil water balance of the entire root zone. Soil water evaporation is based on Ritchie's two-step evaporation approach (Ritchie, 1972; Allen *et al*., 1998). Since the dual K_C approach was abandoned, AquaCrop uses an empirical approach to estimate T_C and E_S based on the CC curve and therefore, has been reported by Paredes *et al.* (2015) to produce poor estimates of daily E_S and available soil water content thus affecting crop water use (ET_C).

4.4.2.2 Case study 2: Brazil (2013-16)

More recently, Battisti *et al*. (2017) tested the AquaCrop using default, partial and fully calibrated parameter sets to simulate soybean yield under both irrigated and rainfed conditions. The main outcomes from this study include the following:

- A full calibration of the model resulted in the most accurate simulation of crop yield when compared to using default and partial calibrated parameters.

- The model cannot simulate soybean yields with high accuracy under severe water deficit conditions, as the model is too sensitive to water deficit conditions.

4.4.2.3 Case study 3: South Africa (2015)

In South Africa, Kunz *et al*. (2015a) used the AquaCrop model to estimate soybean water use, yield and WUE for 5838 homogeneous response zones. The model's default crop parameters for soybean were used, together with fixed values for the planting date (1st November) and planting density (328000 plants ha^{-1}). They produced national scale maps showing the spatial variation in yield and WUE_C of soybean. The maps show that soybean is most water use efficient when produced along the coastal region of KwaZulu-Natal and the Eastern Cape. Hence, rural communities in the Eastern Cape could benefit from growing soybean on underutilized arable land (i.e. commonly known as the former homelands) for biofuel production. Therefore, the expansion of soybean production would boost rural economic growth in the province.

4.4.2.4 Case study 4: Swayimane (2015/16)

A study done by Lembede (2017) at Swayimane (rural farming community in KwaZulu-Natal, South Africa) performed a full calibration (25 crop parameters adjusted) of the AquaCrop model to simulate the effect of mulching and fertilization treatments on crop water use and yield. The model simulated crop yield and biomass well for the non-mulched treatment but performed poorly under the mulched treatment. The model did not account for the complex interactions between the soil and mulch residue that resulted in soil nitrogen deficiency. As a result, the model simulated higher crop yields in comparison to what was observed.

4.4.2.5 Case study 5: Baynesfield (2012/13)

Mbangiwa *et al*. (2019) performed a minimal calibration of the model for soybean grown at Baynesfield (KwaZulu-Natal Midlands). Certain cultivar-specific and non-conservative parameters were "fine-tuned" by the authors to better represent local growing conditions. FAO (2017a; 2017b) provided a list of parameters that should be adjusted for a minimal calibration of the AquaCrop model, since these parameters vary with the selected cultivar and may also be affected by field management and environmental conditions. According to Mbangiwa *et al*.

(2019), their calibration of AquaCrop was shown to simulate CC and crop yield well when compared to observations.

4.5 Summary

Based on studies that involved the AquaCrop model, the water use and yield of grain sorghum (see **Table 3.1**) has been measured at more locations in South Africa than compared to soybean (see **Table 3.2**). Although Lembede (2017) looked at the effects of crop management treatments (i.e. mulch and fertilization) on crop water use and yield at a smallholder farm scale in South Africa, it was noted that the soybean trial was not inoculated, which may have resulted in low crop yields reported in the study. In addition, crop water use was not measured (as runoff was not observed, only soil water content), but rather modelled using AquaCrop. Hence, this study investigated the effects of inoculation and fertilization on soybean WUE_C.

AquaCrop has been applied both internationally and locally to simulate water use and yield of different soybean cultivars grown under different crop management options and environmental conditions. The accuracy in simulating soybean water use and yield varied, depending on the level of calibration undertaken by the authors. For this study, a minimal calibration of AquaCrop was performed by adjusting certain parameters related to crop growth and phenology. The next section discusses the methods that were used in estimating crop water use and yield of inoculated compared to non-inoculated soybean grown under rainfed conditions.

5. METHODOLOGY

In this chapter, the approach that was taken to achieve the aims and objectives of this study are outlined. The methodology includes a description of the following: experimental site selected for this study, the planting material used for the trial, the experimental design, the agronomic practices performed, data collection pertaining to crop water use and yield as well as linking field measurements to modelling. Lastly, the statistical analysis performed on observed crop water use and yield data is also given.

5.1 Experimental Site

A field trial was conducted from November 2018 to April 2019 at Swayimane High School (29°31'09.25"S; 30°41'38.77"E; elevation 878 m a.s.l.), located near Wartburg (**Figure 5.1**) in the province of KwaZulu-Natal, South Africa. Based on climatic records from January 2001 to December 2017, annual rainfall ranges from 575 and 1085 mm, with an average of approximately 830 mm. The mean annual air temperature is approximately 17.9ºC (Smith, 2006). Monthly averages of maximum and minimum air temperatures are 24.0ºC and 11.8ºC, respectively. Soil textures in this region are mainly fertile clay loams, with medium drainage due to the presence of impermeable clay layers at deeper depths (Smith, 2006).

Figure 5.1: A Satellite-derived image obtained from Google Earth® (dated 23/03/2018) showing the location of the trial site within Swayimane High School

5.2 Plant Material

Three soybean *(Glycine max*) varieties, *viz*. PAN1521R, LS6161R and CAPG3 were purchased from PANNAR seeds, Link Seed and Capstone Seeds respectively, were planted at the experimental site. The agronomic characteristics pertaining to the three crop varieties are given in **Table 5.1.** The varieties selected for this study differ in respect to growth types (i.e. determinant and indeterminant), where PAN1521R and CAPG3 are indeterminant crops, which continues to develop (i.e. increase in plant height) even when flowering to pod formation has occurred. LS6161R is a determinant crop that stops developing once flowering or pod formation occurs. The three varieties also differ in relative maturity (i.e. the time taken for each variety to reach flowering and thus harvest maturity). LS6161R is an early maturing variety (i.e. it takes ~128 to 160 days to mature), PAN1521R is an early to medium maturing variety (i.e. ~140 to160 days) and CAPG3 is a late maturing variety (i.e. ~160 to 180 days) (see **Table 5.1**).

Table 5.1: Agronomic characteristics of the three varieties (i.e. PAN1521R, LS6161R and CAPG3) grown under rainfed conditions and two inoculation treatments

Agronomic characteristics	PAN1521R	LS6161R	CAPG3
Growing season (MG*)	5.7	6.1	6 to 6.2
Growth type	Indeterminant	Semi-Determinant	Indeterminant
Relative maturity	Early	Early-medium	Medium-late
Pod height (9 = Excellent, 1 = Poor)	9	9	9
Standability (9 = Excellent, 1 = Poor)	9	9	9
Shattering resistance (9 = Excellent, 1 = Poor)	9	9	9
Relative number of days to 50% flower	46 to 75	43 to 74	58 to 70
Relative number of days to 50% harvest maturity	128 to 160	140 to 160	160 to 170

Note= *Maturity Group

All three varieties are genetically modified to have excellent pod height (i.e. the pods are formed much higher from the ground), standability (i.e. it prevents the crop from lodging or falling over) and shattering resistance, which is thus very important as it prevents early shattering of pods which thus prevents loss of seed yield at harvest (see **Table 5.1**). All three varieties are also genetically modified to withstand glyphosate, i.e. an active ingredient found in Roundup®

herbicide (PANNAR, 2006). Non-hybrid varieties, as listed in the Agricultural Research Council (ARC) 2017/18 cultivar guide (de Beer and Bronkhorst, 2018), proved difficult to obtain from the seed companies mentioned above.

5.3 Experimental Design

The trial design used for this study was a split-plot arranged in a randomised complete block design, with plots replicated three times (refer to **Figure 5.2**). The main factor was inoculation (i.e. inoculation compared to non-inoculation) and the sub-factor comprised of three soybean varieties. There were 18 plots in total, where each plot was 6 m by 3.15 m in size. Hence, the total trial area was 451 m^2. Each plot contained 7 rows as follows: 2 inner (i.e. experimental) rows where all measurements were conducted over the growing season; 1 row for destructive sampling; 2 outer or border rows on either side (i.e. 4 rows in total), which were not considered due to edge effects.

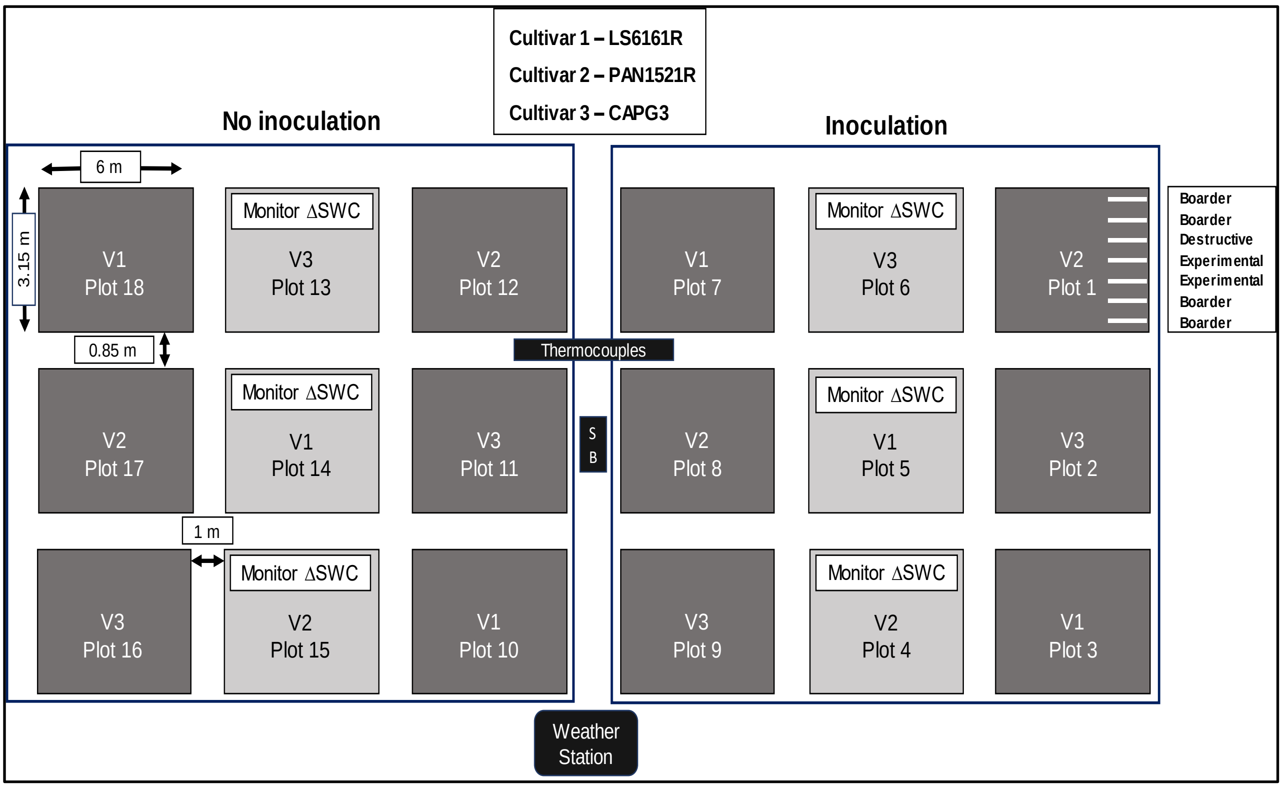


Figure 5.2: Diagram of proposed trial design to investigate the effects of inoculation on water use and yield of three soybean cultivars at Swayimane

Watermark sensors (Irrometer, Riverside CA, USA) were installed in plots 4 to 6 and 13 to 15 at depths 0.15, 0.30, 0.60 and 1.00 m to monitor changes in soil moisture over the growing season. Four CS650 soil moisture sensors (Campbell Scientific, Utah, USA) were placed in plot 14 at the same four depths as the Watermark sensors. This was done to calibrate the Watermarks, but due to budget constraints, CS650 sensors were not installed in any other plot. Soil thermocouples were also installed in plots 5 and 14 for soil surface temperature monitoring. All sensors were connected to a CR1000 data logger (Campbell Scientific Inc., Logan, Utah, USA) that was installed in the middle of the trial in a strong box (SB in **Figure 5.2**).

For surface runoff measurements, 1 m by 1 m aluminium square grids were placed down the slope of the trial in plots 7, 11 and 4 as well as to represent the three planted varieties. An automatic weather station is situated below the trial plots for continuous monitoring of climatic parameters (i.e. rainfall, solar irradiance, air temperature, relative humidity, wind speed and direction) throughout the growing season.

5.4 Agronomic Practices

A detailed account of the land preparation, planting, treatments and chemicals used during the season are discussed in the subsections that follow.

5.4.1 Site preparation

Land preparation was completed prior to planting and involved ploughing and disking the field. Hand hoes were used to manually remove weeds and for turning the soil to ensure a smooth seedbed. Weeding was also done at critical periods during the season. Fencing was installed around the experimental site to protect the plots from animals such as cows. The above-mentioned tasks were completed with the help of staff from Ukulinga research farm as well as contracted labour from the local Swayimane community.

5.4.2 Planting

According to Nieuwenhuis and Nieuwelink (2002), PANNAR (2006) and DAFF (2010a), a planting date from mid-October to November is recommended for soybean to achieve optimum crop development. However, a planting up to December in warmer areas have shown to be viable as well (de Beer and Bronkhorst, 2018). An inter-row and intra-row spacing of 0.4 to 0.9 m and 0.05-0.15 m respectively is recommended to achieve a planning population of 200000 to 400000 plants per hectare. The three cultivars were planted on the 19th of November 2018 with an inter-row and intra-row spacing of 0.45 m and 0.07 m, respectively. This achieved the desired planting population density of 317460 plants ha^{-1}.

Planting rows were opened using hand hoes and seeds were individually sown (not broadcasted) at a depth of 0.03 m. This negated the need to thin the trial after emergence, which can damage the establishing crop and thus, affect crop development (DAFF, 2010a). However, PAN1521R variety did not germinate in the required germination period (i.e. 7-10 days). A germination test undertaken in the laboratory (refer to **Figure 9.1** in **Appendix 1**) was unsuccessful due to the poor quality of seed material obtained from PANNAR. Therefore, measurements were only performed on the other two varieties (i.e. LS6161R and CAPG3) in plots 2, 3, 5, 6, 7, 9, 10, 11, 13, 14, 16 and 18 throughout the season. Once the crops emerged, gaps that appeared between rows were sown with seeds to achieve the target planting density.

5.4.3 Treatments

For this study, the two treatments used in the experiment were inoculation and fertilization. The application of these two treatments under field conditions is explained next in more detail.

5.4.3.1 Inoculation

The benefits of inoculating soybean seed prior to planting were explained in **Subsection 3.3.2**. In total, 50 ml of liquid inoculant (*Bradyrhizobium japonicum*) supplied by Link Seed was added to a 16 L knapsack and mixed thoroughly with water. The mixing was done in a cool shady place to prevent exposure of the bacterium to the elements. The inoculant was applied in the furrows with the seed and fertilizer to only one-half of the trial (see **Figure 5.2**). The furrows were then immediately covered to prevent exposure of bacteria to the climatic conditions, which could render the treatment ineffective over the growing period.

5.4.3.2 Fertilization

The importance of applying fertilizers to an inoculated crop was explained in **Subsection 3.3.3**. Prior to the commencement of the trial, soil samples at a depth of 0.15 m were taken from each of the 18 plots for soil fertility analysis. The analysis was undertaken by the Soil Analytical Service Laboratory located at the Cedara College of Agriculture in KwaZulu-Natal. Based on the results, 0 kg ha^{-1} of N, 60 kg ha^{-1} of P and 80 kg ha^{-1} of K was recommended for the trial site. Single superphosphate (P; 10.5%) and potassium chloride (K; 50%) fertilizers were applied at optimum rates using a broadcasting method to all plots once off. The trial did not require a further top dressing of fertilizer during the growing season.

5.4.4 Chemicals

Prior to planting, the trial site was sprayed with Dual Gold, a pre-emergent herbicide to control weeds. A dilution rate of 60 ml of herbicide per 16 L of water was used. The trial site was sprayed at flowering using Kemprin insecticide (a/l cypermethrin) using a total dilution rate of 185 ml of insecticide per 16 L of water. The immediate area surrounding the trial site was sprayed with Gramoxone (150 ml per 10 L of water) to control weed growth during the growing season. To prevent an outbreak of soybean rust, the trial site was sprayed during floral initiation (February) using Artea fungicide spray at a dilution rate of 29 ml per 16 L of water. A second application was applied 21 days later floral initiation.

5.5 Data Collection

For this study the variables such as weather parameters, crop water use, soil water content as well as crop growth and phenology were measured and monitored throughout the growing season. These variables will be described in more detail below.

5.5.1 Weather data

Daily climatic data such as rainfall (mm), solar irradiance (MJ m^{-2}), air temperature (˚C), relative humidity (%) and wind speed (m s^{-1}) was recorded over the growing season using an automatic weather station (AWS) installed at the trial site. All sensors on the AWS were purchased from Campbell Scientific Africa, Somerset West, RSA. Air temperature and relative humidity were measured using an HMP50 sensor. Rainfall was measured by a tipping bucket rain gauge (Texas Electronics TE525, Texas, USA). Wind speed was measured using a cup anemometer (03101-L RM Young, Washington, USA) that was installed 2 m above the soil surface. Solar irradiance was measured using a LI200S pyranometer (Retraska, USA). Measurements were recorded at 15-minute intervals, then averaged to hourly and then daily by a CR1000 data logger (Campbell Scientific Africa). Missing data, due to a battery failure, was patched using observations from another station situated 4.5 km away (i.e. Bruyns Hill, 29°25'S; 30°41'E; 990 m a.s.l.). Daily ET_O values were calculated by the data logger program that's based on the FAO 56 approach.

5.5.2 Crop water use

The variables that were calculated over the growing season include: actual evapotranspiration (ET_C), reference crop evapotranspiration (ET_O), crop coefficients, water use efficiency (WUE_C) and soil water content, are explained next.

5.5.2.1 Reference evapotranspiration

The FAO Penman-Monteith method (see **Subsection 4.1.1**) was used to estimate ET_O at a daily timestep from hourly data. However, ETo values calculated by the data logger were unrealistically low, due to incorrect inputs of latitude, longitude and height of instruments into the program. Daily ET_O values were then calculated using FAO's ET_O Calculator software, which was obtained from the Internet (http://www.fao.org/nr/water/eto.html).

5.5.2.2 Actual evapotranspiration

Crop ET_C was estimated as a residual of the soil water balance equation (see **Subsection 4.1.2 Equation 3**) as described by Dastorani and Poormohammadi (2012). Daily precipitation was measured by a tipping bucket rain gauge, whereas weekly runoff was observed from the three runoff plots. Soil moisture content was measured daily using the Watermark sensors. ET_C was determined for the two varieties under both inoculation treatments.

5.5.2.3 Crop coefficients

The water use (or crop) coefficient (K_C) is commonly used to estimate crop water requirements at different development stages. According to Allen *et al*. (1998), K_C is defined as the ratio of the potential evapotranspiration (ET_C in mm) of the crop to reference evapotranspiration (ET_O in mm).

$$K_C = \frac{ET_C}{ET_O}$$ **Equation 7**

K_C values obtained from the international literature may not represent local growing conditions (Ortega-Farias *et al*., 2009; Kunz *et al*., 2015a). For this reason, crop coefficients were estimated for both varieties. Crop coefficients were calculated at a monthly timestep (i.e. From November to April) for both varieties grown under rainfed conditions and each inoculation treatment.

However, it is worth noting that crop coefficients were derived for non-standard (i.e. rainfed) conditions (Allen *et al*., 1998), as the crop may have experienced water stress during their growing season. Thus, ET_C was simulated using the AquaCrop model. The model can only determine monthly crop coefficients till physiological maturity as the model only simulates crop growth till maturity and not harvest.

5.5.3 Soil water content

5.5.3.1 Volumetric water content

As noted in **Subsection 4.1.3**, soil water content was continuously monitored throughout the growing season at four depths (i.e. 0.15, 0.30, 0.60 and 1.00 m) using two different types of sensors. Due to budget constraints, only four CS650 reflectometers were installed in plot 14 only (see **Figure 5.2**), along with four Watermark sensors that aided in their calibration. Watermark sensors were also installed in plots 4, 5, 6, 13, 14 and 15. Gravimetric samples were taken in plots 6 and 13 to represent both varieties and treatments.

The Watermarks measured soil electrical resistance. Electrical resistance from Watermarks and volumetric water content from the CS650s was recorded in 15-minute intervals, which was then integrated to hourly and then daily datasets over the growing season. Using the method described by Allen (2000), electrical resistance (Ω) was converted to matric potential (kPa) using soil temperature from the CS650 as a temperature correction factor. Soil matric potential (kPa) was then converted to volumetric water content ($m^3\ m^{-3}$) using the method described by Varble and Chavez (2011). Gravimetric soil moisture content (%) was also determined (see **Subsection 4.1.3.3**), which required soil dry bulk densities for conversion to volumetric water content.

5.5.3.2 Soil texture and fertility analysis

Soil texture and fertility measurements are important as they indicate soil water availability and nutrient status across the study site. Studies **(**e.g. Tarekegn *et al*., 2017; Islam *et al*., 2018) have shown that soil texture and fertility analysis should be done prior to planting in order to determine if soil conditions are appropriate for planting and whether fertilizer amendments are needed to create optimum conditions for plant development. Soil samples are generally augured at multiple depths for soil texture analysis, while the top 0.15 m is augured for soil fertility analysis (Mokoena, 2013; Lembede, 2017; Islam *et al*., 2018). These soil samples are usually sent to an analytical service laboratory for soil texture and fertility analysis.

Prior to planting, soil samples augured at four depths (0.15, 0.30, 0.60 and 1.00 m) were taken in each of the 18 plots and were sent to the Soil Analytical Service Laboratory (Cedara College of Agriculture, KwaZulu-Natal) for soil fertility and textural analysis. The soil textural analysis shown in **Table 5.2** indicated that the top 0.3 m is dominated by sandy clay loam, which transitions into a sandy clay for the next 0.7 m.

Table 5.2: Soil particle size distribution and textural classes for the different depths at the experimental site

Soil profile depth	Coarse silt and sand	Fine silt	Clay	Soil textural class
	(0.02 to 2 mm)	(0.002 to 0.02 mm)	(< 0.002 mm)	
m	%	%	%	
0.15	47.80	19.30	33.00	Sandy Clay Loam
0.30	50.50	16.00	33.50	Sandy Clay Loam
0.60	49.00	16.80	34.00	Sandy Clay
1.00	46.50	12.50	41.00	Sandy Clay

5.5.3.3 Soil water retention

Soil water retention parameters can be estimated from soil texture (see **Subsection 3.3.5**). For example, the SPAW model (Saxton and Rawls, 2006) can also be used to provide soil water retention parameters from particle size distribution and organic matter content. However, a common method for determining soil water retention parameters is the outflow pressure method (Kunz *et al.*, 2015a; Lembede, 2017; Mokonoto, 2019), which is detailed in Lorentz *et al*. (2001). This method requires undisturbed soil cores and is therefore labour intensive, which means human error can result in poor results.

A 1 m by 1 m pit was dug prior to planting in order to obtain undisturbed soil cores at four depths to determine soil dry bulk density. The cores were placed in an oven for 24 hours at a constant temperature of 105°C to dry and then weighed to determine mass of solids. The length and diameter of each core was measured in order to calculate the volume of soil, from which soil bulk density was calculated. Saturation (SAT) was then calculated from dry bulk density. The undisturbed soil cores were also used to determine soil water retention parameters using the controlled outflow pressure method (see **Subsection 9.2**) in the soil water laboratory at UKZN. Outputs from the outflow pressure apparatus (see **Subsection 9.2** in **Appendix 2**) were used to create soil water retention curves via the Van Genuchten equation (Van Genuchten, 1980). From these curves, volumetric water content (%) at FC and PWP was estimated at a pressure head of -33 kPa and -1500 kPa, respectively. However, according to Lorentz (2019), estimates of soil water retentions parameters at lower soil depths with increasing clay content can be difficult to obtain and can result in errors in data.

5.5.4 Crop phenology

Measurements of crop phenology were observed in each of the 12 plots at the trial site over the growing season, starting at crop establishment (i.e. time for which 90% emergence had occurred in at least 50% of each plot). Throughout the growing season, weekly measurements of chlorophyll content index and stomatal conductance were taken on six plants (i.e. three plants in each of the two experimental rows). Each of these measurements is discussed next in more detail.

5.5.4.1 Chlorophyll content index

The SPAD-502 Plus Chlorophyll Meter (Konica Minolta, Osaka, Japan) was used to estimate leaf chlorophyll content. The chlorophyll meter transmits two different wavelengths through the leaf, which are affected by the chlorophyll content (Danner *et al*., 2015). Measurements were taken between 11h30 and 13h30 from the adaxial surface of newly formed and healthy leaves of unstressed plants at different growth stages were used.

5.5.4.2 Stomatal conductance

Transpiration is mainly affected by soil water content, but also by vapor pressure gradient, temperature and wind (Mabhaudhi, 2012). Transpiration is considered a productive use of water as it contributes to biomass development and crop yield (WMO, 2008). Stomatal conductance provides an indication of crop transpiration rate as it measures the diffusion of carbon dioxide (CO_2) and water vapour into and out of the plant (Mabhaudhi, 2012). Stomatal closure results in reduced stomatal conductance is therefore the first indicator of plant water stress in most plants (Cornic and Massacci, 1996). On the other hand, high stomatal conductance indicates adequate crop transpiration and therefore, a healthy crop, i.e. not nutrient or water stressed. Stomatal conductance was measured weekly using a steady-state Leaf Porometer (model SC-1, Decagon Devices, Pullman, Washington) between 11h30 and 13h30 on warm, sunny days (not during cool, rainy weather) and on the abaxial surface of young healthy leaves that are fully photosynthetically active as suggested by Mabhaudhi (2012).

5.5.5 Crop growth

Measurements of crop growth were observed in each of the 12 plots at the trial site over the growing season, starting at crop establishment (i.e. time for which 90% emergence had occurred in at least 50% of each plot). Throughout the growing season, weekly measurements of plant height, leaf number and leaf area index were taken on six plants (i.e. three plants in each of the two experimental rows). Each of these measurements is discussed next in more detail.

5.5.5.1 Plant height

Plant height (PHT) was defined as the distance from the soil surface to the tip of the youngest developing leaf (before floral initiation stage) or the tip of the growing panicle (after floral initiation). It was measured weekly using a tape measure (e.g. Chimonyo *et al*., 2016; Hadebe *et al.*, 2017).

5.5.5.2 Leaf number

Leaf number (LN) was counted for fully unfolded, expanded and photosynthetically active (50% green leaf area) leaves from establishment onwards (Mabhaudhi *et al*., 2014). A fully formed leaf is defined as when the first (unifoliolate) leaves are visible without dissecting the plant (du Plessis, 2008; Hadebe, 2015). Leaf number was counted weekly for leaves that only showed more than 50% green leaf area.

5.5.5.3 Leaf area index

Leaf area index (LAI) is defined as the ratio of leaf area (one side or upper leaf surface) per unit ground area (LI-COR, 2009). It was measured using a LAI-2200 Portable Leaf Area Meter (Li-COR, USA), a non-destructive technique based on radiation measurements (LI-COR, 2009). The technique involved taking measurements above and below canopy to obtain interception of light by the canopy, from which LAI was estimated. Although this method does not require calibration, it can be affected by the presence of weeds and/or cloud cover (LI-COR, 2009). For this reason, the trial site was kept weed free and measurements were preferably taken on sunny days.

5.5.5.4 Canopy cover

It is important to note that canopy cover (CC) was not measured directly in the field, however, measured LAI values (see **Subsection 5.5.5.3**) were used to compute the diffuse non-intercepted radiation (DIFN), from which *CC* development was calculated using **Equation 8** as follows (Mabhaudhi *et al*., 2014):

$$\boldsymbol{CC}\ (\%) = \mathbf{100}\ \times (\mathbf{1} - \boldsymbol{DIFN}) \qquad \textbf{Equation 8}$$

5.5.6 Phenological development

Phenological development was observed at different crop development stages, such as time to: emergence, flowering, formation, leaf senescence and maturity. The duration of flowering was observed. A phenological stage occurred when at least 50% of plants in each plot exhibited the required characteristic (Mabhaudhi *et al.*, 2014). For example, time to leaf senescence was defined as when at least 10% of leaves had senesced without any new formation of leaves to replace them (Mabhaudhi *et al*., 2014). Time to maturity was defined in terms of physiological maturity when at least 50% of leaves had senesced. Phenological growth periods were initially recorded in calendar days, then converted to growing degree days using Method 3 as proposed by McMaster and Wilhelm (1997).

5.5.7 Biomass accumulation

Destructive sampling was undertaken every fortnight and a total of 12 plants were sampled (i.e. one from each plot). Roots were removed from the plants in the field, before being transported to the laboratory for total above ground biomass determination. Plants samples were oven dried at a constant temperature of 80˚C for 24 hours, then weighed to constant mass (g) to obtain the total above ground dry biomass.

5.5.8 Final biomass and seed yield

Plants were harvested sequentially as the crops matured at different periods. Variety LS6161R was harvested first, followed by CAPG3. Ten representative plants were selected from each plot (i.e. five plants from each of the two experimental rows) in order to determine final biomass and seed yield. Harvested plants were then placed in a greenhouse to air dry, then weighed to determine total dry biomass. Pods were manually shelled to determine seed yield. The harvest index was then calculated using **Equation 4** in **Subsection 4.2.3**.

5.5.9 Seed oil content and biodiesel yield

Soybean seed oil content were determined using a hexane extraction process (Meyer *et al.*, 2008) for each plot, then averaged for both LS6161R and CAPG3 varieties grown under the two inoculation treatments. In the laboratory, seeds were first crushed into a fine powder. Approximately 1 g of the powdered sample was homogenised with hexane solvent, then placed in a water bath at 50˚C for approximately ten minutes to initiate reaction of the solvent (i.e. extraction of seed oil). The mixture was then passed through a glass-wool filter to trap solid particles, with the liquid collected in small glass vials. These vials were then placed into a vacuum set at 35˚C for three and a half hours to vaporize the hexane solvent leaving behind the oil. The recovered oil was weighed and used to determine the percentage of oil recovered (% w/w). From this, the theoretical biodiesel yield was determined using **Equation 5** in **Subsection 4.3** for both varieties and inoculation treatments.

5.6 Crop Water Use Efficiency

Using crop water use estimated via the soil water balance equation (see **Subsection 4.1.2**), the following two metrics were calculated for both varieties grown under the two inoculation treatments:

- crop water use efficiency (see **Subsection 3.1**) using final seed yield (see **Subsection 4.2.2**), and
- water use efficiency of biodiesel production (as defined in **Subsection 4.3** 4.3was calculated using the theoretical biodiesel yield (see **Subsection 4.3**).

5.7 Statistical Analysis

A statistical analysis of observed data is required to highlight patterns of significance between the varieties and treatments considered in this study. Statistical analyses of crop growth, final biomass, grain yield and harvest index were undertaken using the GenStat® Version 17 (VSN

International, UK). An analysis of variance (or ANOVA) was used to analyse the data to determine the differences between the two inoculation treatments and varieties. Statistical indicators such as the least significant difference (LSD) were used to separate means at the 5% level of statistical significance. In addition, the coefficient of variation (CV) was used to determine the variation within the data, i.e. if CV > 30%, this may indicate an error in data collection. The Turkey multiple range test was used to separate means at the 5% significance level. It is a more appropriate statistical tool as compared to the Duncan multiple range test as it can analyse finer differences between datasets.

5.8 Linking Field Measurements to Modelling

The AquaCrop model was used to provide simulations of crop water use and yield for both soybean varieties grown under rainfed conditions and two inoculation treatments. For this study, a minimal calibration of the model was performed based on observations at the trial site as discussed below.

5.8.1 AquaCrop inputs

AquaCrop requires a climate (*.CLI) and soils (*.SOL) input file of daily data to run, as well as a crop parameter (*.CRO) file and a file (*.CO2) of ambient CO_2 concentration. The default CO_2 concentration file was used, which is based on measurements from Mauna Loa (19.536°N; 155.576°W; altitude 3394 m a.s.l.).

5.8.1.1 Climate data

All input climate files required by the model, except for ET_O that was obtained using the FAO ET_O calculator, were obtained from the automatic weather station (AWS) located at the trial site. Missing data (due mainly to battery issues) were infilled with data from a weather station situated 4.5 km away from the site (Bruyns Hills; 29°25'S; 30°41'0"E; 990 m a.s.l), as was done by Lembede (2017).

5.8.1.2 Soils data

AquaCrop requires inputs of soil depth, soil water retention characteristics and saturated hydraulic conductivity (K_{SAT}). Although soil depth and soil water retention parameters (see **Subsection 5.5.3.3**) were determined for four depths, they were depth weighted to produce values for two soil horizons based on similar textures (see **Table 5.2** in **Subsection 5.5.3.2**) as follows;

- 0.30 m sandy clay loam (A-horizon), and
- 0.70 m a sandy clay soil (B-horizon).

Estimates of FC, PWP and SAT were determined using the outflow pressure method as discussed in **Subsection 5.5.3.3**. Values for depths 0.15 and 0.30 m were weighted to represent the upper soil horizon. Measurements at depths 0.60 and 1.00 m represented the lower soil horizon. K_{SAT} was determined using the SPAW model and was weighted for the two soil horizons as shown in **Table 5.3** below. The model also required the initial soil water condition, which was derived from CS650 measurements on the day of planting.

Table 5.3: Soil texture and soil water retention parameters averaged for the two soil horizons in AquaCrop

Soil texture	**Thickness**	**PWP[1]**	**FC[2]**	**SAT[3]**	**K_{SAT}[4]**
	m	**Volumetric (%)**			**mm day^{-1}**
Sandy clay loam	0.3	13.5	37.8	58.5	123.5
Sandy clay	0.7	21.0	39.3	55.3	60.8

Note: 1=Permanent wilting point; 2= Field capacity; 3= Saturation; 4=Saturated hydraulic conductivity

5.8.1.1 Planting date and density

The planting date and planting density for this study were the 19th of November 2018 and 317460 plants ha^{-1} respectively. These values were then used as inputs required by the AquaCrop model.

5.8.2 AquaCrop calibration

As noted in **Subsection 4.4.2.5**, Mbangiwa *et al*. (2019) used AquaCrop to simulate the water use and yield of soybean grown at the Baynesfield Estate (KwaZulu-Natal, Midlands) in the 2012/13 season. The authors calibrated the model using observations of LAI to estimate canopy cover development. AquaCrop was then used to convert phenological growth stages observed in calendar days to thermal time, i.e. in growing degree days. A very similar calibration approach was followed in this study as detailed next.

5.8.2.1 Canopy cover

The seedling leaf area (in cm^2) was measured at emergence and together with planting density as inputs, was used by the model to compute initial canopy cover (CC_O), as described by Raes *et al*. (2009). Measured LAI values (see **Subsection 5.5.5.3**) were then used to compute the diffuse non-intercepted radiation (DIFN), from which CC development was calculated using **Equation 8** (see **Subsection 5.5.5.4**). The model calculated the canopy growth coefficient (i.e. the increase in CC per degree day) from observations of maximum canopy cover percentage (CCx) and the time taken to reach CCx (similar to time from sowing to flowering).

5.8.2.2 Crop growth and phenology

For a partial calibration of AquaCrop, Steduto *et al*. (2012; see Table 2 on p 44) provided a list of crop parameters that should be adjusted to reflect local cultivars and growing conditions. This list included, *inter alia*:

- maximum rooting depth (Zr_{max}),
- the time required to reach Zr_{max},
- the time to reach certain phenological growth stages (e.g. emergence, flowering, canopy senescence and maturity), and
- the duration of flowering.

More recently, FAO (2017a; 2017b) provided a similar list of cultivar-specific and non-conservative parameters that should be "fine-tuned" to better represent local growing conditions as follows:

- Planting and management: planting method (direct or transplanting), planting density (which will affect initial and maximum canopy cover) and time to reach 90% emergence.
- Crop growth: initial canopy cover, maximum canopy cover and maximum rooting depth.
- Crop phenology: time to maximum canopy cover, time to flowering, time to senescence, time to maturity, time to maximum rooting depth and flower duration.
- Soil profile: thickness and texture of each soil horizon as well as soil water retention parameters and saturated hydraulic conductivity of each soil layer.
- Management options: field practices related to soil fertility stress, weed management as well as the use of mulching to conserve soil water and contouring to reduce runoff.

In this study, the maximum rooting depth was observed by digging a trench between rows in a plot. The distance from the topsoil to the maximum depth at which roots were visible was then measured. The maximum rooting depth was determined at flowering and physiological maturity. The time when Zr_{max} was reached was determined when the maximum rooting depth based on observations was constant, which was taken to be around flowering to physiological maturity. The phenological growth parameters were initially observed in calendar days (CD) and then converted in the model to growing degree days (GDD).

5.8.2.3 Field management

AquaCrop's field management options do not consider inoculation, only soil fertility stress. The soil fertility option does not express what type and proportion of nutrients were applied to the trial. Hence, assumptions were made to better represent the two inoculation treatments. The non-limiting option in AquaCrop was selected to represent the inoculated and fertilized plots in the trial. For the non-inoculated treatment, a few iterations were performed to change the fertility stress from optimum to moderate, i.e. where certain nutrients (e.g. N) were deficient. These two fertility options were used to simulate crop water use and yield, which were then compared to observations.

The model simulations were performed under rainfed (i.e. non-standard) conditions in order to determine actual crop water use, which could then be compared to field-based measurements.

Under the management option in AquaCrop, the irrigation option was also invoked to relieve plant water stress. This represented standard (i.e. non-stressed) conditions and allowed for the determination of maximum crop water use, from which crop coefficients were calculated. According to Allen *et al*. (1998), crop coefficients should be determined for non-stressed growing conditions. There are three irrigation methods in AquaCrop, *viz*. 1) net irrigation water requirement, 2) irrigation schedule, and 3) generation of irrigation schedule. For both varieties and inoculation treatments, the net irrigation water requirement was selected, where irrigation would occur when the soil water content dropped to 50% of plant available water.

5.8.3 AquaCrop output

The outputs of the model were then used to calculate WUE of both crop and biodiesel production and monthly K_C values for both soybean varieties (i.e. LS6161R and CAPG3). The monthly K_C values were derived for non-stressed conditions for both varieties and inoculation treatments using the model outputs of ET_C and ET_O (see **Equation 7** in **Subsection 5.5.2.3**). Monthly crop coefficients were also derived for stressed conditions (i.e. dryland) from simulated output and compared to observed values. The performance of the model was assessed statistically as discussed next.

5.8.4 Model evaluation

Model evaluation is essential for determining the accuracy of model simulates in comparison to observations. This evaluation helps to assess the robustness of the model calibration. Canopy cover (derived from observed LAI) and observed biomass were plotted and compared against the simulations using the following statistical indicators: Pearson correlation coefficient (R) and its square (R^2), root mean square error (RMSE), and Willmott's index of agreement (D) (Paredes *et al.*, 2015).

R^2 simply measures the dispersion of observed compared to predicted data. R^2 ranges from 0 to 1, with 1 indicating an excellent correlation. However, this statistical indicator can be misleading as a model can over- or under-estimate and still produce high R^2 values (Krause *et al*., 2005). Therefore, other statistics are needed.

Root mean square error or RMSE quantifies the extent of differences between observed compared to simulated data trends and ranges from zero to positive infinity. One limitation of this statistic is that it does not differentiate between over- and under-estimation. Therefore, errors are squared, with more weight given to higher values than lower values in the time series (Legates and McCabe, 1999).

Normalised root mean square or NRMSE quantifies differences between observed compared to simulated data trends in %. It is calculated as the ratio of RMSE to the mean of observations. A model simulation is termed excellent when NRMSE $\leq$ 10%, good if 10% $<$ NRMSE $\leq$ 20%, acceptable if 20% $<$ NRMSE $\leq$ 30% and poor if NRMSE $>$ 30% (Jamieson *et al*., 1991).

Willmott's D-index of agreement measures the degree to which simulations approach observations. The D-index ranges from 0 to 1, with 1 being a good agreement and 0 being a poor agreement. This statistic overcomes the insensitivity of R^2 to under- and over-estimations by the model (Willmott, 1982).

6. RESULTS AND DISCUSSION

In this chapter, the results obtained in this study are presented and discussed. This section begins with an overview of the weather conditions during the growing season, followed by the results related to crop water use, soil water content and retention parameters, crop growth and phenology, grain yields and biodiesel yields, crop water use efficiency and biodiesel use efficiency. Thereafter, the modelling of crop water use and yield using AquaCrop is presented, with simulated results compared to observations to assess model performance.

6.1 Weather Conditions

According to DAFF (2010a), soybeans can produce high yields in areas that receive seasonal rainfall ranging from 500 to 900 mm that is well distributed, assuming all other conditions are optimal. The total amount of rainfall (mm) received during the growing period was 581.2 mm.

There were several periods of dry spells (i.e. < 2 mm) throughout the growing period, which can be seen in **Figure 6.1**. However, these periods were not considered long enough to hamper critical development stages. According to DAFF (2010a), adequate moisture is essential once flowering occurs, up to pod formation. Initial flower development began at 71 and 79 days after sowing (DAS) for LS6161R and CAPG3, respectively. Whereas, pod formation began at 88 and 95 DAS for LS6161R and CAPG3 respectively. Therefore, it can be shown in **Figure 6.1** that water stress did not occur during these critical development periods and thus, final yield potential was not affected. In addition, the effects of crop water stress would result in yellowing of leaves and stunted growth, which were not observed during the growing season.

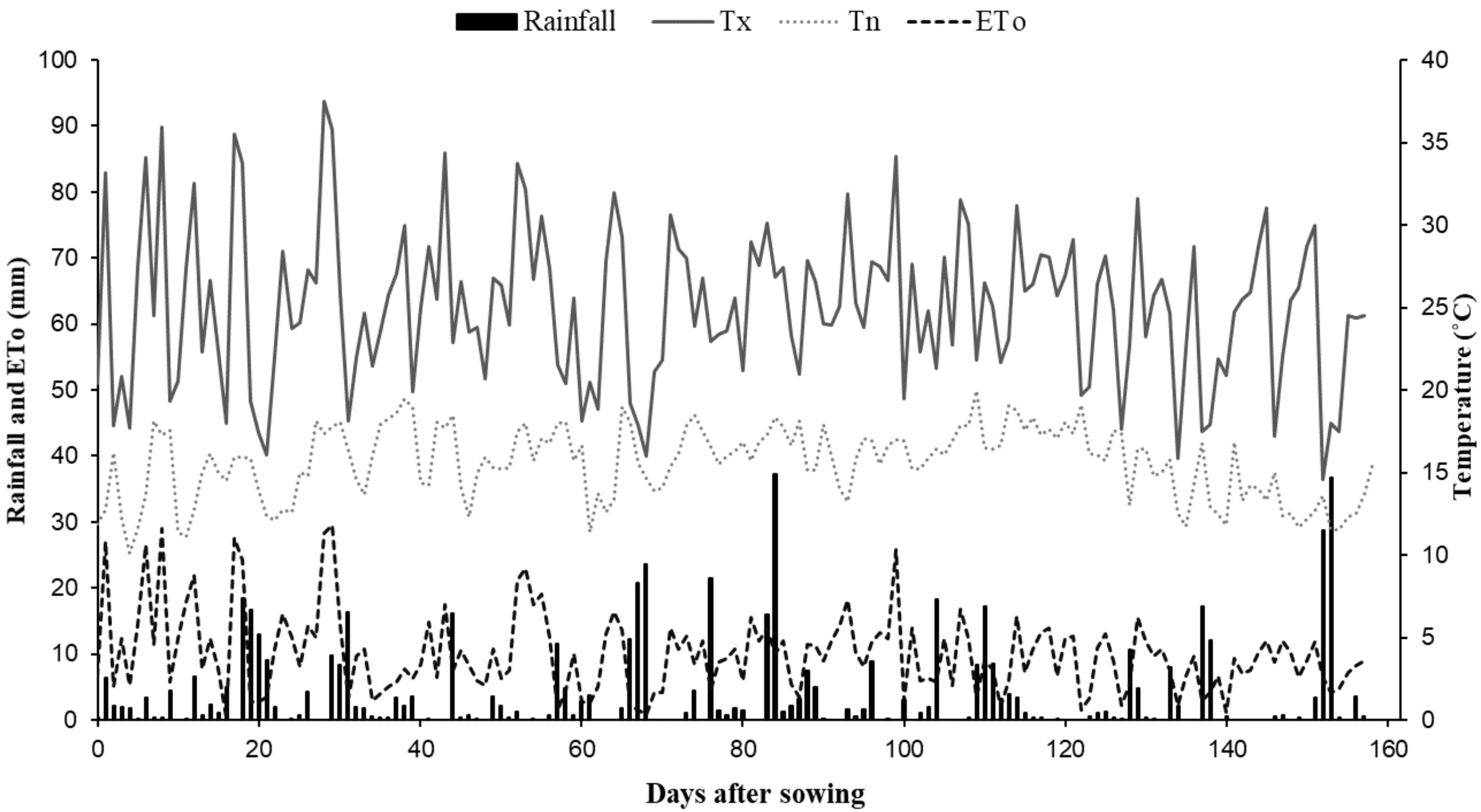


Figure 6.1: Variation in rainfall (mm), reference evapotranspiration (mm) and maximum and minimum air temperature (˚C) from 17th November 2018 to 25th April 2019 at Swayimane

The daily average air temperatures for the trial site ranged from 15.6 to 25.1˚C. According to DAFF (2010a), soybean can develop optimally when air temperatures range from 25 to 30˚C and still can grow well when the daily temperatures are above 13˚C throughout the growing season but are less tolerant to very cold temperatures below 13˚C. Very cold temperatures (i.e. below 13˚C) and very warm temperatures (i.e. in excess of 30˚C) can delay flowering or cause flower abortion that can ultimately affect crop yield (PANNAR, 2006; DAFF, 2010a). In this study, the daily maximum and minimum air temperatures (i.e. Tx and Tn respectively) did not exceed 35˚C, nor dropped below 15˚C for the majority of the growing season (refer to **Figure 6.1**). As a result, the daily air temperatures did not hamper flower development as well as pod formation in both varieties of soybean and thus did not affect crop yield potential.

Daily totals of solar irradiance (R_S) ranged from 0.69 to 28.93 MJ m^{-2}, while ET_O ranged from 0.1 to 11.8 mm. During the growing season, Rs and ET_O were frequently reduced due to cloud cover. ET_O was higher during periods where there was little or no cloud cover and where Tx approached 30˚C or more. During these conditions, the crop was more likely to experience water stress. Soil water evaporation prior to canopy closure is also influenced by these high temperatures. During initial crop development, there is reduced canopy closure, which results in the soil surface being exposed to high air temperatures and therefore high soil water evaporation.

6.2 Crop Water Use

For this subsection the results pertaining to soil water retention parameters and soil water content will be given. Thereafter, it is followed by the results of Actual ET and lastly ending with crop coefficients determined for this study.

6.2.1 Soil water retention

The soil dry bulk density and soil water retention parameters determined from the outflow pressure method were compared to values derived from the SPAW (Soil-Water-Air-Water) model (Saxton and Rawls, 2006). The SPAW model only utilizes inputs of soil texture and organic matter content, but more importantly, is based on soils from the US. The results in **Table 6.1** show that the SPAW model tends to overestimate dry bulk density and certain soil water retention parameters and thus, values determined from the laboratory were used in this study. Overall, the overestimation of soil water retention parameters estimated from SPAW model when compared to the observed soil water retention parameters for this study were not large. However, it was noted that there was an error in PWP estimate observed from the pressure cells in the laboratory at 1.00 m soil depth as the difference in PWP from the 0.60 m to 1.00 m soil depth was 11.5%, which was significant. According to Lorentz (2019), the error in estimating PWP at 1.00 m soil depth is due to the increasing clay content determined at this depth, therefore, it is difficult to accurately measure soil water retention parameters at this soil depth. As a result, the PWP plotted for the 1.00 m soil depth graph in **Appendix 4** in **Subsection 9.4** is incorrect as the PWP value plotted is high, resulting in the VWC of soil moisture sensors trends falling below the PWP, which should not be the case especially at this soil depth. SPAW estimates of soil bulk density are high indicating the soil is compacted, which is not the case considering land preparation was done by hand and not heavy farm machinery. Soil bulk density is an important parameter required to convert from gravimetric water content to volumetric water content (VWC). When the SPAW estimates of bulk density were used, VWC was overestimated when compared to measurements from the CS650 sensors. Therefore, it is important to determine soil bulk density from the field, rather than using modelled values.

Table 6.1: Estimation of soil water retention parameters using two methods i.e. the SPAW model and the outflow pressure method

Method	**Soil water characteristics**	**Units**	**Depth (mm)**			
			150	**300**	**600**	**1000**
SPAW model	Saturation (SAT)	% vol	48.2	44.3	44.6	45.0
	Field capacity (FC)	% vol	34.4	32.6	33.1	36.9
	Permanent wilting point (PWP)	% vol	22.0	21.3	21.5	25.4
	Soil bulk density	g m^{-3}	1.4	1.5	1.4	1.4
	Saturated hydraulic conductivity (K_{SAT})	mm day^{-1}	152.4	94.6	89.9	31.7
Outflow pressure	Saturation (SAT)	% vol	58.5	58.4	57.9	52.8
	Field Capacity (FC)	% vol	36.7	38.9	40.5	38.1
	Permanent wilting point (PWP)	% vol	12.4	14.6	15.2	26.7
	Soil bulk density	g m^{-3}	1.1	1.1	1.2	1.2

The saturated hydraulic conductivity (K_{SAT}) could not be determined in the soil water laboratory at UKZN due to faulty equipment. Therefore, values were derived using the SPAW model, which produced acceptable estimates when compared to default values suggested in AquaCrop user manual (FAO, 2018). K_{SAT} ranged from 152.4 mm day^{-1} at 0.15 m, which decreased to 31.7 mm day^{-1} at 1.00 m, due to increasing clay content with depth.

6.2.2 Soil water content

6.2.2.1 CS650 probes

The soil water content measured by the CS650 soil moisture probe at four depths, together with daily rainfall measured during the growing season, is shown in **Figure 6.2.** The PWP and FC plotted in **Figure 6.2** is the minimum PWP of the topsoil (i.e. 12.4%) and the maximum FC of the subsoil (i.e. 40.5%). The initial soil moisture and soil temperature (i.e. measured by the CS650) for the topsoil in the trial was about 18.5% and 19.1°C, respectively. **Figure 6.2** highlights the largest variation in soil water content at 0.15 m, which is due to the frequent wetting and drying cycles resulting from interactions of soil temperature, rainfall and wind speed at the soil surface.

The initial soil moisture content at planting was low, which could have influenced seed germination. Several seeds from both varieties did not germinate fully, therefore gap filling was undertaken to achieve the desired plant density. During maximum canopy development (i.e. 96 and 103 DAS for LS6161R and CAPG3 for the inoculation treatments respectively), the topsoil water content did not fluctuate as much as it did prior to full canopy closure. The full canopy development meant that the topsoil was shaded from the harsh climate, which reduces the overall soil water evaporation as result there would be an increase in soil water content in the soil profile.

The soil moisture content at 0.6 m depth was mostly above 50% of plant available water (i.e. FC – PWP) throughout the growing season. The maximum rooting depth observed at flowering and physiological maturity was 0.62 m, which indicates that the varieties may not have been severely affected by water stress due to soybean's long tap root system (DAFF, 2010a).

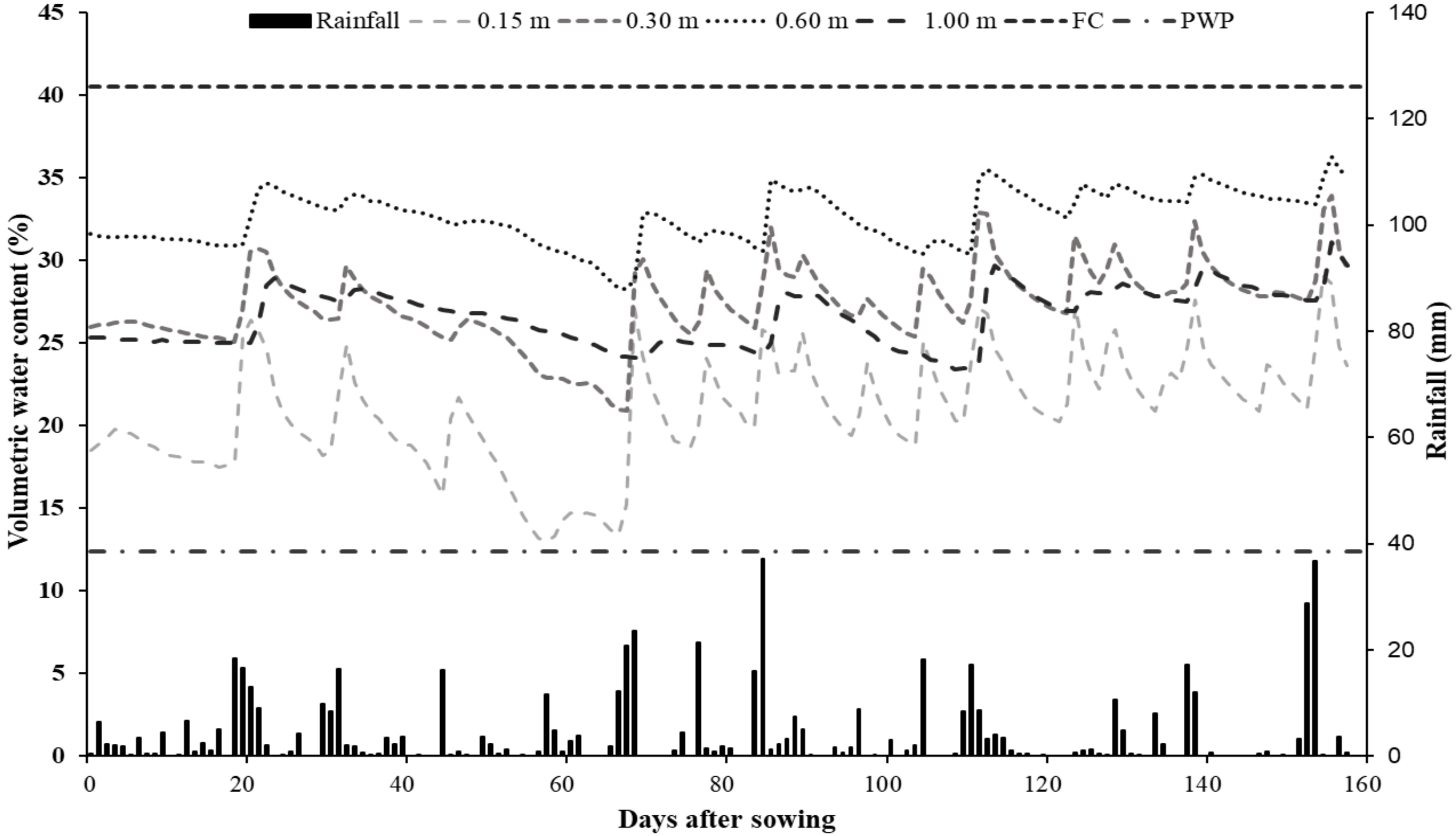


Figure 6.2: Soil water content measured by the CS650 at depths 0.15, 0.30, 0.60 and 1.00 m from 17th November 2018 to 25th April 2019 at Swayimane

6.2.2.2 Watermark sensors

Regression equations were obtained by plotting VWC (CS650 probes) against corresponding pressure head (Watermark sensors) for each depth. The regression graphs are given in **Appendix 3** in **Subsection 9.3** and show low R^2 values that indicate relatively poor correlation between the two sensor types, especially for the topsoil (i.e. 0.15 m depth).

The poor correlations were due to large periods of missing data which occurred at each of the four depths. Missing data was caused by damage of the wires connecting sensors to the power unit. The damage occurred when hand hoes were used to initially prepare the trial site for planting and during frequent weeding sessions over the growing season. The missing data was patched using CS650 data located near the Watermarks. Although the Watermarks are considerably cheaper than the CS650s, they require calibration and are not ideally suited to accurate estimation of VWC.

After the calibration of the Watermarks, the Watermarks were plotted against the VWC from the CS650s as well as the VWC obtained from the gravimetric samples taken bi-weekly at the field (see **Appendix 4** in **Subsection 9.4**). It is important to note that the soil bulk density used for the conversion was taken from plot 14, where the CS650s were installed. Ideally, soil bulk density should have been obtained for plots 6 and 13, where the gravimetric samples were taken. However, this was not done due to labour constraints. This explains the poor correlation between VWC of the gravimetric samples when compared to that obtained from the two sensors (i.e. Watermarks and CS650s), especially at the 0.15 and 1.00 m depths. The topsoil at the trial site has been disturbed in previous seasons due to ploughing and disking involving a tractor. From this study, it is recommended that soil bulk densities are measured using undisturbed cores and not estimated via the SPAW model. In addition, the soil cores should be obtained from the sample plots where the gravimetric sampling is done.

6.2.3 Actual ET

Crop water use (ET_C) was estimated using the soil water balance method as discussed in **Subsection 4.1.2**, where inputs of P and R in **Equation 3** were assumed to be constant for the two treatments. ΔS represents the difference between the soil water content measured at planting (i.e. initial soil moisture) and at physiological maturity (i.e. final soil moisture), which was different for both varieties as they were observed to mature at different periods. The ET_C values obtained for both varieties and inoculation treatments are presented **Table 6.2**. However, the calculated ET_C was not significantly different ($P<0.05$) across varieties and between the two inoculation treatments.

Table 6.2: Actual evapotranspiration (ET_C) determined for two soybean varieties (LS6161R and CAPG3) grown under rainfed conditions and two inoculation levels

Treatment	Varieties	Evapotranspiration (ET_C in mm)
Inoculation	LS6161R	481
	CAPG3	508
Non-inoculation	LS6161R	482
	CAPG3	519

The ET_C estimates were higher for the CAPG3 variety than for LS6161R for both inoculation treatments. ET_C estimates given in **Table 3.2** (see **Subsection 2.4.2**) for other soybean studies in South Africa under rainfed conditions meant that for this study, the water use of LS6161R falls within this range, however, it is was not the case for CAPG3. The higher water use calculated for CAPG3 was due to the higher leaf numbers and LAI, which meant more stomata and thus higher transpiration rates. The higher water use of CAPG3 is also due to its longer crop cycle of 143 days compared to 135 days for LS6161R. Therefore, it is difficult to compare crop ET_C between these varieties due to their differences in crop season lengths. **Figure 6.4** (see **Subsection 6.3.1**) shows the stomatal conductance measured for the two varieties and inoculation treatments, it can be seen that there were two periods of crop water stress, hence, the crop ET_C observed for the two varieties and inoculation treatments do not represent the maximum water use as the trial was not irrigated, which is therefore not ideal.

The planting density used for this study were higher as compared to studies done by Lembede (2017) and therefore there was a good canopy closure as opposed to Lembede (2017) studies. From Lembede (2017) studies, it was noted that there was higher soil water evaporation on the non-mulched treatment, which may have resulted in less water available for transpiration and hence, reduced yield. The good canopy closure for this study as opposed to Lembede (2017) meant that soil water evaporation was minimized resulting in more water available for crop uptake (i.e. increase transpiration) of the CAPG3 variety due to more shading of the soil (i.e. due to high leaf numbers) as compared to the LS6161R for both inoculation treatments. Therefore, the overall ET_C would be higher for the CAPG3 variety as compared to the LS6161R variety for both inoculation treatments.

As highlighted by Richard *et al*. (2011), the measurement of parameters in the soil water balance equation does have limitations which reduces the confidence in crop ET_C values produced. The soil water balance does not partition ET_C into soil water evaporation (E) and transpiration (T_C) and therefore, it is difficult to determine the productive water use (i.e. excluding E) of each variety. Another major source of error in determining crop ET_C using this method, is uncertainty in drainage from the soil depth sampled or any upward movement of water into the soil depth sampled. According to Richard *et al*. (2011), these errors in drainage are often difficult to detect and therefore it means applying parametric modelling to estimate these deep-water fluxes. However, due to budget constraints for this study installing soil water sensors for more than a meter to detect these deep fluxes where not feasible, therefore capillary rise and deep drainage were assumed to be negligible for this study, however, it may have affected the accuracy of crop ET_C determined for the two varieties and inoculation treatments. It is thus shown that these two parameters can likely impact the accurate determination of crop ET_C using the soil water balance method. However, the soil water balance method is still an effective and cheaper method (i.e. if projects budgets are limited) and is well suited to small scale studies. On the other hand, the use of lysimeters or micrometeorological techniques (e.g. surface renewal and eddy covariance) are expensive, require larger fetch areas and knowledge of complex software applications (e.g. EddyPro).

6.2.4 Crop coefficients

As discussed in **Subsection 5.5.2.3,** the crop coefficient (Kc) is defined as the ratio of actual evapotranspiration (ET_C) to the reference crop evapotranspiration (ET_O) as seen in **Equation 7**. The Kc were calculated using weekly estimates of crop ET_C derived using the soil water balance method (see discussed in **Subsection 4.1.2**) and ET_O derived from FAO's ET_O Calculator utility, for which monthly K_C for both varieties and inoculation treatments under rainfed conditions were calculated. Monthly K_C values for both varieties and inoculation treatments under rainfed conditions are shown in **Table 6.3**. For rainfed conditions, CAPG3 generally exhibits higher crop coefficients than LS6161R for both inoculation treatments. It is worth noting that April K_C values for both varieties and inoculation treatments are higher than expected. This is difficult to understand considering soybean typically drops its leaves once senescence begins, indicating a marked drop in transpiration rate. However, it is important to note that both varieties were shown to reached physiological maturity at the 2^{nd} and 10^{th} of April for LS6161R and CAPG3 respectively. Therefore, monthly K_C estimated for April for both varieties at maturity was done in order to be comparable with the AquaCrop, which simulates crop growth only up till physiological maturity. Therefore, these few days resulted in high monthly K_C's determined for both varieties and inoculation treatments. In addition, Mbangiwa *et al*. (2019) also reported high crop coefficients at the end of the season for soybean.

Table 6.3: Monthly observed crop coefficients (K_C) determined for two soybean varieties (LS6161R and CAPG3) grown under rainfed conditions and two inoculation levels

Treatment	**Variety**	**Monthly crop coefficients (K_C)**					
		Nov	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**
Inoculation	LS6161R	0.44	0.97	0.93	0.99	0.82	0.92
	CAPG3	0.33	0.85	0.95	1.03	0.87	1.05
Non-Inoculation	LS6161R	0.32	0.96	0.94	1.01	0.91	0.97
	CAPG3	0.43	0.87	0.97	1.02	0.95	1.08

6.3 Crop Phenology

For this subsection, the results pertaining to chlorophyll content index and stomatal conductance that were measured throughout the growing season are presented and discussed.

6.3.1 Chlorophyll content index

Chlorophyll content index (CCI) was measured as an indicator of both plant health as well as its ability to capture photosynthetically active radiation (see **Subsection 5.5.4.1**) (Devnarain *et al.*, 2016). The statistical analysis of CCI showed a significant difference (P<0.05) between varieties, but no significant difference (P>0.05) between inoculation treatments. However, there were significant differences (P<0.05) in the interaction between varieties and inoculation treatments. The low CV as seen in **Figure 6.3b**, does indicate the data collected is good and does not exceed >30%, which would indicate poor data collection and may affect the results presented. Both varieties and inoculation treatments had a similar trend in CCI as seen in **Figure 6.3**.

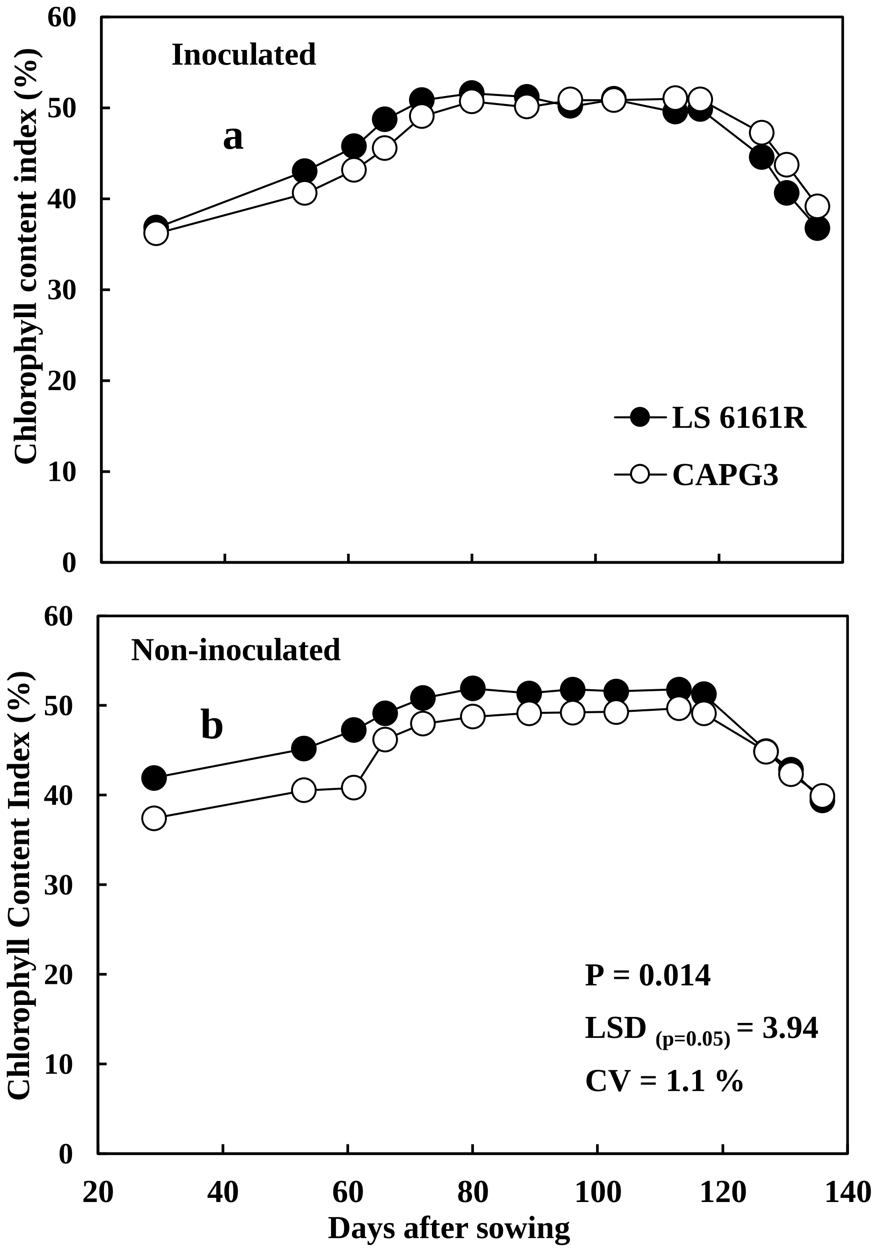


Figure 6.3: Chlorophyll content index for two soybean varieties (LS6161R and CAPG3) grown under (a) inoculation and (b) non-inoculation treatments and rainfed conditions

The LS6161R variety had a slightly higher CCI than CAPG3, in particular for the non-inoculated treatment (see **Figure 6.3b**). This may indicate that the latter variety was affected more by N deficiency. In addition, LS6161R may be better adapted to N deficient conditions, which is deemed an attractive adaptation mechanism for smallholder farming. N promotes leaf development and is a vital component of chlorophyll (IFA, 1992; Olivar *et al*., 2014). A healthy crop would indicate high transpiration or productivity (i.e. higher SC), which results in higher biomass and thus crop yields produced, which was observed for both varieties under the inoculation treatment but not the non-inoculation treatment due to a deficiency in soil N.

6.3.1 Stomatal conductance

Stomatal conductance (SC) was significantly different ($P<0.05$) between varieties as well as between inoculation treatments. However, the interaction between the two inoculation treatments and varieties was not significant different ($P>0.05$). The CV determined for SC as seen in **Figure 6.4**, does show that the SC data collected is good as the CV doesn't not exceed 30%. From **Figure 6.4a and b**, SC was lowest on approximately 47 and 103 DAS for both varieties and inoculation treatments, which coincided with high relatively humidity levels that were close to 80% (i.e. the air was saturated) (refer to **Figure 6.5**), hence, there was no gradient for transpiration, which will result in the low observed SC.

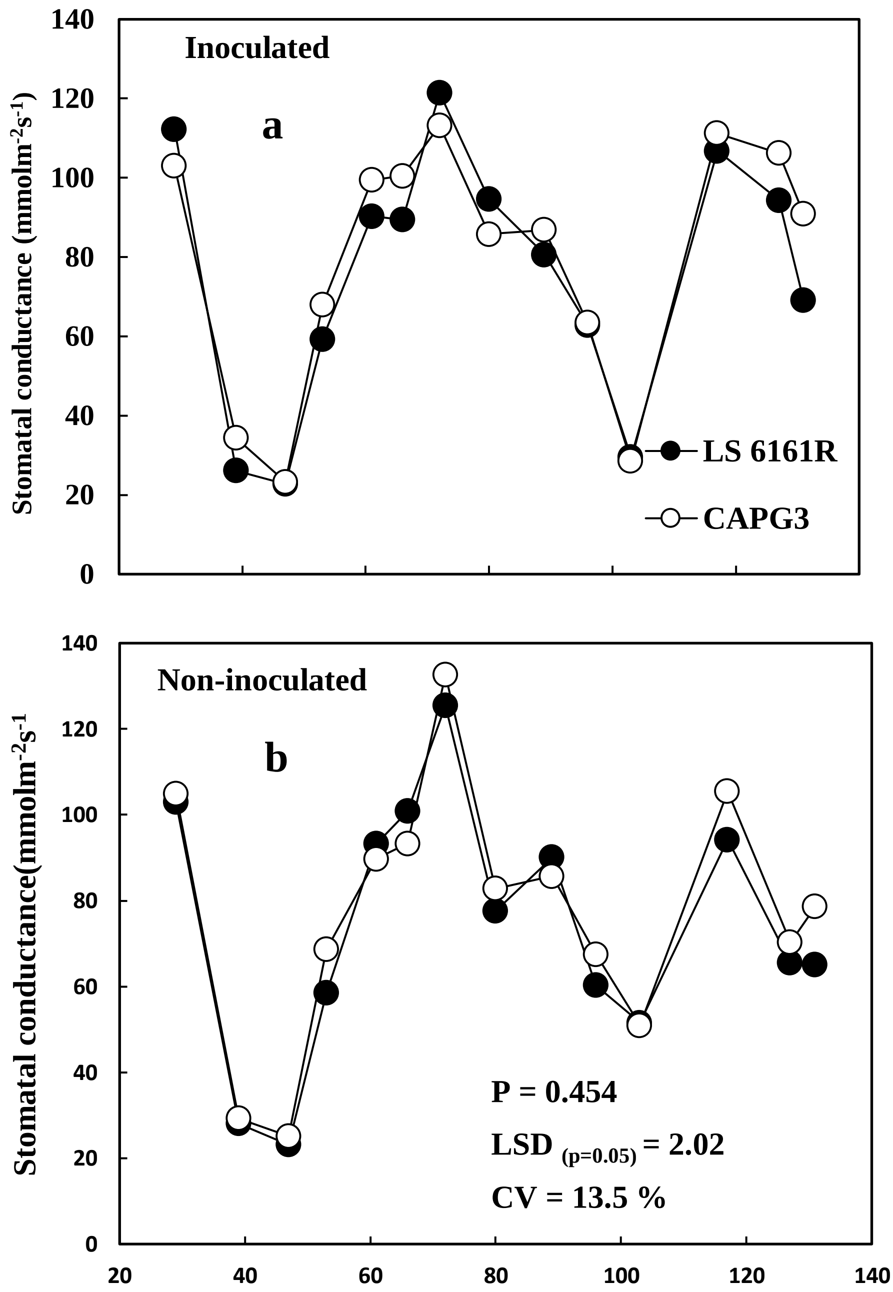


Figure 6.4: Stomatal conductance for two soybean varieties grown under (a) inoculation and (b) non-inoculation treatments and rainfed conditions

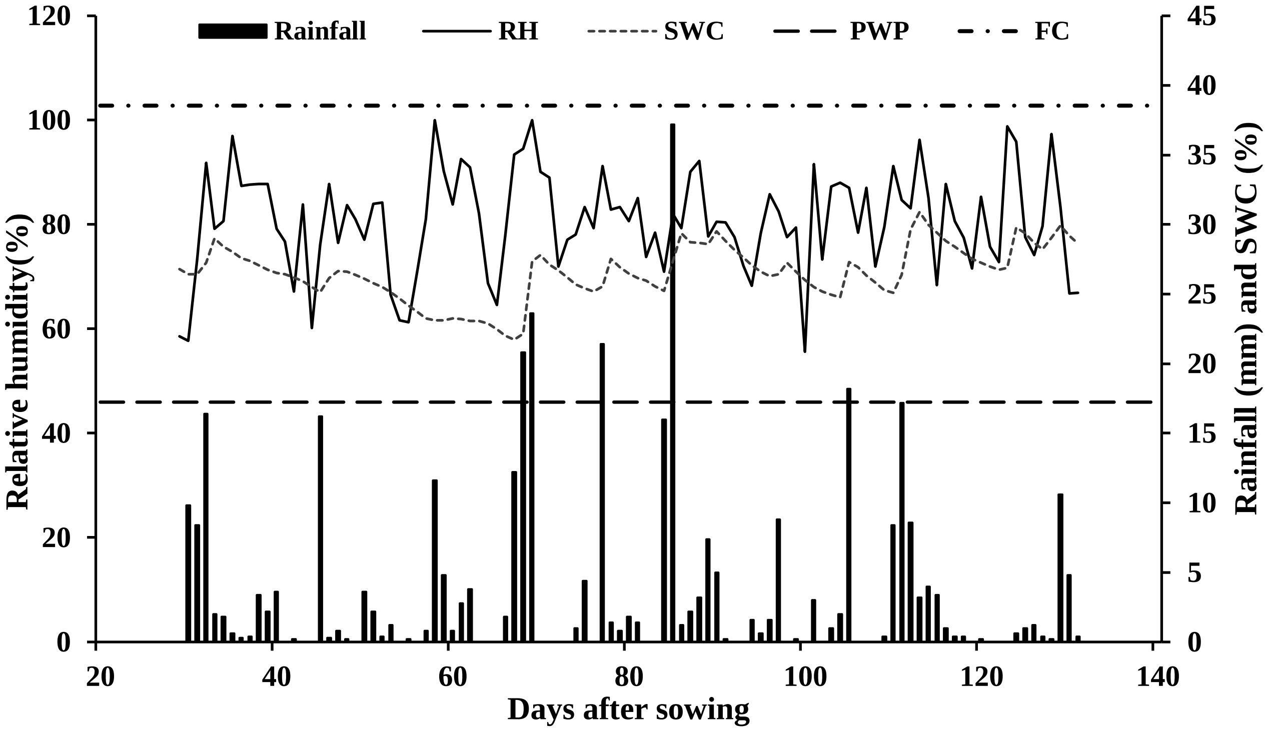


Figure 6.5: Relative humidity (RH%), rainfall (mm), soil water content (SWC%), field capacity (FC%) and permanent wilting point (PWP%) over the 2018/19 growing season at Swayimane

From day 47, SC increased steadily and reached a maximum on day 72 for both varieties and inoculation treatments. This period coincided with the period of flowering and pod formation (i.e. 71 to 88 DAS) for LS6161R, during which reduced transpiration can result in reduced yield (as discussed in **Subsection 5.5.4.2**). For CAPG3, flowering occurred from 79 to 95 DAS, during which time SC and thus transpiration decreased. This may explain the lower yield produced by this variety when compared to LS6161R. Approximately 117 DAS as seen in **Figure 6.4a** and **b**, the CAPG3 variety is shown to have a higher SC than the LS6161R variety for both inoculation treatments due to the LS6161R senescing faster, hence, losing more leaves at that stage (i.e. 117 DAS) as compared to the CAPG3, which senesces much later and keeps on developing. In **Figure 6.4a**, the reduction in SC towards the end of the growing season is due to senescence, i.e. 130 to 135 DAS for both LS6161R and CAPG3, respectively. The SC is influenced by increased LN and which thus affects the number of stomata found on the leaves thus influencing transpiration rates, which influences biomass as well as crop yields.

6.4 Crop Growth

For this subsection, the results pertaining to plant height, leaf number and leaf area index that were measured throughout the growing season are presented and discussed.

6.4.1 Plant height

The inoculated varieties were slightly taller than the non-inoculated varieties. Furthermore, CAPG3 was taller than the LS6161R variety for both inoculation treatments as seen in **Figure 6.6**. However, there were no statistical differences (P>0.05) in plant height (PHT) between varieties, inoculation treatments and the interaction between inoculation treatments and varieties (**Figure 6.6**). The CV determined for measured PHT for the two varieties and inoculation treatments as seen in **Figure 6.6** indicates that the PHT measured were good and consistent throughout the growing season.

For inoculated treatment, the maximum PHT was 0.71 and 0.73 m at 136 DAS for LS6161R and CAPG3, respectively. For the non-inoculated treatments, maximum PHT was 0.66 and 0.68 m at 136 DAS for LS6161R and CAPG3, respectively as seen in **Figure 6.6a** and **b**. This suggests that the inoculation treatment did not significantly influence PHT for both varieties. However, the differences of maximum PHT between varieties and inoculation treatment reported above does show that the influence of the genetic traits of both varieties (i.e. growth rate) on PHT are different. Since LS6161R is a semi-determinant variety (see **Subsection 5.2**), PHT will stop increasing once pod formation (i.e. approximately 71 to 88 DAS) is reached and also reaches maximum crop height faster than CAPG3. The CAPG3 variety is an indeterminant variety that continues to develop (i.e. increase PHT from 96 DAS onwards) even once flowering pod formation is reached and which reaches maximum crop height much later as seen in **Figure 6.6**.

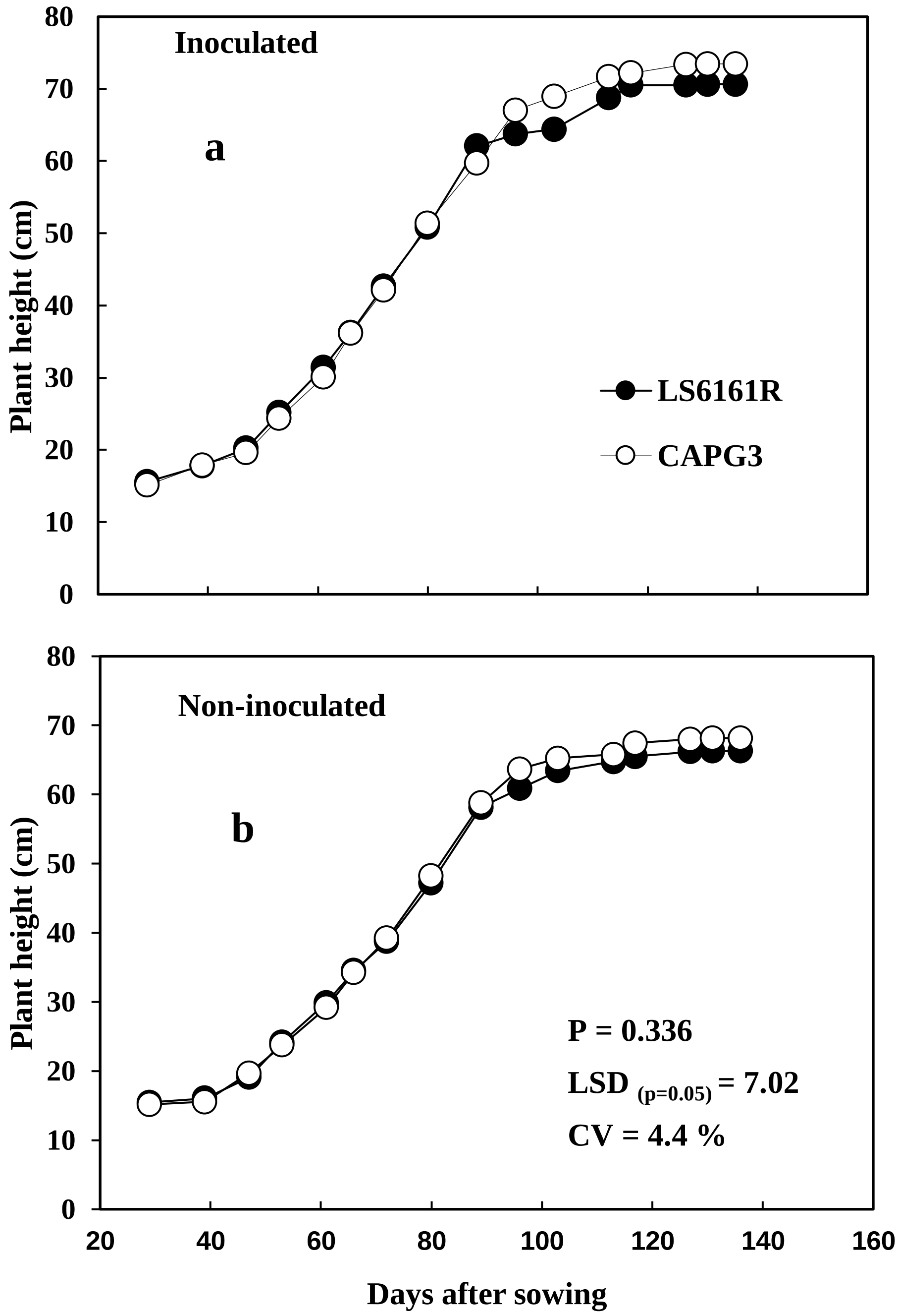


Figure 6.6: Plant height for two soybean varieties (LS6161R and CAPG3) grown under (a) inoculated and (b) non-inoculated treatments and rainfed conditions

6.4.2 Leaf number

From **Figure 6.7**, the CAPG3 variety produced more leaves than LS6161R under both inoculation treatments. However, leaf number (LN) was not statistically significant (P>0.05) between varieties, inoculation treatments and the interaction between inoculation treatments and varieties.

The CV determined for LN for both varieties and inoculation treatments as seen in **Figure 6.7** is low, which shows that the data collected was good and consistent. The maximum LN values measured were 139 and 157 at 89 DAS for LS6161R and CAPG3 respectively under inoculation treatment. The maximum LN values measured were 128 and 138 at 89 DAS for LS6161R and CAPG3 respectively under non-inoculation treatment.

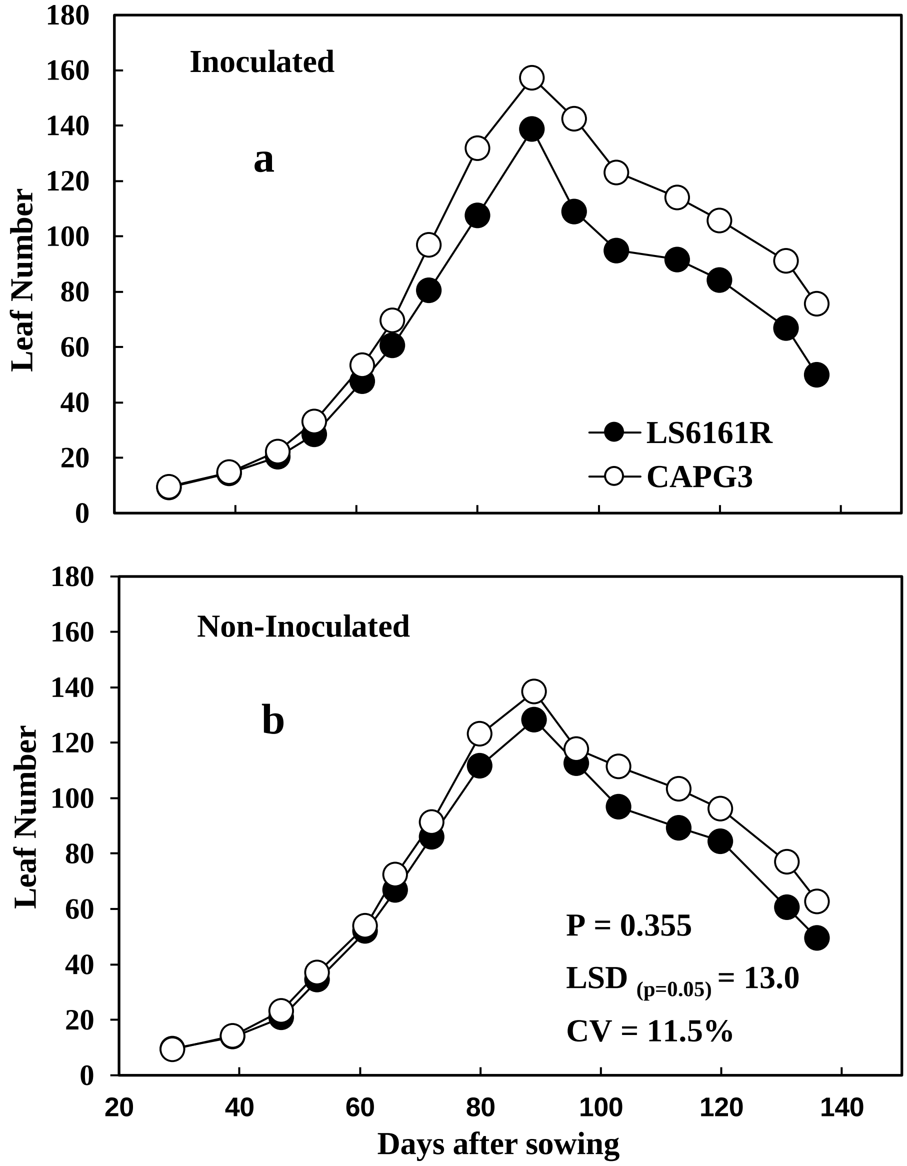


Figure 6.7: Leaf number for two soybean varieties (LS6161R and CAPG3) grown under (a) inoculation and (b) non-inoculation treatments and rainfed conditions

The decrease in LN measured for the LS6161R variety could be due to the difference in relative maturity of each variety, where the LS6161R is an early maturing variety, which will produce less leaves as it develops faster as compared to the CAPG3 which matures late. The genetic modification of the varieties may not be bred to produce high leaf numbers or biomass (i.e. CAPG3 variety), but rather high crop yields (i.e. LS6161R variety). This trend can be seen for both inoculation treatments (refer to **Figure 6.7**). However, it can be seen that there is a slight difference in LN between inoculation treatments. The inoculation treatment supplies N to both varieties and together with fertilizers (i.e. P and K), it will help improve leaf development as compared to the non-inoculation treatment. These slight differences between varieties and treatments of LN have an influence on the LAI and stomatal conductance, where higher leaf numbers results in high LAI and thus more stomata found on the leaves resulting in higher transpiration rates that will increase productivity of the plant (i.e. to produce higher biomass and thus seed yields).

6.4.3 Leaf area index

As shown in **Figure 6.8**, the leaf area index (LAI) for both LS6161R and CAPG3 was not significantly different ($P>0.05$) between varieties and inoculation treatments. The interaction between the two inoculation treatments and varieties was also not significantly different ($P>0.05$). The CV determined for the LAI data collected for both varieties and inoculation treatments as seen in **Figure 6.8**, does show there is good data collection of LAI as well as consistency in measurement of LAI in the field. The maximum LAI measured were 5.8 (at 103 DAS) and 4.6 m^2 m^{-2} (at 96 DAS) for CAPG3 and LS6161R respectively under the inoculation treatment. The maximum LAI measured were 5.1 and 5.0 m^2 m^{-2}, which both occurred at 89 DAS for CAPG3 and LS6161R respectively under the non-inoculation treatment. The LAI values for both varieties and inoculation treatments follow a similar trend with low values at planting, which peaked from flowering to pod formation and decreased after senescence.

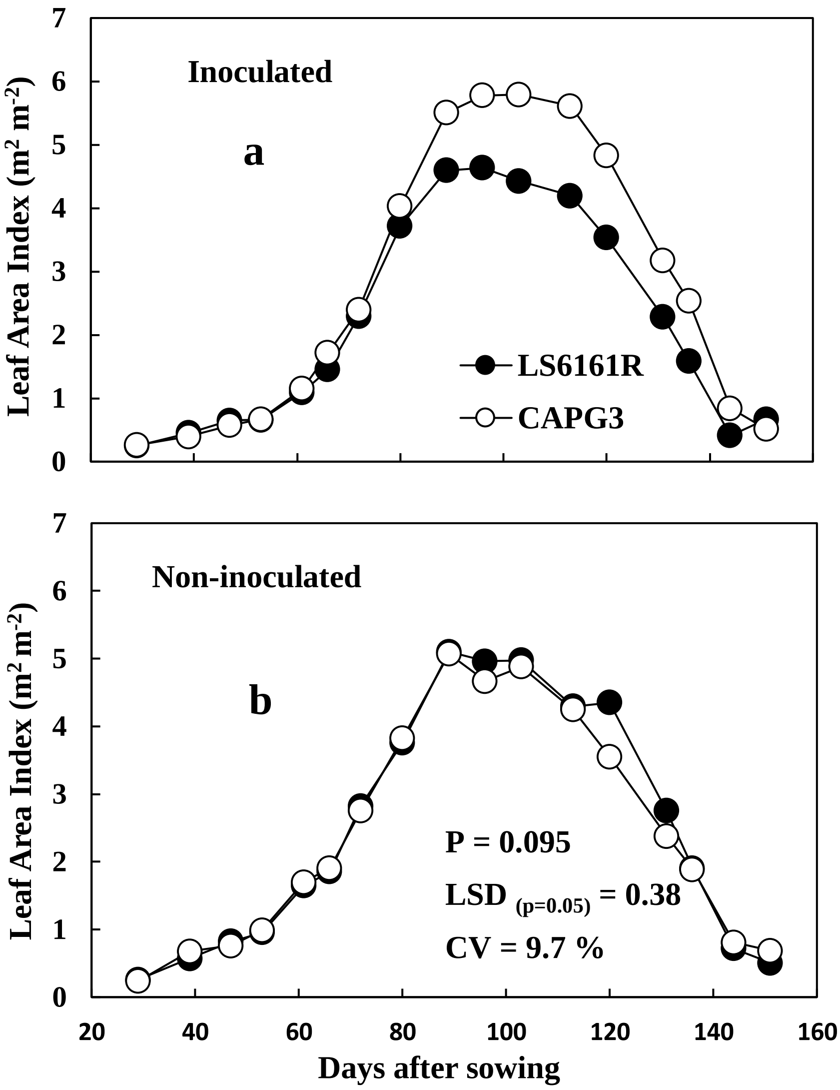


Figure 6.8: Leaf area index for two soybean varieties (LS6161R and CAPG3) grown under (a) inoculation and (b) non-inoculation treatments and rainfed conditions

The LN and PHT values measured for both varieties and inoculation treatments, showed that the CAPG3 produced more leaves at 89 DAS (refer to **Figure 6.7a**) as well as having a slightly higher PHT under the inoculation treatment (refer to **Figure 6.6a**) as compared to the LS6161R variety. As a result, the CAPG3 will have higher LAI due to the variety being taller and having more leaves as a result of the inoculation treatment (i.e. improved crop development) (refer to **Figure 6.8a**). The higher LAI will highly influence ETc in turn influencing biomass and thus seed yields. However, it is noted that the LS6161R variety produced lower LAI values as compared to the varieties under the non-inoculation treatment. Reasons why LS6161R did not respond to the combined inoculation and fertilizer application as expected are as follows:

- Ineffective application of inoculant at planting - Since the inoculation was sprayed via knapsack, it could have been rendered inactive by wind drift and/or failing to cover the seed with soil immediately after spraying.
- The variety could be genetically modified to produce fewer leaves and less biomass, but higher yield, as shown by the leaf number in **Figure 6.7** (see **Subsection 6.4.2**), biomass production in **Figure 6.11** (see **Subsection 6.7.1**) and crop yield in **Figure 6.11** (see **Subsection 6.7.2**). However, no attempt was made to verify this with the seed supplier.

6.4.4 Canopy cover

With respect to CC for both LS6161R and CAPG3, there were significant differences (P>0.05) across all factors (see **Figure 6.9**). Maximum values of CC approached 100% for both varieties under the two inoculation treatments. It is important to note that CC is derived from LAI (see **Equation 8** in **Subsection 5.5.5.4**) and thus, the two variables are directly proportional to one another. Hence, they follow similar trends with low values at planting, which peaked from flowering to pod formation, then decreased after senescence. It can also be seen that a low CV was obtained for the LAI and CC measurements (e.g. as seen in **Figure 6.9**). This emphasizes the importance of accurate LAI measurements as poor measurements of LAI may result in poor estimates of CC. It is important to note that CC can also be influenced by planting density (i.e. inter and intra-row spacing), which may be the cause for the CC to vary for both varieties and treatments. The planting density used for this study was 317460 plants ha^{-1}, while a planting density of 266667 plants ha^{-1} was used by studies done by Lembede (2017) (see **Appendix 5 in Subsection 9.5**), which does explain the higher LAI and thus CC for this study as compared to Lembede (2017) study.

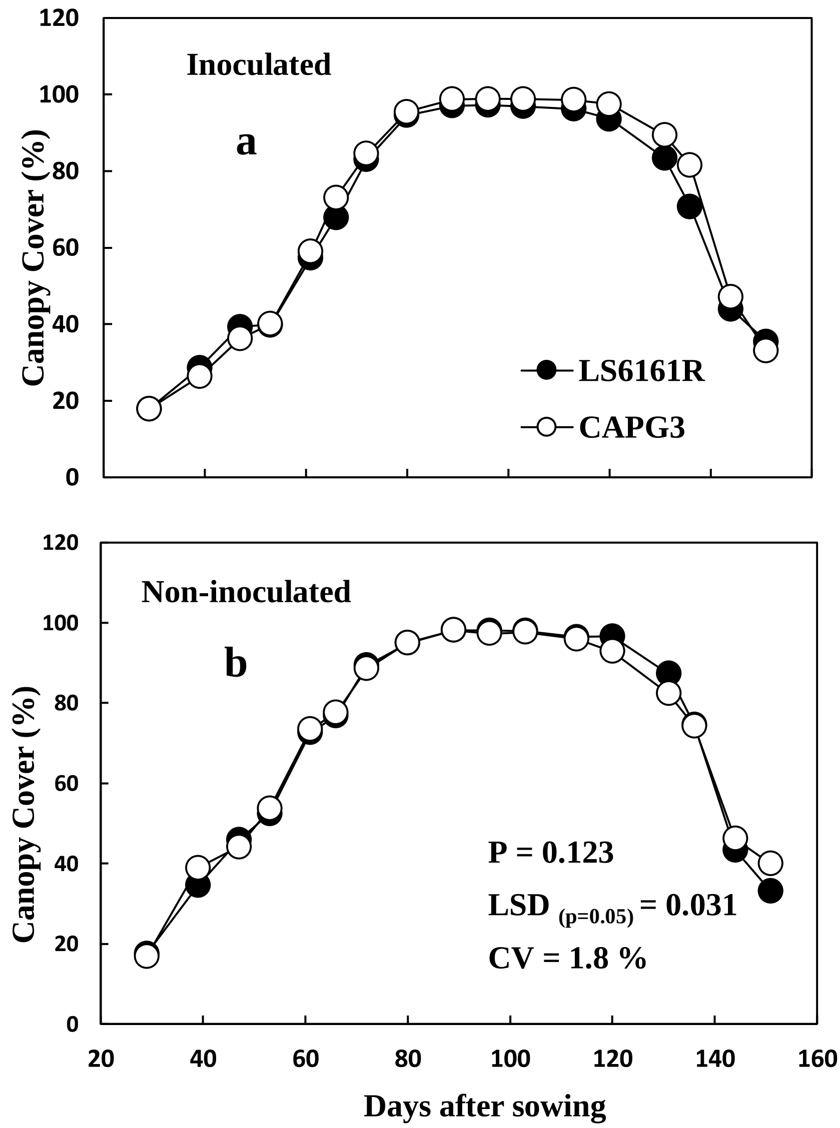


Figure 6.9: Canopy cover for two soybean varieties (LS6161R and CAPG3) grown under (a) inoculation and (b) non-inoculation treatments and rainfed conditions

6.5 Phenological development

Phenological dates were not observed on a per plot basis, due to labour constraints needed to observe and measure the large number of plants in the trial. Therefore, the statistical analysis to determine significance between varieties, inoculation treatments and the interaction between inoculation treatments and varieties was not performed. Thus, a phenological stage has observed when at least 50% of plants in each plot exhibited the required characteristic (Mabhaudhi *et al.*, 2014). The phenological dates were observed for both varieties in calendar days throughout the growing season, then converted to growing degree days using the method by McMaster and

Wilhelm (1997) in the AquaCrop model. The results that LS6161R developed faster (by approximately one week) from flowering to maturity when compared to the CAPG3 variety. The faster crop development of the LS6161R is due to its genetic makeup, considering it is early maturing whereas CAPG3 is a late maturing variety.

6.6 Biomass accumulation

Accumulated biomass shown in **Figure 6.10** represents the total above ground biomass of each variety measured bi-weekly under both inoculation treatments. The **Figure 6.10a** shows that the inoculated varieties produced higher accumulated biomass when compared to the non-inoculated varieties. In addition, CAPG3 produced more biomass than LS6161R, due mainly to high leaf number (and thus greater leaf area) as shown in **Figure 6.7** in **Subsection 6.4.2** and **Figure 6.8** in **Subsection 6.4.3** respectively. It thus shows with a greater LAI, there would be more stomata present in the leaf enabling greater transpiration rates (i.e. higher SC observed as seen **Figure 6.4**) therefore increasing productivity of the plant to produce more biomass (leaves plus stems/ and or grain) and thus crop yields.

At 117 DAS, biomass growth peaked at 65.0 and 77.4 g for inoculated LS6161R and CAPG3, respectively, which is consistent with the same period in which SC increased for the inoculated CAPG3 variety as seen in **Figure 6.4a.** In comparison, the non-inoculated treatment produced values of 52.1 and 69.6 g for LS6161R and CAPG3, respectively. Furthermore, **Figure 6.10a** and **b** highlighted a rapid increase in biomass growth for CAPG3 from 103 to 117 DAS, which is due to the variety forming pods and with its high LN at the same stage (see **Figure 6.7**), it would thus produce high biomass. For LS6161R under both treatments, a lower biomass resulted from lower LN. However, this shows that pod mass makes up most of the biomass at the same development stage, since the LS6161R variety matures early as compared to CAPG3 and therefore loses all of its leaves. However, overall there was no statistical difference ($P>0.05$) across varieties and inoculation treatments, as well as the interaction between inoculation treatments and varieties. The CV obtained for biomass measure for both varieties and inoculation treatments is high as seen in **Figure 6.10**, which may be due to inconsistency in selecting individual plants for destructive sampling throughout the season.

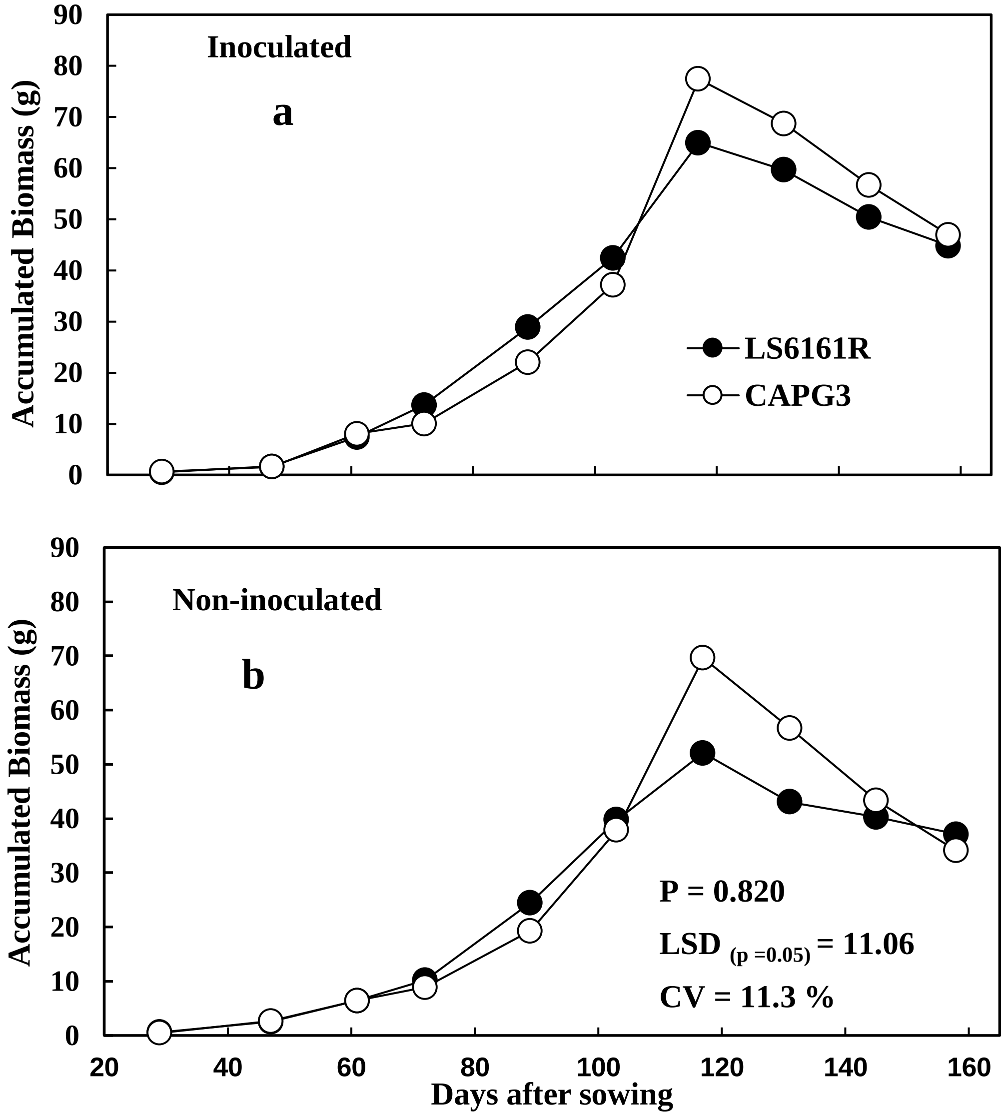


Figure 6.10: Accumulated biomass for two soybean varieties (LS6161R and CAPG3) grown under (a) inoculation and (b) non-inoculation treatments and rainfed conditions

6.7 Final Biomass and Seed Yield

In this subsection the results pertaining to final biomass, seed yield and harvest index determined for both varieties and two inoculation treatments are presented below.

6.7.1 Biomass at harvest

Only the pods and stem contribute to the final biomass and is therefore lower than the accumulated biomass, which includes the leaf mass. At harvest, the average number of pods per plant under the inoculation treatment was 58 and 51 for LS6161R and CAPG3 respectively. As expected, lower values were obtained from plants that were not inoculated. The higher number of pods, combined with the heavier pod mass as shown in **Table 6.4**, produced more biomass under inoculation treatment. The LS6161R produced heavier and more pods, which reflects the higher biomass produced for this variety, however, it thus also shows that the CAPG3 responded well to the inoculation as shown by the improvement in pod mass from 21.97 to 25.61 g per plant.

Table 6.4: Final pod mass, pod numbers and biomass determined for two soybean varieties (LS6161R and CAPG3) grown under rainfed conditions and two inoculation levels

Treatment	**Variety**	**Dry pod mass**	**Pod number**	**Final biomass**
		g plant^{-1}	**per plant**	**t ha^{-1}**
Inoculation	LS6161R	27.68	58	8.68
	CAPG3	25.61	51	8.48
Non-Inoculation	LS6161R	26.72	55	8.33
	CAPG3	21.97	48	7.40

The inoculated treatment produced final biomass values of 8.68 and 8.48 t ha^{-1} for LS616R and CAPG3, respectively. As expected, these values were higher than those obtained from the non-inoculated treatment, i.e. 8.33 (LS616R) and 7.40 (CAPG3) t ha^{-1} (**Table 6.4**). The increase in pod mass, pod numbers and final biomass as result of the inoculation treatment correlates well with what is reported by studies done by Singh (2005), Schulz *et al.* (2005), Mokoena (2013) and Siyeni (2016).

6.7.2 Seed yield

The statistical analysis of final biomass across all factors was not significantly different ($P>0.05$) as shown in **Figure 6.11**. However, seed yield was significantly different ($P<0.05$) between varieties, but not significantly different between inoculation treatments and the interaction between inoculation treatments and varieties. The CV determined for both final biomass and seed yield can be seen in **Figure 6.11** for both varieties and inoculation treatments. The CV does not indicate the accuracy of data, which in this case shows that the final biomass and thus seed yield data collected was poor due to the harvesting method used for this study as explained in

Subsection 4.2.2. As expected, the inoculated treatment produced higher soybean yields at harvest of 4.59 and 4.35 t ha^{-1} for LS6161R and CAPG3, respectively (**Figure 6.11**). As discussed in **Subsection 3.3.3,** inoculation together with application of P and K nutrients should increase crop yields, as was observed in the trial for both varieties.

The higher seed yields observed for this study as opposed to Lembede (2017) could be due to the high planting density used and good crop management practices (see **Subsection 9.5**). A smaller inter-row spacing resulted in higher canopy closure for both varieties (refer to **Figure 6.9**) causing a reduction in solar irradiance reaching the soil surface and thus, reduced soil water evaporation. With less soil water evaporation, more soil water was available to the crop, which probably reduced crop water stress in key development stages (e.g. during flowering and pod formation) and thus, improved the final yield. The advantages of minimizing weed growth and reducing competition for resources are well documented in the available literature.

The average soybean yields reported by DAFF (2010a) for South Africa range from about 2.5 to 3 t ha^{-1}. On smallholder farms, low yields are attributed to, *inter alia*, incorrect application and concentration of fertilizers and no seed inoculation. Although the yields obtained in this study are considered high, they show that with better crop management practices (e.g. inoculation, fertilization, weeding as well as application of herbicides, insecticides and fungicides), smallholder farmers should produce higher yields, thus reducing the yield gap between smallholder and commercial farms.

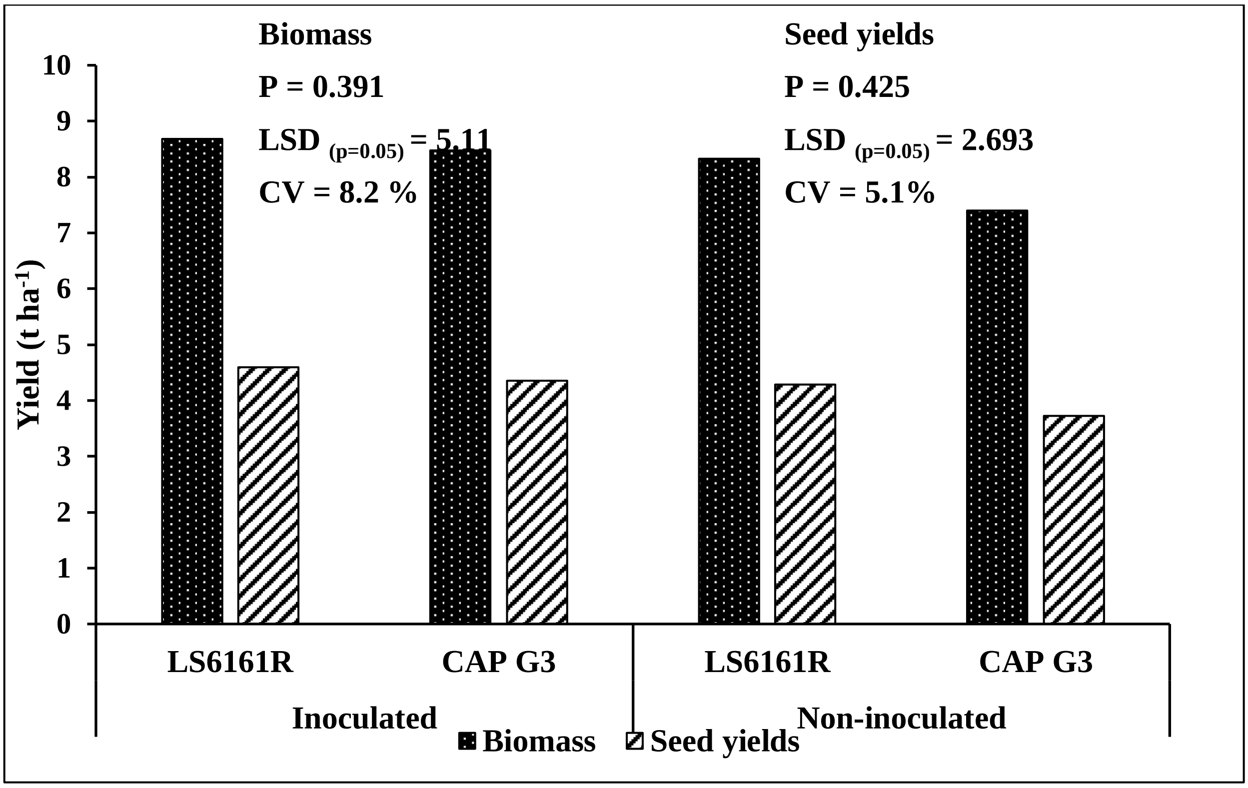


Figure 6.11: Biomass and seed yields for two soybean varieties (LS6161R and CAPG3) grown under rainfed conditions and two inoculation levels

6.7.3 Harvest index

Harvest index (HI) represents the ratio of crop yield to final biomass. As shown in **Figure 6.12**, values ranged from 48 to 51% and thus, were quite similar between treatments and varieties. Hence, differences in HI were not significantly different (P>0.05). The CV determined for the harvest index for both varieties and treatments is 5.4% and does not reflect the accuracy of the harvest index determined due to the poor seed yield and biomass data collected. As reported in the literature (e.g. Donatelli *et al.*, 1997; Cui and Yu, 2005; Steduto *et al.*, 2012; Islam *et al.*, 2018), HI ranges from 25 to 40% owing to the relatively low yields of soybean in comparison to its high biomass production. HI is generally used as an indicator of sampling error with respect to crop yield and biomass (Donatelli *et al.*, 1997). The HI values reported in **Figure 6.12** are considered large, due mainly to the high yields that resulted from the sampling method adopted in this study (as explained in **Subsection 4.2.2**).

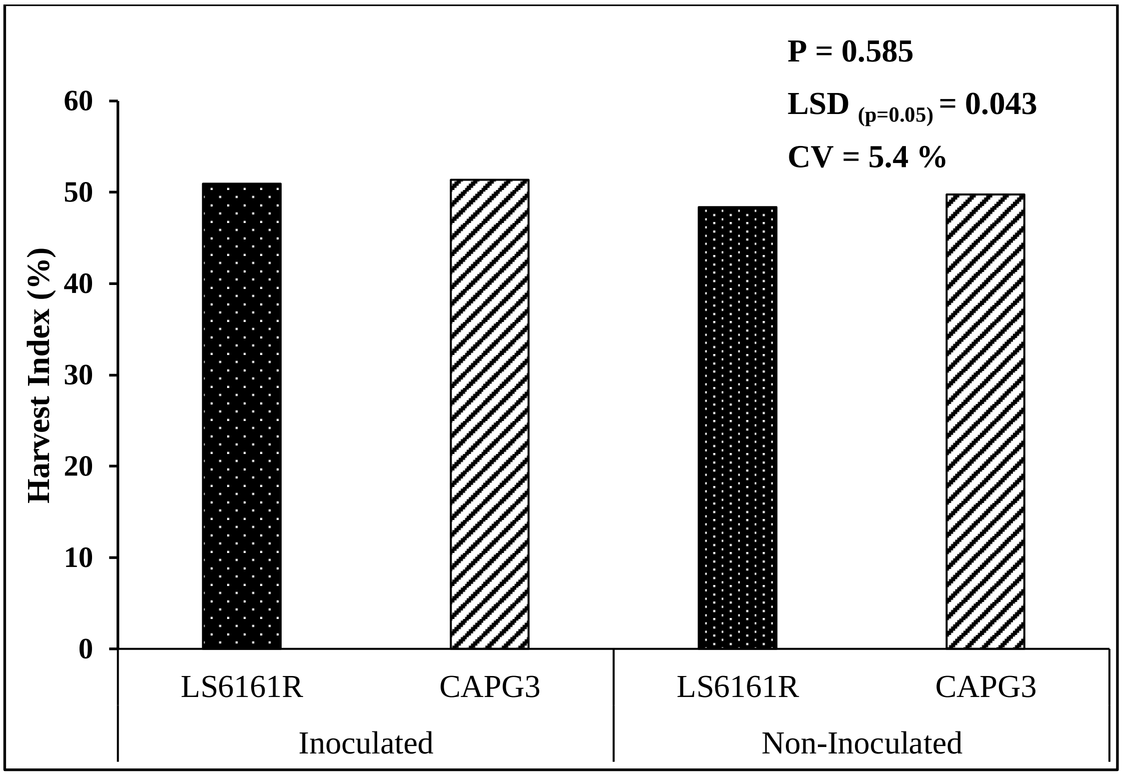


Figure 6.12: Harvest index of two soybean varieties (LS6161R and CAPG3) grown under rainfed conditions and two inoculation levels at Swayimane in the 2018/19 season

6.8 Water Use Efficiency

The crop water use and yield results determined in this study were then used to calculate the crop water use efficiency for both crop and biofuel production. The usefulness of this metric is also discussed in this subsection.

6.8.1 WUE of crop production

In this study, WUE_C is defined as the ratio of crop yield (kg ha^{-1}) to ET_C (m^3) as mentioned in **Subsection 3.1**. WUE values calculated for inoculated LS6161R and CAPG3 were 0.95 and 0.86 kg m^{-3}, respectively. For the non-inoculated treatment, figures of 0.89 and 0.72 kg m^{-3} were obtained for LS6161R and CAPG3, respectively. Hence, LS6161R is more water use efficient than CAPG3 for both inoculation treatments. The CAPG3 variety produced less yield and used more water when compared to LS6161R. CAPG3 produced more accumulated biomass (see **Figure 6.10** in **Subsection 6.6**) as it directed more of assimilates towards leaf and stem development. In comparison, LS6161R produced less biomass and therefore more assimilates were available for seed production. Hence, LS6161R is better suited for biodiesel production at both smallholder and commercial scale farming.

Estimates of WUE_C obtained in previous soybean studies are shown in **Table 3.2** (see **Subsection 3.2.2**). The WUE_C shown in this table for Baynesfield vary because of the different techniques used to measure soybean's water use, which ranges from 347-520 mm. The WUE_C's reported in this study for Swayimane are similar to those obtained by Masanganise (2019) at Baynesfield. By definition, this metric is sensitive to crop yield, which in turn is strongly influenced by various crop management practices. According to Kunz *et al*. (2015a), the comparison of WUE_C values is very difficult, due to the different techniques used to measure crop water use and yield, as well as differences in the scale of each study. Therefore, the usefulness of this metric is questionable, especially for comparative purposes.

6.8.2 WUE of biodiesel production

Water use of biodiesel production (WUE_B) represents the ratio of biodiesel yield (L ha^{-1}) to crop water use (m^3 ha^{-1}). The statistical analysis of biodiesel yield was not performed because single data points were calculated across varieties and between inoculation treatments. The seed oil content determined for both varieties under the two inoculation treatments is shown in **Table 6.5**. CAPG3 possibly exhibited a higher oil content than LS6161R for both treatments. The CAPG3 variety is known for its high seed oil content and quality (de Beer and Bronkhorst, 2018), assuming it receives all essential nutrients during the growing season. According to Nolte (2007), the oil content of soybean seed is typically 18%. Similar figures ranging from 16.7 to 21% were reported by Lembede (2017), which concur with values obtained in this study. Biodiesel yields given in **Table 6.5** show how higher seed oil contents can "offset" lower yields, considering CAPG3 produced a similar quantity to LS616R under inoculation. Therefore, CAPG3 may be better suited for animal feed production, since it produced more biomass and a higher oil content, whereas LS6161R produced more crop yield, making it better suited to biodiesel production.

Table 6.5: Biodiesel yield and WUE of biodiesel production (WUE_B) for two soybean varieties (LS6161R and CAPG3) grown under rainfed conditions and two inoculation levels

Treatment	Variety	Seed oil content	Dry seed yield	Biodiesel yield	Water use (ET_C)	WUE_B
		%	t ha^{-1}	L ha^{-1}	m^3 ha^{-1}	L m^{-3}
Inoculation	LS6161R	17.9	4.59	850	4810	0.18
	CAPG3	18.8	4.35	845	5083	0.17
Non-Inoculation	LS6161R	17.1	4.28	756	4821	0.16
	CAPG3	17.5	3.72	673	5193	0.13

6.9 Modelling Crop Water Use and Yield using AquaCrop

A minimal calibration of AquaCrop was performed where a few crop parameters were adjusted to represent local soybean varieties and growing conditions. The model's performance in simulating canopy cover development was assessed using various statistical indicators such as R^2, RMSE, NRMSE and Willmott's D-index.

6.9.1 Model calibration

6.9.1.1 Adjusted crop parameters

This trial was conducted under rainfed conditions and hence, soil water stress may have affected the attainable biomass production and crop yield. Since growing conditions were not considered optimum in terms of moisture supply, an iterative procedure was not followed to typically adjust model parameters to improve the simulation of canopy cover development and biomass production against observations. Instead, a partial calibration was performed were only a few parameters were adjusted (see **Table 6.7**). According to FAO (2017a; 2017b), the parameters found in **Table 6.7** are required adjustments in order to perform a partial calibration on the model. More specifically, LAI was used to calibrate the CC curve, which resulted in four adjusted parameters, *viz*. CCo, CCx, CGC and CDC (as explained in **Subsection 5.8.2)**.

Table 6.6: Adjustment of canopy cover parameters in AquaCrop to represent both soybean varieties grown at Swayimane in the 2018/19 season

Parameter	LS6161R	CAPG3
Seedling leaf area (cm^2)	5.00	5.00
Initial canopy cover (CCo in %)	1.59	1.59
Maximum canopy cover (CCx in %)	95.00	97.00
Canopy growth coefficient (CGC):		
% day^{-1}	7.4350	8.0960
% GDD^{-1}	0.4957	0.5397
Canopy decline coefficient (CDC):		
% day^{-1}	0.6690	2.9860
% GDD^{-1}	0.0485	0.1972

As noted in **Subsection 5.5.6**, phenological development was observed at different crop development stages. As shown in **Table 6.7** phenological growth periods were initially recorded in calendar days, then converted to growing degree days using Method 3 as proposed by McMaster and Wilhelm (1997).

Table 6.7: Input parameters for the AquaCrop model for soybean obtained from soybean trials at Swayimane, KwaZulu-Natal, South Africa

Parameters	Variety		Units
	LS6161R	CAPG3	
Time to 90% emergence	105	105	GDD
Time to maximum canopy cover	1440	1335	GDD
Time to flowering	1065	1185	GDD
Duration of flowering	255	240	GDD
Time to senescence	1950	2025	GDD
Time to maturity	2025	2145	GDD
Maximum rooting depth (Zr_{max})	0.62	0.62	m
Time to maximum rooting depth (Zr_{max})	1680	1680	GDD

Note: GDD = Growing degree days

As discussed in **Subsection 5.8.2.3**, AquaCrop does not consider inoculation as a field management option, nor can the model user specify what type and concentration of nutrients were applied to the crop. However, the soil fertility option can be changed from poor to non-limiting soil fertility stress. Therefore, the soil fertility stress option was set to 1) non-limiting to represent the inoculated treatment, and 2) moderate to near optimal soil fertility stress to represent the non-inoculated treatment (where only N is assumed deficient). **Table 6.8** shows the adjusted parameters to account for soil fertility stress in AquaCrop.

Table 6.8: Parameters adjusted in AquaCrop to account for soil fertility stress of LS6161R and CAPG3 grown under non-inoculated and rainfed conditons

Parameter	**LS6161R**	**CAPG3**
Considered soil fertility stress for calibration (%)	18	18
Shape factor for the response to soil fertility stress of:		
a) Canopy expansion (%)	1.08	0.79
b) Maximum canopy cover (%)	-0.11	-0.40
c) Crop water productivity (%)	1.18	2.35
d) Canopy cover decline (% day^{-1})	2.74	2.74

In addition, model simulations were performed under rainfed (i.e. non-standard) conditions to mimic field conditions in order to determine actual crop water use. Under the management option in AquaCrop, the irrigation option was also used to calculate the net amount of water applied on days when the crop is water stressed, i.e. when soil water content dropped below 50% of plant available water. This represented standard (i.e. non-stressed) conditions and allowed for the determination of maximum crop water use.

6.9.1.2 Canopy cover development

As shown in **Figure 6.13**, $R^2 \geq 90\%$ and D-index ≥ 0.85 suggest a good correlation between simulated and observed canopy cover development for both inoculated varieties. The model was less successful in predicting CC development for the non-inoculated varieties, with $0.84 \leq R^2 \leq 0.86$ and $0.77 \leq$ D-index ≤ 0.80. However, these two statistics indicate a good correlation because the model consistently underestimates CC. On the other hand, the RMSE and NRMSE statistics indicate a poor correlation, as they are more sensitive to under- and over-estimations.

From **Figure 6.13**, RMSE ranges from 21.8% to 31.5% for both treatments and cultivars. However, Paredes *et al.* (2015) noted that if RMSE is above 10.2%, the estimation error is high, which may indicate problems with the calibration process. The NRMSE also provides a good indication of the accuracy of CC simulation as explained in **Subsection 5.8.4**. The NRMSE for both varieties and treatments were greater than 30%, which indicates that the partially calibrated model estimates CC with poor accuracy under non-standard conditions. Overall, the model adequately explained the variation in observed CC development, but clearly underestimated CC, which resulted in high RMSE and NRMSE values.

The poor simulation of CC for this study is due to the partial calibration performed, which is similar to results obtained by Battisti *et al*. (2017) (refer to **Subsection 4.4.2.2**). Battisti *et al*. (2017) noted that a full calibration is needed in order to produce adequate simulations of CC, which was not done in this study due to non-irrigated conditions. Similar findings were also reported by Paredes *et al*. (2015) (see **Subsection 4.4.2.1**), where a full calibration was also performed to improve accurate simulations of CC. However, Mbangiwa *et al.* (2019) reported a good fit between observed and simulated CC (RMSE=10.50%; R^2=0.83), based on a partial calibration of soybean grown at Baynesfield. This may be attributed to supplemental irrigation of the crop that was carried out at time of establishment and during pod formation growth stage. It is important to note that since the trial was conducted under rainfed (i.e. water stressed) conditions, the model was expected to overestimate canopy development and not underestimate it.

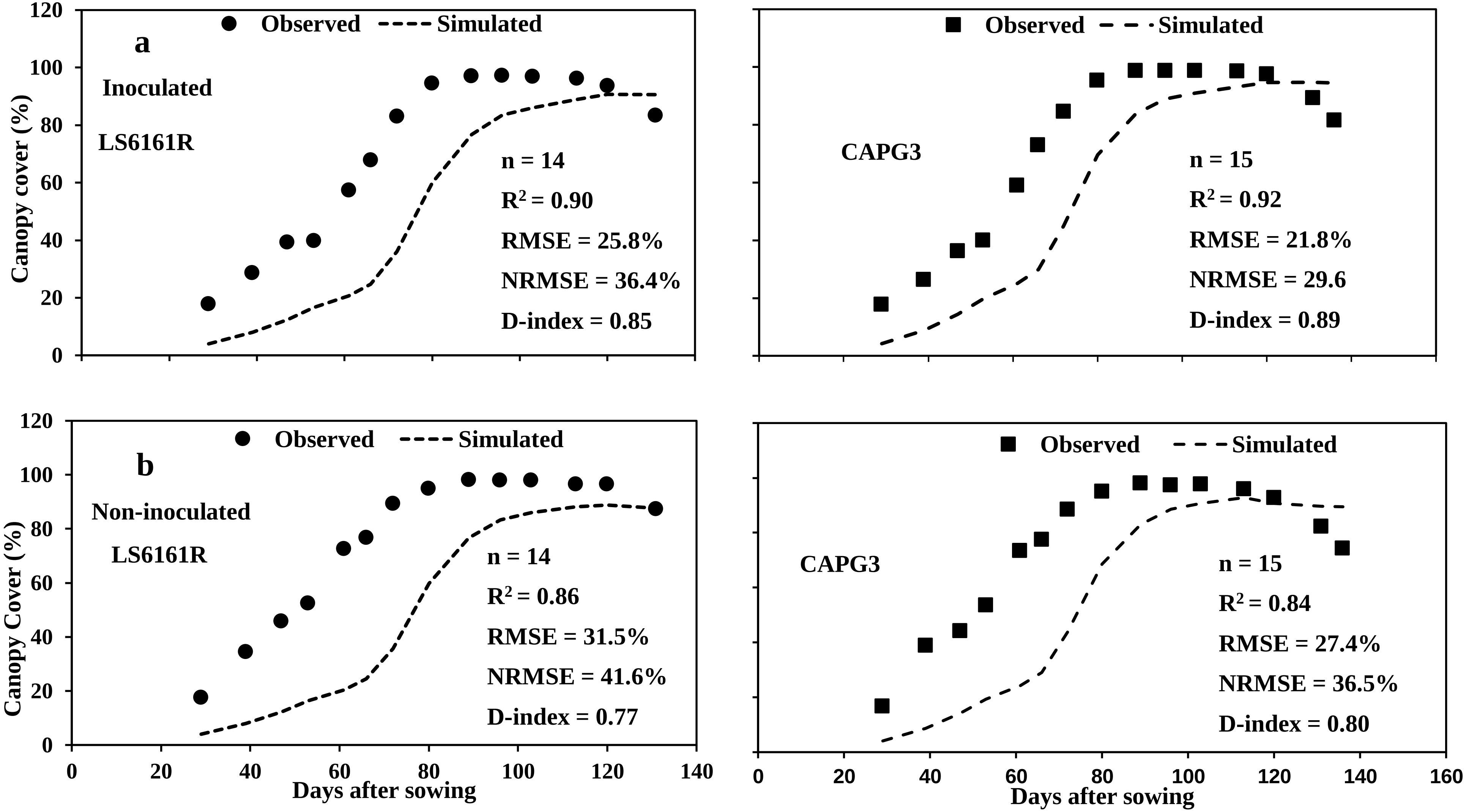


Figure 6.13: Comparison between simulated and observed canopy cover for the two rainfed soybean varieties grown under (a) inoculation and (b) non-inoculation treatments

6.9.2 Model validation

AquaCrop was then validated by comparing simulated and observed biomass production over the growing season. The model's ability to predict the final yield of each variety was also evaluated for each inoculation treatment.

6.9.2.1 Biomass production

With respect to biomass production as shown **Figure 6.14**, a good correlation between simulated and observed results was obtained for both inoculated cultivars, with $0.95 \leq R^2 \leq 0.98$ and $0.95 \leq$ D-index ≤ 0.98. The model was not successful in simulating biomass production for the non-inoculated cultivars (especially CAPG3), with $0.95 \leq R^2 \leq 0.97$ and $0.63 \leq$ D-index ≤ 0.92. For both inoculated cultivars, the RMSE ranges from 0.7 to 1.3 t ha^{-1}, while the RMSE for both non-inoculated varieties were higher ($1.3 \leq$ RMSE ≤ 1.6 t ha^{-1}). However, for both varieties and inoculation treatments, NRMSE indicates a poor simulation of biomass production, i.e. NRMSE > 30%. Overall, the simulation of biomass production indicates the model overestimates observations, with greater deviations towards the end of the season. For the non-inoculated treatment, the simulations are poorer and this could be due to the assumptions made with regard to soil fertility stress (see **Subsection 5.8.2.3**). These results correlate with those reported by Paredes *et al*. (2015) (see **Subsection 4.4.2.1**) and Battisti *et al*. (2017) (see **Subsection 4.4.2.2**). Therefore, a partial calibration done for this study resulted in poor simulations of both biomass production and CC development.

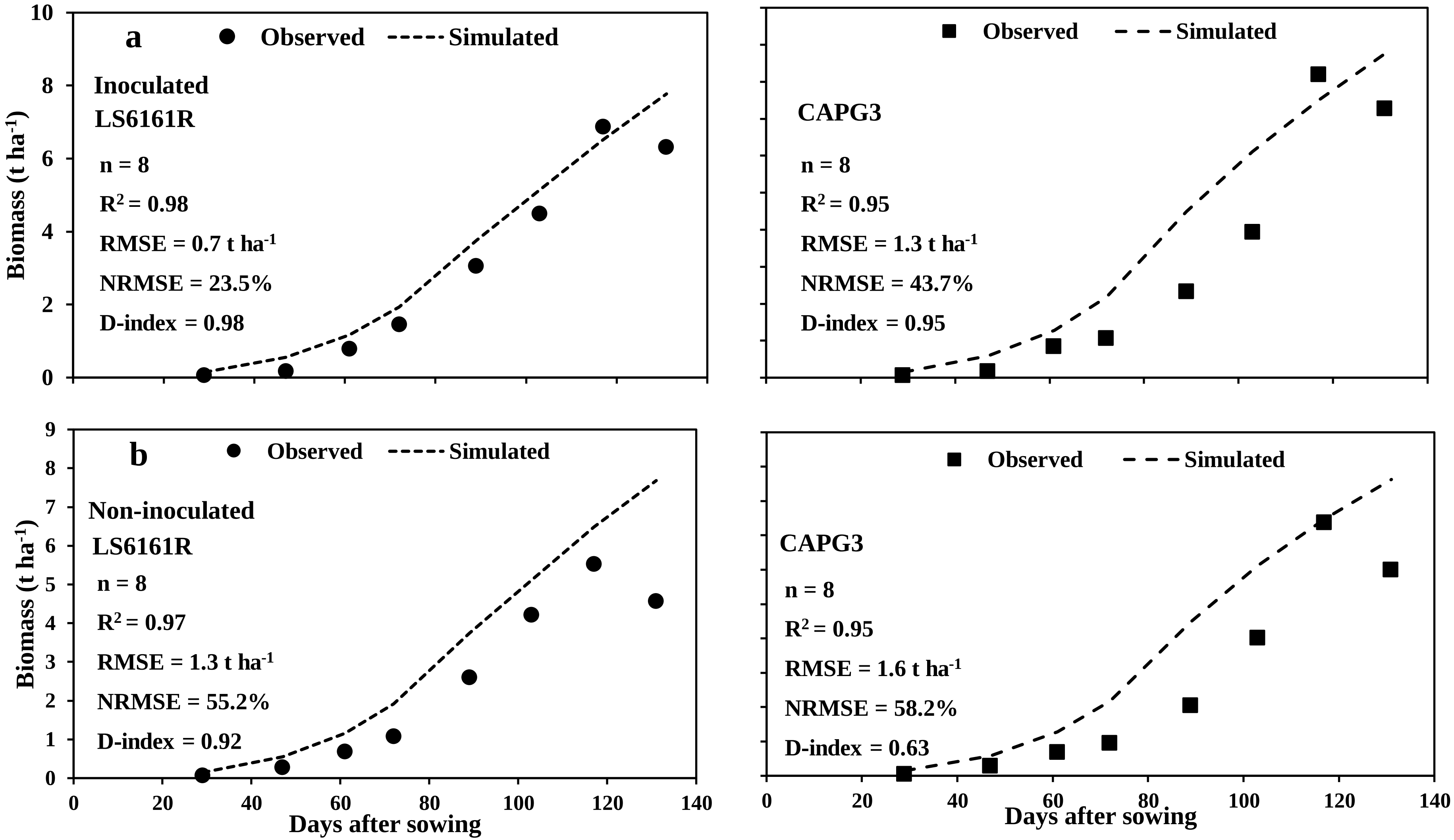


Figure 6.14: Comparison between simulated and observed biomass for the two soybean varieties grown under (a) inoculation and (b) non-inoculation treatments and rainfed conditions

6.9.2.2 Final biomass and seed yield

AquaCrop was run to simulate final biomass and seed yields as well as the harvest index for both varieties and inoculation treatments. Model output was then compared to observations as shown in **Table 6.9**. The results show that there is underestimation of seed yield by the model when compared to observations, except for the non-inoculated CAPG3 where the model adequately simulated the final seed yield (3.7 compared to 3.7 t ha^{-1}).

From **Table 6.9**, the results show that when compared to observations, the model overestimated biomass production of CAPG3 for both the inoculated (8.5 compared to 9.8 t ha^{-1}) and non-inoculated (7.4 compared to 9.6 t ha^{-1}) treatments. However, the model underestimated biomass production of LS6161R for the inoculated (8.7 compared to 8.1 t ha^{-1}) and non-inoculated (8.3 compared to 8.0 t ha^{-1}) treatments. The over- and under-estimation may be due to the assumptions made to account for these treatments, as well as due to a partial calibration of the model.

The harvest index results from **Table 6.9** show that the model underestimated values for both varieties and inoculation treatments. As explained in **Subsection 4.2.2**, the harvesting method used in this study resulted in high yields and thus, large HI values that are above the range of 25-45% reported in the literature. Therefore, the model simulates HI well, as the default reference HI parameter value of 0.40 was used in this study.

Table 6.9: Simulated versus observed data for biomass and seed yields, as well as the harvest index for two soybean varieties grown under rainfed conditions and two inoculation levels

	Treatment	Variety	Seed yield	Final biomass	Harvest index
			t ha^{-1}	**t ha^{-1}**	**%**
Observed	Inoculation	LS6161R	4.6	8.7	51.4
		CAPG3	4.4	8.5	50.9
	Non-inoculation	LS6161R	4.3	8.3	49.8
		CAPG3	3.7	7.4	48.4
Simulated	Non-limiting soil fertility stress	LS6161R	3.2	8.1	39.5
		CAPG3	3.8	9.8	38.8
	Moderate soil fertility stress	LS6161R	3.1	8.0	38.8
		CAPG3	3.7	9.6	38.5

6.9.2.3 Crop water use

AquaCrop simulations of crop water use (ET_C) for both varieties and inoculation treatments are shown in **Table 6.10** below. When soil fertility stress is non-limiting (i.e. representing the inoculated treatment), simulated ET_C correlated well with observed data for both varieties. Although there is some underestimation of observed ET_C, these differences are not considered large. As explained by Paredes *et al.* (2015) in **Subsection 4.4.2.1**, the over- and under-estimation of crop water use is due to the abandonment of the dual crop approach in AquaCrop. Similar findings were also reported by Battisti *et al.* (2017) in **Subsection 4.4.2.2**. According to Mbangiwa *et al.* (2019), AquaCrop simulated a water use of 420 mm for soybean based on a WUE_C of 1.14 kg m^{-3} and final simulated yield of 4.79 t ha^{-1} grown at Baynesfield during the 2012/13 season. This value compared well with the water use reported by Kunz *et al.* (2015a) of 469 mm that was obtained using the surface renewal technique (see **Table 3.2**). The latter method accounts for the evaporation of intercepted water, whereas the model does not simulate this process, which could explain the difference of 49 mm.

Table 6.10: Comparison between simulated and observed crop water use for the two soybean varieties grown under rainfed conditions and two inoculation levels

	Treatment	Variety	Crop water use (ET_C)	
			mm	**m^3 ha^{-1}**
Observed	Inoculation	LS6161R	481	4810
		CAPG3	508	5083
	Non-inoculation	LS6161R	482	4821
		CAPG3	519	5193
Simulated	Non-limiting soil fertility stress	LS6161R	464	4640
		CAPG3	481	4881
	Moderate soil fertility stress	LS6161R	463	4630
		CAPG3	478	4780

AquaCrop estimates surface runoff using the Soil Conservation Service (SCS) method (Raes *et al*., 2012). The initial abstraction (I_a) was changed from 0.20S (version 4 or below; Raes *et al.*, 2012) to 0.05S (version 5 or above; FAO, 2018), based on research by Woodward *et al.* (2003). Soils with a high curve number will have a small potential storage (S) and may generate a large amount of runoff. Curve number (CN) values were calibrated from a combination of soils, land cover classes, land management treatments and hydrological conditions. The soils were divided into four hydrological soil groups according to permeability and infiltration rates (Hawkins, 1978). For each of the aforementioned combinations, an "average" CN was selected for each scenario and thus, CNs were derived for catchments in the USA.

The SCS method was adapted for South African hydrological conditions by Schmidt and Schulze (1987). The soil hydrological groups were increased to seven to account for the wide range of soil types as depicted by the South African Binomial Soil Classification method (MacVicar *et al*., 1977). Further work was also done to adjust the CN to account for antecedent soil moisture (Schmidt and Schulze, 1987; Schulze, 2012). Therefore, the surface runoff generated from the AquaCrop model is based on the CN approach, which considers the hydrologic soil groups and properties. Hence, model users should use CN derived by Schmidt and Schulze (1987) for use in AquaCrop.

6.9.2.4 Profile water content

The profile water content (PWC) simulated by AquaCrop for a total depth of 1 m was compared to that obtained from the CS650 and Watermarks sensors in plot 14. As discussed in **Subsection 6.2.2.2**, the CS650s provided a more accurate and reliable estimate of soil water content than the Watermark sensors. The Watermarks had patches of missing data, which was infilled using data from the CS650s, which explains certain close correlations shown in **Figure 6.15**. This figure indicates that the model underestimates PWC in the early stages of crop development (i.e. approximately 0 to 50 DAS), as well as in the late stages of development (i.e. approximately 110 to 150 DAS). On the other hand, the model overestimates PWC during the mid-season, i.e. approximately 60 to 100 DAS. The same patterns were reported by Paredes *et al*. (2015) and Lembede (2017) for soybean, as well as in other studies for barley (Pereira *et al.*, 2015) and maize (Paredes *et al*., 2014). According to Paredes *et al*. (2015), the reasoning for the biased estimation of PWC is likely due to AquaCrop abandoning FAO's dual crop coefficient approach that resulted in transpiration and soil water evaporation being too dependent on the CC curve.

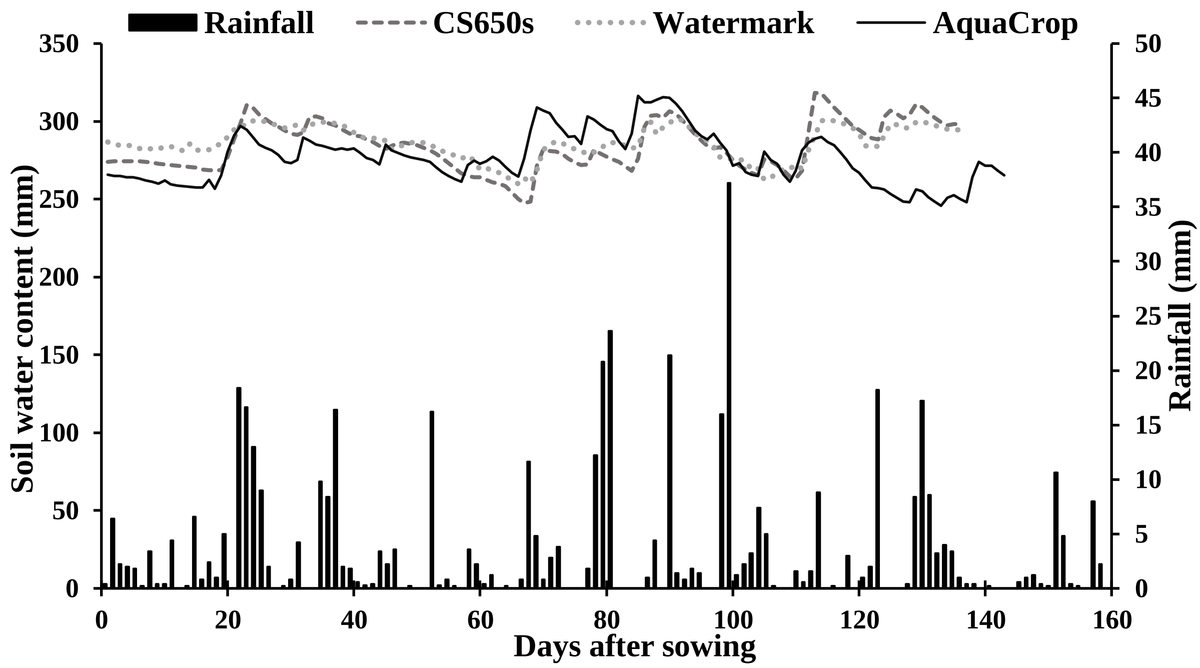


Figure 6.15: Comparison between the simulated soil water content from the AquaCrop model and observed soil water content from two sensors (i.e. CS650s and Watermark) throughout the growing season

As noted, AquaCrop overestimated PWC during the flowering to pod formation stage (i.e. approximately 75 to 90 DAS), when CC reached its maximum of approximately 97%. Owing to the relatively large rainfall events observed during this period, there would have been high interception loss resulting in decreased infiltration and thus, lower soil water content. However, AquaCrop does not account for interception loss, which means more rainfall will infiltrate the soil, contributing to higher profile water content.

6.9.3 Model application

Model simulations of crop water use and yield were performed under rainfed conditions in order to mimic field conditions. Model output was used to determine actual crop water use, from which water use efficiency of both crop and biodiesel production was calculated as well as monthly crop coefficients. The irrigation option in AquaCrop was also used to artificially relieve any water stress that occurred during the growing season, i.e. when soil water content dropped below 50% of plant available water. This represented standard (i.e. non-stressed) conditions and allowed for the determination of maximum crop water use, from which monthly crop coefficients were calculated. These simulated values were then compared to those obtained from observed data (as shown in previous Subsections).

6.9.3.1 Biodiesel yield

Biodiesel yield was calculated using **Equation 5** (see **Subsection 4.3**) with inputs of crop yield simulated by AquaCrop and seed oil content determined in the laboratory. These values were then compared to those obtained using measured yields. When compared to the CAPG3 variety, the theoretical biodiesel yields simulated for LS6161R correlated well with that obtained using measured yields for both inoculation treatments. These biodiesel yields are much higher than those reported by Lembede (2017), which ranged from 123 to 289 L ha^{-1}. This is due to the lower crop yields obtained in the 2015/16 season of 1.6 to 1.76 t ha^{-1} when compared to the higher yields obtained in this study. According to Mbangiwa *et al.* (2019), the observed and simulated soybean yields for Baynesfield were 5.28 and 4.79 t ha^{-1}, respectively. These correspond to theoretical biodiesel yields of 981 and 890 L ha^{-1} respectively, assuming an oil content of 18%. The biodiesel yields reported for this study (**Table 6.11**) are lower than the biodiesel yields produced at Baynesfield, which is expected due to the high seed yields at Baynesfield. This illustrates the sensitivity of seed yield on calculations of biodiesel yield. The biodiesel results for this study also show that even though CAPG3 produced higher seed oil content (%), it still produces less biodiesel yield. This further supports its potential use for animal feed production, while LS6161R has more potential for use in biodiesel production.

Table 6.11: Simulated versus observed data of biodiesel yield for the two soybean varieties grown under rainfed conditions and two inoculation levels

	Treatment	**Variety**	**Seed oil content**	**Biodiesel yield**
			%	**L ha^{-1}**
Observed	Inoculation	LS6161R	17.9	850
		CAPG3	18.8	845
	Non-inoculation	LS6161R	17.1	756
		CAPG3	17.5	673
Simulated	Non-limiting soil fertility stress	LS6161R		593
		CAPG3		738
	Moderate soil fertility stress	LS6161R		547
		CAPG3		669

6.9.3.2 Water use efficiency

The simulated WUE results given in **Table 6.12** correlate well with observed values for both varieties and both inoculation treatments. AquaCrop tends to underestimate WUE_C and WUE_B, since the model simulated lower yields and lower crop ET_C under rainfed conditions. Simulated results of crop WUE_C indicate that CAPG3 is more water use efficient than LS6161R. This contradicts observations that showed LS6161R utilizes less water to produce higher crop yields under the inoculation treatment, which makes it more WUE_C than CAPG3 variety.

Table 6.12: Comparison between simulated and observed water use efficiency of crop (WUE_C) and biodiesel production (WUE_B) for the two soybean varieties grown under rainfed conditions and two inoculation levels

	Treatment	Variety	WUE_C kg m^{-3}	WUE_B L m^{-3}
Observed	Inoculation	LS6161R	0.95	0.18
		CAPG3	0.86	0.17
	Non-inoculation	LS6161R	0.89	0.16
		CAPG3	0.72	0.13
Simulated	Non-limiting soil fertility stress	LS6161R	0.68	0.13
		CAPG3	0.78	0.15
	Moderate soil fertility stress	LS6161R	0.67	0.12
		CAPG3	0.78	0.14

6.9.3.3 Crop coefficients

K_C values derived from AquaCrop simulated ET_C for both rainfed (i.e. water stressed) and irrigated (optimal) conditions are also shown in **Table 6.13**. In general, K_C obtained from maximum ET_C under irrigated conditions are higher than those derived for rainfed conditions. This is expected and highlights the fact that rainfall over the growing season was not sufficient to meet crop water demand. The K_C simulated in November under irrigation is shown to be much higher than the value observed under rainfed conditions as a result of higher soil water evaporation. The highest simulated K_C for both varieties were in February under no soil fertility stress (i.e. optimum ET_C), which is expected as compared to K_C under soil fertility stress.

Table 6.13: Simulated crop coefficients (K_C) determined for two soybean varieties (LS6161R and CAPG3) for dryland and irrigated conditions, as well as two fertility stress levels

Type of conditions	Treatment	Variety	Monthly crop coefficients (K_C) Nov	Dec	Jan	Feb	Mar	Apr
Dryland	Non-limiting fertility stress	LS6161R	0.42	0.79	0.72	1.07	0.97	0.87
		CAPG3	0.42	0.79	0.73	1.07	0.95	0.88
	Soil fertility stress	LS6161R	0.42	0.79	0.72	1.07	0.96	0.85
		CAPG3	0.42	0.79	0.73	1.07	0.94	0.86
Irrigated	Non-limiting fertility stress	LS6161R	0.74	0.96	1.04	1.09	1.04	0.97
		CAPG3	0.74	0.96	1.05	1.08	1.02	0.95
	Soil fertility stress	LS6161R	0.74	0.95	1.02	1.07	1.04	1.05
		CAPG3	0.74	0.95	1.03	1.06	1.03	1.00

For rainfed conditions, similar crop coefficients were simulated by AquaCrop for both fertility options. However, when irrigation was applied to relieve water stress, K_C in March increased from 0.97 to 1.04 for LS6161R. This shows that the two varieties grown under rainfed conditions were water stressed and therefore, produced lower monthly K_C values. It is strongly recommended that for the simulation of crop coefficients by AquaCrop, the irrigation option is invoked as well as the non-limiting fertility option, in order to derived values for optimum growing conditions.

6.10 Summary

6.10.1 Benefits of inoculation

Inoculation was shown to significantly improve CC, SC and CCI for both CAPG3 and LS6161R varieties. With addition of soil N together with P and K fertilizer application, crop development should significantly improve (i.e. increase in leaf number and improved chlorophyll content). Improved crop development results in higher LAI, which results in more stomata and therefore increased transpiration rates (i.e. higher SC). Higher transpiration rates will result in increased biomass production and crop yields, as was observed in this study and reported by other studies (e.g. Mokoena, 2013; Siyeni, 2016). The inoculation treatment was also shown to improve pod numbers and pod mass, which thus contributed to the higher crop yields. Studies done by Singh (2005), Schulz *et al*. (2005), Mokoena, (2013), Khonje (2016) and Siyeni (2016) have shown that inoculation of seeds with a compatible rhizobia bacterium (i.e. *Bradyrhizobiurn*) increased plant height, pod numbers, nodules, biomass and crop yields. According to Javaid and Mahmood (2010), inoculation of soybean has the potential of increasing dry crop yield, nitrogen yield, and residual N levels.

However, the inoculation treatment did not significantly improve PHT, LN and accumulated biomass, which may be due to human error in its application prior to planting. However, inoculation should still be used, especially for smallholder farmers ensuring that correct application of the treatment is performed as it reduces fertilization cost and can be excellent for rotational crops such as maize, since N can be stored in the soil for the next crop. However, emphasis must be placed on the correct application of the inoculant, which should be done in conjunction with P and K application. According to Singh (2005) and Schulz *et al*. (2005), inoculation can also improve seed oil content of soybean, which may result in higher biodiesel yields, as demonstrated in this study.

6.10.2 LS6161R compared to CAPG3

The two varieties (i.e. LS6161R and CAPG3) considered in this study differ in genetic traits, considering LS6161R is an early maturing variety, whilst CAPG3 is a late maturing variety. As shown in **Table 5.1** in **Subsection 5.2**, LS6161R matured a week earlier when compared to CAPG3. The two varieties under the inoculation treatment produced significantly different results for CC, SC and CCI. Inoculated CAPG3 was shown to produce higher SC, PHT, LN and LAI, which resulted in higher accumulated biomass produced as compared to the LS6161R variety. However, the LS6161R variety produced higher CCI under the non-inoculation treatment as compared to CAPG3, which shows its adaptability to soil N deficiency, the latter considered an attractive adaptation mechanism for smallholder farming. The LS6161R variety was also shown to produce higher total biomass and seed yields, which in turn produced higher biodiesel yields whilst utilizing the least water, making it more WUE_C. On the other hand, the CAPG3 variety produced lower final biomass and seed yields, which in turn produced lower biodiesel yields (even though seed oil content was higher than LS6161R) but utilized more water (i.e. least WUE_C). Therefore, the LS6161R variety is possibly suited for biodiesel production due to its high crop yields and high WUE_C, whereas the CAPG3 variety is possibly suited for animal feed production due to its high biomass production and seed oil content. It is important to note that management practices which affect seed oil content need to be further investigated.

6.10.3 AquaCrop modelling

Model evaluation is a critical step that assesses the accuracy of model simulations. When compared to observations, AquaCrop did not simulate crop yields, final biomass and water use as well as expected. The model both under- and over-estimated observations, which is likely due to the minimal calibration conducted in this study.

Deviations between model simulations and observations may be due to the following:

- According to Battisti *et al*. (2017), AquaCrop is highly sensitive to soil water deficit and therefore may produce poor estimates of crop water use and yield.
- The abandonment of the dual Kc approach resulted in poor simulations of crop yields and soil water content over the growing season, which was raised by Paredes *et al*. (2015).
- Evaporation of intercepted water is not accounted for in the model, which will therefore affect AquaCrop's soil water balance. Since the model utilizes a water-driven growth engine, this may result in higher biomass and yield simulations.

In this study, deviations between model simulations and observations may also be due to various assumptions that were made to overcome certain limitations in the model. These include the following:

- The model cannot account for inoculation and therefore, assumptions pertaining to the soil fertility stress option were made to represent this management practice.
- The model does not adequately represent actual soil fertility conditions experienced in the field.

7. CONCLUSIONS

In this chapter, a summary of the approaches taken to meet the aims and objectives of this study is given, followed by a synthesis of the main findings of this study. Thereafter, recommendations for future research are given.

7.1 Summary of Approach

The main aim of this study was to estimate the seasonal water use and yield of three soybean varieties grown under dryland conditions. From this, the theoretical biodiesel yield was determined, together with water use efficiency of both crop and biodiesel production. In addition, the response of the varieties to inoculation was also assessed. However, one of the three varieties (PAN1521R) failed to emerge as a result of bad seed quality. Hence, crop water use and yield measurements were completed for only two varieties (LS6161R and CAPG3).

A soybean trial was established at Swayimane and throughout the growing season, measurements of leaf area index (LAI), leaf number (LN), plant height (PHT), chlorophyll content index (CCI) and stomatal conductance (SM) were undertaken. In addition, accumulated biomass production was determined via destructive sampling. Phenological growth periods were recorded for both varieties throughout the growing season as time taken to: emergence, flowering, pod formation, senescence and physiological maturity. From this, the duration of flowering was estimated. The final biomass production and crop yield was estimated from ten plants in each plot across both inoculation treatments. A statistical analysis of observed data was performed using GenStat software.

Crop water use (ET_C) was determined using the soil water balance method, which required inputs of precipitation (P), change in soil moisture (ΔS) and surface runoff (R), while other parameters such as irrigation (I), capillary rise (U) and drainage (D) were considered negligible for the trial site. An on-site automatic weather station (AWS) provided daily measurements of P, whilst R was measured weekly via three runoff plots installed at the site. In addition, ΔS was determined daily at four depths using Watermark sensors calibrated by CS650 probes. The

Watermarks were installed in three plots in each of the two treatment blocks (inoculation compared to non-inoculation).

Water use efficiency (WUE) was determined using crop water use and dry seed yield. Biodiesel yield was estimated using seed yield and the seed oil content. The latter was determined using a hexane solvent extraction process in a laboratory. The WUE_B of biodiesel production was calculated from biodiesel yield and crop ET_C. Crop coefficients (K_C) were determined using weekly estimates of ET_C and reference evapotranspiration (ET_O), which were then averaged to produce monthly K_C values.

The AquaCrop model was used to simulate the water use and yield of the two soybean varieties for each inoculation treatment. Canopy cover development was estimated from weekly measurements of leaf area index, then used to perform a minimal calibration of the model. Crop parameters describing the different phenological growth stages were also adjusted, based on observations in calendar days. These parameter values were then converted to thermal time using AquaCrop and inputs of daily maximum and minimum temperature. The latter was measured at 15-minute intervals by the AWS, from which daily values were calculated. Daily reference evapotranspiration was derived using FAO's ET_O Calculator, using inputs of daily incoming solar radiation, relative humidity (maximum and minimum) and wind speed measured by the AWS. Based on observations at flowering and physiological maturity, the maximum rooting depth was set to 0.62 m.

Two of the three soil water retention parameters (i.e. SAT and FC) required by AquaCrop were determined at four soil depths using the outflow pressure method in the soil laboratory at UKZN. Soil water retention curves were then derived and used to estimate PWP at 1500 kPa. Saturated hydraulic conductivity was modelled using SPAW, with inputs of soil texture and organic carbon content obtained by the Soil Analytical Service Laboratory at the Cedara College of Agriculture.

Monthly crop coefficients were calculated from simulations of crop water use by AquaCrop for both rainfed (i.e. water stressed) and irrigated (i.e. optimum) conditions. Assumptions pertaining to the soil fertility stress option in AquaCrop were made to represent both inoculation treatments. Crop yield simulated by AquaCrop was used to calculate biodiesel yield, as well as the WUE_B of crop and biodiesel production. AquaCrop was also used to simulate profile water content averaged for 1-meter soil depth. Results obtained from simulated crop yield and water use were then compared to that obtained using observed data.

7.2 Summary of Findings

It was shown that statistically there were no significant differences between the two inoculation treatments with respect to measured crop water use and yield. However, inoculation together with fertilization is still considered beneficial. Inoculation of seed prior to planting, together with application of P and K nutrients, produced better crop growth in terms of LAI, CC, CCI, SC, LN, PHT and biomass production. In conclusion, it is recommended that soybean is inoculated and that all farmers adopt good management practices in order to improve crop yield and thus WUE_C.

When compared to the LS6161R variety, inoculated CAPG3 produced significant differences with regard to certain crop growth and phenology parameters such as higher PHT, LN, LAI, CC, biomass production, as well as higher oil content. However, LS6161R produced higher biomass production and seed yield at harvest when compared to the CAPG3 variety. Hence, the latter variety produced less biodiesel yield, despite the seed yielding a higher oil content. CAPG3 also exhibited a higher crop water use and thus, less WUE_B. Therefore, CAPG3 could be potentially used for animal feed production due to its high production of biomass and oil content, whilst LS6161R may be better suited to biodiesel production due to its higher crop yield and better WUE_B.

Soil water retention parameters measured in the laboratory were more representative of the study site when compared to values derived from the SPAW model. SPAW tends to overestimate certain parameters such as soil bulk density. Poor estimates of PWP determined using the pressure outflow method at the 1.00 m soil depth was obtained, due to increasing clay content at this depth. Measurement of soil bulk density is important for the conversion of soil moisture content to volumetric water content of gravimetric samples. It is thus important that soil bulk density be representative of the experimental site. As seen in this study, volumetric water content from gravimetric samples were consistently higher when compared to the other soil moisture sensors used in this study.

AquaCrop simulations also showed that the CAPG3 variety produced higher yields and higher ET_C than LS6161R, which is contrary to observations. Hence, CAPG3 was more WUE in terms of both crop and biodiesel production as compared to LS6161R, which also contradicted observations. Overall, the model produced poor simulations of crop water use and yield, due to the partial calibration undertaken in this study. A full calibration was not done as the trial was conducted under non-standard (i.e. dryland) conditions. The poor simulation of CC and biomass production correlates well with findings by Battisti *et al*. (2017). They showed that the use of default parameters and a partial calibration results in poor model performance when predicting CC development, biomass production and crop yield. Therefore, a full calibration is needed to ensure better accuracy of model simulation of crop yields and water use. According to Paredes *et al*. (2015), the abandonment of the dual K_C approach in AquaCrop has hampered the model's ability to accurately partition transpiration and soil water evaporation. The dual K_C approach provides more accurate estimates of crop ET_C when computed on a daily basis. This will, in turn, improve simulations of crop water use and yield as the model is based on a water-driven growth engine.

The simulation of profile water content throughout the growing period was considered adequate, except during the flowering to pod formation stage. During this period when biomass production is highest, the model overestimated the soil profile's water content, which may be due the model's inability to 1) account for evaporation of intercepted rainfall, and 2) abandonment of the dual crop coefficient approach. The AquaCrop model has other limitations such as not accounting for inoculation. In addition, the soil fertility option in the model is too simplistic, i.e. user cannot specify the type and quantity of each macronutrient applied to the field, which thus affects the simulation of crop yields and water use as highlighted in this study.

7.3 Recommendations for Future Research

As per the findings of this study, the following recommendations for future research are made.

- One of the aims of this study was to quantify the benefits of inoculation on soybean WUE. The trial should be repeated for a second season to validate the results obtained in this study.
- In addition, the benefits of both inoculation and mulching on soybean WUE could be assessed in future studies.
- The trial could also be repeated using other soybean varieties. However, it is strongly recommended that seed germination tests are conducted prior to planting.
- Watermark sensors are not ideal for monitoring changes in soil water content. Therefore, it is highly recommended that future studies allocate sufficient budget to purchase the more expensive CS650 probes, which provide more accurate estimates of soil water content.
- It is recommended that soil bulk density is measured using undisturbed soil cores and not estimated using the SPAW model.
- Soil water retention parameters should also be determined from undisturbed cores via the outflow pressure method and not estimated using SPAW.
- The soil fertility option in AquaCrop should be modified to account for the type and quantity of nutrients on biomass production and crop yield.
- The AquaCrop model should also be modified to account for evaporation of intercepted rainfall by the developing canopy cover.
- The adoption of the dual crop coefficient approach in AquaCrop would also result in improved estimates of daily crop ET_C (and hence crop yield).
- It is also recommended that a full calibration of the crop model should be done using the same varieties and treatments under standard conditions (i.e. irrigated).
- Other studies have found that inoculation had a significant impact on biomass and crop yield, but not in this study. Therefore, the experiment should be repeated in another agro-ecology, which may show the benefits of inoculation.
- PAN1521R is suited to a wide range of growing conditions (i.e. from cold to warm areas). Hence, the water use and yield of this cultivar should be studied further in order to determine its suitability for biodiesel production.

- In order to improve biodiesel production, it is recommended that management practices which affect seed oil content need to be further investigated.

8. REFERENCES

ALLEN, R. 2000. Calibration for the Watermark 200SS. Soil Water Potential Sensor to fit the 7-19-96 "Calibration #3" Table from Irrometer. [Internet]. Available from: http://www.kimberly.uidaho.edu/water/swm/calibration_watermark2.pdf [Accessed 04 October 2019]

ALLEN, R.G, PEREIRA, L.S, RAES, D and SMITH, M. 1998. Crop evapotranspiration-Guidelines for computing crop water requirements. FAO Irrigation and Drainage Paper 56. Food and Agriculture Organization, Rome, Italy. [Internet]. Available from: http://www.fao.org/3/X0490E/X0490E00.htm [Accessed 04 March 2018]

ATABANI, A.E, MAHLIA, T.M.I, BADRUDDIN, I.A, MASJUKI, H.H, CHONG, W.T and LEE K.T. 2013. Investigation of physical and chemical properties of potential edible and non-edible feedstocks for biodiesel production, a comparative analysis. Renewable and Sustainable Energy Reviews 21: 749-755.

BATTISTI, R, SENTELHAS, P.C and BOOTE, K.J. 2017. Inter-comparison of performance of soybean crop simulation models and their ensemble in southern Brazil. Field Crops Research 200: 28-37.

BELLALLELOUI, N. BRUNS, H.A. ABBAS, H.K. MENGISTU, A, FISHER, D.K and REDDY, K.N. 2015. Agricultural practices altered soybean seed protein, oil, fatty acids, sugars, and minerals in the Midsouth USA. Agricultural Practices and Seed Nutrition 6: 1-14.

BENIZRI, E, BAUDOIN, E and GUCKERT, A. 2010. Root colonization by inoculated plant growth-promoting rhizobacteria. 557-574.

BLANCHARD, R, RICHARDSON, D.M, O'FARELL, P.J and VON MALTITZ, G.P. 2011. Biofuels and biodiversity in South Africa. South African Journal of Science 107: Art. #186, 8 pp.

BORGES, R and MALLARINO, A.P. 2000. Grain yield and early growth and nutrient uptake of no-till soybean as affected by the phosphorus and potassium placement. Agronomy Journal 92: 380-388.

BRENT, A. 2014. The agricultural sector as a biofuels producer in South Africa. Understanding the Food Energy Water Nexus. WWF-SA, RSA.

CAMPBELL SCIENTIFIC. 2012. CS650 and CS655 Water Content Reflectometers Revision Manual: 1. Campbell Scientific, Inc, USA. [Internet]. Available from: https://s.campbellsci.com/documents/es/manuals/cs650.pdf [Accessed 30 June 2019]

CHARD, J. 2002. Watermark soil moisture sensors: Characteristics and operating instructions. Utah State University, Utah, USA. [Internet]. Available from: https://www.researchgate.net/publications/237805713_WATERMARK_SOIL_MOISTURE_SENSORS_Characteristics_and_Operating_Instructions [Accessed 28 April 2019]

CHIMONYO, V.G.P, MODI, A.T and MABHAUDI, T. 2016. Water use and productivity of a sorghum-cowpea-bottle gourd intercrop system. Agricultural Water Management 165: 82-96.

CORNIC, G and MASSACCI, A. 1996. Leaf photosynthesis under drought stress. In: Baker NR, ed. Photosynthesis and the environment. The Netherlands: Kluwer Academic Published. [Internet]. Available from:
https://scholar.google.com/scholar?q=Cornic+G,Massacci+A.+1996++Leaf+photosynthesis+under+drought+stress.+In:+Baker+NR,+ed.++Photosynthesis+and+the+environment+.+The+Netherlands:+Kluwer+Academic+Publishers.+
[Accessed 23 April 2019]
CUI, S.Y and YU, D.Y, 2005. Estimates of relative contribution of biomass, harvest index and yield components to soybean yield improvements in China. Plant Breed 124: 473-476.
DAFF. 2010a. Soya beans production guideline. Department of Agriculture, Forestry and Fisheries, RSA. [Internet]. Available from:
http://www.nda.agric.za/docs/brochures/soya-beans.pdf
[Accessed 28 March 2019]
DAFF. 2010b. Sorghum production guideline. Department of Agriculture, Forestry and Fisheries, RSA. [Internet]. Available from:
https://www.nda.agric.za/docs/Brochures/prodGuideSorghum.pdf
[Accessed 28 March 2019]
DANNER, S.A, OBSTER, M, LOCHERER, M, HANK, T and RICHTER, K. 2015. Measuring leaf chlorophyll content with the Konica Minolta SPAD-502 Plus: Theory, Measurement, Problems, Interpretations. EnMAP Field Guides Technical Report, GFZ Data Services. Potsdam, Germany.
DASTORANI, M.T and POORMOHAMMADI, S. 2012. Evaluation of water balance in a mountainous upland catchment using SEBAL approach. Water Resources Management 26: 2069-2080.
De Beer, A.S and Bronkhorst, L. 2018. Soybean cultivar recommendations. ARC-Grain Crops Institute, Potchefstroom, RSA. [Internet]. Available from:
http://www.arc.agric.za/arc-gci/Documents/Soybeans/Soja17-18.pdf
[Accessed 19 November 2019]
DEVNARAIN, N, CRAMPTON, B.G, CHIKWAMBA, R, BECKER and O'KEEDY, M.M. 2016. Physiological responses of selected African sorghum landraces to progressive water stress and re-watering. South African Journal of Botany 103: 61-69.
DME (Department of Minerals and Energy). 2007. Biofuels Industrial Strategy for the Republic of South Africa. Pretoria, RSA. [Internet]. Available from:
http://www.gov.za/documents/biofuels-industrial-strategy-republic-south-africa
[Accessed 15 April 2019]
DoE (Department of Energy). 2012. Regulations regarding the mandatory blending of biofuels with petrol and diesel. Government Gazette No. 35623, 23 August 2012. Amendment no. R.671 of the Petroleum Products Act of 1977, Pretoria, RSA. 3-7. [Internet]. Available from:
http://www.info.gov.za/view/DownloadFileAction?id=173022
[Accessed 29 August 2019]
DoE (Department of Energy). 2014. Position paper on the South African biofuel's regulatory framework the first phase of the implementation of the biofuel's industrial strategy. [Internet].

Department of Energy, Pretoria, RSA. Government Gazette No. 538 of 2014: 15 January 2014, No. 37232, Pretoria, RSA. [Internet]. Available from:
http://www.energy.gov.za/files/policies/Draft_position_paper_on_the_SA_Biofuels_Reg_Frmwrk.pdf
[Accessed 15 April 2019]
DONATELLI, M, STÖCKLE, C, CEOTTO, E and RINALDI, M. 1997. Evaluation of Crop System for cropping systems at two locations of northern and southern Italy. European Journal of Agronomy 6: 35-45.
DONBURG, V, VAN VUUREN, D, VAN DE VEN, G, LANGEVELD, H, MEEUSEN, M, BANSE, M, VAN OORSCHOT, M, ROS, J, VAN DEN BORN, G, AIKING, H, LONDO, M, MOZAFFARIAN, H, VERWEIJ, P and FAAIJ, A. 2010. Bioenergy revisited: key factors in global potentials of bioenergy. Energy and Environmental Science 3: 258-267.
DU PLESSIS, J. 2008. Sorghum production. Pretoria, RSA. Department of Agriculture, RSA. [Internet]. Available from:
https://www.nda.agric.za/publications/
[Accessed 13 April 2019]
DWS (Department of Water and Sanitation). 2016. Policy position on water use in bio-fuel production in South Africa. Department of Water and Sanitation (DWS), Pretoria, RSA.
EL BASSAM, N. 2010. Energy Crops Guide. Handbook of Bioenergy Crops, Chapter 10: 335-359. Earthscan, London, Washington, DC.
FAGERIA, N.K, BARBOSA FIHLHO, M.P and DA COSTA, J.G.C. 2001. Potassium-use efficiency in genotypes. J. Plant Nutrition. 24: 1937-1945
FAO (Food and Agriculture Organization). 2015. Yield gap analysis of field crops-Methods and case studies, by Sadras, V.O, Cassman, K.G.G, Grassini, P, Hall, A.J, Bastiaanssen, W.G.M, Laborte, A.G, Milne, A.E, Sileshi, G and Steduto, P. FAO Water Reports No. 41, Rome, Italy.
FAO (Food and Agriculture Organization). 2016. AQUASTAT website. Food and Agriculture Organization, Rome, Italy. [Internet]. Available from:
https://www.fao.org/nr/water/aquastat/countries_regions/Profile_segments/ZAF-WU_eng.stm
[Accessed 15 June 2019]
FAO (Food and Agriculture Organization). 2017a. Book I: Understanding AquaCrop. Food and Agriculture Organization, Rome, Italy. [Internet]. Available from:
http://www.fao.org/3/a-i6051e.pdf
[Accessed 25 June 2019]
FAO (Food and Agriculture Organization). 2017b. Book II: Running AquaCrop. Food and Agriculture Organization, Rome, Italy. [Internet]. Available from:
http://www.fao.org/3/a-i6052e.pdf
[Accessed 25 June 2019]
FAO (Food and Agriculture Organization). 2018. AquaCrop version 6.0-6.1 reference manual. Food and Agriculture Organization, Rome, Italy. [Internet]. Available from:
http://www.fao.org/3/a-br248e.pdf
[Accessed 25 June 2019]

FARAHANII, H.J, IZZI, G and OWEIS, T.Y. 2009. Parameterization and evaluation of the AquaCrop model for full and deficit irrigated cotton. Agronomy Journal 101: 469-476.

FAGERIA, N.K, BARBOSA, FIHLHO, M.P and COSTA, J.G.C. 2001. Potassium-use efficiency in genotypes. Journal of Plant Nutrition 24: 1937-1945.

FERMONT, A and BENSON, T. 2011. Estimating Yield of Food Crops Grown by Smallholder Farmers: A Review in the Uganda Context. IFPRI Discussion Paper 01097. Development Strategy and Governance Division. Uganda.

GARY, C, JONES, J.W and CHAMITCHIAN, M. 1998. Crop modelling in horticulture: state of the art. Scientia Horticulture 74**:** 3-20.

GHEEWALA, S.H, BERNDES, G and JEWITT, G. 2011. The bioenergy and water nexus. Biofuels, Bioproducts & Biorefining, 5: 353-360.

GREILER, Y. 2007. Biofuels, opportunity or threat to the poor? [Internet]. Swiss Agency for Development and Cooperation. Available from:
http://www.ddrn.dk/filer/forum/File/Issue_Paper_Biofules.pdf
[Accessed 15 April 2019]

GYANESHWAR, P, KUMAR, G.N, PAREKH, L.J and POOLE, P.S. 2002. Role of soil microorganisms in improving P nutrition of plants. South African Journal of Plant and Soil 245: 83-93.

HADEBE, S.T. 2015. Water use of selected sorghum (*Sorghum bicolor* L. Moench) genotypes MSc dissertation in Crop Science, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Pietermaritzburg, RSA. [Internet]. Available from:
https://researchspace.ukzn.ac.za/bitsream/handle/10413/12925
[Accessed 25 January 2019]

HADEBE, S.T, MABHAUDHI, T and MODI, A.T. 2017. Water use of sorghum (*Sorghum bicolor* L. Moench) in response to varying planting dates evaluated under rainfed conditions. Water South Africa Volume. 43 Number 1.

HARDARSON, G, GOLBS, M and DANSO, S.K.A. 1989. Nitrogen fixation in soybean (*Glycine max* L. Merrill) as affected by nodulation patterns. Soil Biology and Biochemistry 21: 783-787.

HAWKINS, R. 1978. Runoff curve numbers with varying site moisture. Journal of Irrigation and Drainage Division, American Society of Civil Engineers 104: 389-398.

HENG, L.K, HSIAO, T, EVETT, S, HOWELL, T and STEDUTO, P. 2009. Validating the FAO AquaCrop model for irrigated and water deficient field maize. Agronomy Journal 101: 488-498.

HILLEL D. 1998. Environmental soil physics. Academic Press, New York, USA. [Internet]. Available from:
http://www.ipni.net/publication/bettercrops.nsf/0/E90E04A957EA624285257980007CD63C/$FILE/Better%20Crops%201998-3%20(lo%20res).pdf
[Accessed 27 May 2019]

HUGHES, S, PARTZSHC, L and GASKELL, J. 2007. The Development of Biofuels Within the Context of the Global Water Crisis. Sustainable Development Law & Policy, Springer 77: 58-62.

IFA (International Fertilizer Industry). 1992. World fertilizer user manual. International Fertilizer Industry Association, Paris. [Internet]. Available from:
http://agris.fao.org/agris-search/search.do?recordID=XF2015040862
[Accessed 28 May 2019]

IRMAK, S, PAYERO, J.O, VAN DE WALLE, B, REES, J. ZOUBEK, G, MARTIN, D.L, KRANZ, W.L, EISENHAUER, D.E and LEININGER, D. 2016. Principles and operational characteristics of watermark granular matrix sensor to measure soil water status and its practical applications for irrigation management in various soil textures. University of Nebraska, Lincoln, USA. Extension division of the Institute of Agriculture and Natural Resources, EC783 publication.

ISLAM, M.S, AHMED, M, HOSSAIN, M.S, AKTER, H and AKTAR, S. 2018. Response of soybean to Rhizobium biofertilizer under different levels of phosphorus. Progressive Agriculture 28: 302-315.

JANDA, K, KRISTOUFEK, L and ZILBERMAN, D. 2012. Biofuels: policies and impacts. Agriculture Journals, 58: 372-386.

JAMIESON, P.D, PORTER, J.R and WILSON, D.R. 1991. A test of computer simulation model ARC-WHEAT1 on wheat crops grown in New Zealand. Field Crops Research 27: 337-350.

JAVAID, A. and N. MAHMOOD. 2010. Growth, nodulation and yield response of soybean to biofertilizers and organic manures. Pakistan Journal of Botany 42: 863-871.

JEWITT, G.P.W, WEN, H.W, KUNZ, R.P and VAN ROOYEN, A.M. 2009. Scoping Study on Water Use of Crops/Trees for Biofuels in South Africa. Water Research Commission (WRC), Pretoria, RSA. WRC Report No. 1772/1/09.

KARUKU, G.N. GACHENE, C.K.K, KARANJA, N, CORNELIS, W, VERPLANCKE, H and KIRONCHI, G. 2012. Soil hydraulic properties of a nitisol in Kabete, Kenya. Tropical and Subtropical Agroecosystems 15: 595-609.

KHOMO, T.L. 2014. Spatial assessment of optimum and sub-optimum growing areas for selected biofuel feedstocks in South Africa. MSc dissertation in Hydrology. School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Pietermaritzburg, RSA.

KHONJE, D.J. 2016. Adoption of the rhizobium inoculation technology for pasture improvement in sub-Saharan Africa. FAO corporate document repository. [Internet]. Available from:
http://www.fao.org/wairdocs/ilri/x5536e/x5536e1g.htm
[Accessed 14 June 2019]

KNOTHE, G. 2010. Biodiesel and renewable diesel: A comparison. Progress in Energy and Combustion Science 36: 364-373.

KOTZE C (2012a) DoE's mandatory blending regulations welcomed by grain producers. Engineering News 32(06), 21-27 September 2012, p 67. Creamer Media (Pty) Ltd, Johannesburg, RSA. [Internet]. Available from:
http://www.engineeringnews.co.za/article/does-mandatory-blending-regulations-welcomed-by-grain-producers-2012-09-21
[Accessed 22 September 2019]

KRAUSE, P, BOYLE, D.P and BÄSE, F. 2005. Advances in geosciences comparison of different efficiency criteria for hydrological model assessment. Advances in Geosciences 5: 89-97.

KUNZ, R.P, MENGISTU, M.G, STEYN, J.M, DOIDGE, I.A, GUSH, M.B, DU TOIT, E.S, DAVIS, N.S, JEWITT, G.P.W and EVERSON, C.S. 2015a. Assessment of biofuel feedstock production in South Africa: Synthesis report on estimating water use efficiency of biofuel crops (Volume 1). Water Research Commission (WRC), Pretoria, RSA. WRC Report No. 1874/1/15. [Internet]. Available from:
http://www.wrc.org.za/wp-content/uploads/mdocs/1874-1-151.pdf
[Accessed 23 May 2019]

KUNZ, R.P, MENGISTU, M.G, STEYN, J.M, DOIDGE, I.A, GUSH, M.B, DU TOIT, E.S, DAVIS, N.S, JEWITT, G.P.W and EVERSON, C.S. 2015b. Assessment of biofuel feedstock production in South Africa: Technical report on the field-based measurement, modelling and mapping of water use of biofuel crops (Volume 2). Water Research Commission (WRC), Pretoria, RSA. WRC Report No. 1874/2/15. [Internet]. Available from:
http://www.wrc.org.za/wp-content/uploads/mdocs/1874-2-151.pdf
[Accessed 03 June 2019]

KUNZ, R.P, DAVIS, NS, THORNTON-DIBB, S.L.C, STEYN, J.M, DU TOIT, E.S and JEWITT, G.P.W. 2015c. Assessment of biofuel feedstock production in South Africa: Atlas of water use and yield of biofuel crops in suitable growing areas (Volume 3). Water Research Commission (WRC), Pretoria, RSA. WRC Report No. TT 652/15. [Internet]. Available from:
http://www.wrc.org.za/wp-content/uploads/mdocs/TT%20652-15.pdf
[Accessed 15 June 2019]

KUSTAS, W.P and AGAM, N. 2013. Energy and evapotranspiration partitioning in a desert vineyard. Agricultural and Forest Meteorology 218-219: 277-287.

LAMPTEY, S, AHIABOR, B.D.K, YEBOAH, S and ASAMOAH, C. 2014. Response of soybean (*Glycine max*) to Rhizobial inoculation and phosphorus application. Journal of Experimental Biology and Agricultural Sciences volume-2.

LEGATES, D.R and MCCABE, G.J. 1999. Evaluating the use of "goodness-of-fit" measures in hydrologic and hydroclimatic model validation. Water Resources Research 35: 233-241.

LEGGETT, M, DIAZ-ZORITA, M, KOIVUNEN, M, BOWMAN, R, PESEK, R, STEVENSON, C and LEISTER, T. 2017. Soybean response to inoculation with Bradyrhizobium Japonicum in the united states and Argentina. Agronomy Journal 109: 1031-1038.

LEIGH, R.A and JONES, R.G.W. 1984. A hypothesis relating critical potassium concentrations from growth to the distribution and function of this ion in the plant-cell. New Phytopathology 97: 1-13.

LEMBEDE, L. 2017. Estimating water use and yields of soybean (*Glycine max.*) under mulch and fertiliser in rainfed conditions on KwaZulu-Natal. MSc dissertation in Hydrology. School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Pietermaritzburg, RSA.

LETETE, T, VON BLOTTNITZ, H. 2012. Biofuel Policies in South Africa: A critical analysis. In: Janssen, R and Rutz, D (eds.). Bioenergy for sustainable development in Africa, 191-199. Springer, Dordrecht.

LI-COR. (2009) LAI-2200 Plant Canopy Analyzer. Instruction manual. LI-COR, Inc. Lincoln, Nebraska, USA. Publication Number 984-10633. [Internet]. Available from: http://www.licor.co.za/manuals/LAI-2200_Manual.pdf [Accessed 24 April 2019]

LORENTZ, S. GOBA, P and PRETORIUS J. 2001. Hydrological processes research: experiments and measurements of soil hydraulic characteristics. Water Research Commission (WRC), Pretoria, RSA. WRC Report No. 744/1/01.

LORENTZ, S. 2019. Personal communication. Associate Professor, Centre for Water Resources Research (CWRR), University of KwaZulu-Natal (UKZN), Pietermaritzburg, RSA.

MABHAUDHI, T. 2012. Drought tolerance and water-use of selected South African landraces of taro (*Colocasia esculenta* L. Schott) and Bambara groundnut (*Vigna subterranea* L. Verdc). PhD theses in Crop Science, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Pietermaritzburg, RSA.

MABHAUDHI, T, MODI, A.T and BELETSE, Y.G. 2014. Parameterisation and testing of the FAO AquaCrop model for a South African Bambara groundnut landrace. Agronomy Journal 106: 243-251.

MACVICAR, C, DE VILLIERS, J, LOXTON, R, VERSTER, E, LAMBRECHTS, J, MERRYWEATHER, F, LE ROUX, J, VAN ROOYEN, T and HARMSE, H.V.M. 1977. Soil Classification - A Binomial System for South Africa. Department of Agricultural Technical Services, Soil and Irrigation Research Institute, Pretoria, RSA.

MALIK, M.A, CHEEME, M.A, KHAN, H.Z and WAHIG, M.A. 2006. Growth and yield response of soybean (*Glycine max*.) to seed inoculation and varying phosphorus levels. Journal of Agricultural Research 44: 47-53.

MAONGA, B.B, MAGANGA, A.M and KANKWAMBA, H. 2015. Smallholder farmers willingness to incorporate biofuel crops into cropping systems in Malawi. International Journal of Food and Agricultural Economics 3: 87-100.

MAPHOSA, T.M. 2015: Growth, Nodulation and yield response of promiscuous and non-promiscuous soybean cultivars to inoculation in different soil types under glasshouse and field conditions. MSc dissertation in Agriculture Agronomy. School of Agricultural and Environmental Sciences, University of Limpopo, RSA.

MASANGANISE, J. 2019. Evapotranspiration of soybean and grain sorghum in a semi-arid climate. PhD thesis in Agrometeorology. School of Agricultural, Earth and Environmental Sciences, College of Agriculture, Engineering and Science, University of KwaZulu-Natal Pietermaritzburg, RSA. Submitted for examination.

MBANGIWA, N.C, SAVAGE, M.J and MABHAUDHI, T. 2019. Modelling and measurement of water productivity and total evaporation in a dryland soybean crop. Agricultural and Forest Meteorology 266-267: 65-72.

MCMASTER, G.S and WILHEM W.W. 1997. Growing degree-days: one equation, two interpretations. Agricultural and Forest Meteorology 87: 291-300.

MENGISTU, M.G., EVERSON, C.S, MOYO, N.C and SAVAGE, M.J. 2014. The validation of the variables (evaporation and soil moisture) in hydro meteorological models. Water Research Commission (WRC), Pretoria, RSA. WRC Report No. 2066/1/13.

MEYER, R.F, STRAUSS, P.G and FUNKE, T. 2008. Modelling the impacts of macro-economic variables on the South African biofuels industry. Agrekon 47: 1-19.

MOKOENA, T.Z. 2013. The effect of direct phosphorous and potassium fertilization on soybean (*Glycine max* L.) yield and quality. MSc dissertation in Agricultural Economics, Faculty of Natural and Agricultural Sciences, University of Pretoria, RSA.

MOKONOTO, O. 2019. Parameterisation and validation of AquaCrop for sugarcane and sugarbeet in South Africa. MSc dissertation in Hydrology, School of Agricultural, Earth and Environmental Sciences. University of KwaZulu-Natal, Pietermaritzburg, RSA.

MOLDEN, D, OWEIS, T, STEDUTO, P, BINDRABAN, P, HANJRA, M.A and KIJNE, J. 2010. Improving agricultural water productivity: between optimism and caution. Agricultural Water Management 97: 528-535.

MUTEGI, J and ZINGORE, S. 2014. Boosting soybean production for improved food security and incomes in Africa. The International Plant Nutrition Institute (IPNI), sub-Saharan Africa program.

NOLTE, M. 2007. Commercial biodiesel production in South Africa: A preliminary economic feasibility study. MSc dissertation (unpublished). Department of Process Engineering, University of Stellenbosch, Cape Town, RSA

NIUWENHUIS, R AND NIEUWELINK, J. 2002. Cultivation of soya and other legumes. [Internet]. Available from:
https://publications.cta.int/media/publications/downloads/1119_PDF.pdf
[Accessed 25 April 2019]

NJERU, E.M, MAINGI, J.M, CHERUIYOT, R and MBURUGU, G.N. 2013. Managing soybean for enhanced food production and soil bio-fertility in smallholder systems through maximized fertilizer use efficiency. International Journal of Agriculture and Forestry 3:191-197.

OLIVIER, F.C, JERMAINE, C. and SINGELS, A. 2014. Increasing water use efficiency of irrigated sugarcane production in South Africa through better agronomic practices. South African Sugarcane Research Institute. Water South Africa 38:77-87

ORTEGA-FARIAS, S, IRMAK, S and CUENCA, R.H. 2009. Special issue on evapotranspiration measurement and modelling. Irrigation Science 28: 1-3.

PANNAR. 2006. Soybean production guide. Pannar Seed (Pty) Ltd, Greytown, RSA. [Internet]. Available from:
http://www.pannar.com/assets/documents/soybeans.pdf
[Accessed 10 August 2019]

PAREDES. P, DE MELO-ABREU, J.P, ALVES, I and PEREIRA, L.S. 2014. Assessing the performance of the FAO AquaCrop model to estimate maize yields and water use under full and deficit irrigation with focus on model parameterization. Agricultural Water Management 144: 81-97.

PAREDES. P, WEI, Z, LIU, Y, XU, D, XIN, Y, ZHANG, B and PEREIRA, L.S. 2015. Performance assessment of the FAO AquaCrop model for soil water, soil evaporation, biomass and yield of soybeans in North China Plain. Agricultural Water Management 152: 57-71.
PEREIRA, L.S, PAREDES, P, RODRIGUES, G.C and NEVES, M. 2015. Modelling barley water use and evapotranspiration partitioning in two contrasting rainfall years. Assessing SIMDualKc and AquaCrop models. Agricultural Water Management 159: 239-254.
RAES, D, STEDUTO, P, HSIAO, T.C and FERERES, E. 2009. AquaCrop-the FAO crop model to simulate yield responses to water: II. Main algorithms and software description. Agronomy Journal 101: 438-447.
RAES, D, STEDUTO, P. HSIAO, T and FERERES, E. 2012. AquaCrop Version 4.0 Reference Manual. FAO, Rome, Italy. [Internet] Available from:
http://www.fao.org/nr/water/aquacrop
[Accessed 15 April 2019]
RAHMAN, M.D, M, MOSTAFIZ, S.B, PAATERO, J.J and LAHDELMA, R. 2014. Extension of energy crops on surplus agricultural lands: A potentially viable option in developing countries while fossil fuel reserves are diminishing. Renewable and Sustainable Energy Reviews 29: 108-119.
REN, X, ZHANG, P, CHEN, X, GUO, J and JIA, Z. 2016. Effect of Different Mulches under Rainfall Concentration System on Corn Production in the Semi-arid Areas of the Loess Plateau. Scientific Report 6, 19019. [Internet]. Available from:
http://www.nature.com/articles/srep19019
[Accessed 2 February 2018]
RICHARD, G.A, LUIS, S.P, TERRY, A.H and MARVIN, E.J. 2011. Evapotranspiration information reporting: I. Factors governing measurement Accuracy. Agricultural Water Management 98: 99-920.
RITCHIE, J.T and BASSO, B. 2008. Water use efficiency is not constant when crop water supply is adequate or fixed: the role of agronomic management. European Journal of Agronomy 28: 273-281.
ROBY, M.C, MARIA, G, FERNANDEZ, S, EMILY, A, H, FERNANDO, E.M and VAN LOOCKE, A. 2017. Biomass sorghum and maize have similar water-use-efficiency under non-drought conditions in the rain-fed Midwest U.S. Agricultural and Forest Meteorology 247: 434-444.
RURANGWA, E, VANLAUWEC, B and GILLER, K.E. 2018: Benefits of inoculation, P fertilizer and manure on yields of common bean and soybean also increase yield of subsequent maize. Agriculture, Ecosystems and Environment volume 261:219-229
SAAB, M.T.A, TODOROVIC and ALBRIZIO, R. 2015. Comparing AquaCrop and Crop System models in simulating barley growth and yield under different water and nitrogen regimes. Does calibration year influence the performance of crop growth models? Agricultural Water Management, 147: 21-33.
SALVAGIOTTI, F, CASSMAN, K.G, SPECHT, J.E, WALTERS, D.T and WEISS, A. 2008. Nitrogen uptake, fixation and response to fertilizer N in soybeans: A review. Agronomy and Horticulture-Faculty Publications. 133. [Internet]. Available from:
https://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1133&context=agronomyfacpub

[Accessed 23 August 2019]
SAXTON, K.E and RAWLS, W.J. 2006. Soil water characteristic estimates by texture and organic matter for hydrologic solutions. Soil Science Society of America Journal 70: 1569-1578.
SCHMIDT, E and SCHULZE, R. 1987. Flood Volume and Peak Discharge from Small Catchments in Southern Africa Based on the SCS Technique. Water Research Commission (WRC), Pretoria, RSA. WRC Project TT 31/87.
SCHULZE, R.E, ANGUS, G.R and GUY, R.M. 1995. Soils. In: Schulze RE (Ed), A Text to Accompany the ACRU 3.00 Agrohydrological Modelling System, Chapter 5, AT5-1 to AT5-28. Water Research Commission (WRC), Pretoria, RSA. WRC Report TT 69/9/95.
SCHULZE, R.E. 1995. Hydrology and Agrohydrology: A Text to Accompany the ACRU 3.00 Agrohydrological Modelling System. Water Research Commission (WRC), Pretoria, RSA.WRC Report TT 69/9/95.
SCHULZE, R.E. and MAHARAJ, M. 2007. Soya bean yield estimation. In: R.E. Schulze (ed), South African Atlas of Climatology and Agrohydrology. Water Research Commission (WRC), Pretoria, RSA. WRC Report No. 1489/1/06.
SCHULZE, R. 2012. Mapping hydrological soil groups over South Africa for use with the SCS-SA design hydrograph technique: methodology and results. 16th SANCIAHS National Hydrology Symposium, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal. Pietermaritzburg, RSA.
SCHULZ, T. J, THELEN, K.D. and D. WANG. 2005. Effect of *Bradyrhizobium japonicum* inoculant on soybean growth and yield. Department of Crop and Soil Sciences, Michigan State University, East Lansing, Michigan, USA. [Internet]. Available from:
https://scisoc.confex.com/crops/2005am/techprogram/index.html
[Accessed 19 April 2019]
SINCLAIR, T. R. and V. VADEZ, 2002. Physiological traits for crop yield improvement in low N and P environments. South African Journal of Plant and Soil 245: 1-15.
SINGH, M.S. 2005. Effect of Bradyrhizobium Inoculation on Growth, Nodulation and Yield Attributes of Soybean. Agricultural Reviews 26: 305-308.
SIYENI, D. 2016. Effects of rhizobia inoculation and phosphorus fertilizers on nodulation and yield of soybean (*Glycine max*) in Dedza Kasungi and Salima districts of Malawi. MSc dissertation in Agronomy, Bunda College of Agriculture, University of Malawi.
SMITH, B. 2006. The farming handbook. University of KwaZulu-Natal Press, Pietermaritzburg, RSA.
SPARKS, G.D. 2010. The economic feasibility of on-farm biodiesel production in KwaZulu-Natal, South Africa. MSc dissertation in Agricultural Economics, University of KwaZulu-Natal, Pietermaritzburg, RSA.
STATISTICS SOUTH AFRICA. 2007. National Accounts - water accounts for South Africa: 2000. [Internet]. Available from:
https://www.nda.agric.za/docs/statsinfo/Censcoagric2007.pdf
[Accessed 19 June 2019]
STEDUTO. P. 2006. Biomass water productivity: comparing the growth engines of crop. Rome, Italy. [Internet]. Available from:

http://www.distagenomics.unibo.it/wuemed/P_steduto_biomass.pdf
[Accessed 22 April 2019]
STEDUTO, P, HIAO, T.C, FERERES, E and RAES, D. 2012. Crop yield response to water. FAO Irrigation and Drainage Paper No. 66, Rome, Italy. [Internet]. Available from: http://www.fao.org/docrep/016/i2800e/i2800e00.htm
[Accessed 21 March 2019]
STEVENS, J.B. 2007. Adoption of irrigation scheduling methods in South Africa. PhD in Agricultural Economics, Department of Agricultural Economics, Faculty of Natural and Agricultural Science, University of Pretoria, Pretoria, RSA.
SWANEPOEL, P.A, BOTHA, P.R, TRUTER, W.F, KAREN, A, J and SURRIDGE-TALBOT. A. 2010. The quantification of biological nitrogen fixation as affected by soil organic matter. MSc in (Agric): Pasture Science, Department of Agricultural Economics, Faculty of Natural and Agricultural Science, University of Pretoria, Pretoria, RSA.
TAREKEGN, M.A and KIBRET, K. 2018. Effects of Rhizobium, Nitrogen and Phosphorus Fertilizers on Growth, Nodulation, Yield and Yield Attributes of Soybean at Pawe North-western Ethiopia.
TIWANI, P.S, JOSHNI, O.P and BILLORE, S.D.2001. Reliable yield potential of soybean varieties at farm level in India. In; Souvenir. Harnessing soya potential for health and wealth. India soybean forum. SOPA 108-112.
TODOROVIC, M, ALBRIZIO, R, ZIVOTIC, L, SAAB, M.A, STOCKLE, C AND STEDUTO P. 2009. Assessment of AquaCrop, Crop Systems, and WOFOST Models in the Simulation of Sunflower Growth under Different Water Regimes. Agronomy Journal 101: 509-521.
UHLENBROOK, S. 2007. Biofuel and water cycle dynamics: What are the related challenges for hydrological processes research? Hydrological Processes 21: 3647-3650.
VAN GENUCHTEN, M.T.H. 1980. A closed-form equation for predicting the hydraulic conductivity of unsaturated soils. Soil Science Society American Journal 44: 892-898.
VARBLE, J.L and CHÁVEZ, J.L. 2011. Performance evaluation and calibration of soil water content and potential sensors for agricultural soils in eastern Colorado. Agricultural Water Management 101: 93-106.
VOLOUDAKIS, D, KARAMANOS, A, ECONOMOU, G, KALIVAS, D, VAHAMIDIS, P, KOTOULAS, V, KAPSOMENAKIS, J and ZEREFOS, C. 2015. Prediction of climate change impacts on cotton yields in Greece under eight climatic models using the AquaCrop crop simulation model and discriminant function analysis. Agricultural Water Management 147: 116-148.
WATSON, H.K, GARLAND, G.G, PURCHASE, B, JOHNSON, F.X, GRIFFEE, P and DERCAS, N. 2008. Bioenergy for Sustainable Development and Global. Competitiveness: the case of Sugar Cane in southern Africa. Thematic Report 1. Stockholm Environment Institute, Stockholm, Sweden.
WHITE, R. 2003. Soils for fine wines. Oxford University Press, New York, USA.WILLMOTT. CJ. 1982. Some comments on the evaluation of model performance. Bulletin American Meteorological Society 63: 1309-1313.
WILLMOTT, C.J. 1982. Some comments on the evaluation of model performance. Bulletin American Meteorological Society, 63: 1309-1313.

WIN, M, NAKASATHIEN S and SAROBOL, E. 2010. Effects of phosphorus on seed oil and protein contents and phosphorous use efficiency in some soybean varieties. Kasetsart Journal of Natural Science 44: 1-9.
WMO (Word Meteorological Organisation). 2008. Guide to Hydrological Practices Volume I Hydrology-From Measurement to Hydrological Information WMO-No. 168 (Sixth edition). Geneva, Switzerland.
WOODWARD, D, HAWKINS, R, JIANG, R, HJELMFELT, A, MULLEM, J and QUAN, Q. 2003. Runoff Curve Number Method: Examination of the Initial Abstraction Ratio. World Water and Environmental Resources Congress. 1-10.
ZELEKE, K.T and WADE, L.J. 2012. Evaporation estimation using soil water balance, weather and crop data. In Evapotranspiration-Remote Sensing and Modelling. International Technology. [Internet]. Available from: http://www.intechopen.com/books/evapotranspirationremote-sensing-and-modeling/evapotranspiration-estimation-using-soil-water-balance-weather-and-crop-data [Accessed 28 April 2018]

9. APPENDICES

9.1 Appendix 1: Seed germination test of PAN1521R variety

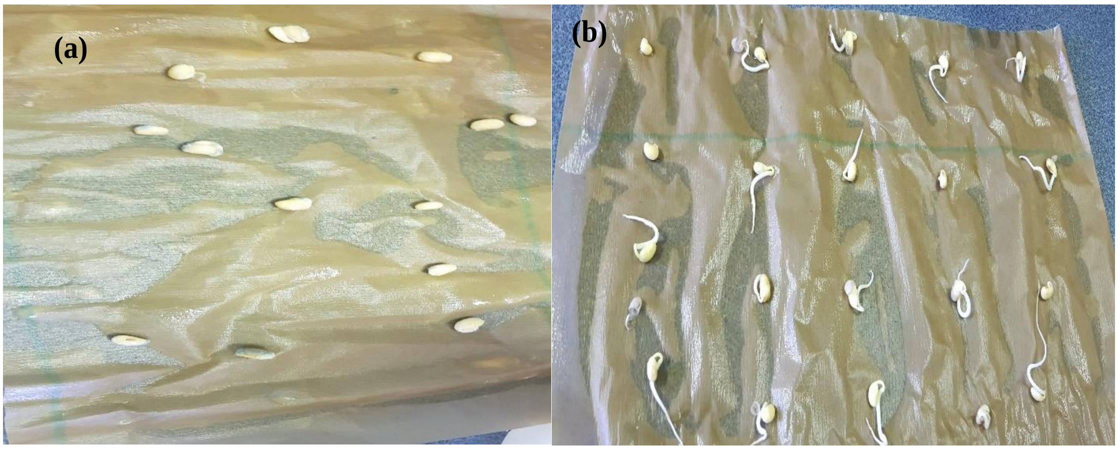


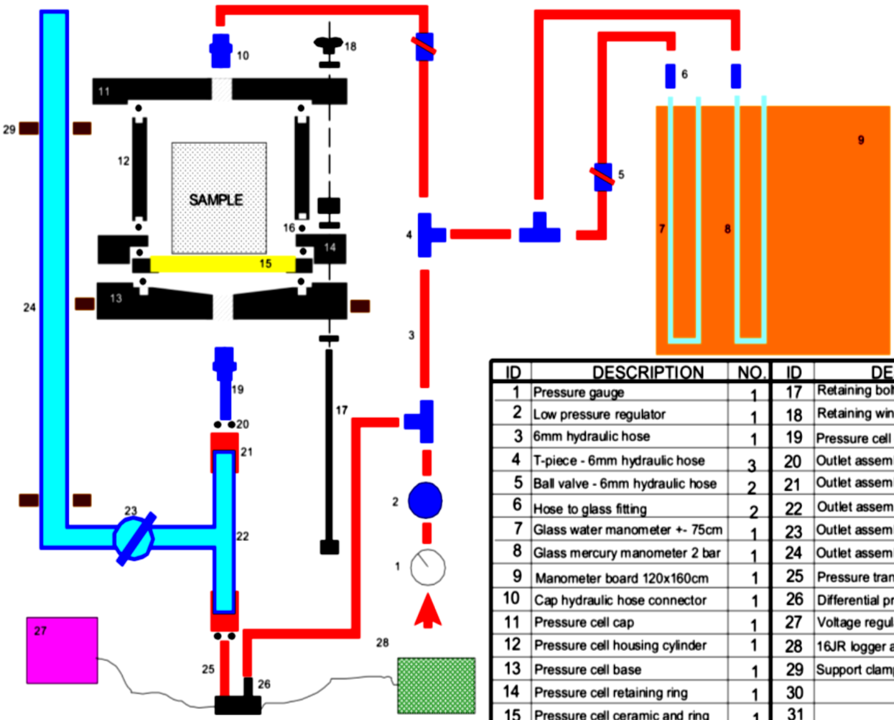
Figure 9.1: (a) PAN1521R poor seed germination after planting (b) PAN1521R good seed germination with new batch of seeds

9.2 Appendix 2: Procedure for determining soil water retention parameters using the outflow pressure method

Soil water retention parameters (i.e. PWP, FC and SAT) were estimated using the outflow pressure method shown in **Figure 9.2** below. A detailed procedure is documented in Lorentz *et al*. (2001; 2004). In this procedure, undisturbed soil cores obtained at four depths (i.e. 0.15, 0.30, 0.60 and 1.00 m) from the experimental site were used. The soil cores were initially placed on porous ceramic disks and placed in deionized water for a period of at least 24 hours. This was done to prevent any air bubbles from potentially disrupting the flow of water during measurements. The samples were then carefully placed into four low-pressure metal chambers. Each chamber was connected to glass burettes to measure drainage rates for a given pressure. At each pressure rate, the volume of water drained from the chamber into the glass burettes must equilibrate, after which a measurement reading was taken from the burette before increasing the pressure again. The system is designed to measure soil water parameters between 0 and 1 bar (or 100 kPa). The pressure is increased at set intervals once the volume of water in the burette (or burette readings) equilibrates and measurement readings are taken until the system reaches 1 bar. The amount of water that is available for plant use in the root zone is retained at matric potentials of between -10 to -1500 kPa. A low matric potential or pressure (e.g. 0 kPa) applied in the beginning meant water drained out faster than at a higher matric potential (33 kPa), which corresponds to saturation (i.e. porosity) and the field capacity respectively.

However, matric potentials of -1500 kPa is where plants commonly wilt (Schulze *et al.*, 1995). The outflow pressure system cannot be used for such high pressures and therefore each soil core was carefully removed from the chambers and weighed immediately and then transferred to a pressure pot that operated at 15 bars (-1500 kPa) of pressure to ascertain the wilting point. However, before placing the soil cores in the pressure pot, a ceramic disc that can withstand pressures of 15 bars was saturated with water for over 24 hours. A similar approach using a glass burette was used, where the water was allowed to drain out of samples until an equilibration state was reached. Once equilibrium was reached (i.e. no more water dripped out into the burette), the soil sample was immediately weighed, then oven dried for 48 hours and finally re-weighed again.

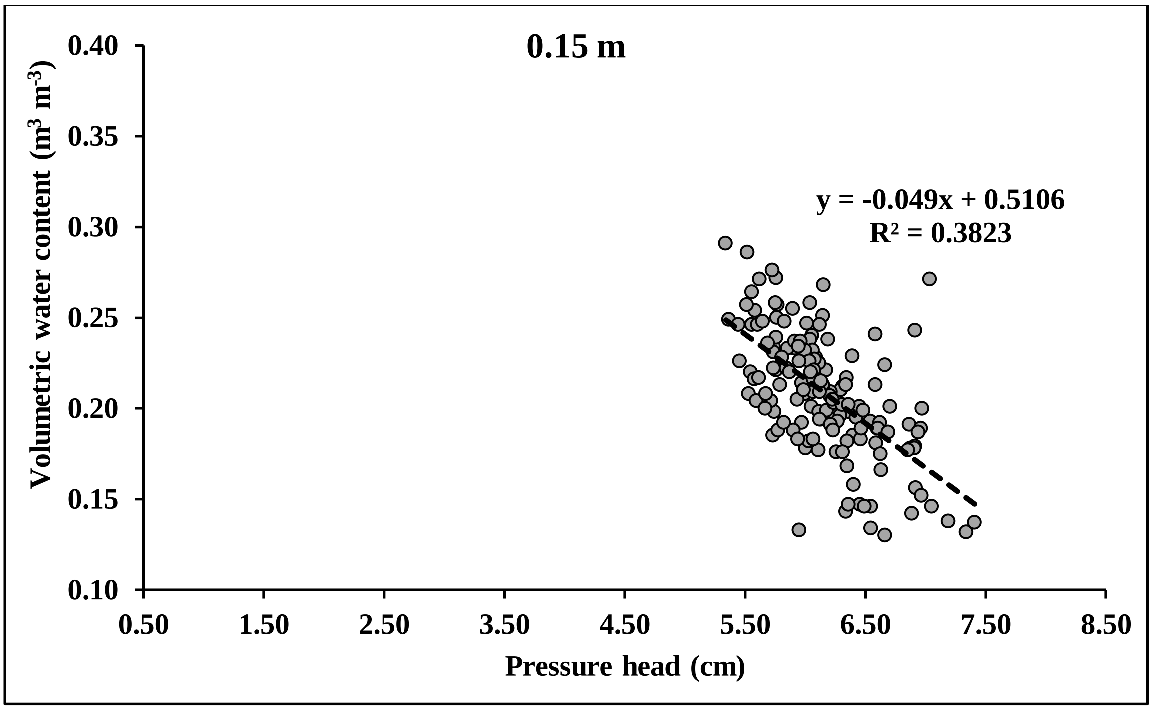
These measurements were then used to calculate the dry bulk density for each of the four soil depths. The porosity was then calculated from the dry bulk density, where ρb is the soil bulk density (g cm^{-3}), i.e. the ratio of the dry mass of soil core (g) to the volume of each soil core (cm^3). Using a Microsoft Excel spreadsheet, calculations were done using porosity, bulk density and readings recorded from the burette to obtain the soil water content values for each of the four depths at the different applied pressures. Soil water retention curves were then determined, where values corresponded to the soil water content at saturation (i.e. porosity), field capacity and permanent wilting point were then obtained.

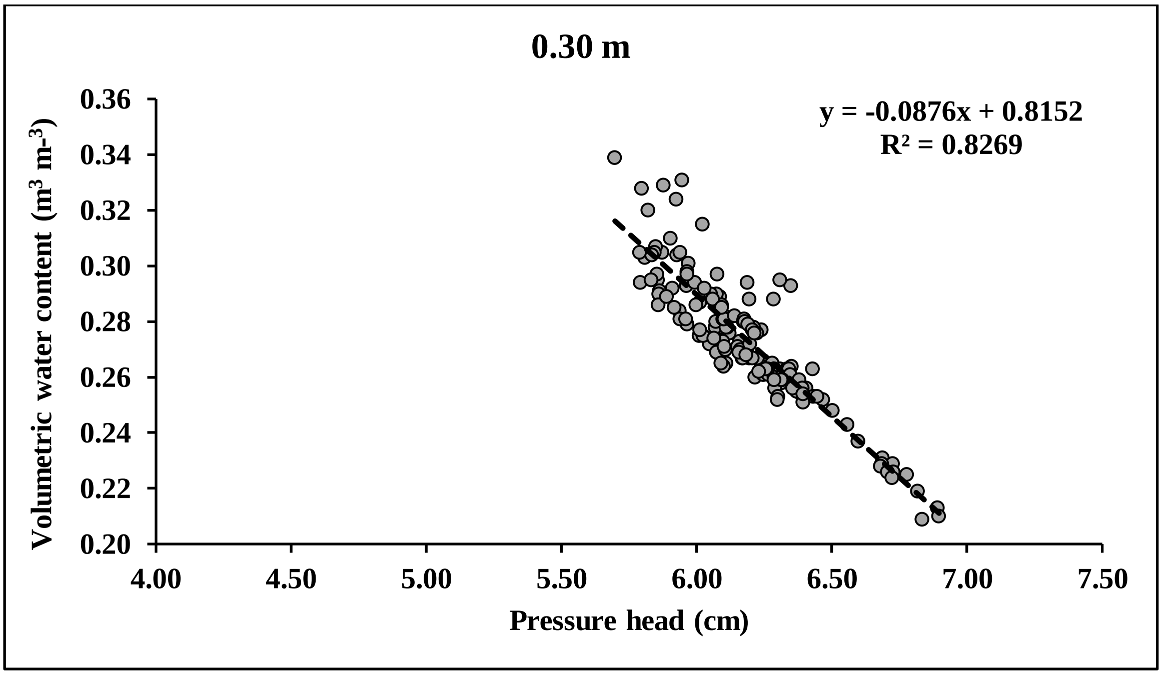


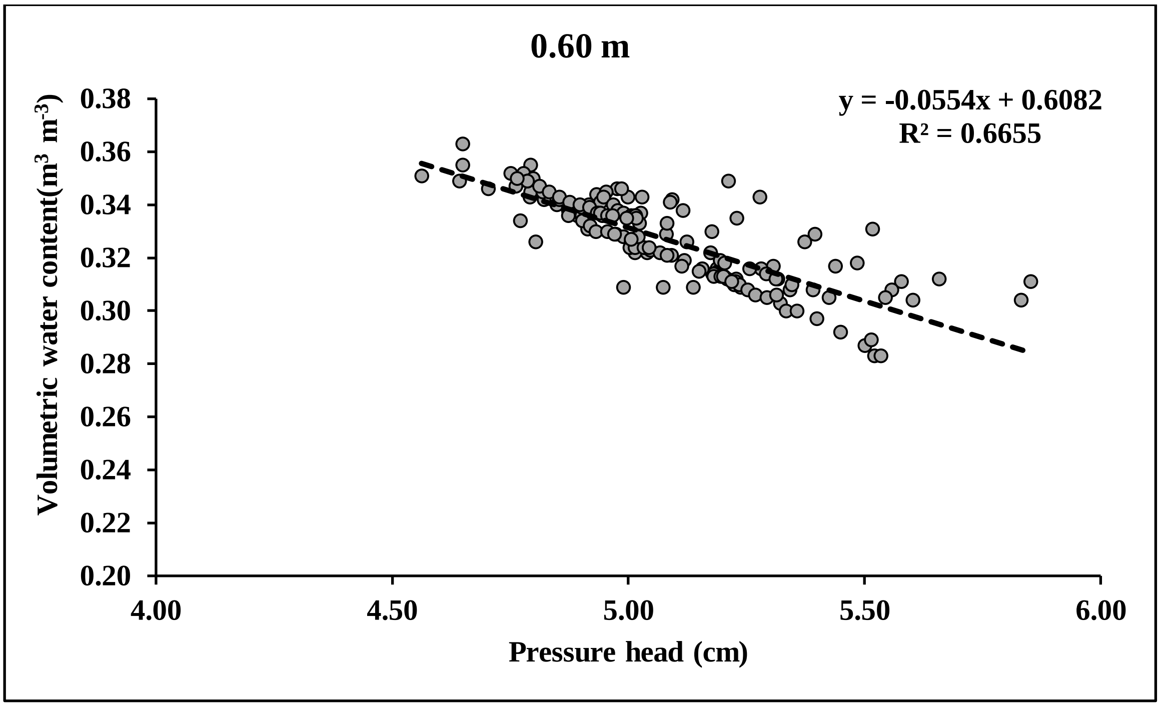
ID	DESCRIPTION	NO.	ID	DESCRIPTION	NO.
1	Pressure gauge	1	17	Retaining bolt, washers and nut	6
2	Low pressure regulator	1	18	Retaining wing nut and washer	6
3	6mm hydraulic hose	1	19	Pressure cell outlet port	1
4	T-piece - 6mm hydraulic hose	3	20	Outlet assembly O-ring	2
5	Ball valve - 6mm hydraulic hose	2	21	Outlet assembly retaining cap	2
6	Hose to glass fitting	2	22	Outlet assembly glass ports and T	1
7	Glass water manometer +- 75cm	1	23	Outlet assembly vacuum stop-cock	1
8	Glass mercury manometer 2 bar	1	24	Outlet assembly burette (50ml)	1
9	Manometer board 120x160cm	1	25	Pressure transducer hydraulic hose	1
10	Cap hydraulic hose connector	1	26	Differential pressure transducer	1
11	Pressure cell cap	1	27	Voltage regulator 6V and distribution	1
12	Pressure cell housing cylinder	1	28	16JR logger and distribution boards	1
13	Pressure cell base	1	29	Support clamps	4
14	Pressure cell retaining ring	1	30		
15	Pressure cell ceramic and ring	1	31		
16	Pressure cell O-ring	4	32		

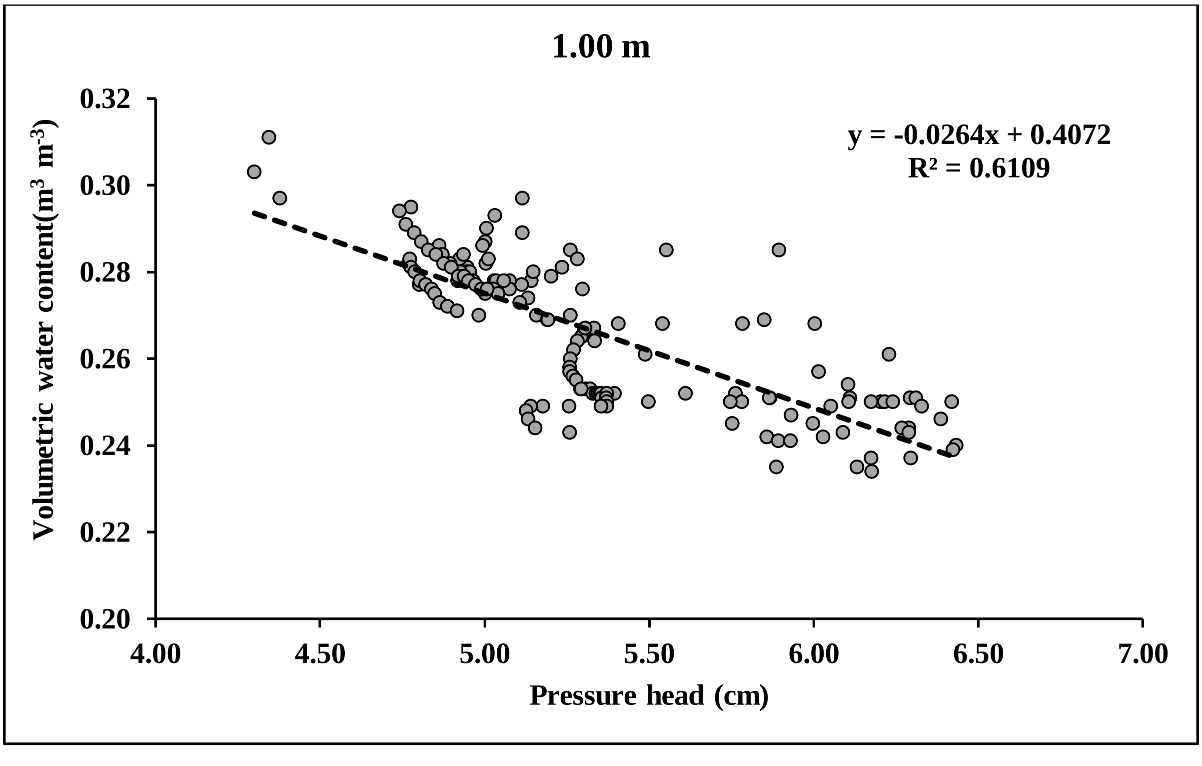
Figure 9.2: Diagram of the structure of the controlled outflow method (source Lorentz *et al*., 2001)

9.3 Appendix 3: Regression curves obtained from the relationship between CS650 volumetric water content ($m^3\ m^{-3}$) and Watermark matric potential (kPa) at four soil depths (i.e. 0.15, 0.30, 0.60 and 1.00 m)

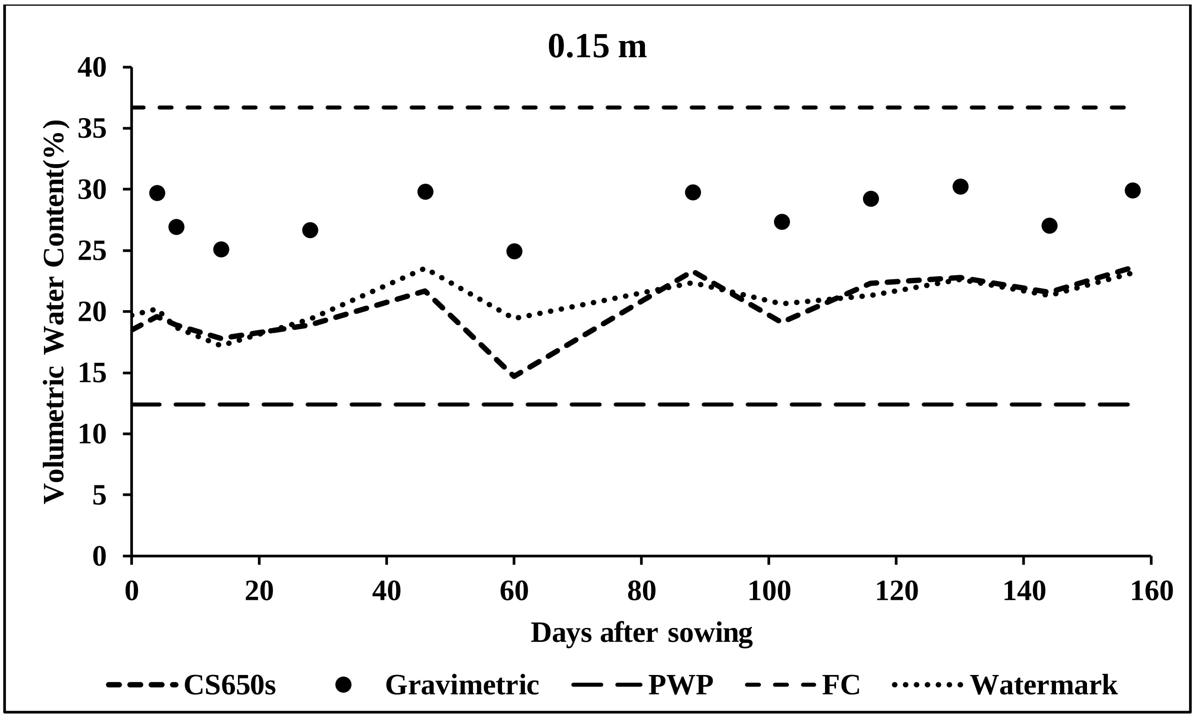


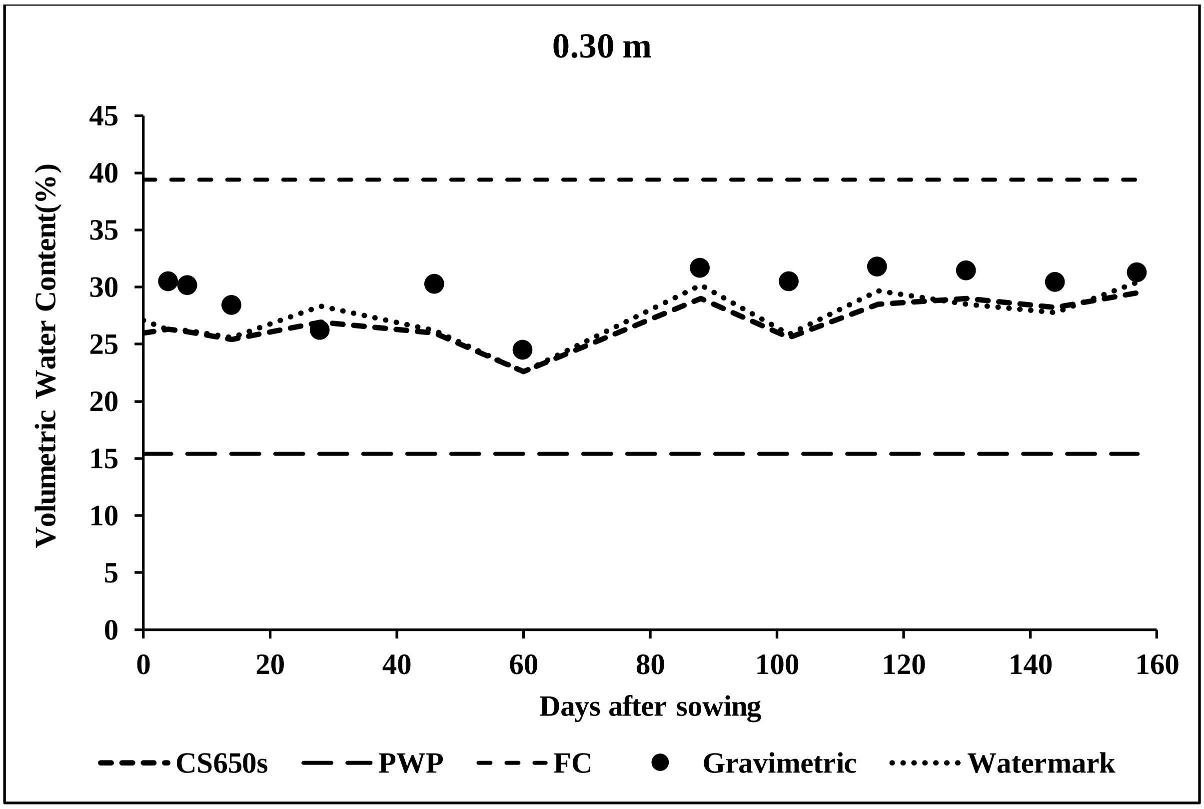


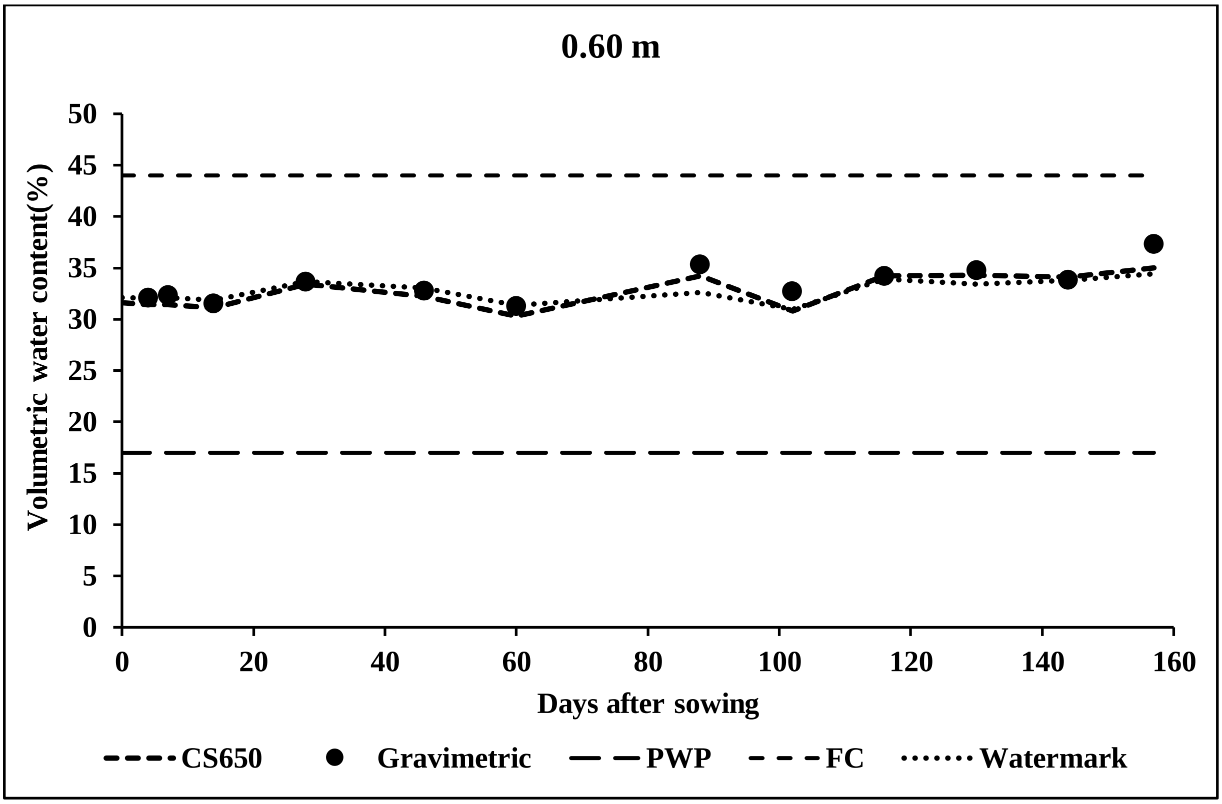

0.60 m
y = -0.0554x + 0.6082
R² = 0.6655
Volumetric water content(m3 m-3)
0.38
0.36
0.34
0.32
0.30
0.28
0.26
0.24
0.22
0.20
4.00
4.50
5.00
5.50
6.00
Pressure head (cm)

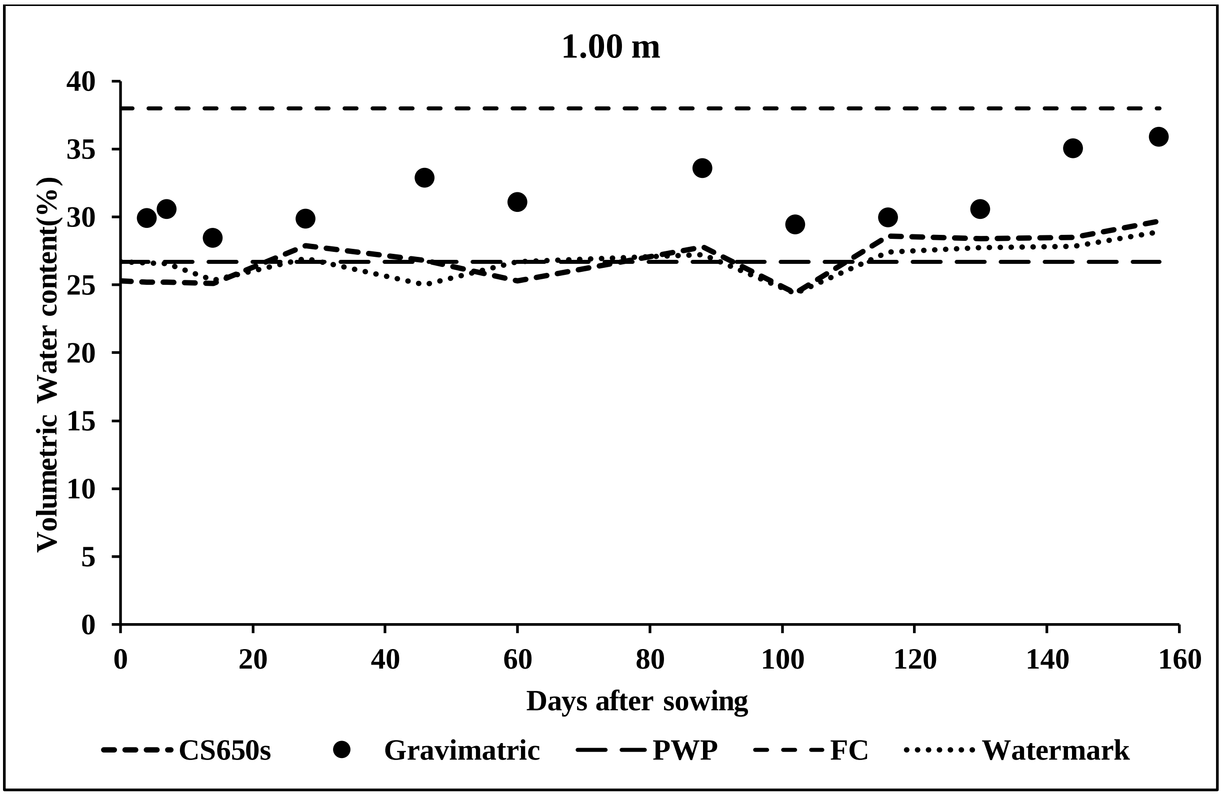

1.00 m
y = -0.0264x + 0.4072
R² = 0.6109
Volumetric water content(m3 m-3)
0.32
0.30
0.28
0.26
0.24
0.22
0.20
4.00
4.50
5.00
5.50
6.00
6.50
7.00
Pressure head (cm)

9.4 **Appendix 4:** Comparison of soil water content between gravimetric, CS650s and Watermarks at the various soil depths (i.e. 0.15, 0.30, 0.60 and 1.00) (PWP error at 1. 00 m soil depth)






0.60 m
Volumetric water content(%)
Days after sowing
CS650
Gravimetric
PWP
FC
Watermark


1.00 m
Volumetric Water content(%)
Days after sowing
CS650s
Gravimatric
PWP
FC
Watermark

9.5 **Appendix 5**: Image of the soybean trial at Swayimane during the 2018/9 season