Influence of elevated CO_2 on the growth, yield and photosynthesis of sugarcane

C Malan

22775366

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Supervisor:	Dr JM Berner
Co-supervisor:	Dr PDR van Heerden
Assistant Supervisor:	Dr A Eksteen

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ABSTRACT

Elevated CO_2 levels could possibly increase the water use efficiency of important agricultural crops. The world´s most productive crops are C_4 plants, for example, maize, sugarcane, sorghum and amaranth. C_4 plants are generally less affected by elevated CO_2 conditions than C_3 plants due to the differing internal CO_2 concentrating mechanism. It's been theorized that C_4 species evolved in an environment with a high CO_2 concentration. This would increase the water use efficiency of the plants when compared to C_3 plants. Various authors suggested that an elevated CO_2 environment reduces the stomatal conductance of plants thereby delaying the effect of water deficit. This will in turn stimulate the biomass yield via stress avoidance. The objective of this study was to determine the direct effects of elevated CO_2 on the physiology, growth, sugar production and yield of two sugarcane varieties. Speedlings of varieties NCo376 and N31 were grown in open-top chambers. The CO_2 levels were controlled at 400 ppm (ambient) and 750 ppm (elevated) in 12 open-top chambers for a period of seven months. Soil water deficit conditions were avoided through irrigating frequently. The effects of CO_2 treatment on photosynthesis, stomatal conductance, chlorophyll *a* fluorescence, biomass and stalk sucrose content was determined. Different varietal responses were observed during the trial. A reduction of 40% for N31 and 30% for NCo376 in stomatal conductance was observed. In spite of this, the increased CO_2 conditions did not have an effect on the sugar production, cane quality, green leaf area and dry biomass. The elevated CO_2 treated plants also had a higher fluorescence intensity than the control plants during the vegetative growth stages, therefore indicating that the sugarcane was responsive to the elevated (750 ppm) CO_2 during this period. However, no effect on the rate of photosynthesis could be demonstrated. Overall it can be concluded that elevated CO_2 conditions in the absence of soil water deficit lowered the stomatal conductance in sugarcane, but no changes, positive or negative were observed in the biomass and sugar yield.

Key terms: Biomass yield, chlorophyll *a* fluorescence, elevated CO_2, photosynthetic efficiency, plant growth, stomatal conductance, sugarcane

OPSOMMING

Die toename in atmosferiese CO_2 vlakke kan verskillende uitwerkings op verskillende gewasse hê. Oor die algemeen word dit aanvaar dat C_4 plante minder beïnvloed word deur verhoogde CO_2 vlakke as C_3 plante, weens die gespesialiseerde CO_2-konsentrasie meganismes van C_4 plante. Van die wêreld se mees produktiefste gewasse is C_4 plante, byvoorbeeld, mielies, suikerriet, sorghum en amaranthus. Die voordeel van verhoogde CO_2 vlakke is dat dit heelwaarskynlik die water gebruik effektiwiteit verbeter van plante. Verskeie outeurs het al voorgestel dat 'n verhoogde CO_2 omgewing die huidmondjie geleiding van plante verlaag en sodoende die onnodige verlies van water uit die plant verminder. Die verminderde water verlies tesame met die hoër interne CO_2 konsentrasie kan lei tot 'n toename in biomassa en opbrengs. Die doel van hierdie studie was om die direkte invloed van verhoogde CO_2 op die fisiologie, suikerproduksie en opbrengs van twee suikerriet variëteite te bepaal. Saailinge van die NCo376 en N31 variëteite was in ooptopkamers ("open-top chambers") gegroei. Die suikerriet was begas teen 400 dpm (kontrole) en 750 dpm (verhoogde CO_2 vlak) CO_2 vir 'n tydperk van sewe maande. Die suikerriet was gereeld besproei om sodoende enige water tekorte te verhoed. Die invloed van die verhoogde CO_2 vlakke op fotosintese, huidmondjie- geleidingsvermoë, chlorofil *a* fluoressensie, biomassa en sukrose inhoud was bepaal. 'n Verhoogde CO_2 omgewing het gelei tot 'n verlaging in die huidmondjie-geleidingsvermoë van 40% vir N31 en 30% vir NCo376. Ten spyte hiervan het die verhoogde CO_2 vlakke nie 'n invloed op suikerproduksie, die rietkwaliteit, die groen blaaroppervlakte en die droë biomassa gehad nie. Die plante wat aan die verhoogde CO_2 vlakke blootgestel was, het ook 'n hoër fluoressensie intensiteit gehad in vergelyking met die kontrole, maar geen effek was waargeneem op die tempo van fotosintese nie. Hierdie studie het bevind dat die verhoogde CO_2 vlakke, in die afwesigheid van enige grondvog tekorte, die huidmondjie-geleidings vermoë verlaag het, maar geen toename in die biomassa of sukroseinhoud was waargeneem nie.

Sleutelwoorde: Biomassa opbrengs, chlorofil *a* fluoressensie, fotosintetiese doeltreffendheid, huidmondjie geleiding, plantegroei, suikerriet, verhoogde CO_2

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List of abbreviations

3-PGA- 3-phosphoglycerate

ABA- Abscisic acid

ADP- Adinosine- diphosphate

A_n- Assimilation rate

ARC- Agricultural Research Council

ATP- Adinosine- triphosphate

CO_2- Carbon dioxide

CAM- Crassulacean acid metabolism

C_i- Internal CO_2 concentration

dpm- deeltjies per miljoen

E- Transpiration rate

FAS- Fertilizer Advisory Service

G_s- Stomatal conductance

Mt- Megatons

NADPH- Nicotinamide adenine dinucleotide phosphate

NUE- Nitrogen use efficiency

NUpE- The ability of the plant to take up the nitrogen supplied

NUtE- The ability of the plant to assimilate and remobilise the N taken up

NWU- North West University

OTC- Open-top chamber

OCE- Oxygen evolving complex

PAR- Photosynthetic active radiation

PCR- Photosynthetic carbon reduction

PEP- Phosphoenolpyruvate

PEPC- Phosphoenolpyruvate carboxylase

PPDK- Pyruvate orthophosphate dikinase

ppm- Parts per million

PS- Photosystem

PSI- Photosystem one

PSII- Photosystem two

PVC- Polyvinyl chloride

Q_A^-- Quinone A

Q_B^-- Quinone B

RHMax- Maximum relative humidity

RHMin- Minimum relative humidity

Rubisco- Ribulose-1,5- bisphosphate carboxylase / oxygenase

SASRI- South African Sugarcane Research Institute

SPAD- Soil plant analysis development

TDM- Total dry plant biomass

TMax- Maximum temperature

TMin- Minimum temperature

TVD- Top visible dewlap

UTP- Uridine triphosphate

UV- Ultraviolet

WUE- Water use efficiency

CHAPTER 1 INTRODUCTION

1.1 General introduction

At present there are various arguments describing what the effect of global warming would be on the earth's environment. One such argument made by those denying man-made global warming, is that elevated levels of atmospheric CO_2, which generally is caused by activities such as deforestation and the burning of fossil fuels (Aljazairi & Nogués, 2015) is actually beneficial for the environment. This argument is based on the logic that if CO_2 is needed for the growth of plants, then more of it would be beneficial and that crops could be expected to become more productive.

The "more is better" viewpoint opposes the way things work in nature. If crops are exposed to too much of a specific element that is considered good for their growth, this could in fact lead to the reverse of the expected outcome. Under controlled environments, however, it is possible to increase the growth of plants with elevated levels of CO_2. Nevertheless once one substance is increased, the requirements for the other substances should also be increased (Taub, 2010). For example, more carbon is made available to the plants when exposed to elevated CO_2 levels, but the levels of other resources such as minerals or water which are found in the soil are not increased (Taub, 2010). Increased photosynthesis and growth as a response to elevated CO_2 could therefore be limited under conditions of low water and mineral availability. CO_2 alone cannot sustain plants and they obtain the bulk of their substances from water and organic or inorganic material. It is easy to increase the water and fertilizer requirements in a controlled environment, but not in large scale field operations where the crop requirements are less controlled.

Elevated levels of atmospheric CO_2 will undoubtedly have a direct effect on the chemistry, development, metabolism and photosynthesis of plants, independent of any climatic changes (de Souza *et al*., 2008; Ziska, 2008; Taub, 2010; Bourgault *et al*., 2016). As photosynthetic organisms, plants have the ability to chemically reduce carbon as they take up CO_2. Not only does it provide the plant with stored chemical energy, but it also supplies the carbon skeletons for the organic molecules that create the structure of a plant. The overall oxygen, hydrogen and carbon assimilated during photosynthesis is responsible for ± 96% of the total dry biomass of a plant (Marschner, 1995). Therefore photosynthesis can be described as the heart of the nutritional metabolism of a plant and by enhancing the CO_2 that is available for photosynthesis, immense changes in the physiology and growth of plants would be expected (Taub, 2010).

The most common effect of elevated CO_2 on plants is an increase in the rates of photosynthesis and reduced stomatal conductance (Ainsworth & Rogers, 2007; Bourgault *et al*., 2016).

Ainsworth and Rogers (2007) found that the photosynthetic rates of the leaves of a variety of plants (for example, sorghum and maize) can be increased with an average of 40% when exposed to CO_2 concentrations between 475-600 ppm (parts per million). CO_2 is also responsible for the regulation of the stomata through which gasses are exchanged with the environment. The open stomata will allow CO_2 to diffuse into the leaves for photosynthesis and at the same time it provides a passageway for water vapour to diffuse out of the leaves. In order to maintain low rates of water loss and high photosynthetic rates, the plants need to regulate the opening and closure of the stomata (Taub, 2010). In the presence of elevated levels of CO_2 plants tend to regulate the internal CO_2 concentration (C_i) in the substomatal cavity by increasing the closure of the stomata (reducing stomatal conductance), thereby decreasing the loss of water. Hence, it would be expected that the overall water usage by the plant will decrease. In addition the extent of the general effect of CO_2 will rely on how it affects other water using components, for example, leaf temperature, plant morphology and size (Taub, 2010). Decreased water usages by plants could in itself have an effect on the hydrological cycle of ecosystems as both water runoff and the soil moisture levels would increase under elevated CO_2 conditions (Leakey *et al*., 2009).

Given that stomatal conductance and photosynthesis are crucial to the carbon and water relations of plants, various secondary effects on the physiology of plants may be seen under elevated CO_2. The chemical composition of the plant tissue can be altered by elevated levels of CO_2. The leaf non-structural carbohydrates (sugars and starches) will become more abundant per unit leaf area due to the increased photosynthetic rates (Ainsworth & Long, 2005; Ainsworth, 2008; Taub, 2010). Other elements such as nitrogen tend to decrease under elevated CO_2 conditions (Aljazairi & Nogués, 2015). This decrease in nitrogen can be explained by several factors including the decreased uptake of minerals from the soil due to lower stomatal conductance, the dilution of nitrogen from increased carbohydrate concentrations and plants taking up less water (Taub & Wang, 2008). Lastly it can be due to a decreased assimilation rate of nitrate into natural compounds (Bloom *et al*., 2010).

The nitrogen status of a plant is also closely related to the protein concentrations in plant tissues. Therefore changes in the nitrogen status will likely have crucial effects on species present in the higher trophic levels. Plant tissue tends to have a lower protein content when cultivated in elevated levels of CO_2 (Zvereva & Kozlov, 2006). It is likely that insect herbivores may consume more plant tissue in order to compensate for the reduced food quality (Stiling & Cornelissen, 2007; Taub, 2010). The protein concentrations of wheat, rice, barley and potato tubers, decreased by 5–14% when grown in experiments associated with elevated CO_2 (Taub *et al*., 2008). Minerals such as calcium, phosphorus and magnesium may also be reduced due to elevated CO_2 (Loladze, 2002; Taub & Wang, 2008).

Another important factor is that different plant species will respond differently to elevated levels of CO_2. It is evident that the atmospheric CO_2 concentration is increasing. Since 1958, the CO_2 concentration has risen, globally, by approximately 40 ppm (Madan *et al*., 2014). Many important agricultural crops will be affected significantly, ecologically and economically by these changes and the effects of elevated CO_2 would also vary from one plant species to another. The photosynthetic type is one of the most crucial determining factors of species variances in response to elevated CO_2. The majority of plant species use a photosynthetic pathway known as C_3 photosynthesis. Other species would either use the CAM (crassulacean acid metabolism) pathway or the C_4 photosynthetic pathway. Tropical and sub-tropical grasses, including several important crops for example, maize, sugarcane and sorghum, make use of the C_4 process (Reddy *et al*., 2010).

Various authors assumed that C_4 photosynthesis was saturated at current atmospheric CO_2 levels and that it would be less affected by increased levels of CO_2 (Ziska & Bunce., 1997; Ziska *et al*., 1999). There are clear functional and anatomical differences between C_3 and C_4 plants. For example, photosynthesis occurs in both the mesophyll cells and the bundle sheath cells in C_4 plants. This would allow C_4 plants to reach maximum rates of photosynthesis at current ambient CO_2 levels (Ghannoum *et al*., 2000). Due to the high CO_2 concentration that is already present within the bundle sheath cells, any increases in the atmospheric CO_2 concentrations above the current levels will have minor effects on the photosynthetic rates of C_4 species. This assumption, however, has been contradicted during the last decade. Many authors concluded that the photosynthetic rate of C_4 plants can also be raised by elevated CO_2 concentrations, therefore implying that the differences between C_3 and C_4 species are not as pronounced (Ainsworth & Rogers, 2007). In addition, C_4 species will respond directly to elevated CO_2 by decreasing stomatal conductance. This could lead to some indirect maintenance of photosynthesis through avoiding water stress in the presence of drought conditions (Leakey, *et al.* 2009).

In contrast to C_4 species, C_3 species may be particularly capable of responding to elevated CO_2 (Rogers *et al*., 2009). Photosynthesis only occurs in the mesophyll cells in C_3 plants (Ghannoum *et al*., 2000). Under elevated CO_2, an alteration in the internal balance between the carbon obtained via enhanced photosynthesis and nitrogen can occur (for most plants). Compared to other plant species, C_3 species show greater enhancement of photosynthesis and growth in response to elevated CO_2 (Rogers *et al*., 2009). Decreases in tissue nitrogen concentrations of C_3 species are also smaller under elevated CO_2 (Jablonski, *et al*. 2002; Taub, *et al*. 2008).

One of the world's most important food-producing C_4 crops is sugarcane. It has a long history for the production of energy, food, and co-products. It also provides ± 75% of the sugar used for human consumption (de Souza *et al*., 2008). Sugarcane grows across a vast region and can be

found in both tropical and subtropical regions, implying that sugarcane has a significant global footprint (Moore *et al*., 2014). During the 2013–2014 crop year in Brazil, roughly nine million hectares of sugarcane produced 659 megatonnes (Mt) of harvested cane and 38 Mt of sugar (Marin *et al*., 2016). The biggest producers of sugarcane are Brazil, India, China and Thailand (Jonker *et al*., 2016). The high photosynthetic efficiency and high biomass production makes sugarcane an ideal crop for both food production and the co-production of non-fossil based chemicals and energy products. Sugarcane has also become widely known for its use in the production of energy and ethanol as biofuel (Pierre *et al*., 2014). The production of ethanol from sugarcane in other countries, for example Brazil, was 24 billion litres in 2012-2013 (Jonker *et al*., 2016).

1.1.1 Problem statement

The effects of elevated CO_2 on the growth, physiology and metabolism of plants are characterized well, but the theoretical expectations are sometimes not met by experimental data (Ainsworth & Rogers, 2007). For example, the photosynthetic stimulation and reduced stomatal conductance observed in CO_2 rich environments may be variable and subjected to environmental changes (Ainsworth & Rogers, 2007). Vu *et al*. (2006) found significant changes concerning the chlorophyll content, protein, sucrose metabolism, photosynthetic enzyme activities and gas exchange when sugarcane is exposed to elevated CO_2 levels under well-watered conditions. However, contrasting reports do exist wherein the level of photosynthetic stimulation differs. For example, in some studies sugarcane varieties subjected to double ambient CO_2 levels led to a 35% stimulation in photosynthesis whereas other studies obtained results of 10% or less (Vu *et al*., 2009, de Souza *et al*., 2008). Stokes *et al*. (2016) however, found no significant, direct stimulation of total biomass and photosynthetic rate in sugarcane. These differences could not yet be explained and as a result more questions are left unanswered as to whether elevated CO_2 would increase the biomass yield and sucrose production in sugarcane.

1.1.2 The aim of this study

Sugarcane is an important source of renewable energy, thus it is important to know how it will respond to elevated CO_2 concentrations. The aim of this study therefore, was to determine the direct benefits of elevated CO_2 concentrations on two contrasting sugarcane varieties.

1.1.3 Objectives

The objectives of this study were:

- To determine the physiological responses of sugarcane to elevated CO_2,

- To assess the effect of elevated CO_2 levels on the growth of sugarcane,
- And lastly to investigate whether elevated levels of CO_2 will result in higher sugar production.

1.1.4 Hypothesis

Elevated CO_2 in the absence of any soil water deficit will increase the photosynthetic efficiency of NCo376 and N31 sugarcane varieties, which will result in growth stimulation and higher stalk sucrose content.

CHAPTER 2 LITERATURE REVIEW

2.1 Morphology and Development of Sugarcane

The morphological features of sugarcane (*Saccharum* spp. hybrids) are closely associated with the specialized ability to synthesize, transport and accumulate high concentrations of sucrose. By comparing these features it is believed that certain characteristics could be related to the high productivity of sugarcane (Rae *et al*., 2014).

2.1.1 Morphology

The morphology of sugarcane closely resembles that of the *Poaceae* family. The shoot contains a stalk consisting of nodes, internodes and an attached leaf per internode (Figure 2-1a) (Rae *et al*., 2014). Sugarcane can reproduce sexually from seed or asexually via lateral buds of seed-cane. In terms of agricultural production, sugarcane is produced primarily via asexual propagation where the stalk is divided into smaller segments (setts). The early growth of these setts are usually described as vigorous whilst the growth of seedlings are more cereal like (grasses, such as, maize, sorghum and wheat), but once the plants age further, no visual differences can be distinguished between the two methods of propagation (Rae *et al*., 2014).

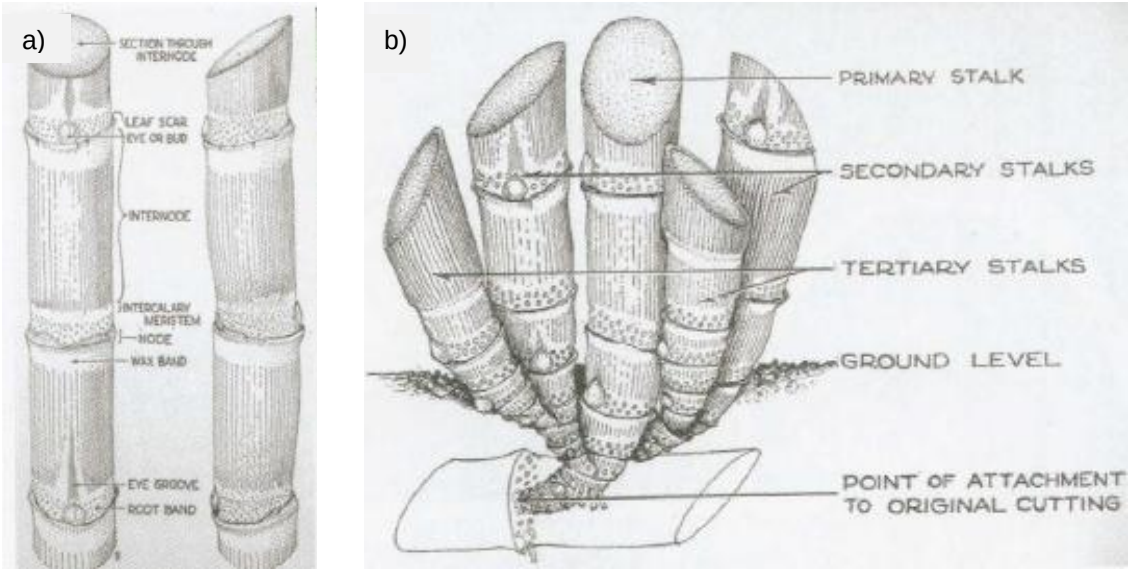


Figure 2-1: The morphology of sugarcane (a & b). a) The stalk, nodes and internodes. b) The primary, secondary and tertiary tillers (Rae *et al*., 2014).

Once setts are planted, the meristematic cells in the buds obtain a critical water content, specifically required for sprouting (van Dillewijn, 1952). After which the buds will sprout to form the primary stalks. The apical meristems are known to produce the primary stalks, whereas the secondary tillers or stalks are usually produced by the underground auxiliary buds found on the

main stem (Figure 2-1b) (Rae *et al*., 2014). This process is called tillering (van Dillewijn, 1952; Jones *et al*., 1990). Stalks can also develop from the lateral buds of seed-cane. Once the seed-cane is planted, a primary shoot may form from each bud.

The capacity of the plant to store sucrose is effected by the anatomy of the stalk, with specific regard to the internode volume. For example, plants that are grown at lower temperatures will usually store less sucrose due to decreased internode volumes (caused mainly by shorter internodes) (Rae *et al*., 2014).

The internodes may also display different colours depending on the climate that the plant is grown in and the specific variety that is being used (Rae *et al*., 2014). In this study two varieties were used. The stalks of N31 may exhibit a yellow to green colour with black and green patches and the internodes are usually long. N31 also has thin internodes compared to NCo376 which has medium to thick internodes (South African Sugarcane Research Institute, 2006a). The internodes of NCo376 are also yellow in colour, but once exposed to light they may change to a green colour (South African Sugarcane Research Institute, 2006b). Cracks are also commonly found on the stalks. Two types of cracks exists, the first being small and harmless which are confined to the epidermis and secondly growth cracks which are deep and harmful. Growth cracks increases water loss and it can host various diseases (Rae *et al*., 2014).

The leaf is characterised by a sheath and a blade which is separated by a blade joint (Rae *et al*., 2014). The developing leaves are tightly rolled around each other and they appear from the buds located at the nodes of a sugarcane stalk. Leaf appearance will begin with the formation of the leaf primordia, a miniature stem with its growing point and primordia of leaves and roots. A critical water content, however, is required in order to drive cell division (van Dillewijn, 1952; Scarpella *et al*., 2010). Cell division drives the appearance of the leaf, but once the shape of the leaf is established, cell expansion drives leaf growth (Scarpella *et al*., 2010). The maturation of the cells is described as basipetally, thus the cells found near the tip of the leaf will mature first, whilst the cells near the base are still in an immature stage. The leaves are alternately attached to the stalk depending on the nodes (Rae *et al*., 2014). The leaf blade of N31 is medium to narrow in width. The top of the midrib (N31) is white in colour whilst the underside may display a yellow colour (South African Sugarcane Research Institute, 2006a). N31 possesses no auricle (ear- shaped appendages located at the upper part of the sheath margins). The blade of NCo376 is medium in width. Chlorotic blotches can also be found on the lower surface of the midrib. Auricles are present on NCo376. The sheath covers the stalk completely by extending over an internode (South African Sugarcane Research Institute, 2006b). Sugarcane leaves grow successively, senescing old mature leaves being replaced with new leaves higher up the stalk.

The root system will develop soon after planting the seed or setts or in the ratoon (emerging crop following harvest). Once again a certain critical water content needs to be attained before they can sprout (van Dillewijn, 1952). As soon as these conditions are met, the root primordia around the nodes of setts will start producing thin and highly branched sett roots (Smith *et al*., 2005). A sequence of root types can be characterized by their origin (Bonnett, 2014). When a seed is planted, the primary root will develop from the embryo after a few days of germination, but when a sett is planted, the root primordia around the base of the internode is seen as the initial source of roots and not the buds on the stalks (Smith *et al*., 2005; Bonnett, 2014; Rae *et al*., 2014).

Two different kinds of roots can be distinguished. The first roots to emerge are the sett roots. Sett roots are thin and branched which will sustain the growing plant until the new shoots develop sufficiently to produce the shoot root system. The lifespan of the sett roots grown in pots vary between varieties (Bonnett, 2014). The sett roots will usually grow for six to 15 days, thereafter it will senesce and disappear by 60 to 90 days (Smith *et al*., 2005,). The shoot roots are also formed from the root primordia (from the lower, relatively unexpanded internodes) and develop into the main root system of the plant. Shoot roots are much thicker and fleshier than sett roots and they are not as branched as the sett roots (Smith *et al*., 2005; Bonnett, 2014; Rae *et al*., 2014).

The root system has the ability to remain active for several months after harvesting the crop, therefore contributing to the growth of the ratoon crop (Bonnett, 2014). The ratoon crop will continue to grow indefinitely. A new shoot root system emerges or new shoots develop from the ratoon crop (Bonnett, 2014). Sugarcane is known to produce numerous tillers and each new tiller that is formed has the ability to produce its own sett roots followed by its own shoot roots. The continuous production of roots is of great importance, because it helps the plant to adjust to the changing conditions (Smith *et al*., 2005).

Various factors have an influence on the development of the root system. For example, the temperature at which the plants are grown can either induce or cease root growth. Root growth is induced at 15°C and inhibited at 10°C (Bonnett, 2014). The availability of water also has an influence on the growth pattern of the developed root system at different depths of soil during the root development (de Silva *et al*., 2011).

2.1.2 Development

The development of the sugarcane plant is described by four phases known as the germination phase, the tillering phase, the grand growth phase and lastly the ripening (maturation) phase (Ramesh, 2000; Silva *et al*., 2008). Germination and tillering is of great importance as they form

the foundation of a good crop (Bonnett, 2014). Good germination will assemble the base of an acceptable stand of crop and adequate tillering will in turn provide the crop with the suitable number of stalks required for a good yield (Bonnett, 2014). The formative phase (known as the tillering and grand growth phases) has been described as the critical demand period for water, due to the fact that 70-80% of the cane yield is produced (Silva *et al*., 2008; Ramesh, 2000). During this time it is of great importance to avoid any water stress.

The germination of sugarcane buds and seeds are influenced by both internal and external factors (Bonnett, 2014). The external factors include soil fertility, soil moisture, aeration and soil temperature (Smit, 2009; Bonnett, 2014). The internal factors are the sett moisture, the bud health, the sett nutrient status and the sett reducing sugar content. Germination induces the activation and the consequent development of the vegetative bud (Bonnett, 2014).

According to Smit (2009) the optimum temperature for the development of the bud is around 30°C and 35°C, while the base temperatures (the temperature at which plant growth is zero) are between 16°C and 18°C. On the other hand Pierre *et al*. (2014) found that the optimum temperatures for seed germination ranged from 27°C to 30°C, while the base temperatures for germination ranged between 11.2°C and 16.4°C. It is important to note that the optimum and minimum temperatures would vary between the various sugarcane varieties. If the temperature and humidity is too high during the germination of the seed, it is possible that burn type lesions can develop on the seedlings (Caieiro *et al.,* 2010). The availability of water will also have an influence on the germination of the bud or seed. A low water potential will slow the process of germination down, due to fact that the seed will have a difficulty extracting water from its surroundings (Pierre *et al*., 2014). This in turn will influence the process of germination greatly (Pierre *et al*., 2014).

Tillering refers to the ability of a single seedling to produce multiple side shoots from the auxiliary buds, therefore contributing to the number of millable stalks in sugarcane (Vasantha, *et al*., 2010; Bonnett, 2014). The tillering process determines the field stand along with the maximum millable cane at harvest and is regulated by a few factors including hormones, the nature of the varieties and the specific environment (Vasantha *et al*., 2010). The rate of tiller initiation is dependent on different light and temperature conditions (Bonnett, 2014), different varieties (Singels *et al*., 2005), crop age (Smit, 2009) and row spacing (Smit & Singels, 2006).

The specific time at which the tillers are produced will determine the percentage of the tillers that would die due to senescence (Vasantha *et al*., 2010). It should be noted that the tillers that are produced two to three months after planting usually do not contribute to the millable harvest (Prochazkova & Wilhelmova, 2007; Vasantha *et al*., 2010) and up to 50% of these stalks senesce. These tillers are different from the tillers that are produced early in the growth season

as they have a low sucrose content, which would lead to a reduction in the overall sucrose yield at harvest (Bonnett, 2014). These tillers would senesce due to the shading of young stalks by older stalks in competition for light (van Dillewijn, 1952; Jones *et al*., 1990; Inman- Bamber, 1994) or due to increased levels of ABA (abscisic acid) which initiates the premature senescence process (Vasantha *et al*., 2010).

The grand growth phase is an important component of yield development. The grand growth phase can be described as the process wherein stem elongation occurs and the intercalary meristem produces cells that subsequently expands (Bonnett, 2014). The internode starts to expand, once the leaf is attached at the base and fully unfolded (van Dillewijn, 1952). By the time the four next youngest leaves are fully unfolded the elongation is completed at the individual cell and internode level. The expansion of the cells and the elongation of the leaves are largely dependent on the plant water relations (hydraulic and biochemically) as well as on the temperature. Inman-Bamber *et al*., (2008) found that the expansion rate for high sucrose varieties was slower than for the varieties with low sucrose content. High sucrose varieties tend to divert more photo-assimilates to sucrose storage than the varieties with lower sucrose content under water stressed conditions (Bonnett, 2014). The expansion rate of the plants will also respond strongly to changes in the temperature when subjected to water deficit conditions. Internodes would cease expansion once the sugarcane is exposed to lower temperatures and reduced water availability (Bonnett, 2014). It was found that expansion rate of the internodes followed temperatures between 14° and 25°C very closely, while the rate of expansion dropped during higher temperatures or during the hottest part of the day (Bonnett, 2014). Therefore it was concluded that the base temperature should be between 16° and 18°C and that high temperatures exceeding 36°C can inversely decrease the expansion rate of the internodes (Bonnett, 2014).

The primary goal of international efforts is to increase sugarcane yield through increased sucrose content. In the case of sugarcane the stalk is the harvestable organ that contains the sugar. The structural development of the stalk occurs during stem elongation, but the accumulation of the sucrose occurs in the lower internodes while the internodes at the top of the stalk continue to expand (Bonnett, 2014). Sugarcane accumulates extraordinary quantities of sucrose. Sucrose consists of glucose and fructose subunits and is a soluble disaccharide. Sucrose is found in the translocation stream (phloem) and is the most common form of sugar (Hopkins & Hüner, 2009). In sugarcane sucrose is stored in the vacuoles of specialized storage cells. Sucrose is synthesized in the cytosol of photosynthetic cells, during which the enzyme sucrose phosphate synthase and sucrose phosphate phosphatase and sucrose synthase is provided (Rolland *et al*., 2002). This reaction does not occur spontaneously and therefore

glucose is required with the nucleotide uridine triphosphate (UTP) rather than ATP (adinosine triphosphate) in order to activate this reaction (Rolland *et al*., 2002; Hopkins & Hüner, 2009).

Elevated CO_2 will in general increase the net photosynthesis and biomass production of plants (Farrar & Williams, 1991). In contrast, C_4 plants are relatively unresponsive to elevated CO_2, in terms of biomass accumulation and carbohydrate partitioning, due to the CO_2-concentrating processes already present within the leaf (Farrar & Williams, 1991). In the long-term, increase in the carbon fixation observed for C_3 plants may not be maintained and the initial yield of CO_2-enriched plants may not mirror the initial photosynthetic response (Farrar & Williams, 1991).

There are numerous stages describing the partitioning of the photosynthetic products. In terms of a developing leaf, the photosynthetic assimilate is partitioned between exported material and further leaf growth or temporary storage within the leaf (Gifford & Evans. 1981). Assimilate exported is partitioned between different sinks, and within these sinks the incoming carbon is partitioned between different chemical constituents (Gifford & Evans. 1981). Sinks could be storage, elongation, or meristematic sinks. The characteristics of storage sinks depend on the type of product that is stored, for example, sucrose, starch, proteins, or lipids (Gifford & Evans. 1981).

In terms of controlled-environment studies, there is no well-defined trend that clearly states what the effect of CO_2 enrichment is on the distribution of dry matter between organs, with the exception of tubers, which become a bigger proportion of plant dry weight at higher levels of CO_2 (Lawlor & Mitchell, 1991). The increase in the proportion of total dry mass in tubers at increased CO_2 during field studies was confirmed for sweet potato and for carrot and radish, but, significant effect of elevated CO_2 in cotton and soybean was found (Lawlor & Mitchell, 1991). On the other hand, Chaudhuri, Kirkham & Kanemasu (1990) found a variable response in winter wheat to elevated CO_2, although it generally declined with CO_2 in water-stressed plants. Ackerson *et al*., (1984) found no significant changes in the partitioning of dry matter among the leaves, stems or pods due to CO_2 enrichment on soybean. Other studies involving sugarcane also found no significant increases in the partitioning of the photosynthetic products due to elevated CO_2 levels (Stokes *et al*,. 2016).

2.2 Mineral requirements of sugarcane

Understanding plant nutrition is of great importance for the implementation of sustainable nutrient management (Kingston, 2014). One of the main challenges for agriculture is to satisfy the rising need for food, energy and fibre, and at the same time maintaining the soil productivity (Gopalasundaram *et al*., 2012). Under intensive farming the nutrient turnover is significantly high and the nutrients are easily removed from the soil via plant uptake or soil erosion. In order to

restore the soil fertility, these nutrients need to be supplied effectively to the soil (Gopalasundaram *et al*., 2012).

Sugarcane produces high biomass yields and would consequently demand large amounts of moisture, sunlight and nutrients (Gopalasundaram *et al*., 2012). The amount of nutrients removed from the soil, will vary from soil to soil, as well as between different varieties. According to Gopalasundaram *et al*., (2012) an estimate of 0.56-1.20 kg of N, 0.38-0.82 kg of P_2O_5, 1.00-2.50 kg of K_2O, 0.25-0.60 kg of Ca, 0.20-0.35 kg of Mg, 0.02-0.20 kg of Na and 2.0-2.7 kg of SO_4 is removed from the soil for every ton of sugarcane produced. Large amounts of nutrients are removed from the soil due to the continuous cultivation of sugarcane and therefore a decline in sugarcane yield can be expected due to nutrient depletion (Kingston, 2014). Soil compaction, acidification, and changes in the biological components in the soil can also decrease sugarcane yield (Gopalasundaram *et al*., 2012; Kingston, 2014).

The nutrient requirement of sugarcane can be divided into macro nutrients and micro nutrients (Kingston, 2014). Three of the macro nutrients are nitrogen, phosphorus and potassium. These are the primary macro nutrients, while the secondary macro nutrients are sulphur, magnesium and calcium. The micro nutrients include, copper, zinc, iron, manganese, boron, molybdenum and chloride. Due to the focus point of this study, only the primary macro nutrients (N, P and K) will be discussed here.

Nitrogen (N) is an important contributor to the productivity of a farming system, ensuring optimum yields (Thorburn *et al*., 2011). Nitrogen influences both the quality and yield of sugarcane. It also increases the leaf area index, early canopy closure as well as the rate of photosynthesis (Gopalasundaram *et al*., 2012). Nitrogen plays an important role in the synthesis of nucleic acids, proteins (Kingston, 2014) and is a promoter of tillering and suckering (shoots that grow from the base or from adventitious buds in the roots). This will lead to an increase in sugarcane yield due to the increased number of tillers and yield attributes, for example, stalk length, stem diameter and the number of millable stalks (Gopalasundaram *et al*., 2012; Kingston, 2014). The amount of nitrogen applied depends on the soil type, crop duration and water availability in the various sugarcane-producing countries and can vary between 50 and 300 kg N ha^{-1} (Gopalasundaram *et al*., 2012). However, up to 65% of the supplied nitrogen is not used by the crop (Hajari *et al*., 2015). The nitrogen use efficiency (NUE) of plants is described as very complex and can be influenced by various factors, for example, the uptake of nitrogen from the soil, assimilation into amino acids that store nitrogen and the transport of nitrogen from source to sink tissue (Hajari *et al.*, 2015). Therefore there is a need to select plants that will utilize the nitrogen more efficiently. The NUE is usually expressed as the ratio of the total plant nitrogen biomass and the total nitrogen supplied. The NUE of plants can be distinguished into two sub-components, the first describing the ability of the plant to take up the

nitrogen supplied (NUpE) and the second which describes the ability of the plant to assimilate and remobilise the N taken up (NUtE) (Hajari *et al*., 2015). If the amount of nitrogen available for the plants decreases, various plant deficiency symptoms can be expected. For example, yellowing of the leaves, retarded growth, stalks with a smaller diameter and premature senescence of the older leaves (Gopalasundaram *et al*., 2012; Kingston, 2014). In the case of excess nitrogen application, sugarcane tends to become succulent and soft which will become more prone to pests and diseases (Gopalasundaram *et al*., 2012). The amount of sucrose stored in the stalk will also decrease (Kingston, 2014).

The availability of phosphorus (P) depends on the fixation of native and applied phosphorus. It leads to the hastened development of shoot roots and tiller production, stalk weight and stalk population (Gopalasundaram *et al*., 2012: Kingston, 2014). It plays a crucial part in cell division and heredity transfer (Kingston, 2014). Once optimum amounts of phosphorus are applied increases in the sugar content and purity of the juice can be expected (Gopalasundaram *et al*., 2012). The assimilation of carbon also depends on the assimilation of phosphorus and is required for energy-rich bonds, for example, ADP (Adinosine- diphosphate) and ATP (Kingston, 2014). Phosphorus also stimulates the maturation of crops (Kingston, 2014). Phosphorus deficiencies will usually result in reduced growth, reduced number of tillers and limited root development (Gopalasundaram *et al*., 2012). The leaves are narrow, thin and short and can turn dark-green to blue-green (Kingston, 2014). The excessive use of phosphorus might affect the uptake of other elements, for example, copper and zinc (Kingston, 2014).

Potassium (K) is one of the most essential elements and fulfils various important roles (Gopalasundaram *et al*., 2012). It regulates the uptake of water and influences the leaf stomatal opening and closing. It maintains the turgidity of cells and the formation of proline during moisture stress (Gopalasundaram *et al*., 2012; Kingston, 2014). Potassium also plays a major role in the synthesis and translocation of proteins and carbohydrates and in the accumulation of sucrose (Kingston, 2014). It increases stalk diameter, stalk volume and weight per stalk, drought and disease resistance and reduces lodging (Gopalasundaram *et al*., 2012). Deficiencies in potassium can be seen first in the older leaves and leaf margins due to its translocation to actively growing immature tissue (Gopalasundaram *et al*., 2012, Kingston, 2014). The tips of the leaves become brown with necrotic spots. Growth is reduced and the stalks are thin (Kingston, 2014). If potassium is present in excess amounts, problems may occur in the processing of sugar, for example it may increase the ash content in the cane juice and raw sugar which will reduce the recovery of sugar crystals in the factory (Kingston, 2014).

2.3 The difference between C_3 and C_4 photosynthesis

The PCR (Photosynthetic carbon reduction cycle) or Calvin-Benson cycle is used by all plants for the fixation of CO_2 (Fridlyand & Scheibe, 1999; Lara & Andreo, 2011; Sage *et al*., 2014). A three-carbon compound known as phosphoglycerate (3-PGA) is produced during this process (Figure 2-2), which is catalysed by Rubisco (Ribulose-1, 5-bisphosphate carboxylase/ oxygenase) and is therefore described as the C_3 cycle (Reddy *et al*., 2010). Plants using this photosynthetic pathway exclusively are named C_3 species. A common complication with the C_3 cycle is that Rubisco is used to catalyses two opposing reactions known as carboxylation and oxygenation (Portis & Parry, 2007). In terms of the oxygenation reaction the movement of carbon is directed through the photorespiratory pathway, which can cause losses as high as 30% of the carbon fixed (Long *et al*., 2006). High temperatures and drought are some of the environmental factors that can result in an increase in the oxygenase reaction (Lara & Andreo, 2011).

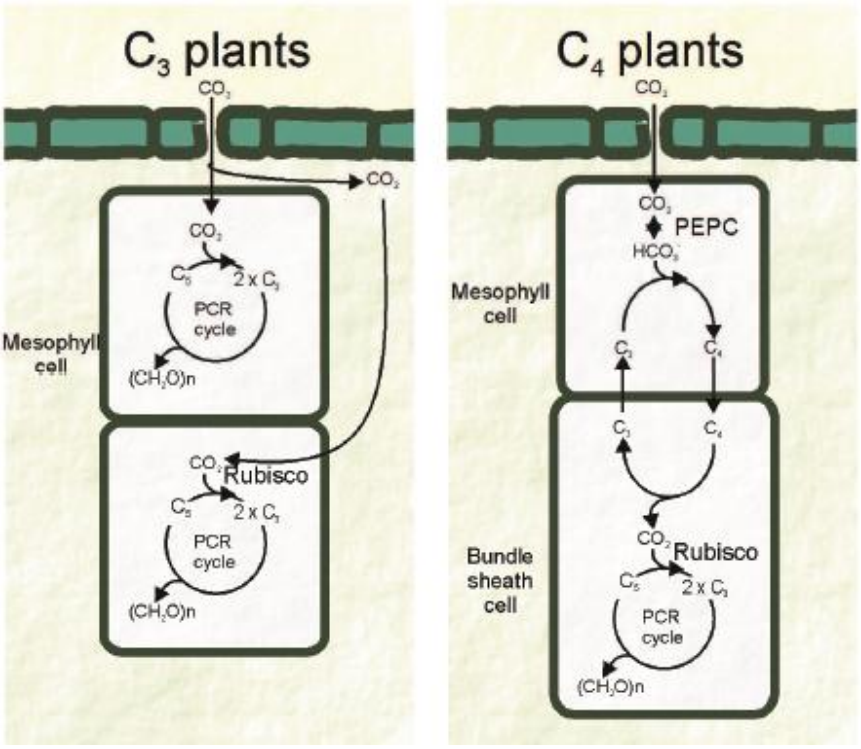


Figure 2-2: A representation of the carbon fixation pathways of C_3 and C_4 plants (Lara & Andreo, 2011).

The C_4 photosynthesis pathway overcomes the limitation of photorespiration by improving the photosynthetic efficiency and minimizing the water loss in warm and/or dry environments (Sage *et al*., 2014). It is often said that C_4 photosynthesis is an adaptation of the C_3 pathway from which C_4 species originated. Generally C_4 species are found in warmer climates than C_3 species (Lara & Andreo, 2005). The majority of C_4 plants can be found in the tropics and warm

temperate zones, where high temperatures and light intensities are present (Moore *et al*., 2014). C_4 plants, therefore exhibit higher photosynthetic and growth rates under these conditions due to the availability of more water, as well as the effective use of carbon and nitrogen (Lara & Andreo, 2005; Sage *et al*., 2014).

C_4 crops are described as some of the world´s most productive crops which include maize (*Zea mays*), sugarcane (*Saccharum* spp hybrids) and sorghum (*Sorghum bicolor*) (Lara & Andreo, 2011). Furthermore, some of the most troublesome weeds for example, nutgrass, barnyard and crabgrass, are also C_4 species. Although C_4 plants only represent a small portion of the world's plants species, they contribute about 20% to the global primary productivity because of highly productive C_4 grasslands (Ehleringer *et al*., 1997). The C_4 photosynthetic pathway can be found in roughly half of the grass and sedge species, but very few of the dicotyledonous species exhibit the C_4 photosynthetic pathway (Lara & Andreo, 2011). Due to their various influences on global productivity, C_4 plants have attracted the awareness of many researchers.

By elevating the CO_2 concentration at the site of Rubisco, photorespiration is suppressed in C_4 plants, because the activity of the oxygenase reaction is inhibited (Uzilday *et al*., 2014). In order to achieve this, C_4 plants utilizes a biochemical CO_2 pump which relies on the spatial separation of the CO_2 fixation and assimilation (Figure 2-2). The Kranz anatomy is found in C_4 species, in which the mesophyll and bundle sheath cells collaborate to fix CO_2 (Edwards *et al*., 2004; Sage *et al*., 2014; Heckmann, 2016). During the carboxylation of PEP (phosphoenolpyruvate) via PEPC (phosphoenolpyruvate carboxylase), four carbon containing organic acids are produced in the cytosol of the mesophyll cells (Lara & Andreo, 2011; Sage *et al*., 2014). The C_4 compounds are then relocated to the bundle sheath cells where they are decarboxylated to form CO_2. Thereafter the CO_2 is assimilated via Rubisco in the PCR cycle (Lara & Andreo, 2011). In addition, three carbon containing organic acids (C_3) are released during the decarboxylation reaction, which returned to the mesophyll cells to regenerate PEP via the enzyme PPDK (pyruvate orthophosphate dikinase) (Sage *et al*., 2014).

It's been speculated that C_4 species evolved in an environment with a high CO_2 concentration (Lara & Andreo, 2011). This would increase the water and nitrogen efficiency of the plants when compared to C_3 plants. In general C_4 plants have greater CO_2 assimilation rates than C_3 plants for a given leaf nitrogen content (Ghannoum *et al*., 2011). This can be explained by the fact that C_4 plants assign less nitrogen (4-21%) to Rubisco and more to thylakoid and other protein components (Evans & von Caemmerer, 2000), whereas C_3 plants will allocate as much as 30% of the nitrogen to the Rubisco protein (Lara & Andreo, 2011). The reason for the low nitrogen requirement of C_4 plants is as a result of their CO_2- concentrating mechanism. This mechanism

increases the bundle sheath CO_2 concentration, therefore inundating the Rubisco protein in normal air. This will stop photorespiration to a certain point (Lara & Andreo, 2011). In C_3 plants Rubisco will only operate at approximately 75% of its ability (Sage *et al*., 2008) therefore losing 25% of the fixed carbon to photorespiration (Lara & Andreo, 2011). Therefore C_3 plants must synthesise more Rubisco and at the same time they have to have a greater nitrogen demand in order for their photosynthetic rates to be equal to those of C_4 plants (Lara & Andreo, 2011).

The Rubisco requirement for CO_2 to prevent photorespiration is temperature sensitive and it will increase once the temperature starts to increase (Long, 1991). Therefore the greatest difference in the nitrogen use efficiency between C_4 and C_3 photosynthesis can be seen at high temperatures. In C_4 plants energy loss due to photorespiration is eliminated, but additional energy is required to operate the C_4 cycle (Lara & Andreo, 2011). An additional 2 ATP molecules are required for every CO_2 assimilated in C_4 plants (Lara & Andreo, 2011). Therefore, when compared to C_3 photosynthesis; C_4 plants have an increased energy requirement (Lara & Andreo, 2011). Nevertheless, when C_3 plants are exposed to temperatures greater than 25°C, more light energy is diverted into photorespiration which surpasses the extra energy that is required for the assimilation of CO_2 in C_4 plants (Hatch, 1992; Long, 1999).

C_4 plants are rarely found in cold environments and their distribution is correlated with the rainfall in specific areas (Ghannoum *et al*., 2011; Lara & Andreo, 2011). C_4 plants have a lower performance in colder environments and are poorly competitive against C_3 plants in cold conditions (Sage & McKown, 2006). The current hypothesis for the inadequate performance of C_4 plants is that C_4 photosynthesis is limited by Rubisco's competency at lower temperatures (Sage, 2002; Kubien *et al*., 2003).

2.4 Climate change

Climate change would probably have the most profound effect on the production of important agricultural crops. Crop production depends on the natural processes that are found in the field and would therefore be greatly influenced once an alteration occurs in the climatic conditions (Zinyengere *et al*., 2014). By the end of this century, it is predicted that the atmospheric CO_2 concentration could reach anywhere between 421 ppm and 936 ppm (IPCC, 2013). Once the CO_2 concentration increases it can be expected that the temperatures would also increase which would result in lower rainfall. This automatically raises a few concerns in terms of the productivity, as well as the sustainability of the cropping systems (Berg *et al*., 2013). However, these changes are caused by human activities which are essentially blameable for the recent increases in the global mean temperatures. These activities would place additional strain on the

global food security system as crop production would have to increase immensely to keep up with the growing demand (Berg *et al*., 2013).

It is expected that the temperatures will rise by 1.2 to over 2°C by the end of this century (IPCC, 2013). Temperature changes would cause additional deviations in the annual rainfall leading to a predicted 20% decrease per year along with a predicted 20% reduction in soil moisture (Schiermeier, 2008). In terms of plants, a reduction in the stomatal conductance and transpiration is expected when exposed to elevated CO_2 concentrations. This will automatically reduce the latent heat loss thereby increasing the leaf temperatures (Lara & Andreo, 2011). Plants will therefore experience an increase in the temperature and water deficit conditions. This would have an influence on the production of crops and the biodiversity of the ecosystem (Thomas *et al*., 2004; Ciais *et al*., 2005). The effect that environmental variables (for example, temperature, soil salinity, water availability and vapour pressure deficit), associated with elevated CO_2 concentrations, would have on the efficiency of photosynthesis has not been documented well in terms of the plants responses to an altering environment (Reddy *et al*., 2010; Lara & Andreo, 2011). Elevated levels of CO_2 will undoubtedly have an effect on the productivity of the ecosystem (Lara & Andreo, 2011). It is thus necessary to understand what effects drought, temperature and CO_2 increases would have on ecosystems (Lara & Andreo, 2011).

2.5 The CO_2 response

Elevated levels of atmospheric CO_2 can increase the photosynthetic ability of plants by means of decreasing photorespiration (Lara & Andreo, 2011). Photorespiration is generally intensified with rising temperatures, however, the negative effects associated with it is known to be much smaller in C_4 plants and CAM plants than in C_3 plants (Lara & Andreo, 2011). Furthermore increased CO_2 levels would normally stimulate C_3 photosynthesis more than C_4 photosynthesis. Ghannoum *et al*. (2000) found a 10-20% increase in the growth of C_4 plants and a 40-45% increase in C_3 plants when doubling the current ambient CO_2 concentration. C_4 photosynthesis has the ability to function at low CO_2 concentrations and simultaneously show remarkable increases in the assimilation of carbon, growth and yields (Lara & Andreo, 2011).

Similar responses were found in sugarcane (increased photosynthetic rate and increased biomass production) when exposed to elevated CO_2 (Vu *et al*., 2006). During carbon assimilation, the enzyme Rubisco has the ability to alter the CO_2 flux at current atmospheric CO_2 concentrations (Long et al., 2004). However, the photosynthetic assimilation of carbon is more or less saturated in C_4 species at the current ambient CO_2 levels. This is due to the fact that HCO_3 is rather used as the main substrate by PEPC instead of CO_2.

The assumption that the CO_2 concentrating mechanism in C_4 plants causes plants to be unresponsive to an enriched CO_2 environment are reflected in the lack of interest to study the effect of elevated CO_2 levels on C_4 plants. Contrasting reports do exist wherein the plants response to elevated CO_2 differ, for example, in sugarcane different photosynthetic rates were found varying from low to higher rates of stimulation. Vu *et al*. (2006) and de Souza *et al*. (2008) found increases in the total biomass yield of sugarcane under elevated CO_2 conditions, whereas Stokes *et al*. (2016) found no biomass increases. Stokes et al. (2016) argued that the increases in sugarcane yield observed by theses authors might have been influenced by the presence of unintentional soil water deficit. Differences can also occur due to the age of the plants, the varieties that were used, the duration of the treatment and the specific experimental techniques that were used (Sage, 2002). Nonetheless, when C_4 species are exposed to increased temperatures and arid conditions, they exhibit positive responses (Sage & Kubien, 2003).

Temperature and CO_2 increases will have various effects on photosynthesis. The process of photosynthesis is thermo-sensitive; therefore a negative effect that can be expected is heat stress (Lara & Andreo, 2011; Grantz, 2014). The process of electron transport (light reaction) and the Calvin cycle (dark reaction) both have thermo-liable elements, particularly photosystem II (light reaction) (Heckathorn *et al*., 2002; Takahashi & Murata, 2005) and Rubisco activase (dark reaction) (Crafts-Brandner & Salvucci, 2002). The process of maintaining Rubisco activated will therefore decrease (Sage *et al*., 2008).

It's predicted that the frequency and severity of droughts will increase in the future. In order to potentially increase the biomass yield and growth of C_4 crops at elevated CO_2, a decrease in water use is required, independent of the effect of increased rates of photosynthesis (Leaky *et al*., 2009). C_4 species use less water than C_3 species under elevated CO_2 conditions. Vu *et al*. (2006) found a significant increase in the water use efficiency of sugarcane when exposed to elevated CO_2. This is explained by the increased CO_2 uptake rates and increased water use efficiency associated with decreased stomatal conductance (Ehleringer *et al*., 1997). C_4 plants will generally also have greater nitrogen use efficiency (Ainsworth *et al*., 2002). Therefore C_3 species would be less competitive than C_4 species due to the increased water use efficiency that would increase the advantage that C_4 plants would have when exposed to drought conditions (Ward *et al*., 1999).

There are still a lot of disagreements regarding the response of sugarcane to elevated CO_2, with specific regard to whether the photosynthetic efficiency and dry biomass yield would increase or not. This study will therefore help to clarify whether elevated CO_2 would have a positive, negative or no effect at all on sugarcane.

CHAPTER 3 MATERIALS AND METHODS

3.1 Trial set-up

This study was conducted at the North West University (NWU) Potchefstroom campus in association with the South African Sugarcane Research Institute (SASRI). An open-top chamber facility was used for these trials (26°40′53″S, 27°5′57″E) (Figure 3-1). OTCs are unique in the sense that various plant species can easily be exposed to controlled levels of air pollutants such as SO_2 and O_3, and drought interaction studies can also be conducted (Heyneke *et al*., 2012b). The open top chambers consist of cylindrical aluminium frameworks which are 2.2 m high and 1.7 m in diameter. The chambers are covered with ultraviolet (UV) stabilised transparent polyvinyl chloride (PVC) sheeting which has a thickness of 400 µm. The sunlight transmission through the PVC sheeting is more than 90% of the photosynthetic active radiation (PAR) (Heyneke *et al*., 2012a). The chamber has a volume of 5 m^3 when covered with the PVC sheeting. A rain cap was fitted to exclude rainfall. Between the top of the chamber and the rain cap there is an opening which allows for the free movement of air through the chamber (Heyneke *et al*., 2012a). For these trials two sugarcane varieties were exposed to two levels of CO_2. Two sugarcane trials were conducted from October 2014 – May 2015, and September 2015 – May 2016. Sugarcane could only be grown from September to May of each year because minimum night temperatures and frost events during winter in Potchefstroom prevented sugarcane growth.

Figure 3-1: Open-top chamber facility at NWU Potchefstroom.

3.1.1 Growth medium

The growth medium consisted of gravel, silica sand and vermiculite. This is the standard practice for SASRI pot trials. The silica sand, vermiculite and gravel were mixed using a 4:1:1 ratio. This mixture was then potted into 10L well drained plastic pots with diameters of 30cm.

3.1.2 Varieties

Plant material of the two varieties was derived from seedcane obtained from the Kearsney research station nursery (S 29º 17'' 48' / E 31º 16'' 06'). Two local contrasting varieties; NCo376, a high sucrose variety and N31, a high biomass variety were used in this study. NCo376 produces good yields on moderate to high potential soils and under favourable growing conditions (South African Sugarcane Research Institute, 2006b). It has a generally high yield and moderate fibre content. N31 has a very high cane yield and high fibre content with low sucrose content. This variety performs excellent on low yield potential and sandy soils (South African Sugarcane Research Institute, 2006a). Single-eyed setts of N31 and NCo376 seedcane were planted in polystyrene seed trays with vermiculite and compost at SASRI in Mount Edgecombe in August 2014 and again in August 2015 (± 150 speedlings). These speedlings were transported to NWU for planting into pots.

3.1.3 CO_2 treatments

In this study the sugarcane was exposed to two CO_2 levels. The control chambers received carbon filtered air. The first CO_2 level was set at the current ambient atmospheric CO_2 concentration of ±400 ppm. The second CO_2 level was kept between 750 and 800 ppm; double that of the ambient air level. The exposure time to elevated CO_2 levels occurred from 08h00 – 17h00. The exposure time matched the day length during which the process of photosynthesis took place. The sugarcane was exposed to these elevated CO_2 levels for seven months.

3.1.4 Insecticide application

Aphids (*Sipha flava*) were found on the sugarcane during trial 1 and 2. The plants were immediately treated with EFEKTO Insecticide Granules (EFEKTO, Bryanston, SA) for the control of aphids, mealybugs, thrips and scale insects. The active ingredient of this insecticide is imidacloprid (chloronicotinyl). This is a systemic insecticide and 15 g were given per pot, as per insecticide label. Thereafter the plants were manually watered to ensure that the insecticide dissolved properly. All pots were treated, irrespective of the presence or absence of aphids.

3.2 Experimental design

3.2.1 Trial 1

A preliminary (pilot) trial was conducted where sugarcane was grown in eight OTCs in order to assess the growth and vitality of the plants under the climatic conditions in Potchefstroom and to optimise the trial protocol to produce vigorously growing, unstressed plants.

3.2.1.1 Trial design

Of the eight OTCs, four were used as the control treatment whilst the remaining four were used for the elevated CO_2 treatment (Figure 3-2). Speedlings of N31 and NCo376 were planted on the 9th of October 2014. One speedling was planted per pot. After planting, four pots of NCo376 and four pots of N31 were placed in one OTC (eight pots per OTC). A total of 64 pots were used during this trial. Once the plants were acclimated to the new environment the CO_2 fumigation was initiated on the 5th of November 2014 and discontinued on the 18th May 2015 (harvest).

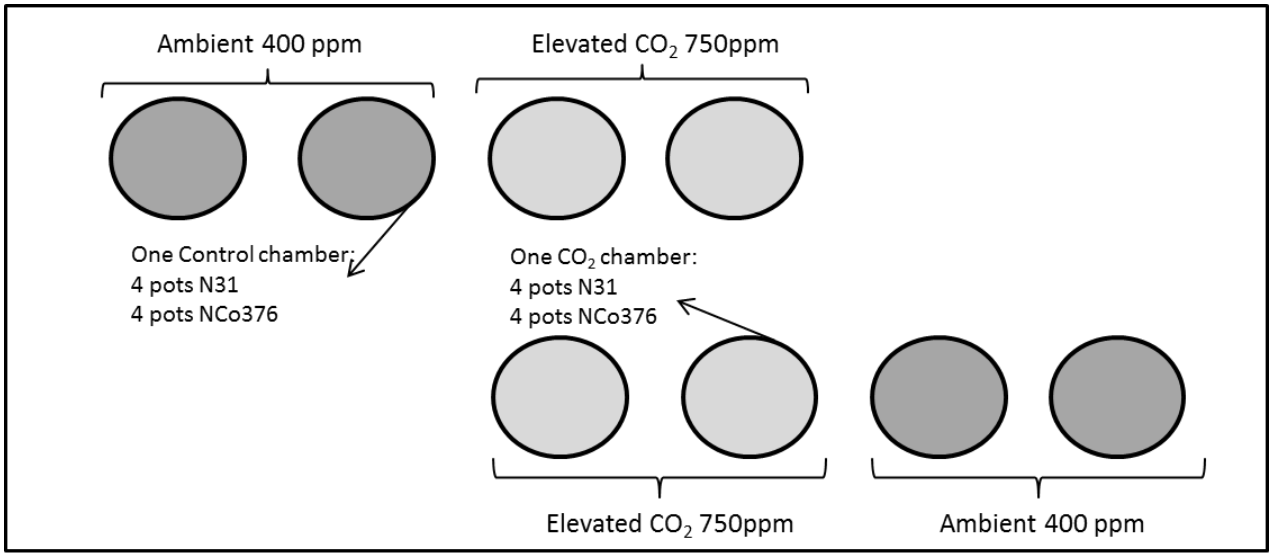


Figure 3-2: The experimental design of the preliminary trial for two sugarcane varieties (N31 and NCo376) grown under elevated and ambient CO_2 conditions at the Potchefstroom OTC facility.

3.2.1.2 Irrigation system

During the preliminary trial a nylon wick irrigation system was used. Four wicks were used per pot. Two wicks were 60 cm long and two were 90 cm long. The nylon wicks were rolled in a clockwise direction at different levels in the soil mixture, with one end protruding through a drainage hole(s) at the base of the pot to ensure uniform wetting of the soil. The pots were placed over buckets which served as water reservoirs. These reservoirs were connected to an irrigation system that refilled the water in the reservoirs twice a day. The pots were placed in a fashion so that they were not in contact with the water in the reservoir.

3.2.1.3 Fertilizer application

The plants were fertilized according to the SASRI Fertilizer Advisory Services (FAS) recommendations (Table 3-1). Each component was weighed and distributed in each pot. The fertilizer was mixed into the growth medium. After the NPK nutrients were applied the Trelmix (The Kendal Group, Howick, SA) solution was mixed with water and applied to each pot. Here after the plants were fertilized once every three weeks.

Table 3-1: Fertilizer application details according to the FAS recommendations.

Nutrient	**Amount applied per pot on the day of planting**	**Amount applied per pot once every 3 weeks**
N	7.5 g LAN	2.5 g LAN
P	15 g Superphosphate	5 g Superphosphate
K	12 g KCl	4 g KCl
Trelmix (Micronutrients)	5 mL Trelmix : 5000 mL water	5 mL Trelmix : 5000 mL water

3.2.2 Trial 2

The performance and practical limitations of the first trial are discussed below and an improved follow-up trial (trial 2) was designed for the main experiment. The limitations of trial 1 and the changes that were introduced in trial 2 are summarized in the following paragraphs.

Number of pots in each chamber

During the first trial, eight pots were placed in each OTC. This created a problem in terms of the space available for growth in the OTC. As the sugarcane elongated, increased competition for solar radiation developed. Hence, certain plants did not receive enough solar radiation. This resulted in reduced growth rates. As an improvement to this method, fewer pots were placed in each OTC and more OTC's were used for the second trial.

Irrigation system

At the harvest of trial 1 in May 2015, it was found that the roots grew through the pot drainage holes into the reservoirs. As a result the roots were submerged in the water and exposed to

poorly aerated conditions, which caused algal growth. The nylon wick system also did not meet the water requirements of the sugarcane. The rate at which water moved into the soil via the wick system was too slow. As a result the plants were exposed to water stress as the above-ground biomass increased. The installation of an automated drip irrigation system, with the ability to vary the quantity and timing of water application, was proposed in order to avoid this problem.

Fertilizer application

Leaf samples of both varieties were taken and sent to SASRI for analysis after four months in the OTC's to assess the nutrition levels of the sugarcane plants. According to the results obtained, the N status of the leaves was very low and this was reflected in the yellow discolouration of the leaves, particularly in variety N31. Alterations in the amount and frequency of the fertilizer application were made after consulting with the soil science department at SASRI. The amount of fertilizer and the frequency at which it was applied was increased.

3.2.2.1 Trial design

Of the twelve OTCs, six were used as the control treatment whilst the remaining six were used as the elevated CO_2 treatment during the second trial (Figure 3-3). Speedlings of N31 and NCo376 were planted on the 21st of September 2015. After planting, three pots of NCo376 and three pots of N31 were placed in one OTC (six pots per OTC in contrast to eight pots during trial 1). A total of 72 pots were used during this trial. Once the plants were acclimated to the new environment the CO_2 fumigation was initiated (1st of November 2015) until harvest on the 24th of May 2016.

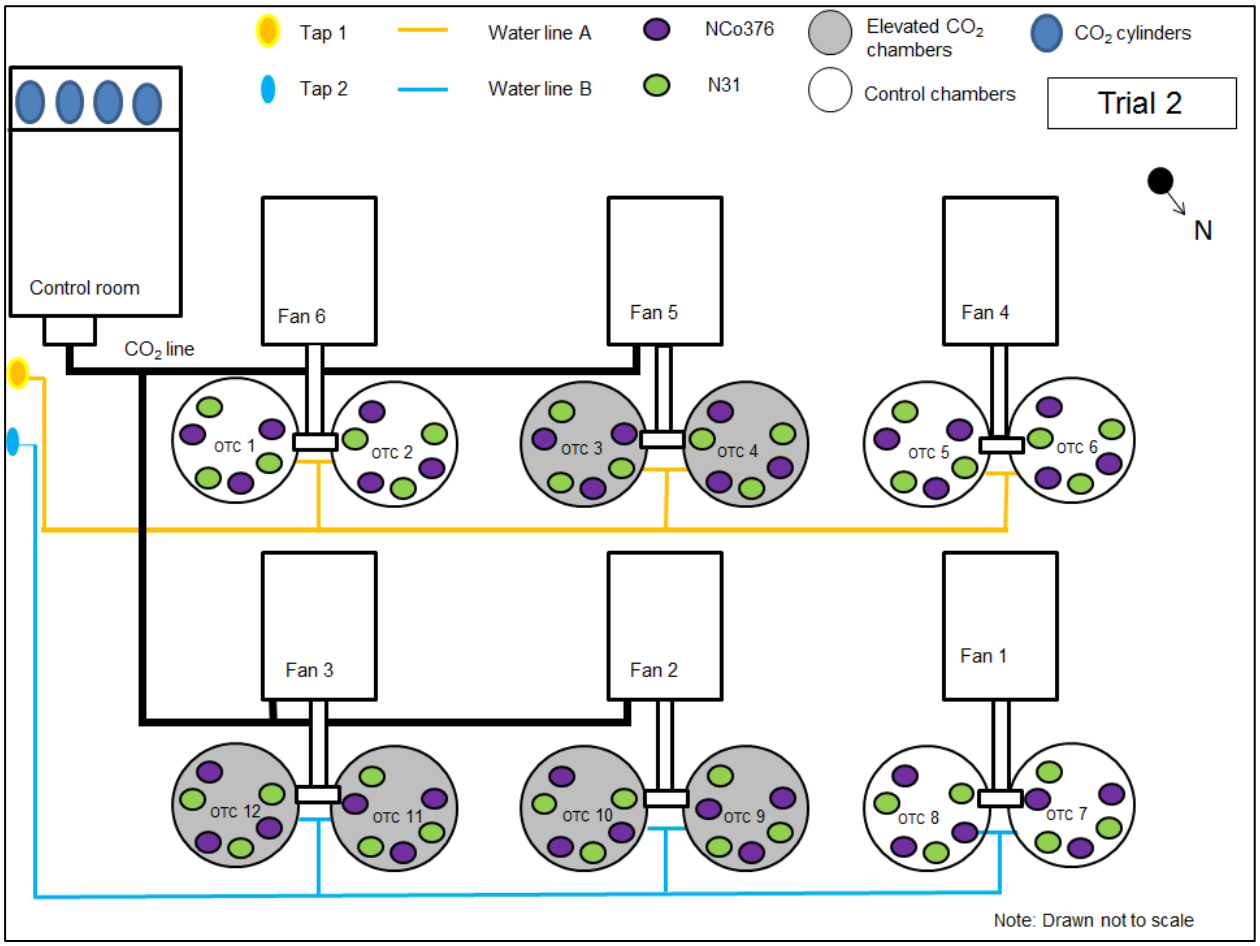


Figure 3-3: The experimental design of the second trial for two sugarcane varieties (N31 and NCo376) grown under elevated and ambient CO_2 conditions at Potchefstroom OTC facility.

3.2.2.2 Irrigation system

An automated dripper irrigation system was installed for the second trial where the pots were watered from the top with plastic drippers. The flow rate was 15 mL of water per minute. During the early stages of growth (September- November) the plants were watered three times a day for 30 minutes via one dripper per pot. The pots therefore received 450 mL water in 30 minutes and 1350 mL water per day. During January (higher temperatures) until the end of the trial, the pots were watered four times a day for half an hour, and received 1800 mL of water per pot per day.

3.2.2.3 Fertilizer application

The fertilizer ratios were applied according to SASRI Fertilizer Advisory Services (FAS) recommendations. The fertilizer ratios that were used during the first trial were changed to ensure that the sugarcane was exposed to necessary nutrient availability. Each component was weighed and distributed in each pot. The fertilizer was mixed into the growth medium. The fertilizer was applied once at planting and thereafter it was applied once a week (compared to

once every three weeks during trial 1). After applying the fertilizer, the pots were irrigated to ensure that the fertilizer dissolves and the nutrients are available to the plants. The application details are shown in Table 3-2.

Table 3-2: Fertilizer application details according to the FAS recommendations.

Nutrient	**Amount to be applied per pot on the day of planting**	**Amount to be applied per pot once every week**
N	7.5 g LAN	5 g LAN
P	15 g Superphosphate	5 g Superphosphate
K	12 g KCl	4 g KCl
Trelmix (micronutrients)	5 mL Trelmix : 5000 mL water	5 mL Trelmix : 5000 mL water

3.3 Measurements

3.3.1 Trial 1

A summary of all the variables measured during the preliminary trial is shown in Figure 3-4. The time intervals of the measurements are also presented. The measurements are categorized into weather data, non-destructive and destructive measurements. The results of the preliminary trial are not presented here as it was used to assess the growth and vitality of the sugarcane in the OTCs. Improvements were also made on the physiological measurements in terms of the frequency of the measurements.

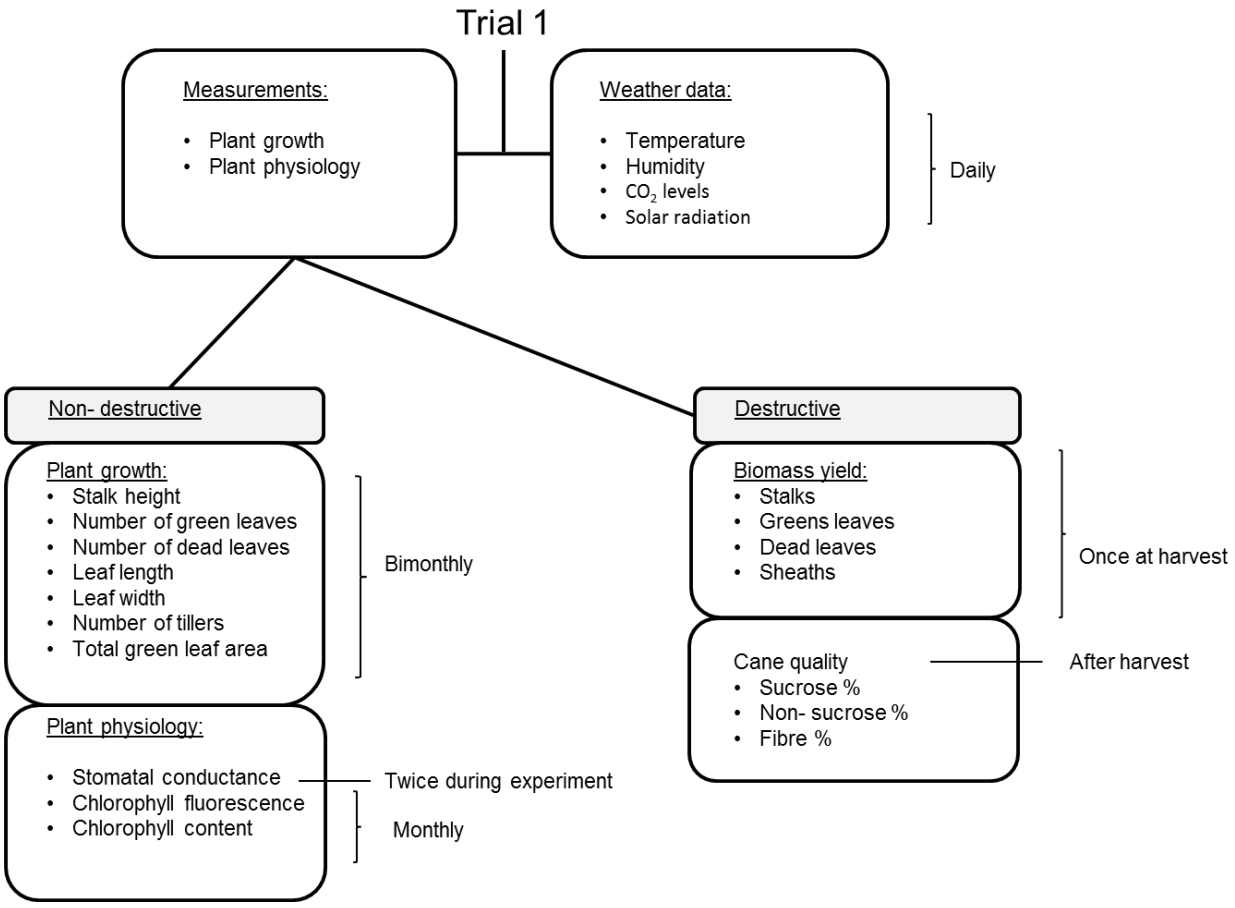


Figure 3-4: A summary of all of the measurements conducted during the first trial.

3.3.2 Trial 2

A summary of all the variables measured along with the time intervals of the measurements for the second trial is shown in Figure 3-5. The measurements are categorized into weather data, non-destructive and destructive measurements. In addition, single leaf photosynthetic and transpiration rate was also measured under the non-destructive measurements. As part of the destructive measurements, the total leaf area and the dry biomass nitrogen (N) content was also determined.

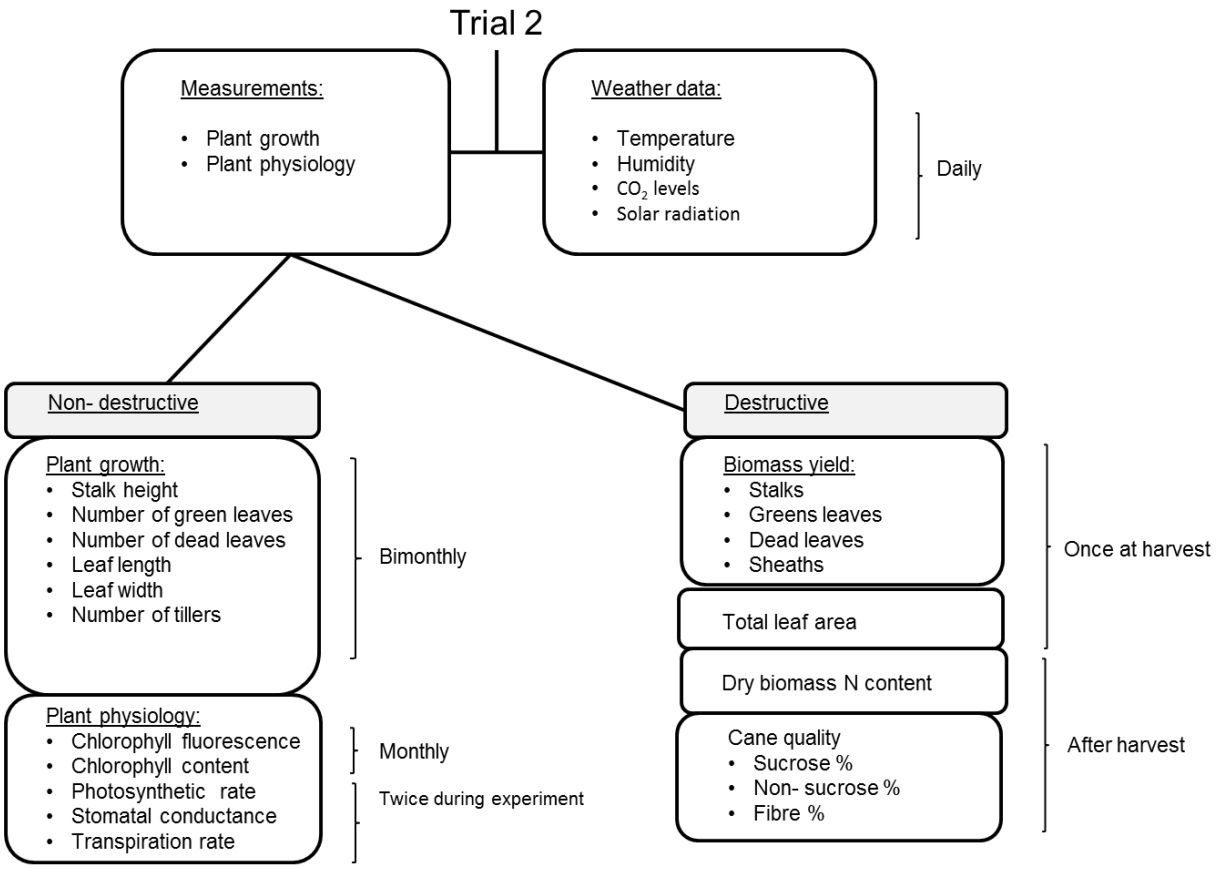


Figure 3-5: A summary of all the measurements taken during trial 2.

3.3.2.1 Weather data

An automated weather station located at the OTC facilities was used to measure the temperature, humidity and solar radiation outside of the OTCs at 5 minute intervals. From this the average hourly temperature and humidity was determined, as well as the solar radiation. The daily maximum and minimum mean temperature and humidity was also recorded. The temperature, humidity and solar radiation were also recorded within the OTCs. A full weather data set was also received from the ARC (Agricultural Research Council, Potchefstroom) climatology department to compare the weather data sets.

The CO_2 levels were recorded using a CO_2 logger (AZ instrument crop, Taiwan). Hourly CO_2 measurements were recorded. The logger also recorded the temperature and humidity levels within the chambers. Two CO_2 loggers were used. One logger was placed in a control chamber whilst the other was placed in an elevated CO_2 chamber. The loggers were rotated between the different chambers to ensure that the CO_2 concentration was at the desired levels in all off the chambers. The data was downloaded using the CO_2 logger software v 1.0.

3.3.2.2 Plant growth

The growth measurements were taken twice a month. A primary tiller was tagged in each pot. The number of dead leaves and green leaves was counted starting from the stalk base upwards to the TVD (top visible dewlap) leaf. The TVD leaf can be described as the youngest fully expanded leaf with a visible dewlap or collar (McCary *et al*., 2005). The stalk height was measured with a tape measurer from the soil surface to the TVD leaf attachment to the stalk. The length and width of the TVD leaf was measured with a tape measurer for each of the tagged tillers. The number of tillers (shoots) present in the pot where counted, excluding the main tiller (primary shoot or stalk). From this the area per green leaf was calculated as a product of leaf width, leaf length and a shape factor (Sinclair *et al*., 2004).

3.3.2.3 Photosynthetic associated responses

Assimilation rate (A_n), Stomatal conductance (g_s) and transpiration (E_n)

Photosynthesis may be seen as the key physiological process involved in air pollution-induced crop losses, since the dry matter of plants is derived from photosynthetic carbon fixation. Measurement of CO_2 assimilation is an effective non-destructive, non-invasive technique for studying the short-term effects of carbon gain of individual organs. This information provides additional information on the long-term carbon gain or final biomass. The approach of measuring photosynthetic gas exchange in plants is in based on Fick's first law of diffusion - which states that the rate of diffusion is directly proportional to the cross-sectional area of the diffusion path and to the concentration or vapour pressure gradient, and that it is inversely proportional to the length of the diffusion path.

To study the CO_2 assimilation in plants, a number of measurements, terms and units are employed (Von Caemmerer & Farquhar, 1981). The CO_2 assimilation rate (A_n) is defined as the amount of CO_2 assimilated per unit leaf area and time (μmol CO_2 m^{-2} s^{-1}), whereas the stomatal conductance (g_s) represents the flow of CO_2 through the stomata (μmol CO_2 m^{-2} s^{-1}). The traspiration rate (E_n) can be defined by using Fick's law, and can be calculated as the ratio (e_i – e_a)/Pr, where e_i and e_a are the vapour pressures of water inside the leaf and in the air, respectively, and Pr is the atmospheric pressure, therefore representing the difference of the water vapour concentration between the inside and the outside of the leaf expressed as a mole fraction.

For the photosynthetic measurements the CIRAS-2 (PP-Systems, Hansatech Instrument Ltd, UK) was used. The CIRAS 2 is a differential and portable combined fluorescence system. It features four independent, non-dispersive infrared gas analysers for accurate, simultaneous measurement of both CO_2 and H_2O fluxes. When measuring the CO_2 assimilation, air is

pumped from the cuvette encompassing a leaf into an infrared gas analyser which continuously measures the CO_2 concentration in the air stream. If the leaf inside the cuvette assimilates CO_2, the CO_2 concentration of the air stream will decrease. The CO_2 assimilation rate is equivalent to the change in the amount of CO_2 in the air stream per unit time. Changes in temperature and pressure are compensated for in the calculation of the CO_2 assimilation rate. The humidity, however, has to be controlled, since a rise in transpiration will cause an increase in the amount of water vapour, resulting in dilution of CO_2 in the air stream (Long & Hällgren, 1993).

During this trial the photosynthetic measurements were conducted over 4 days. The TVD leaf was used for the measurements. This resulted in having 12 readings per chamber. CIRAS measurements were taken in eight open top chambers. The light intensity of the light source inside the leaf cuvette was 2000 mol mol^{-1}, while the flow rate was 300 mL min^{-1}. The control plants were measured at 400 ppm CO_2 and the elevated CO_2 plants were measured at 750 ppm CO_2. The leaves acclimated inside the cuvette for at least 1 minute before the measurements were taken. These measurements were conducted twice (February 2016 and April 2016) during the second trial. The measurements were taken between 10:00 am and 13:00 pm when the photosynthetic rate was at its highest.

Chlorophyll *a* fluorescence

In order to take chlorophyll *a* fluorescence measurements the leaves of the plants need to be dark adapted for at least one hour. Measurements were therefore taken during the night after the plants were exposed to darkness for at least one hour. The Handy PEA fluorimeter (Plant Efficiency Analyzer, Hansatech Instrument Ltd, UK) was used to take the measurements. The only light that is allowed to be used during these measurements is a green light. The green light won't stimulate any photosynthetic activity, whereas other light would stimulate photosynthesis. Measurements were made by placing the leaf clip at a central point on the leaflet between the midrib and the leaf margin on TVD leaves. Three measurements were taken per pot, therefore a total of 216 measurements were taken (3 replicates x 6 pots x 12 chambers). PEA Plus (v 1.10) software was used to calculate the photosynthetic (O-J-I-P) parameters from the variable fluorescence. The chlorophyll *a* fluorescence measurements provide insight into the photosynthetic responses of plants to elevated levels of CO_2 and to which degree the elevated CO_2 will affect the photosynthetic subunits (Krause & Weis, 1991; Govindjee, 1995; Maxwell & Johnson, 2000; Strasser *et al*., 1999, 2000). The JIP-test was used to translate the original fluorescence measurements of O-J-I-P transient into several phenological and biophysical expressions quantifying PSII functions (Strasser *et al*., 2000; Strasser & Tsimilli-Michael, 2001; Cuchiara *et al.,* 2013).

The theory of energy flow in thylakoid membranes describes the inflow and outflow energy of photosynthetic pigments, therefore forming the basis of the JIP-test (Kalajii & Loboda, 2007). The physiological state of the plant, due to physical and chemical environmental conditions, will determine the shape of the OJIP-transient. In order to survive, the plant needs to adapt to these stress conditions, therefore the ability to adapt, is studied through the vitality of the photosynthetic system of the plant (Rout & Das, 2013). The JIP- test allows a separate estimation of the maximum yield of primary photochemistry. It also examines the probability that an electron would move into the electron transport chain beyond Q_A^- (Quinone A). The reduction of the electron transport chain is therefore represented by the OJIP transient. As a result, the OJIP curves can be used to analyse how efficient the plant photosynthesises under conditions of stress. The chlorophyll *a* fluorescence measurements were repeated once a month.

The OJIP transients were normalized between 0.01 and 300 ms to obtain the variable fluorescence between these two points (Table 3-3). Hereafter, the variable fluorescence was normalised to the control to visualize the differences in the redox potential of the light dependant reaction between the different varieties and treatments. Generally positive values indicate that the light reactions in terms of photosynthesis and the electron transport chain are less efficient compared to its corresponding control. Negative values indicate a more efficient reaction in terms of photosynthesis and the electron transport chain. Four functional steps are measured which are known to regulate the activity of the reaction centre complex during the initial stages of photosynthetic activity (Table 3-4). The equations illustrated in Table 3-3 and 3-4 were used in assessing the JIP test for the analysis of chlorophyll *a* fluorescence and the relevant photosynthetic parameters (Strasser *et al.,* 2004).

Table 3-3: The following equations were used in assessing the JIP test for the analysis of chlorophyll *a* fluorescence and the relevant photosynthetic parameters.

Equation	**Description**
$\mathbf{V_t = (F_t - F_{0.01})/(F_{300} - F_{0.01})}$	Variable Fluorescence
$\mathbf{\Delta V_{OP} = V_{treatment} - V_{control}}$	Difference in variable Fluorescence

Table 3-4: The photosynthetic parameters and their descriptions.

	Performance Indexes	**Descriptions**
O phase	$\gamma(RC)/((1-\gamma(RC))$	The ability of the active chlorophyll pigments, to absorb sunlight energy (the absorbance of E_{light}).
J phase	$\varphi(Po)/((1-\varphi(Po))$	Amount of light energy trapped per RC (the trapping of $E_{excitation}$).
I phase	$\Psi(Eo)/(1-\Psi(Eo))$	The efficiency with which an electron will move further than Q_a^- (the conversion of $E_{excitaion}$).
The first three steps contributing to photosynthesis	$PI_{ABS} = [(\gamma_{RC})/(1-\gamma_{RC}] \cdot [(\varphi_{Po})/(1-\varphi_{Po})] \cdot [(\Psi_o)/(1-\Psi_o)]$	Indication of the vitality of a plant. γ Light absorption efficiency. φ Maximum quantum yield of photochemistry. Ψ Dark redox reaction efficiency.
P phase	$\Delta = [(1-V_i)/(1-V_j) – (1-V_i)]$	The success of the reduction of $NADP^+$ to NADPH (the reduction of end e^- acceptors).
A multi-parametic expression of all four steps	$PITotal = PI_{ABS} \cdot \Delta$	PI_{Total} considers the ability of a plant to produce NADPH and it evaluates the total vitality of a plant.

Chlorophyll content

The chlorophyll content of the TVD leaves was measured using a hand-held chlorophyll content meter (Model CCM 300, Opti-science, USA). Dimensionless Soil Plant Analysis Development (SPAD) values were generated to estimate the leaf chlorophyll content. Measurements were made by placing the leaf clip at a central point on the leaf between the midrib and the leaf margin on TVD leaves. Three SPAD measurements were taken on a TVD leaf per pot, therefore a total of 18 measurements were taken per chamber and 216 measurements in total. The SPAD measurements were taken at the end of each month.

3.3.2.4 Biomass sampling

Destructive sampling of the aboveground biomass was done at harvest on the 24th and 25th of May 2016. Plants from six pots per chamber from eight chambers (four ambient and four elevated CO_2 chambers) were harvested (48 pots in total). The pots were sampled destructively by cutting the stalks at the soil surface. The fresh weight of the different biomass components (stalks, green leaves, dead leaves and tops) were determined. Sub samples were taken of each biomass component to determine their fresh: dry mass ratio after they were dried in an oven at 75°C for three days. The stalks were analysed for dry matter content, fibre, sucrose and non-sucrose contents and the mass of each component was determined accordingly by the SASRI cane testing laboratory, Mount Edgecombe.

3.3.2.5 Biomass nitrogen content

After weighing the sub samples, the samples were sent to FAS to determine the dry biomass nitrogen percentage in the stalks, leaves, tops and brown leaf residue. The LECO combustion analyser (TruSpec CN, LECO Corporation, Michigan, USA) was used to determine the nitrogen percentage for each component. The average nitrogen content was verified for the total dry biomass. From this the NUE was determined (NUE= NUpE× NUtE).

3.3.2.6 Crop modelling component

The Canesim sugarcane crop model (Singels & Donaldson, 2000; Singels, 2007) was used to simulate the effect of different CO_2 concentrations on cane and sucrose yield, as well as crop water use of the two sugarcane varieties and to compare simulated results with those observed in trial 2. An elevated CO_2 scenario and a control scenario were simulated in order to compare the two treatments with each other. A range of parameters are entered into the model from which the user can select those relevant to their experiment, for example, the soil parameters includes the soil texture fractions, available water capacity, water content at saturation and the drainage rate for each layer along with the root zone as a whole (Table 3-5). At the same time the soil water retention parameters are also calculated from the soil texture information. The number and thickness of the soil layers is determined by the user by considering the maximum length of the roots.

In terms of weather information, the Canesim model is linked to the SASRI weather database from which the Daily rainfall (mm), shortwave radiation (Srad in MJ m^2), maximum temperature (Tmax in °C), minimum temperature (Tmin in °C) and sugarcane reference evaporation information can be accessed. The atmospheric CO_2 concentration that was used in the simulation was determined from the cropping dates.

The varieties can be selected from a drop down list. In addition, the crop start and harvest dates, row spacing, crop class (plant or ratoon crop) amount of crop residue from previous crop is also required model inputs. The irrigated scenarios and the type of irrigation system that was used should be specified. Canesim also considers the growth phases including, germination, tillering and stalk elongation. The growth development is controlled by the thermal time.

The results are recorded in various reports, which summarize all the cropping scenarios in a projection in terms of the water balance and cane and sucrose yield at harvest. A summary of field inputs used in the simulation, as well as simulated cane and sucrose yield, stalk dry matter and sucrose content, soil water content, extent of lodging, seasonal totals of rainfall, irrigation and crop water use, canopy cover at harvest and the extent of missing weather data are specified in the report.

Table 3-5 specifies the parameters that were used in the model. A simulation was run either with the control CO_2 levels (400 ppm) or the elevated CO_2 levels (750 ppm). The ARC weather data was used for the simulation as a wider range of parameters was provided. The only parameters that were measured at the OTCs were the temperature, humidity and solar radiation. The simulation model however required the wind speed and the total evaporation rate which was not measured at the OTCs.

The plants were not exposed to water deficit conditions and therefore had no water restrictions. The two cultivars, N31 and NCo376, were alternatively used in the simulation model. The row spacing was calculated by converting the surface area that was occupied by the plants in the chambers to meters per square. From this the row spacing was calculated. The type of crop was set as ratoon crop, because speedlings were used for this trial.

Table 3-5: Parameters used during the simulation.

Scenario Properties	
Total available water (mm)	58
Drainage constant	0.24
Crop start date	01-SEP-15
Harvest date	24-MAY-16
Atmospheric CO_2 (ppm)	400 or 750
Weather station	ARC Potchefstroom
Water restriction	Unrestricted
Scheduling method	On demand
Auto irrigation	Yes
Type of irrigation	Surface drip
Refill level	58
Irrigation trigger level (mm)	48
Irrigation cycle (day)	1
Variety	N31 or NCo376
Target amount (mm)	10
Row spacing (m)	1.4
Plant/ Ratoon	Ratoon
Trash layer (t ha^{-1})	0
Simulate lodging	ON

3.4 Data processing and analysis

The dry biomass data was subjected to a two way analysis of variance (complete randomized design) to compare treatments at the 5% (VSN International, 2015).

Mixed models (REML) were used to analyse the plant growth data, photosynthetic parameters, stomatal conductance, photosynthetic rate and chlorophyll content at the 5% significance level (Fischer's Protected LSD) by using GENSTAT® for Windows version 18 (VSN International, 2015).

The photosynthetic (O-J-I-P) parameters from the variable fluorescence were subjected to a one way analysis of variance to compare treatments at the 5% significance level (Tukey test) by using SigmaPlot v12.0 software (Systat Software, Inc., San Jose California USA).

CHAPTER 4 RESULTS

4.1 Weather data

The temperature, relative humidity, solar radiation and CO_2 levels were measured constantly to ensure that the conditions for sugarcane growth would be suitable. The weather data was also used for crop modelling purposes.

4.1.1 Temperature

The temperature was monitored throughout the trial (Figure 4-1) and the daily averages were calculated. The average maximum ambient temperature during the trial was 28.5ºC and the average minimum ambient temperature was 14ºC.

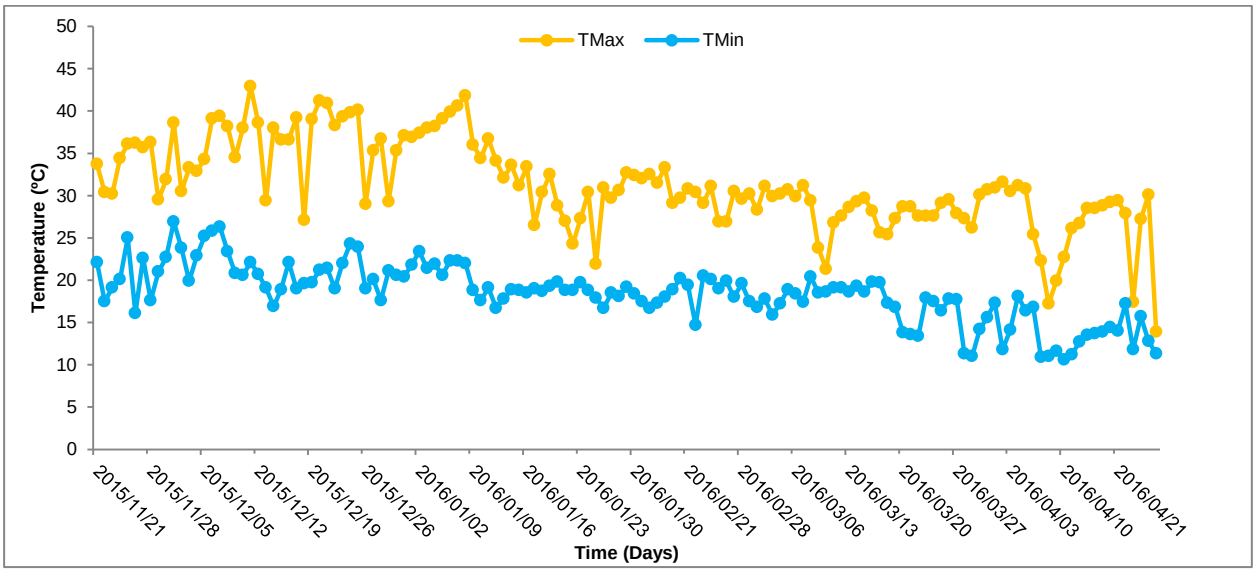


Figure 4-1: The daily maximum (TMax) and minimum (TMin) ambient temperatures during the sugarcane trial at the OTC facility at Potchefstroom.

The average maximum temperature within the chambers during the trial was 31.3ºC and the minimum temperature within the chambers was 18ºC (Figure 4-2). The temperatures inside the OTCs were generally 3 and 4ºC higher than the ambient temperatures.

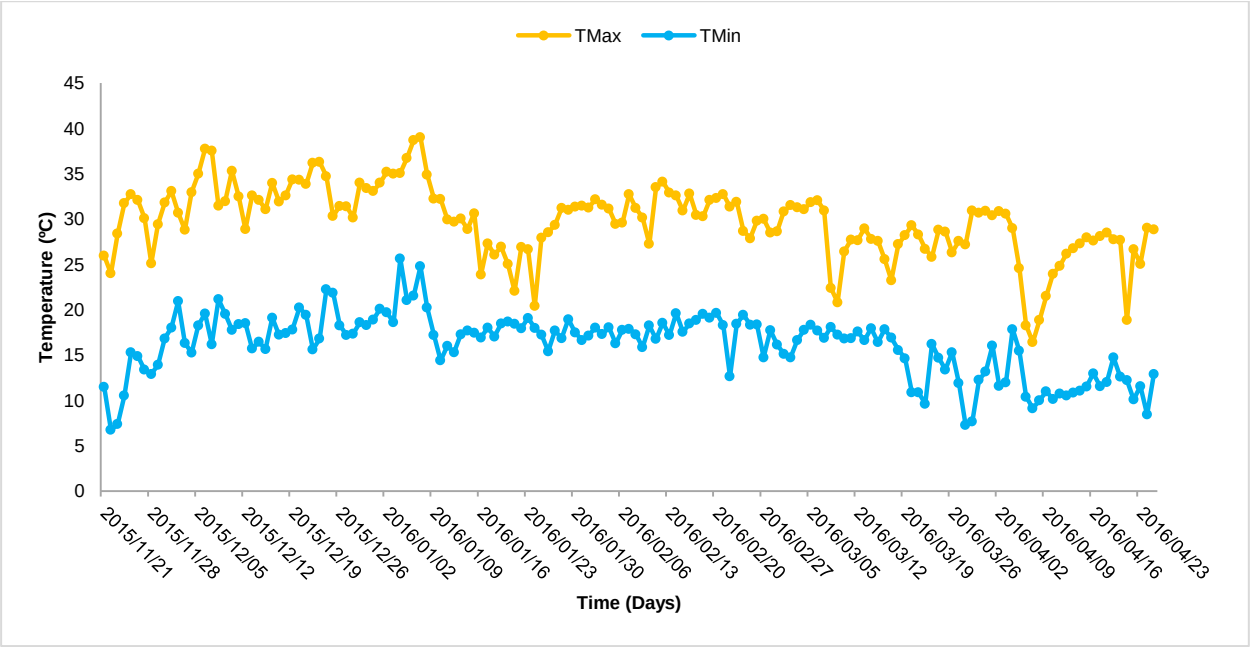


Figure 4-2: The daily maximum (TMax) and minimum (TMin) temperatures within the chambers during the sugarcane trial at the OTC facility at Potchefstroom.

4.1.2 Humidity

The average maximum ambient humidity was 77.21% and the minimum ambient humidity was 26.5% (Figure 4-3).

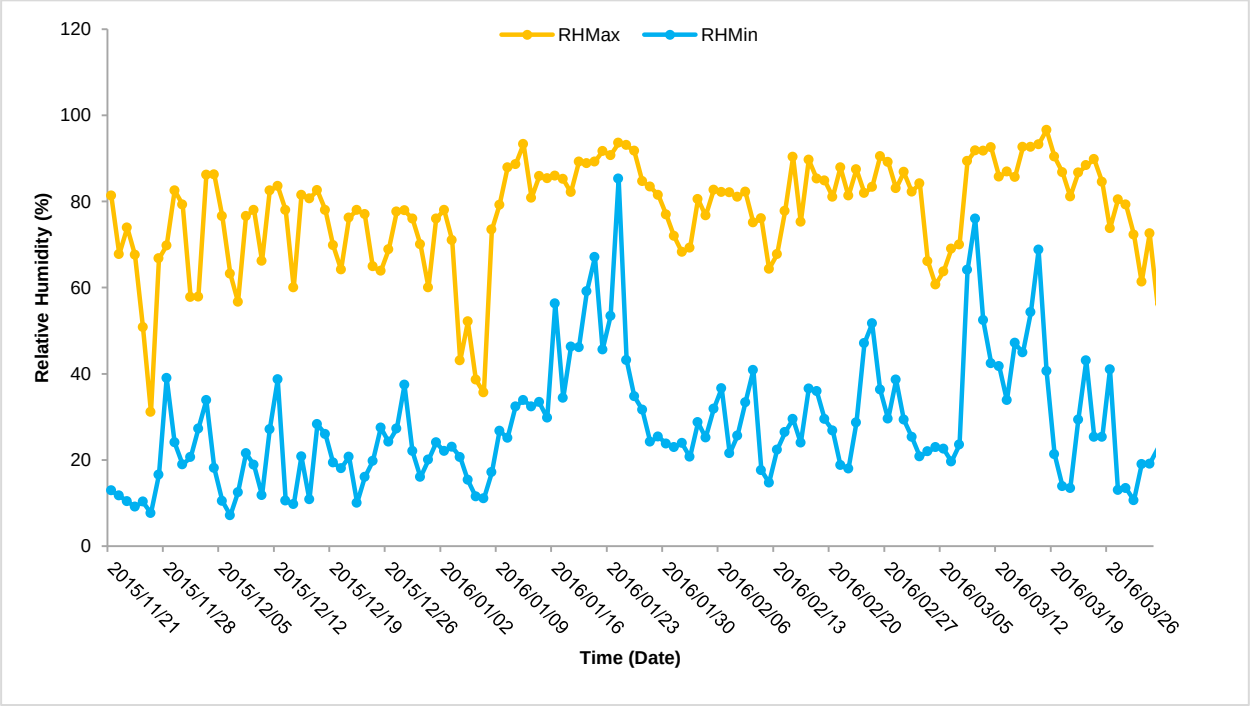


Figure 4-3: The daily maximum (RHMax) and minimum (RHMin) ambient humidity during the sugarcane trial at the OTC facility at Potchefstroom.

The average maximum humidity within the chambers was 87.4% and the minimum humidity within the chambers was 50.5% (Figure 4-4). The humidity within the OTCs was generally 10-20% higher than the ambient humidity.

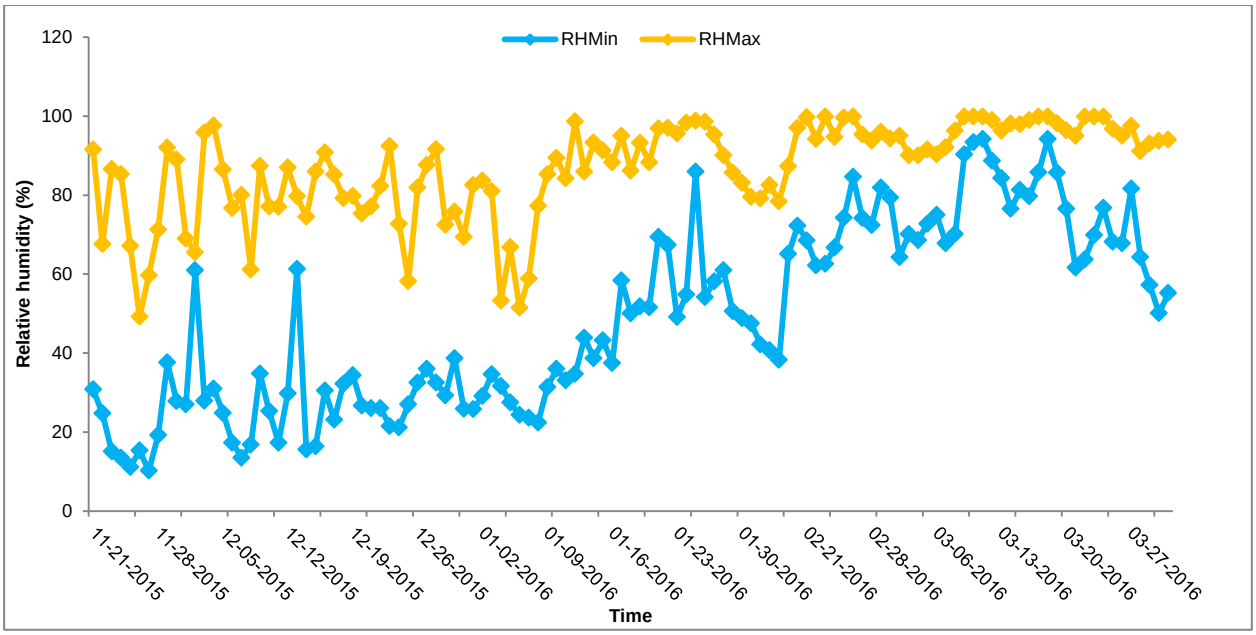


Figure 4-4: The daily maximum (RHMax) and minimum (RHMin) humidity within the chambers during the sugarcane trial at the OTC facility at Potchefstroom.

4.1.3 Solar radiation

On average the external solar radiation was 18.37 MJ m^2 during the trial (Figure 4-5). The zero values were due to an isolated instrument failure.

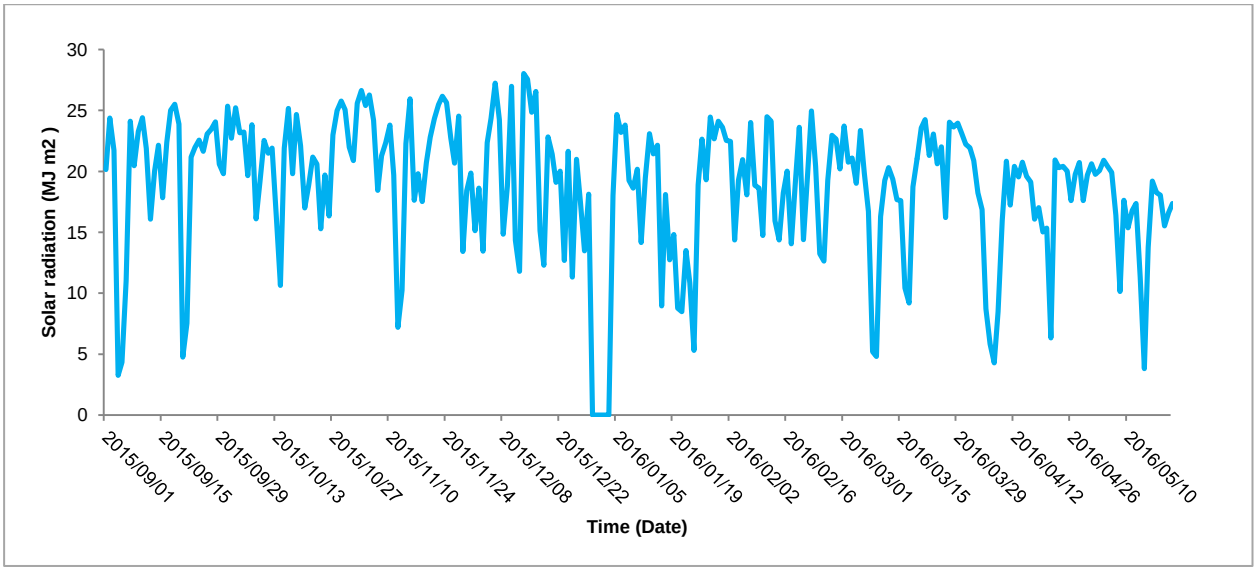


Figure 4-5: The ambient solar radiation during the sugarcane trial at the OTC facility at Potchefstroom.

4.1.4 CO_2 levels

The average daily CO_2 concentration was determined inside the elevated CO_2 chambers, as well as the ambient (control) chambers. The overall average CO_2 concentration in the elevated CO_2 chambers was 745 ppm and the average ambient concentration was 430 ppm (Figure 4-6).

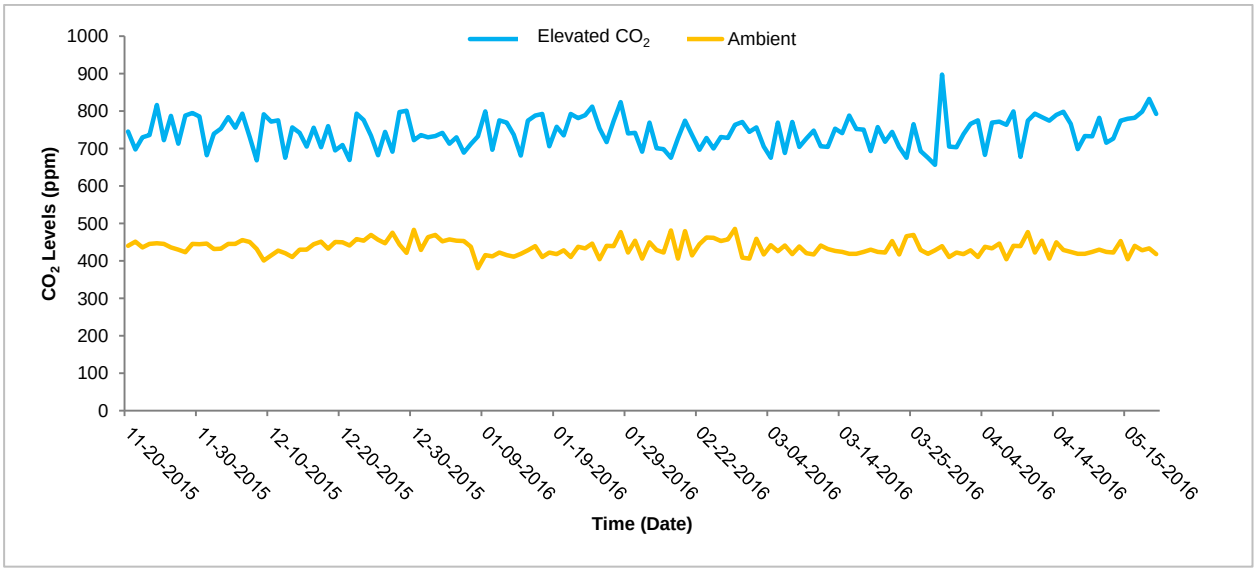


Figure 4-6: The elevated CO_2 and ambient CO_2 levels inside the OTC chambers during the sugarcane trial at the OTC facility at Potchefstroom.

4.1.5 Growth medium status

After the harvest, soils samples were sent to FAS for analysis. An inert growth medium consisting of gravel, silica sand and vermiculite was used for this trial. After harvest samples of

the growth mediums where sent to FAS to evaluate the status of the nutrients available to the plants in the growth medium. According to the analysis the N, K and P levels were not limiting and the acid saturation was low, therefore there were no limiting soil factors for growth (Table 4-1).

Table 4-1: Analysis of the soil samples, according to the SASRI Fertilizer Advisory Services.

Analysis	Unit	Sample value	Comment
pH in calcium chloride		7	
Phosphorus (P) (Resin)	mgL^{-1}	880	High
Potassium (K)	mgL^{-1}	449	Adequate
Calcium (Ca)	mgL^{-1}	2847	Adequate
Magnesium (Mg)	mgL^{-1}	320	Adequate
Sodium (Na)	mgL^{-1}	143	
Exchangeable Acidity (Al+H)	cmol L^{-1}	0,05	
Total cations	cmol L^{-1}	18	
Acid saturation	%	0,28	Not limiting
Exchangeable sodium % (ESP)	%	3	Not limiting
Ca/Mg (Equivalence ratio)		20	Not limiting
Zinc (Zn)	mgL^{-1}	10	Adequate
Copper (Cu)	mgL^{-1}	3,1	Adequate
Manganese (Mn)	mgL^{-1}	20	Adequate
Iron (Fe)	mgL^{-1}	71,5	Adequate
Silicon (Si)	mgL^{-1}	63	Adequate
Nitrogen (N) category	cat	3	
N volatilization	%	3,7	Moderate
Volume weight	mgL^{-1}	1,1	

4.2 Non- destructive measurements

4.2.1 Plant growth

The results for the number of green and dead leaves, stalk height, leaf length- and width and number of tillers are represented here. This set of parameters is easily measured and changes in the growth rate can easily be observed. These plant growth parameters were specifically chosen to evaluate whether elevated levels of CO_2 would increase the growth of sugarcane or not.

Number of green leaves:

According to the data collected for the number of green leaves, no significant differences for both varieties were found between the elevated CO_2 treatment and the control for the first 14 weeks of fumigation (Figure 4-7). After 14 weeks significant differences ($P \leq 0.05$) were

observed between the control and CO_2 treatments for both varieties during the same period as when the highest temperatures were recorded (Figures 4-1 and 4-2). During this period a difference ($P \leq 0.05$) between the two varieties was also observed in which NCo376 displayed a higher green leaf count than N31. On average, the CO_2 treatment of NCo376 had a 29% higher green leaf count than the control treatment, whereas N31 had a 17% higher green leaf count between 16 to 20 weeks of CO_2 fumigation. After twenty weeks of CO_2 fumigation the elevated CO_2 treatment differed with 30% from the control treatment for NCo376 and 17% for N31. During the first 20 weeks all the plants had a high green leaf count, which indicated that the plants had not experienced any water stress. Furthermore, no significant differences were observed between the two varieties beyond 20 weeks until harvest. This could be ascribed to the lower temperatures observed during those weeks which could have retarded the appearance of new leaves.

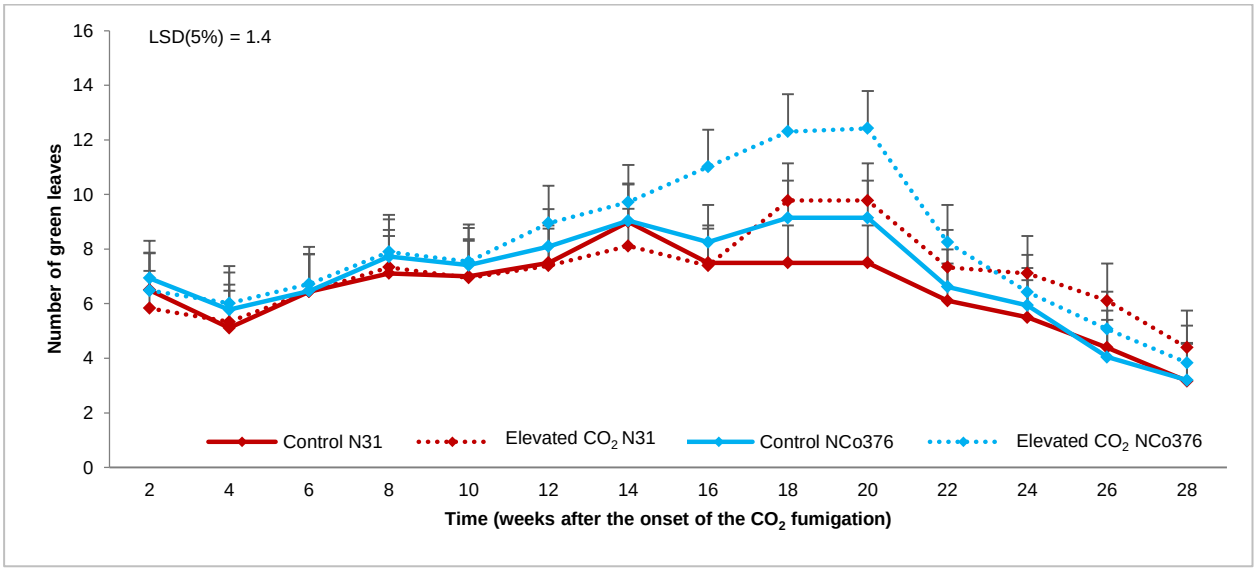


Figure 4-7: The number of green leaves per stalk of two sugarcane varieties (N31 and NCo376), grown under elevated and ambient CO_2 conditions at Potchefstroom OTC facility.

Number of dead leaves:

Significant differences ($P \leq 0.05$) were found for the number of dead leaves between 16 and 20 weeks of CO_2 fumigation for both varieties (Figure 4-8). The elevated CO_2 treated plants for N31 had a 13% lower dead leaf count than the control, whereas NCO376 had a 31% lower dead leaf count than the control. The largest difference ($P \leq 0.05$) between the CO_2 treatment and control was found at 20 weeks of CO_2 fumigation when the elevated CO_2 treated plants of N31 had a 15% lower dead leaf count and NCo376 had a 36% lower dead leaf count than the control. This indicates a higher leaf senescence rate for the control treatments compared to the elevated CO_2 treatments in both varieties. After 20 weeks of CO_2 fumigation a steady increase in the amount of dead leaves was observed. This could be attributed to the minimum temperatures that started to drop during the last 8 weeks before harvest. These low temperatures could have contributed

to the rapid senescence of green leaves, which is a well-known phenomenon in sugarcane during ripening (Inman-Bamber, 1994).

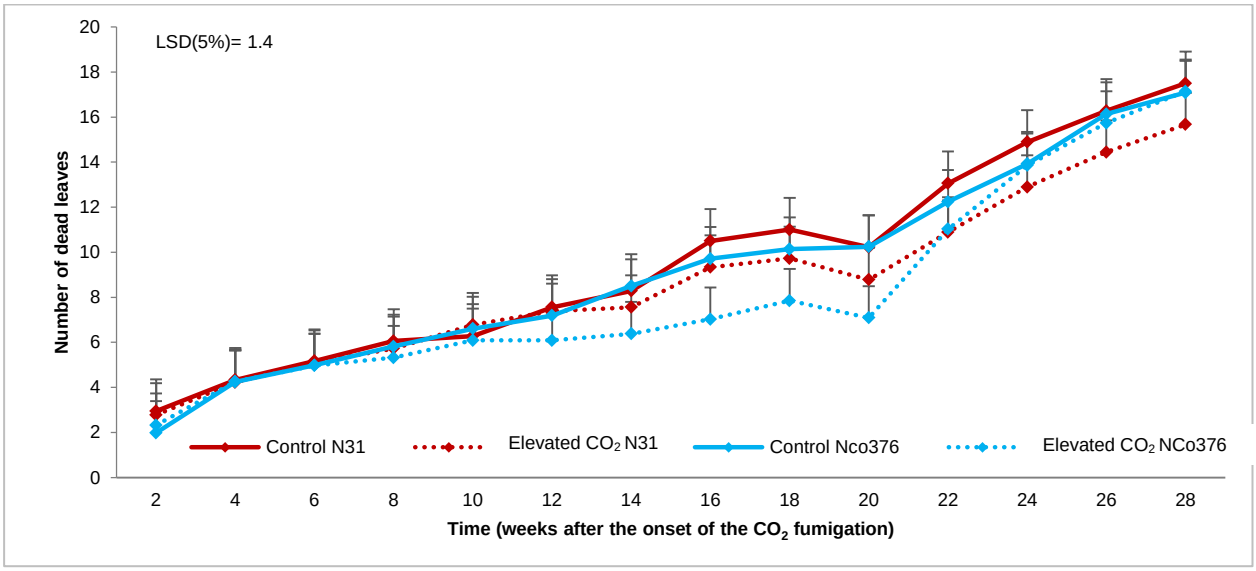


Figure 4-8: The number of dead leaves per stalk of two sugarcane varieties (N31 and NCo376) grown under elevated and ambient CO_2 conditions at Potchefstroom OTC facility.

TVD leaf length:

The TVD leaf length of the CO_2 treated sugarcane varied little from the control (Figure 4-9). Statistical differences ($P \leq 0.05$) were observed between 24 to 28 weeks of fumigation for NCo376, which displayed a difference of 10% between the CO_2 treatment and the control. The plants of NCo376 exposed to elevated levels of CO_2 increased the leaf length after 24 weeks of CO_2 fumigation. The control treatment of NCo376 had a maximum leaf length of 147.8 cm, while the elevated CO_2 treatment displayed a maximum length of 152.7 cm. The control treatment of N31 had a maximum leaf length of 154.7 cm, whereas the elevated CO_2 treatment had a maximum length of 153.1 cm.

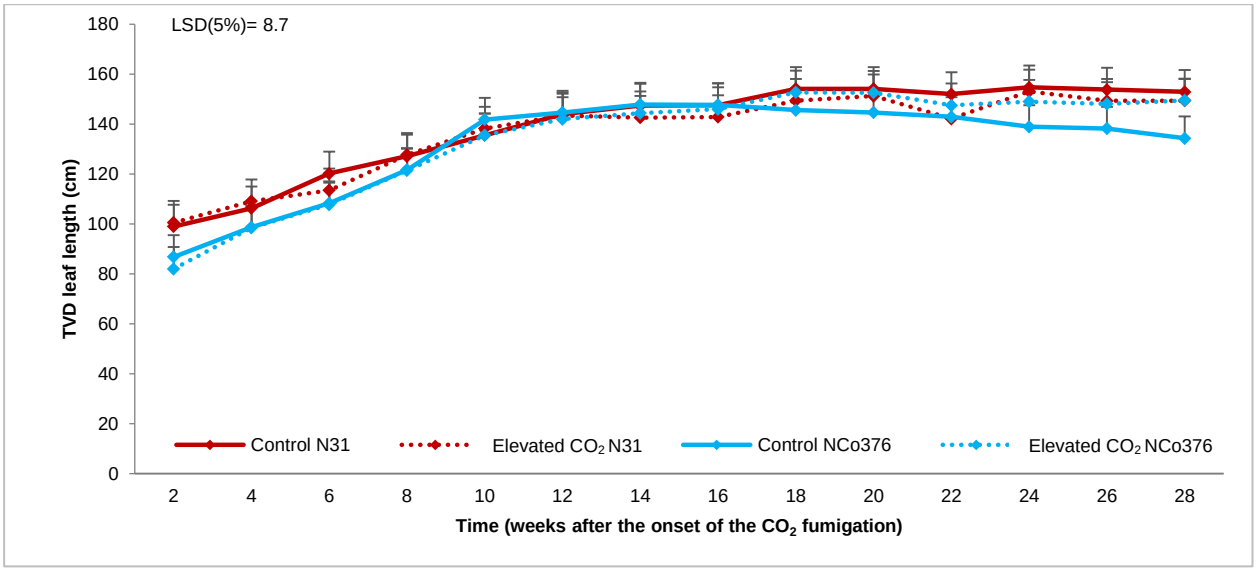


Figure 4-9: The TVD leaf length (cm) of two sugarcane varieties (N31 and NCo376) grown under elevated and ambient CO_2 conditions at Potchefstroom OTC facility.

TVD leaf width:

The two varieties differed significantly ($P \leq 0.05$) from each other in terms of the TVD leaf width. It was observed that N31 had a significantly ($P \leq 0.05$) higher TVD leaf width than NCo376, therefore indicating possible varietal differences (Figure 4-10). The only statistical differences ($P \leq 0.05$) found were for NCo376 at 26 to 28 weeks of CO_2 fumigation, which indicated that the elevated CO_2 treatment had a 19% higher leaf width than the control. The leaf width of the newly emerged leaves decreased from 24 weeks of CO_2 fumigation for the control treatment of NCo376, but not in the elevated CO_2 treatment.

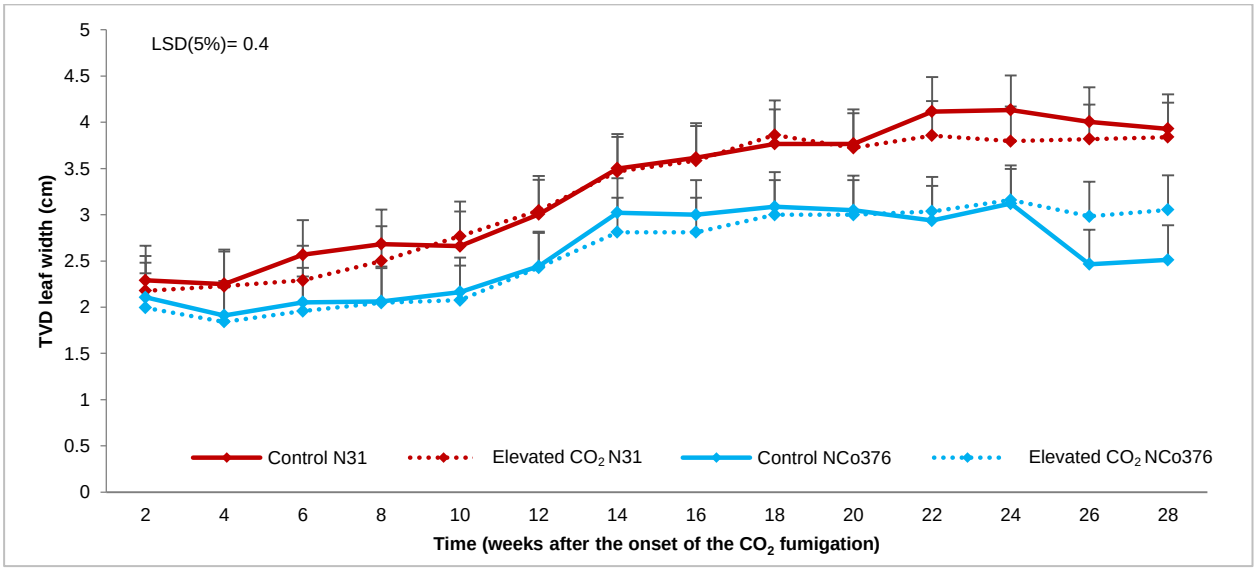


Figure 4-10: The TVD leaf width (cm) of two sugarcane varieties (N31 and NCo376) grown under elevated and ambient CO_2 conditions at the Potchefstroom OTC facility.

TVD leaf area:

The TVD leaf area was calculated by multiplying the TVD leaf length with the TVD leaf width and a shape factor (0.71).The differences between the elevated CO_2 treatment and the control varied little in terms of the CO_2 treatment (Figure 4-11). The only statistical differences ($P \leq 0.05$) found for N31 was at 22 weeks of CO_2 fumigation, where the elevated CO_2 treatment had a 13% lower TVD leaf area than the control treatment. The only differences ($P \leq 0.05$) found for NCo376 were at 26 to 28 weeks after CO_2 fumigation, which indicated that the elevated CO_2 treatment had a 28% higher TVD leaf area than the control.

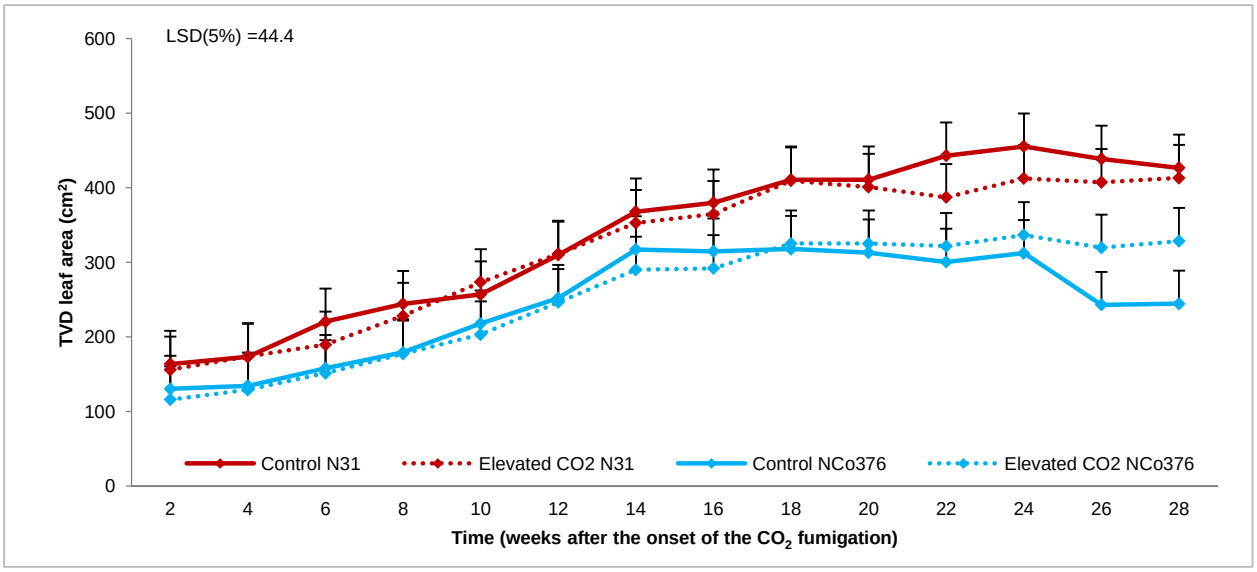


Figure 4-11: The TVD leaf area (cm^2) of two sugarcane varieties (N31 and NCo376) grown under elevated and ambient CO_2 conditions at the Potchefstroom OTC facility.

Stalk height:

It was observed that the stalk height of N31 was greater during the duration of the trial when compared to NCo376. During the first 16 weeks of CO_2 fumigation little variation occurred in the stalk height between the control treatment and the elevated CO_2 treatment for both varieties (Figure 4-12). At 18 weeks of fumigation significant differences ($P \leq 0.05$) between the control treatment and the elevated CO_2 treatment of NCo376 can be seen. The elevated CO_2 treated plants displayed a 13% increase in stalk height compared to the control for NCo376. In terms of NCo376 the control treatment had a maximum stalk height of 163 cm, while the elevated CO_2 treatment had a height of 186.6 cm. The control treatment of N31 had a maximum stalk height of 193 cm, whereas the elevated CO_2 treatment had a height of 201.4 cm, but this difference was not significant. After 22 weeks of CO_2 fumigation the length of the stalks increased at a much slower rate due to cooler temperatures.

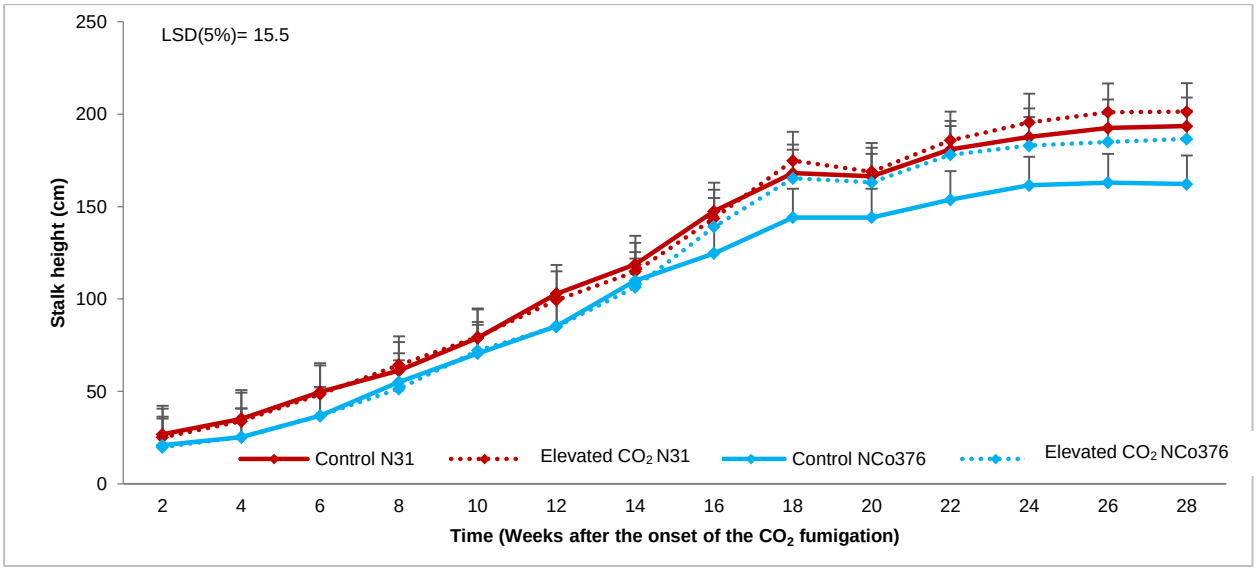


Figure 4-12: The stalk height (cm) of two sugarcane varieties (N31 and NCo376) grown under elevated and ambient CO_2 conditions at the Potchefstroom OTC facility.

Number of tillers:

Throughout the trial, NCo376 produced more tillers per pot than N31. At six and eight weeks of CO_2 fumigation, statistical differences ($P \leq 0.05$) were found between the control and elevated CO_2 treatment for N31 (Figure 4-13). At six and eight weeks of fumigation the control plants had a 32% and 23% higher tiller count per pot than the elevated CO_2 treated plants respectively. In terms of NCo376, significant differences ($P \leq 0.05$) were found between 12 to 20 weeks of CO_2 fumigation and again at 28 weeks of fumigation. In this case the elevated CO_2 treatment had on average a 19% higher tiller count per pot than the control treatment for NCo376.

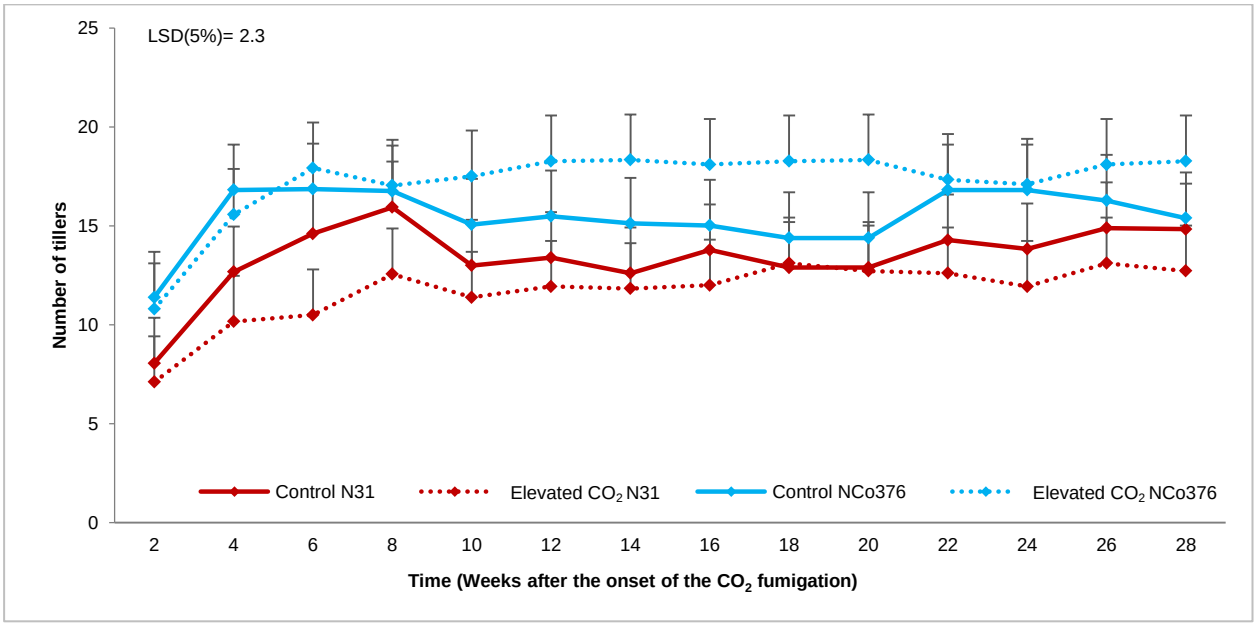
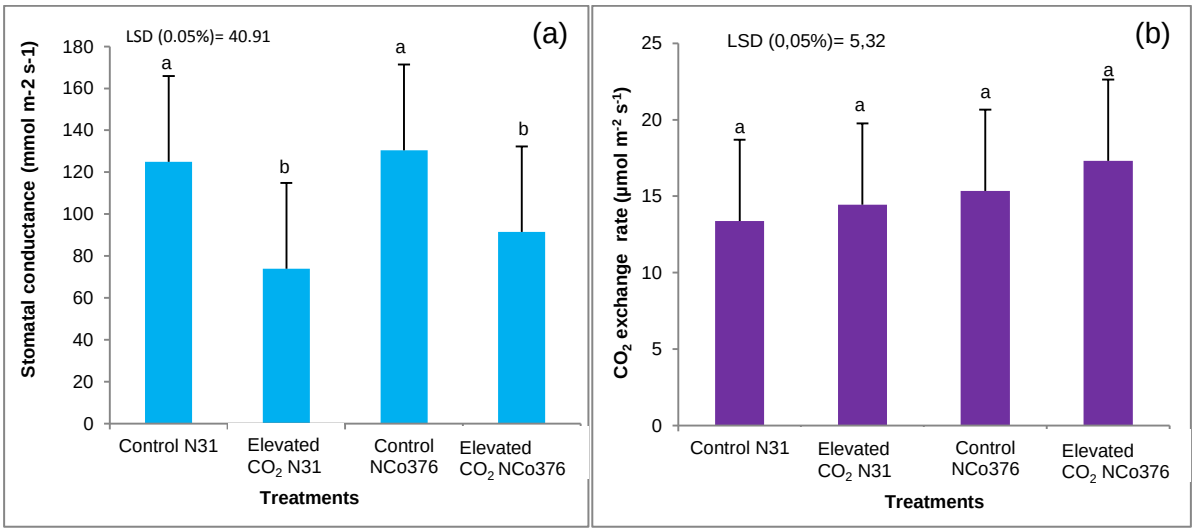


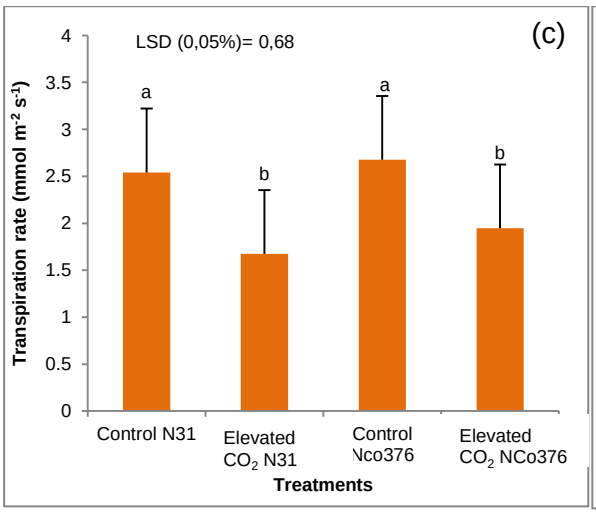
Figure 4-13: The stalk height (cm) of two sugarcane varieties (N31 and NCo376) grown under elevated and ambient CO2 conditions at the Potchefstroom OTC facility.

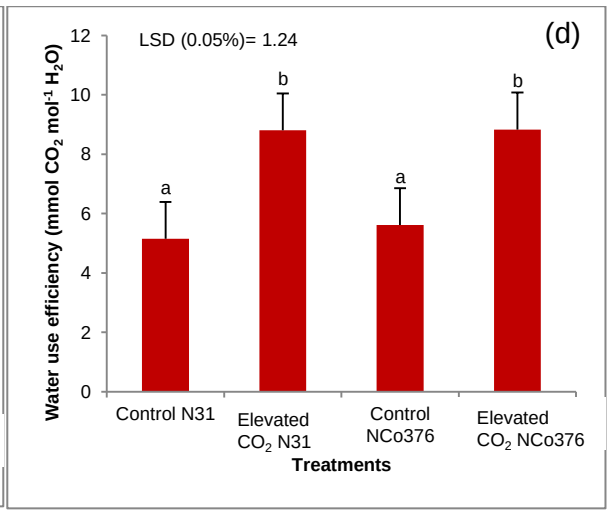
4.2.2 Photosynthetic associated responses

The effects of CO_2 treatment on stomatal conductance (G_s), CO_2 gas exchange rate (A_n), transpiration rate (E), single-leaf water use efficiency (WUE = A_n/E) and internal CO_2 concentration (C_i) were determined by combining the data sets of February and April 2016 in order to obtain an overall average for the trial duration. In terms of variety x treatment interactions pooling of the data sets of February and April were statistically valid. A significant ($P<0.05$) decrease in G_s was found for the elevated CO_2 treatments in both varieties (Figure 4-14a). The G_s of N31 decreased by 41%, whereas that of NCo376 decreased by 30%.

When comparing A_n for the control and the CO_2 treatment, no significant differences were found (Figure 4-14b). Transpiration rate (E) of the plants accords with the G_s data. A significant difference between the control and the CO_2 treatment was found in terms of E (Figure 4-14c). In N31 E decreased with 34%, whereas in NCo376 it decreased with 27% when exposed to the elevated CO_2 conditions. In both varieties single-leaf WUE increased when fumigated with elevated CO_2 (Figure 4-14d). On average the WUE of N31 increased by 71% and 57% for NCo376 when comparing the control treatment and the elevated CO_2 treatment. The C_i differed statistically ($P \leq 0.05$) between the control and elevated CO_2 treatment. N31 had a 67% higher C_i, while NCo376 exhibited a 84% increase when compared to the control treatments (Figure 4-14e). The differences in C_i agree with the G_s and E data, wherein an increase in C_i would initiate the closure of the stomata and therefore cause a decrease in E.







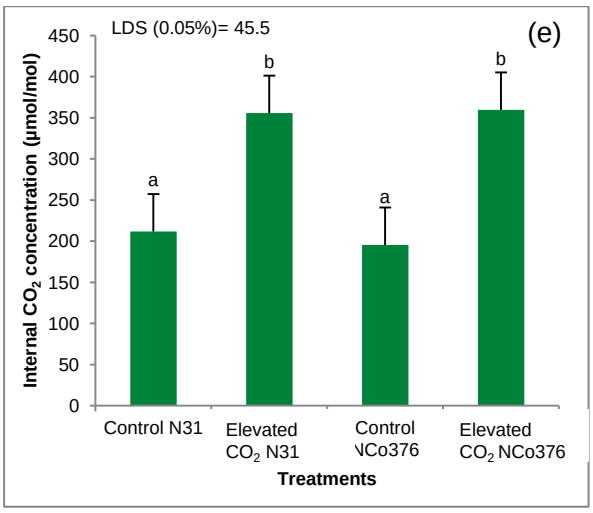


Figure 4-14: The (a) stomatal conductance (G_s), (b) assimilation rate (A_n), (c) transpiration rate (E), (d) water use efficiency (WUE) and (e) internal CO_2 concentration (C_i) of two sugarcane varieties (N31 and NCo376) grown under elevated (750 ppm) and ambient (400 ppm) CO_2 conditions at the Potchefstroom OTC facility. Different letters indicate significant ($P<0.05$) differences between treatments within a variety.

4.2.3 Chlorophyll *a* fluorescence

In this section the combined chlorophyll *a* fluorescence data of the measurements made in February and April 2016 will be shown for the purpose of comparing it (see Chapter 5) with the photosynthesis parameters shown in Figure 4-15.

4.2.3.1 The OJIP curve

The JIP-test provided information of the behaviour and *in vivo* vitality of the photosynthetic apparatus of the different sugarcane varieties. The shape of the O-J-I-P transient is very sensitive to stress caused by changes in environmental conditions (Baker & Rosenqvist, 2004; Strasser *et al*., 2004).

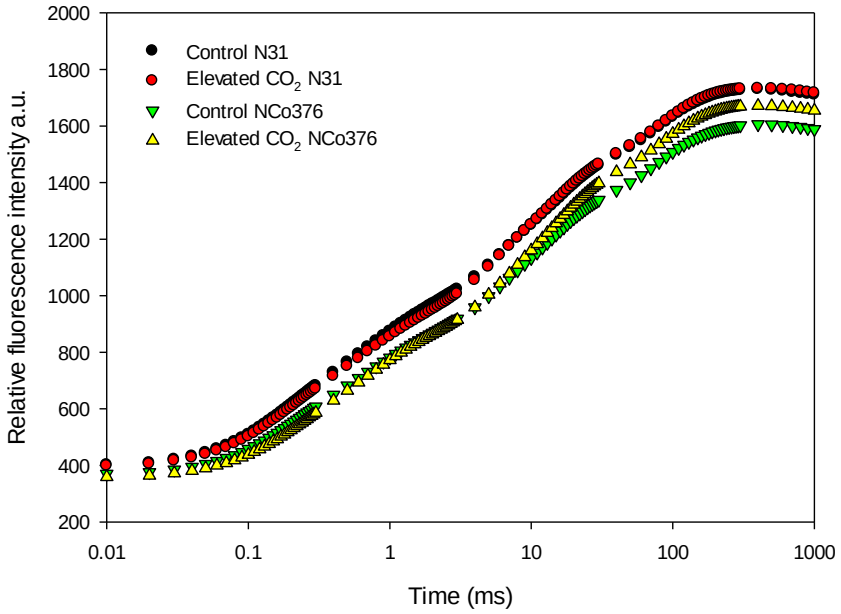


Figure 4-15: The OJIP transients of two sugarcane varieties (N31 and NCo376) grown under elevated (750 ppm) and ambient (400 ppm) CO_2 conditions at the Potchefstroom OTC facility.

Table 4-2: The significant differences found in the multiple turn over events between 30- 300 ms indicating the difference in ranks (measures the degree of similarity between two rankings) and q-value (a positive result is significant) and the p-value (significant difference if below 0.05).

Comparison	**Diff of Ranks**	**q**	**P<0.05**
Control NCo376 vs. Elevated CO_2 NCo376	985	4.105	Yes
Control N31 vs. Elevated CO_2 N31	183	0.763	No

During the trial the elevated CO_2 treatment of NCo376 had a significantly ($P \leq 0.05$) higher fluorescence intensity than the control treatment in the multiple turn over events found between 30 and 300 ms, but no significant differences were found between the two CO_2 treatments for N31 (Figure 4-15 and Table 4-2).The polyphasic rise of the OJIP transient for N31, however, was higher than for NCo376 irrespective of treatment.

4.2.3.2 Difference in relative variable fluorescence

To fully visualize and understand the changes in the redox potential of the OJIP transient, the difference in variable fluorescence is plotted, wherein different Δ-bands are revealed, which describes the flow of electrons through the electron transport chain.

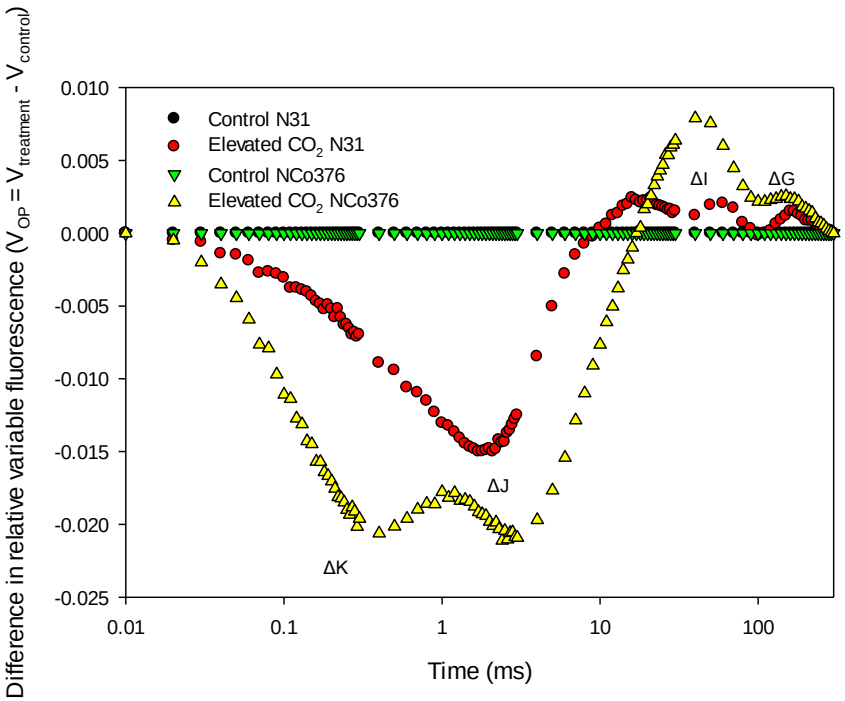


Figure 4-16: The difference in variable fluorescence (ΔV_t = ($V_{Teatment}$ - $V_{Control}$)) of two sugarcane varieties (N31 and NCo376) grown under elevated (750 ppm) and ambient (400 ppm) CO_2 conditions at the Potchefstroom OTC facility.

The ΔK- band (0.3 ms) reveals information regarding the reactions present on the donor side of the PSII reaction centre (Strasser *et al*., 2007). The ΔK- band is related to the inactivation of the oxygen evolving complex (OEC), therefore leading to an imbalance between the electron flow leaving the reaction centre and moving towards the acceptor side and the electron flow coming towards the reaction centre from the donor side of PSII. The difference in variable fluorescence plots in NCo376, in particular, displayed a negative ΔK- band, therefore the flow of electrons between the acceptor side and the donor side of PSII was stimulated by the elevated CO_2 (Figure 4-16).

The ΔJ-band, represents the oxidized primary quinone acceptor Q_A^- (Strasser *et al*., 2007). The presence of a positive ΔJ-band indicates the accumulation of Q_A^- at 2 ms and that the electrons will not move beyond Q_A^- to the secondary quinone acceptor. A negative ΔJ-band indicates the more efficient movement of electrons beyond Q_A^-. Both varieties displayed a negative ΔJ- band, indicating that the movement of electrons beyond Q_A^- was more effective (Figure 4-16).

The ΔI- band (30 ms) represents the transport of electrons between the PSII and PSI reaction centres (Strasser *et al.,* 2007). A slight positive ΔI- band was found for both varieties, which indicates that the transport of the electrons is slightly slower due to the accumulation of plastoquinol (Figure 4-16).

A very slight positive ΔG- band (100 ms) was found for both varieties (Figure 4-16), which implies that the secondary quinone acceptor is in a protonated state due to the accumulation of plastoquinol in the thylakoid membrane (Strasser *et al*., 2007).

4.2.3.3 Photosynthetic parameters

The results for the different photosynthetic parameters are characterised here.

The absorbance of E_{light} describes the ability of the active chlorophyll pigments of a plant, to absorb sunlight energy (Strasser *et al*., 2004). A significant difference ($P \leq 0.05$) was found between the two treatments for N31, during which the plants exposed to the elevated CO_2 had an increased ability to absorb sunlight energy compared to the control treatment (Figure 4-17a). No significant differences were observed for NCo376.

Trapping of $E_{excitation}$ describes the amount of light energy trapped per reaction centre (RC) (Strasser *et al*., 2004). A significant difference ($P \leq 0.05$) was found for N31 during which the elevated CO_2 treatment enhanced the plants ability to trap light energy (Figure 4-17b). No significant differences were observed for NCo376.

The conversion of $E_{excitation}$ to the e^- transport chain, describes the efficiency with which an electron will move further than Q_A^- (Strasser *et al*., 2004). A significant difference ($P \leq 0.05$) was found between the two treatments for N31. In this case the elevated CO_2 stimulated the flow of electrons further than Q_A^- (Figure 4-17c). No differences were observed for NCo376.

The PI_{ABS} is used to indicate the vitality of plants, including the light absorption efficiency, the maximum quantum yield of the photochemistry and the dark redox reaction efficiency (Strasser *et al*., 2004). A significant difference ($P \leq 0.05$) was found in the PI_{ABS} for N31, during which the elevated CO_2 had a higher PI_{ASB} compared to the control (Figure 4-17d). NCo376 however exhibited no significant differences in the PI_{ASB}.

Reduction of end e^- acceptors, describes the success of the reduction of $NADP^+$ to NADPH (Strasser *et al*., 2004). No significant differences were found for both varieties exposed to the two treatments (Figure 4-17e).

PI_{Total} considers the ability of a plant to produce NADPH and it evaluates the total vitality of a plant (Strasser *et al*., 2004). The control treatment of NCo376 had a significantly higher ($P \leq 0.05$) PI_{Total} than the elevated CO_2 treatment, but no differences were found in the PI_{Total} of N31 (Figure 4-17f).

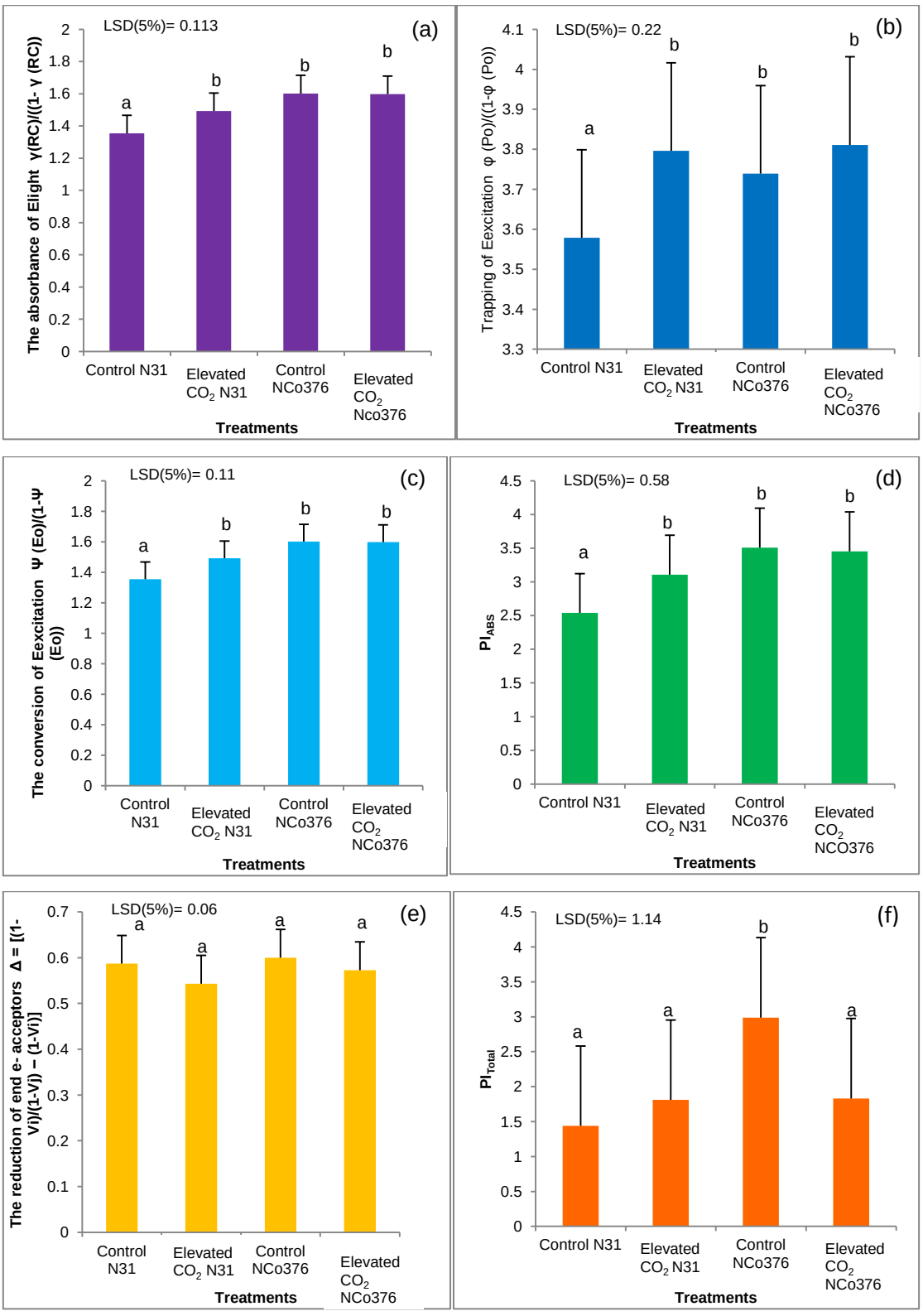


Figure 4-17: The (a) absorbance of E_{light} γ(RC)/((1- γ (RC)) (gs), (b) the trapping of $E_{excitation}$ φ (Po)/((1-φ (Po)), (c) the conversion of $E_{excitation}$ Ψ (Eo)/(1-Ψ (Eo)), (d) the PI_{ABS}, (e) the reduction of end e- acceptors Δ = [(1-Vi)/(1-Vj) – (1-Vi)] and (f) the PI_{Total} of two sugarcane varieties (N31 and NCo376) grown under elevated (750 ppm) and ambient (400 ppm) CO_2 conditions at the Potchefstroom OTC facility. Different letters indicate significant ($P<0.05$) differences between treatments within a variety.

4.2.4 Chlorophyll content

Both varieties responded differently to the elevated CO_2 treatment. Overall NCo376 had a higher chlorophyll content (SPAD units) than N31. It could therefore be possible that varietal differences in terms of the chlorophyll content do exist. The chlorophyll content in November (four weeks after the onset of CO_2 fumigation) displayed significant differences ($P \leq 0.05$) between CO_2 treatments for both varieties (Figure 4-18). The control plants of NCo376 and N31 had respectively a 10% and 19% higher chlorophyll content than the elevated CO_2 treatments. In December (eight weeks after the onset of CO_2 fumigation) the elevated CO_2 treatment in N31 had a 27% higher chlorophyll content than the control. For the remainder of the treatment period there were no significant treatment effects in both varieties.

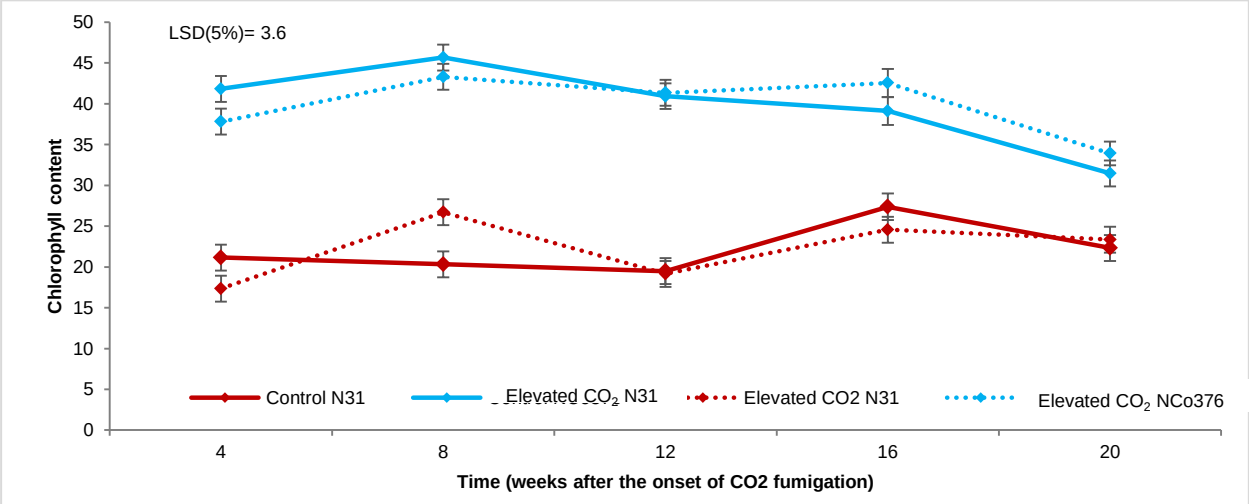


Figure 4-18: The chlorophyll content (SPAD units) of two sugarcane varieties (N31 and NCo376) grown under elevated (750 ppm) and ambient (400 ppm) CO_2 conditions at the Potchefstroom OTC facility.

4.3 Destructive measurements

4.3.1 Total dry biomass

As was noted previously that elevated CO_2 caused a transient increase in green leaf number (Figure 4-7) and stalk height (Figure 4-12) in both varieties after 18 weeks of CO_2 fumigation. However, based on the results of the destructive harvest (May 2016); it was found that elevated CO_2 did not cause a significant change in total above-ground dry plant biomass (TDM) per pot in the two varieties (Figure 4-19). There was also no significant difference in above-ground biomass production per pot between the two varieties.

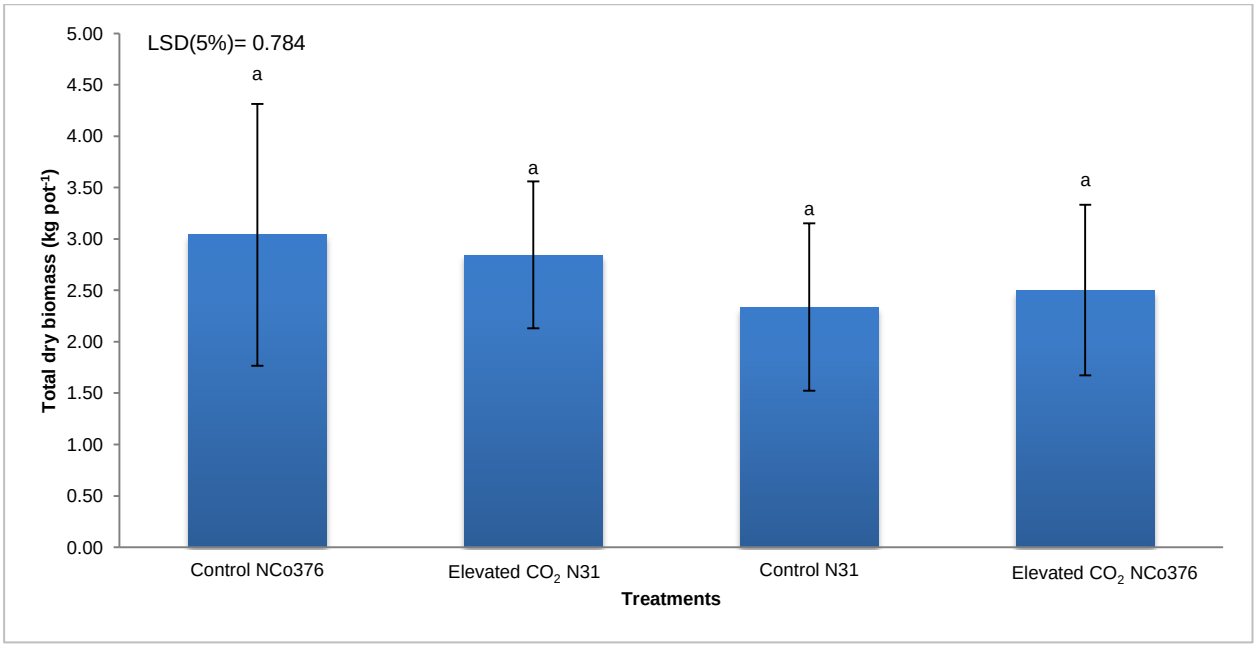


Figure 4-19: The total above-ground dry biomass (kg pot^{-1}) of two sugarcane varieties (N31 and NCo376) grown under elevated (750 ppm) and ambient (400 ppm) CO_2 conditions at the Potchefstroom OTC facility.

4.3.2 Fresh stalk mass

In agreement with above-ground total dry biomass (Figure 4-19) it was found that elevated CO_2 did not cause a significant change in stalk fresh mass per pot in the two varieties (Figure 4-20). There was also no significant difference in stalk fresh mass per pot between the two varieties.

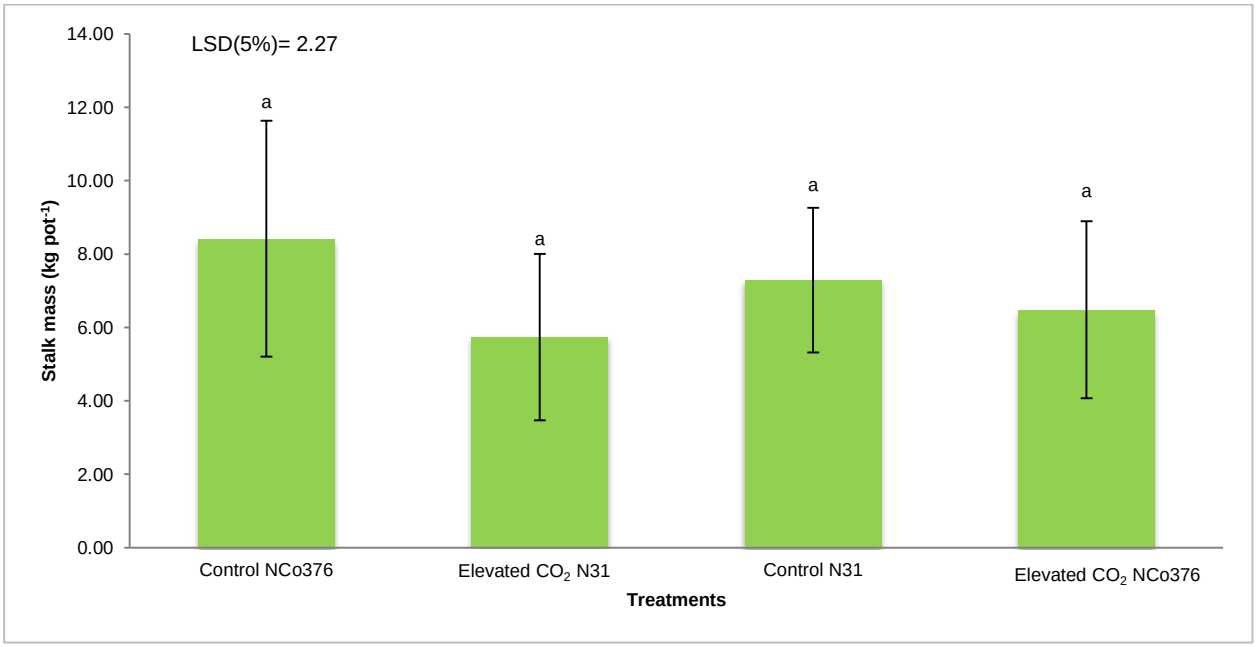


Figure 4-20: The stalk fresh mass (kg pot^{-1}) of two sugarcane varieties (N31 and NCo376) grown under elevated (750 ppm) and ambient (400 ppm) CO_2 conditions at the Potchefstroom OTC facility.

4.3.3 Biomass components

The dry biomass fractions (stalk, green leaves, residue and tops) is represented in (Figure 4-21). There were no differences in the proportion of biomass allocated to the green leaves, tops and residue fractions between CO_2 treatments. A difference between the varieties in terms of increase in growth in response to CO_2 was not expected, but rather changes in the water use. N31 is expected to have a higher biomass variety when compared with NCo376 (without having the elevated CO_2 factor), but due to the large variation found between the biomass per pot, limited any significant differences.

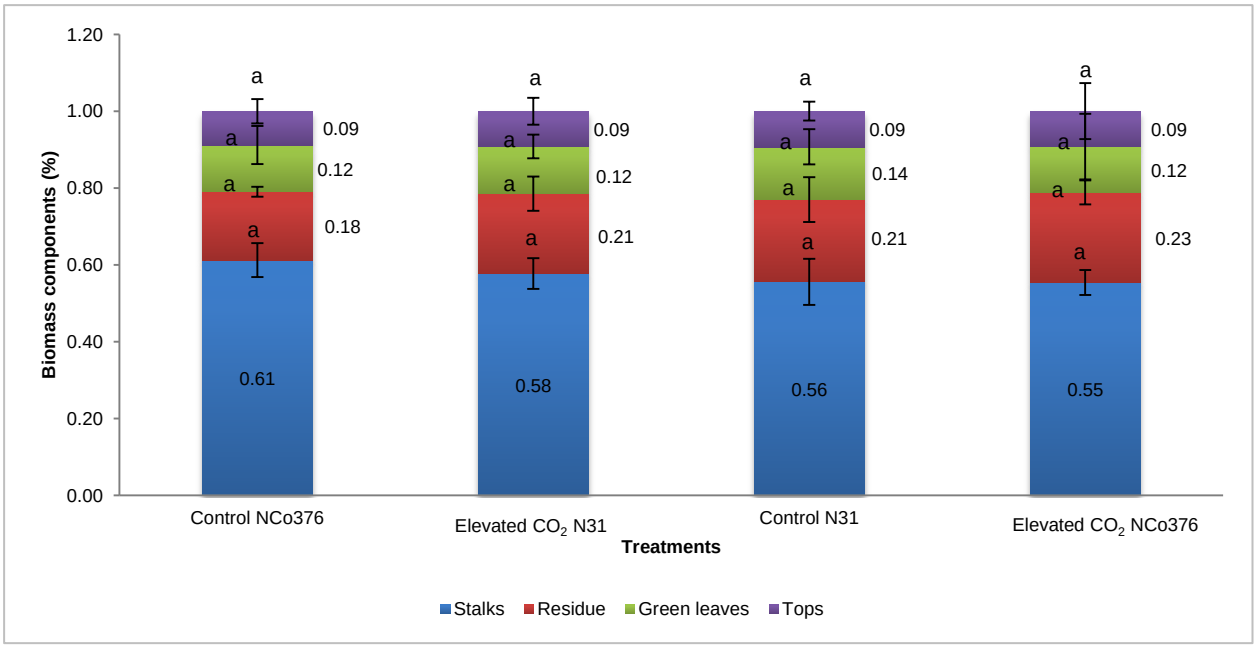


Figure 4-21: The dry biomass components (%) of two sugarcane varieties (N31 and NCo376) grown under elevated (750 ppm) and ambient (400 ppm) CO_2 conditions at the Potchefstroom OTC facility.

4.3.4 Cane quality

The stalk quality fractions (fibre, sucrose and non-sucrose) are represented in (Figure 4-22). No differences in these fractions were found for both N31 and NCo376 between the control CO_2 treatments. It should be noted that these were not mature sugarcane stalks; they were immature and as such, would have had low juice purity and low sucrose levels. The vast difference in stalk quality between the two varieties is observed when the stalks are mature at harvest.

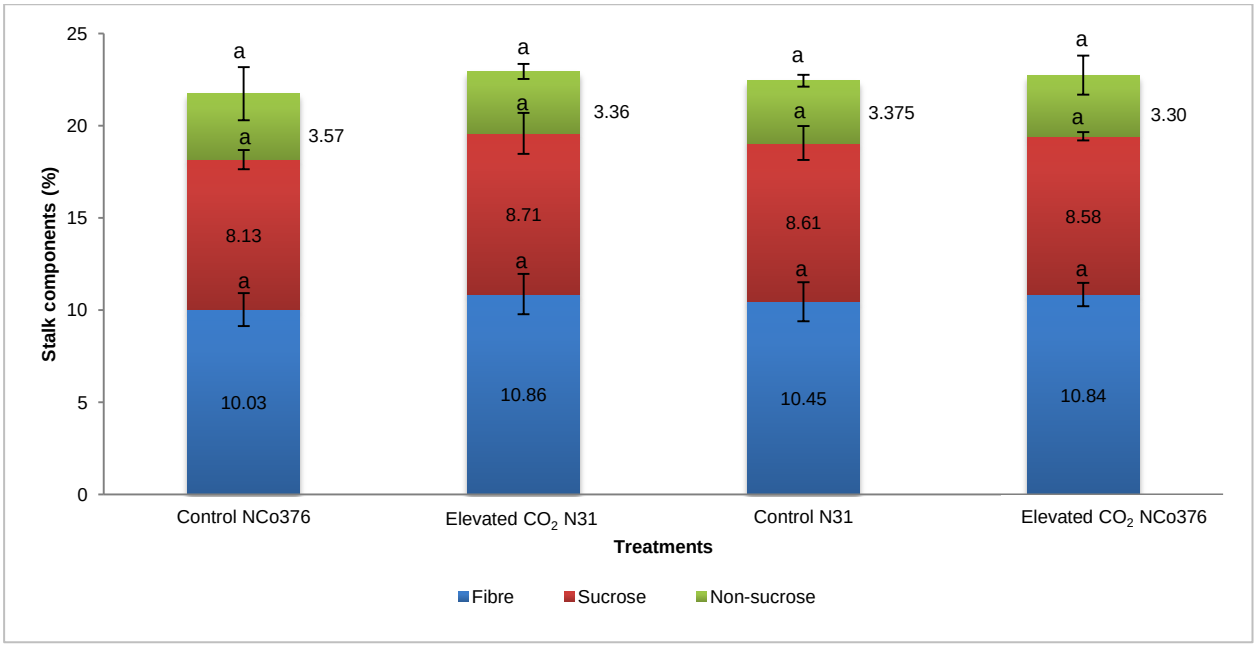


Figure 4-22: The stalk quality components (%) of two sugarcane varieties (N31 and NCo376) grown under elevated (750 ppm) and ambient (400 ppm) CO_2 conditions at the Potchefstroom OTC facility.

4.3.5 Green leaf area at harvest

The green leaf area per pot for NCo376 and N31 at ambient and elevated CO_2 is represented in Figure 4-23. No significant differences in the green leaf area per pot were found for both varieties. At the time of harvest, very few (approximately 3 – 5) green leaves were found on each stalk due to the lower temperatures which facilitated leaf loss and ripening.

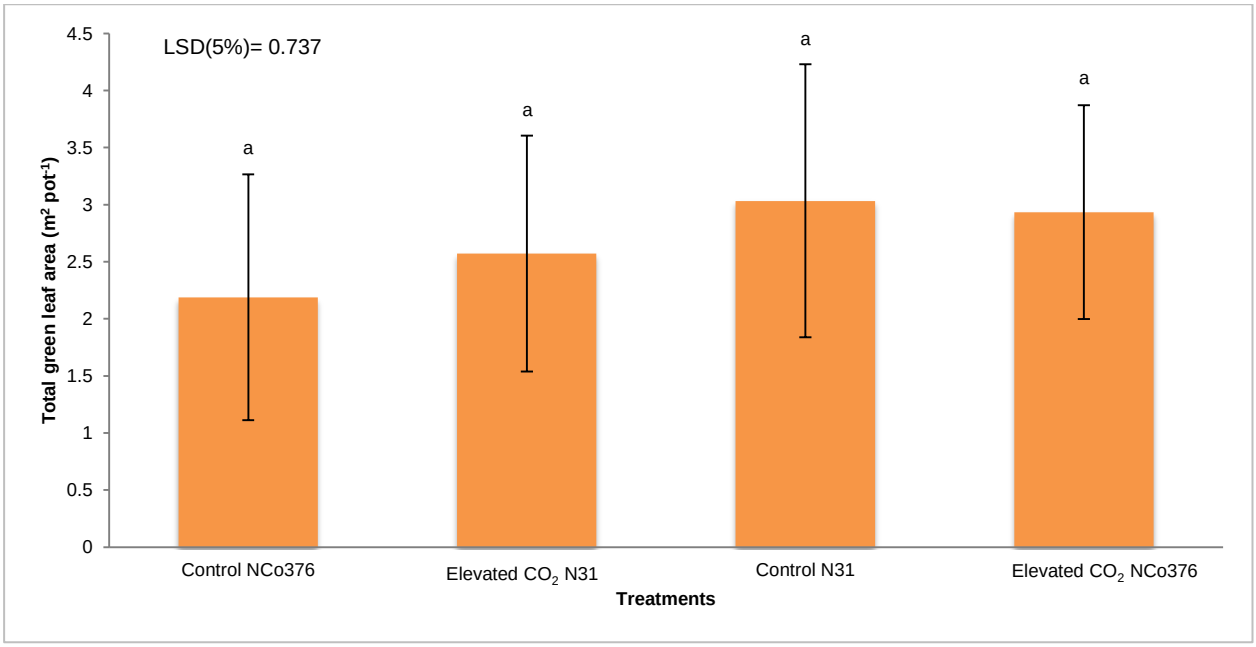


Figure 4-23: The total green leaf area (m^2 pot^{-1}) at harvest of two sugarcane varieties (N31 and NCo376) grown under elevated (750 ppm) and ambient (400 ppm) CO_2 conditions at the Potchefstroom OTC facility.

4.3.6 The dry biomass N content (NUE)

There were no significant differences found in the NUE between the control and elevated CO_2 treatments for both varieties (Table 4-3).

Table 4-3: The nitrogen use efficiency (g/g) of two sugarcane varieties (N31 and NCo376) grown under elevated (750 ppm) and ambient (400 ppm) CO_2 conditions at the Potchefstroom OTC facility.

Varieties	Treatment	Total dry matter (g)	Total Plant N content (%)	NUpE (plant N/N supplied)	NUtE (biomass/plant N)	NUE (NUpE × NUtE)
N31	Control	256.35	2.13	0.0896	120.1044	10.7713[a]
N31	Elevated CO_2	229.61	1.99	0.0840	120.2503	9.6477[a]
NCo376	Control	257.23	2.46	0.1036	106.3929	10.8081[a]
NCo376	Elevated CO_2	229.28	2.01	0.0847	115.9306	9.6336[a]

4.4 Crop modelling component

According to the Canesim model, a marginal increase in the cane and sucrose yield in response to the elevated CO_2 was stimulated in both varieties (Table 4-4). The model also simulated a decrease in crop water use for the elevated CO_2 treated plants of both varieties.

Table 4-4: The results for the Canesim simulation of two sugarcane varieties (N31 and NCo376) grown under elevated (750 ppm) and ambient (400 ppm) CO_2 conditions at the Potchefstroom OTC facility.

Results	Elevated CO_2 N31	Control N31	Elevated CO_2 NCo376	Control NCo376
Cane Yield (t ha^{-1})	76.5	75	86	84.3
Sucrose Yield (t ha^{-1})	8.9	8.7	11.3	11
Crop Water Use (mm)	819	878	780	840

CHAPTER 5 DISCUSSION

The hypothesis that was tested in this study was that elevated CO_2, in the absence of any soil water deficit, will increase the photosynthetic efficiency of sugarcane, which in turn will result in growth stimulation and higher sucrose content.

In this study a transient stimulating effect was observed during the vegetative growth stages of the sugarcane when exposed to 750 ppm CO_2. Exposure of sugarcane to 750 ppm CO_2 resulted in an increase in the number of green leaves compared to the control plants (Figure 4-7). The number of dead leaves produced in the control treatment was higher than that of the elevated CO_2 treatment (Figure 4-8), therefore indicating that the elevated CO_2 stimulated the growth of the plants. The elevated CO_2 also influenced the leaf morphology in terms of leaf width and leaf length (Figure 4-9 and 4-10). For example, NCo376 produced longer leaves when exposed to elevated CO_2 levels. The stalk growth for NCo376 also increased under elevated CO_2 levels (Figure 4-12). The effects of elevated CO_2 seemed to be greatest during the warmer months when the plants were in their vegetative growth stage. When referring to the response of the number of green leaves (both N31 and Nco376), stalk height (NCo376) and TVD leaf area (both N31 and NCo376) to elevated CO_2, the most noteworthy differences were found from February 2016, but as the end of the growing season neared and ambient chamber temperatures decreased, these differences disappeared. No differences, positive or negative, were found in the total biomass yield and stalk quality between the control and elevated CO_2 treated plants at harvest (Figure 4-19 and Figure 4-22).

The NUE (NUpE × NUtE) of plants tend to decrease when exposed to elevated CO_2 (720 ppm) levels (Bloom *et al*., 2010). As the atmospheric CO_2 concentration rises, NO_3 assimilation is inhibited in the plants due to the decrease of plant organic N compounds (Bloom *et al*., 2010). Crops will therefore become depleted of organic nitrogen compounds, including proteins. In this study, no significant differences were found between the elevated CO_2 treated plants when compared to the control plants (Table 4-3).

The higher CO_2 levels did result in significant increases ($P \leq 0.05$) in the C_i for both varieties (Figure 4-14e). The transpiration rate and stomatal conductance, both responded to the elevated CO_2 concentration. The significant increase ($P \leq 0.05$) in the C_i coincided with a reduction in the G_s in both varieties (Figure 4-14a). A similar trend has been observed among a wide variety of both C4 (such as maize) and C3 (such as wheat) species (Vu *et al*., 2006). A reduction in the G_s also coincided with a reduction in E for both varieties, which resulted in an improved WUE of the plants (Figure 4-14c, d). In other studies, the same trend occurred for maize when exposed to a triple-ambient CO_2 concentration, which resulted in a 71% decrease in the G_s and a 225% increase in the WUE when compared to the control plants (Vu *et al*.,

2006). The findings of the present study agree with that of Vu *et al*. (2006) who observed a reduction in both the G_s and E and a 52% increase in the WUE for sugarcane grown under the elevated CO_2 conditions (720 ppm) compared to the control plants (360 ppm). The Canesim model also simulated a reduction in crop water use for the elevated CO_2 treated plants compared to the control plants (Table 4-4). When comparing the simulation with the photosynthetic data observed in this study it is found to be in agreement. The measured reduction in Gs and E supports the simulation by the model that crop water use would decrease under elevated CO_2 growing conditions.

In terms of the fluorescence data, the most significant difference, was found for NCo376 in which the elevated CO_2 treated plants had a higher fluorescence intensity compared to the control plants, however no significant differences were found for N31 (Figure 4-15 and Table 4-2). Associated with this is the PI_{ABS} values in which the elevated CO_2 treated plants for N31 had a significantly ($P \leq 0.05$) higher PI_{ABS} when compared the control plants. This suggests that the vitality of N31 has improved in response to elevated CO_2 levels, however no significant differences were observed for NCo376 (Figure 4-17d). It was also during this period where significant differences in the number of green leaves and stalk height (NCo376) were found. During these two months the elevated CO_2 treated plants displayed an increased number of green leaves and in the case of NCo376 a higher stalk length.

Further analysis of the fast polyphasic fluorescence (OJIP) transient revealed that elevated CO_2 significantly ($P > 0.05$) influenced the redox potential of photosystems I and II of NCo36 (Table 4-2). These differences were especially noticeable in the multiple turnover phase of the OJIP-transient recorded in variety NCo376 under elevated CO_2. No significant differences, however, were found for N31.

Double normalization of the fluorescence rise between 0.01 ms and 300 ms revealed hidden bands within the transient (Strasser *et al*., 2004: Mehta *et al*., 2010). The different bands visually explain what the effect of elevated CO_2 was on the photosynthetic efficiency of sugarcane. The OJIP curves are divided into two phases, namely the single turnover event, which occurs between 0 ms and 2 ms, and the multiple turnover event, which occurs between 2 ms and 300 ms (Vredenberg *et al.*, 2006). The single turnover event describes the photosynthetic light reactions from the point where sunlight is absorbed to where Q_A^- is formed (Vredenberg *et al.*, 2006). During the single turn over event, the elevated CO_2 stimulated the electron flow leaving the reaction centre and moving towards the acceptor side and the electron flow approaching the reaction centre from the donor side of PSII. Therefore the electrons in turn moved beyond Q_A^- to the secondary quinone acceptor. The multiple turnover event describes the reaction further, from Q_A^- to the reduction of $NADP^+$, which is the end electron accepter (Vredenberg *et al.*, 2006). During the multiple turn over events, the elevated CO_2 slightly

decreased the rate at which electrons where transported due to the high accumulation of plastoquinone which lead to a decrease in the reduction of $NADP^+$ to NADPH (Nicotinamide adenine dinucleotide phosphate). However, comparing the results of the multiple turn over event with the photosynthetic parameters (the combined data sets); specifically with the reduction of the end e^- acceptors ($\Delta = [(1-V_i)/(1-V_j) - (1-V_i)]$), indicated that the effect of the elevated CO_2 on the reduction of $NADP^+$ was very small and not significant.

Generally, a decrease in the reduction of CO_2 to sugars during the Calvin cycle will result in a decrease in the reduction of $NADP^+$ to NADPH. Therefore, a decrease in the A_n rate will lead to a decrease in the reduction of $NADP^+$ because of the limited supply of CO_2. In this study however, no significant differences were found in the A_n rate, therefore indicating that the reduction of CO_2 did not require additional energy in the form of NADPH. Baker & Rosenqvist (2004) proposed several explanations to clarify the decrease in NADPH and ATP utilization. These explanations include: a decreases in the carboxylation efficiency, a decrease in the ability to regenerate ribulose 1,5-bisphosphate, a decrease in the supply of CO_2 via the stomata to the sites of carboxylation, or a decrease in the transport of carbohydrates out of the cells.

Previous studies with sugarcane reported a positive response in the plant biomass associated with elevated levels of CO_2 (720 ppm) even in irrigated experiments (Vu *et al*., 2006; de Souza *et al*., 2008). In these experiments a reduction in the G_s and E was reported, which would have led to an improvement in the soil water status due to lower crop water use, as indeed simulated by Canesim in this study. Hence, if any soil water deficit would have been present in the experiments of Vu *et al*. (2006) and de Souza *et al*. (2008), these effects would eventually increase the CO_2 exchange rates of leaves and leaf production rate that would lead to an increase in the assimilation rate and biomass for elevated CO_2 treated plants. The observed increase in biomass and yield is thus likely due to the indirect improvement of photosynthesis through avoiding water stress (Vu *et al*., 2006; de Souza *et al*., 2008). Vu *et al*. (2006) also suggested that the 31% increase in the leaf area and the 55% increase in the stalk yield of the elevated CO_2 treated plants could have been partly due to the enhanced water use efficiency of the plants. de Souza *et al*. (2008) also found a 40% increase in the biomass yield. Stokes *et al*. (2016), however, argued that the increases in sugarcane yield observed by Vu *et al*. (2006) and de Souza *et al*. (2008) could have occurred due to the unintentional exposure of the plants to soil water deficit conditions.

Stokes *et al*. (2016) suggested that if altered levels of CO_2 were to affect sugarcane growth via the alleviation of water stress; then the level of water stress (under the current CO_2 environment) would affect the extent in which the plants would respond to CO_2. Stokes *et al*. (2016) did not report any increases in biomass due to water stress being avoided in their experiments. In this study we did not observe any biomass increases, as no changes in the

carbon partitioning in response to elevated CO_2 were found therefore, which suggests that the sink strength does not change in sugarcane in response to elevated CO_2. Our findings therefore coincide with that of Stokes *et al*. (2016).

The Canesim model however simulated a marginally higher cane yield (t ha^{-1}) and sucrose yield (t ha^{-1}) for the plants exposed to the elevated CO_2 compared to the control plants for both varieties (Table 4-4). Based on the observed biomass data, that showed no stimulation effects due to elevated CO_2, the model appears to slightly overestimate the benefit of elevated CO_2 on cane and sucrose yields (Figure 4-19 and Figure 4-22). Possible reasons for this could be the very shallow soil that had to be selected in the Canesim simulation (to mimic the use of pots in the OTC experiment), and the inability of the model to irrigate the simulated crops more than once a day (compared to four times a day in the OTC experiment). Evaluation of model output revealed that under these conditions the model was not capable of excluding drought stress entirely in the simulated crops. Because decreased crop water use was simulated concurrently in response to elevated CO_2, this would have benefitted these crops leading to marginal increases in simulated yields. The model simulations thus supports the argument that even very mild drought stress in previous experiments could explain the reported increases in sugarcane yields in response to elevated CO_2.

From above discussion it is hypothesised that an improvement in the WUE would be of benefit for sugarcane grown under elevated CO_2 conditions when soil moisture is limiting. The presence of water deficit stress will influences various metabolic processes of the entire plant resulting in reductions in leaf area, root growth and stomatal closure (da Graça *et al*., 2010). Improved WUE associated with elevated levels of CO_2, and associated conservation of available soil water, would therefore help the plants to avoid some of these stresses related to soil water deficit conditions. Elevated levels of CO_2 could possibly delay the effects of soil water deficit and thereby enhance the biomass productivity of sugarcane. Therefore the interactive effects of elevated CO_2 and drought on the biomass yield and sucrose content of sugarcane should be investigated further in order to test this hypothesis, explain conflicting reports in the literature and to increase our ability to model sugarcane productivity in future climates where elevated CO_2 and episodes of soil water deficit are predicted to co-occur.

CHAPTER 6 CONCLUSION

The data from this study elucidated the effect of elevated CO_2 on the photosynthetic efficiency of sugarcane. This study revealed that the leaf photosynthesis of sugarcane responded to the elevated CO_2 levels, with a decreased stomatal conductance and an increased water use efficiency. The TVD leaf area and stalk height of the sugarcane was positively affected early in the trial. However, the proposed biomass and sucrose increase was not observed at harvest. The elevated CO_2 did not have a positive or negative effect on the total dry biomass and sucrose content during this trial. The elevated CO_2 treated plants had a higher fluorescence intensity than the control plants during the vegetative growth stages, therefore indicating that the sugarcane was responsive to the elevated CO_2 (750 ppm) during this period. However, no effect on the rate of photosynthesis could be demonstrated. Areas lacking research is the interactions of leaf and air temperature, as well as the shoot water relations of sugarcane under elevated CO_2 conditions. Further research could also be conducted to evaluate the effect of water stress and elevated CO_2 on sugarcane. This would broaden our understanding of what effect elevated CO_2 would have on sugarcane in different water regimes.

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