GROWTH, LEAF GASEOUS EXCHANGE AND NUTRITIVE VALUE OF SELECTED SUMMER FORAGE LEGUMES AND THEIR CONTRIBUTIONS TO SUCCEEDING WINTER GRASS (*Secale cereale*) IN DISTINCT AGRO-ECOLOGICAL ZONES OF LIMPOPO PROVINCE

MINI-DISSERTATION

Submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

PASTURE SCIENCE

BY

PHILEMON LESETJA LEKGOTHOANE

2022

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PL LEKGOTHOANE 2022

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(School of Agricultural and Environmental Sciences)

at the

UNIVERSITY OF LIMPOPO

SUPERVISOR : PROF K.K AYISI

CO-SUPERVISOR : MRS L.M MOTSHEKGA

2021

DECLARATION

I, Philemon Lesetja Lekgothoane declare that 'Growth, Leaf Gaseous Exchange and Nutritive Value of Selected Summer Forage Legumes and their Contributions to Succeeding Winter Grass (*Secale cereale*) in Distinct Agro-Ecological Zones of Limpopo Province' is my work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references, and that this work has not been submitted before for degree purposes at this or any other institution.

Philemon Lesetja Lekgothoane	20/12/2021
………………………………………..	……………………..
Full Names	**Date**

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DEDICATION

I am dedicating this research to my mother (Maedi) and my two brothers (Maredi and Lesiba)

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LIST OF ABBREVIATIONS AND SYMBOLS

A= Photosynthetic rate (μmolm^{-2}s^{-1})

ANOVA = Analysis of Variance

BNF= Biological Nitrogen Fixation

B= Boron

Ca = Calcium

CCI = Chlorophyll content index

Ci = Sub-stomatal CO_2 (mol m^{-2}s^{-1})

CP = Crude Protein

CPY = Crude protein Yield

Cu = Cupper

°C = Degree Celsius

DAE = Days after emergence

E = Transpiration rate (mmol m^{-2} s^{-1})

Fe = Iron

G = Grams

GHG= Greenhouse gases

gs = Stomatal conductance (mol m^{-2} s^{-1})

K = Potassium

Kg = Kilogram

Kg ha^{-1} = Kilogram per hectare

LSD = Least significant differences

$Mg\ kg^{-1}$ = Milligram per kilogram

Mg = Magnesium

Mn = Manganese

N = Nitrogen

Na = Sodium

NRF = National Research Foundation

P = Phosphorus

RCBD = Randomized Complete Block Design

SAS = Statistical Analysis System

T_{min} = Maximum Temperature (°C)

T_{max} = Maximum Temperature (°C)

Zn = Zinc

ABSTRACT

In South Africa, livestock production is a rapidly growing business in the agricultural sector contributing up to 46.9% of the gross domestic value. The shortage of adequate, good quality forage during the winter months is one of the biggest problems confronting livestock farmers in the Limpopo Province. This study was initiated in 2019 to evaluate the production potential and nutritive value of different summer annual forage legumes, namely sunnhemp (*Crotalaria juncea*), forage cowpea (*Vigna unguiculata*), lablab bean (*Lablab purpureus*), and pigeon pea (*Cajanus cajans*), and their impact on succeeding winter stooling ryegrass (*Secale cereale*), at the University of Limpopo experimental farm Syferkuil and a Cooperative farmers' field at Ofcolaco. The study was evaluated in a randomized complete block design with four replications. Dry matter yield, crude protein, crude protein yield, leaf chlorophyll content, Normalized Difference Vegetative Index (NDVI), and leaf gaseous exchange parameters of forage crops were statistically analysed with Statistical Analysis System (SAS), Enterprise Version 9.4, using the least significant difference (LSD) method for mean comparison.

Pigeon pea biomass accumulation was 57% lower than the average of the three other legumes at Syferkuil. Sunnhemp produced superior biomass ($P<0.05$) compared to the other three species, reaching a peak yield of 3142.4 kg.ha^{-1} and 8970.8 kg ha^{-1} at Syferkuil and Ofcolaco, respectively. Cowpea and lablab produced similar biomass at Syferkuil. The crude protein content of the forage species ranged from 22.91% to 26.82% at Syferkuil and 17.03% to 23.84% at Ofcolaco. Leaf chlorophyll content differed ($P<0.001$) among the forage legume species at both locations with cowpea producing the highest chlorophyll content at Syferkuil, whereas at Ofcolaco, pigeon pea constantly produced the highest chlorophyll compared to other species. Pigeon pea was the only species rated moderately healthy with Normalised Difference Vegetative Index (NDVI) readings at Syferkuil, unlike at Ofcolaco where all forage legumes were rated as very healthy. At Syferkuil, no root nodules were observed among all the forage legumes at all sampling dates but at Ofcolaco, nodules were produced at 44 DAE with cowpea producing the highest, 92.32% higher than the average of sunnhemp, lablab, and pigeon-pea. At this location pigeon pea did not nodulate. The transpiration rate at Syferkuil was significant ($P<0.01$) among the species starting with a low transpiration rate from 24 days after planting and reaching

their peak at 66DAE. Overall, pigeon pea had the highest ($P<0.05$) mean transpiration rate compared to the other species.

At Ofcolaco the forage legume treatment did not have any significant ($P>0.05$) influence on transpiration rate, stomatal conductance and sub-stomatal conductance. The transpiration rate of the species ranged from 0.1 mol m^{-2} s^{-1} to 5.15 mol m^{-2} s^{-1} across all sampling dates whereas stomatal conductance ranged from 0.06 to 5.59 mol m^{-2} s^{-1} at Syferkuil and 0.1 to 5.15 mol m^{-2} s^{-1} at Ofcolaco, across all sampling dates and species. At Syferkuil, the mean stomatal conductance values ranged from 129.75 mol m^{-2} s^{-1} to 374 mol m^{-2} s^{-1} across the sampling dates and species, whereas, at Ofcolaco, the means ranged from 185 mol $m^{-2}s^{-1}$ to 390.25 mol $m^{-2}s^{-1}$.

The succeeding stooling rye produced a similar biomass yield under every preceding forage legume. This can be concluded that all the four forage legumes did not have any effect on the biomass production of stooling rye. However, there appeared to be a tendency of higher biomass production in the grass species grown after pigeon pea and lablab compared to those following sunnhemp and cowpea. Further experiments are required to establish the full benefits of the forage legumes on succeeding forage grass crop.

Based on the results from this study, it was concluded that sunnhemp can be considered as the first choice forage legume at both Syferkuil and Ofcolaco due to its consistently high biomass production, comparable nutrient profile, high crude protein content and high protein yield compared with the other legumes. Though sunnhemp was superior, the other forage summer legumes species studied also managed to produce enough biomass for grazing and had similar nutritive value which was above minimum recommendations. They can therefore be cultivated in the province to meet the constraint of the feed gap in the province. Additional studies at different locations, however, will help to understand the productivity of the species and also to establish the full benefits of the forage legumes on succeeding forage grass crops.

Keywords: Dry matter yield. Chemical composition. Forage legumes

CHAPTER 1: GENERAL INTRODUCTION

1.1 Background of the study

The demand for livestock production and products is on the increase in developing countries in response to rapid population growth, increase in income and urbanization. However, the challenges posed by climate change and environmental degradation in the livestock sector towards 2050 and the management considerations required to maintain sustainability have been highlighted (Thornton, 2010). Climate change is the long-term misbalance of temperature, wind, and rainfall characteristics of a specific region and this are currently constraining agricultural productivity (Fawzy *et al.,* 2020). Climate change has negative impacts on the grazing capacity of rangelands and the nutritional stresses in livestock, further exacerbating the existing vulnerability of pastoral systems (Rust and Rust, 2013). Although the degree and impact of the drought vary across the pastoral groups, drought remains the bigger cause of asset losses and resource degradation leading to poverty (Rust and Rust, 2013). Global model projections indicate that temperature warming in southern Africa in all seasons is likely to exceed average global warming, coupled with drier conditions, especially over in winter months and a high risk of severe droughts (IPCC, 2014).

Livestock production is one of the world's largest users of land resources, either directly through grazing or indirectly through the consumption of fodder and feed grains (Bruinsman, 2003). South Africa has approximately 84% of the surface area available for agriculture. However, a larger part of this available land is not suitable for crop production, with approximately 13% that is arable. In the greater part of South Africa, approximately 70% is suitable only for extensive livestock farming (Scholtz *et al*., 2013). In the arid areas of Africa livestock feed on a low nutritive value of crop residues and veld pastures which rely on fluctuating rainfall. The low productivity of livestock which in turn results in economic losses to livestock owners are the result of poor nutrition especially during dry seasons (Mokolopi, 2019).

Low protein content and high fibre content of the veld due to the effects of climate change limit their efficient utilization by livestock. The value of the veld is influenced by seasonal fluctuations in both quantity and quality (Baloyi *et al*., 2008). Different ways of improving the nutritive value of these low-quality roughages by chemical methods, such as alkalis treatment or the addition of molasses and urea or ammonia have been studied worldwide but such methods are generally not easily adopted by the smallholder farmers (Baloyi *et al*., 2008). The success of the green revolution in the 20th century was the result of the heavy application of fertilizers to increase plant productivity. This was done because most crops which had been cultivated were responsive to the high level of fertilizer particularly nitrogen. Therefore, large amounts of nitrogen fertilizer were applied to the soil in crop production resulting in several environmental issues (Hawkesford, 2014). Legumes offer important opportunities for sustainable grassland-based animal production. They can contribute to important key challenges by increasing forage yield, substituting inorganic N-fertilizer inputs with symbiotic N_2 fixation, mitigating and facilitating adaptation to climate change, as elevated atmospheric CO_2, warmer temperatures, and drought stress periods increase thereby increasing the nutritive value of herbage and raising the efficiency of conversion of herbage to animal protein (Luscher *et al*., 2014).

N_2-fixing by legumes may also provide nitrogen to the succeeding crops through senescent leaves drop and belowground parts. The quantity of N fixed by legumes can be measured but it is difficult to assess accurately the quantity of N supplied by the legume to the non-fixing cereal in legume cereal rotations (Bado *et al*., 2006). Crops following a legume in rotation yield more than many other pre-crops. Even where all crops are fertilized for optimum yield, cereal crops following legume crops are reported to yield 15 to 25% more than cereals grown continuously (Bado *et al*., 2006), due to reductions in diseases and improvements to root growth.

Rapid increase in carbon dioxide (CO_2) concentration in the atmosphere related to other greenhouse gases (GHGs), such as nitrous oxide (N_2O) and methane (CH_4), since the industrial revolution is a major concern with respect to its impact on climate change. There is an urgency to adopt effective measures for mitigating the threat of global climate change (Wang and Alva, 2010). Legumes provide several important services to communities. They deliver important sources of oil, fibres, and protein-rich food and feed while supplying N to agro-ecosystems via their unique ability to fix atmospheric N_2 in symbiosis with the soil bacteria rhizobia, increasing soil carbon content, and stimulating the productivity of the crops that follow (Jensen *et al*., 2012)

1.2 Research problem

The livestock sector is the most rapidly growing agricultural sub-sector contributing up to 46.9% of the gross value of agricultural production in South Africa (DAFF, 2017). The shortage of adequate, good quality forage during the winter months is one of the biggest problems confronting the livestock farmer in Limpopo Province (Mokoboki *et al*., 2002). The most limiting nutrient experienced by livestock grazing on the rangelands is protein. Failure to meet minimum animal nutrition requirements results in a decline in animal productivity through the reduction in multiple births rate, milk production,growth, conception rate and disease resistance (Corson *et al*., 1999). The major constraints that the livestock sector is faced within developing countries is the scarcity of feed resources resulting in low productivity and poor growth and reproduction of animals (FAO, 2011). In the lower rainfall areas of South Africa, where sweet veld is available during winter, feeding of animals is less of a problem due to adequate quality fodder. However, the reduced quantity presents the biggest problem in such areas (Dannhauser, 1991).

Nitrogen (N) is the most commonly deficient mineral nutrient in soils due to its high mobility and demand, often contributing to reduced plant growth, development, and yields (Reckling *et al*., 2014). To deal with this situation, agriculture has been largely reliant on nitrogen fertilizers to maximize crop productivity with about 50% of the nitrogen fertilizers leaching into the aquatic ecosystem and causing significant environmental pollution. Nitrogen can be supplied to plants as inorganic or organic fertilizers. However, fertilizers are an expensive resource for poor subsistence farmers, and their production and excessive usage especially inorganic N fertilizers may pollute the environment through greenhouse gas production (Brown and White, 2010). Summer legumes can be used both for fodder production as protein supplements to grazing livestock, for fodder conservation to reduce the winter fodder gap as well as to improve the soil nitrogen status.

1.3 Motivation of the study

South Africa is modest in terms of international livestock trade (Meissner, 2013). The shortage of feeds and low quality of available feeds have become the major constraints for livestock production. The shortage of feed becomes more severe during long dry periods when green forage is rarely available (Tsegaye *et al.,* 2008). The most common type of animal feeds available are high-fibre feeds, which arelow in nutrient ions such as nitrogen, sulphur, phosphorus, etc. necessary for microbial fermentation. The availability of quality livestock feed is important for improving the productivity of the livestock sector. To solve the problem of feed shortage and increase livestock productivity, it is necessary to introduce high-quality forages with high yielding ability and adaptability to the biotic and abiotic environmental stresses. Annual summer legumes are important in the livestock industry because they are the largest feed (forages) resources, used for grazing and hay production for the animals (Araújo *et al*., 2015). Researchers such as Whitbread *et al*., (2010); Odhiambo, (2011); Gwata and Shimelis, (2013) worked on Lablab bean (*Lablab purpureus*), Sunnhemp (*Crotalaria juncea L*), and Pigeon pea (*Cajanus cajans*) biomass production in Limpopo Province respectively, but not much on their nutritional value was done. Ravhuhali (2010), conducted a study in Limpopo Province where the feeding values of four varieties of cowpea (*Vigna unguiculata*) hay were compared, but this study did not focus on measuring biomass production of those varieties of forage cowpea.

Cowpeas, Lablab, and other forage legumes are fast-growing annual summer legumes that can produce high biomass, support a high carrying capacity and grow quality fodder (Negus, 2014). Sunnhemp is a tall herbaceous annual plant mostly grown in the tropics and probably originated in India. Its main use is for hay production, but other uses of sunnhemp include fibre production as well as green manure (Kessler and Shelton, 1980). Pigeon pea has for centuries been used as a high protein animal feed and grain for human food because it produces a large amount of biomass with high protein content. Its beneficial effect on biomass production and grain yield of a succeeding winter crop such as wheat has been reported (Coleman *et al*., 2015).

Furthermore, these annual summer legumes have the potential to supply nitrogen (N) for their growth and for other plants growing with them in intercropping systems or following them in crop rotation systems, when properly inoculated (Newman and Chambliss, 2007). In their symbiosis with rhizobia bacteria (*Rhizobium leguminosarum*) they can fix N_2 from the atmosphere to the soil. A previous study byStallings (2014), indicated that legumes can replace a substantial amount of N fertilizer for the following crop when utilized as a cover crop. There is little knowledge available regarding the ecosystem services of the selected annual summer forage legumes in short-term rotations with a grass forage such as *Secale cereale* L. (stooling rye). Stooling rye is a tufted annual winter grass species that can grow as tall as 1,5

m. The crop is a valuable winter fodder grass, for hay and silage production as well as a cover crop (Truter *et al*., 2015). Its hay and silage production is extremely important to bridge the winter fodder gap (Ammann and Nash, 2015). This crop requires N of about 30 to 40 kg N/ha to be applied once the seedlings have established and another top-dressing after grazing (Ammann and Nash, 2015). The summer legumes selected for this study can be beneficial for sustainable rangeland-based animal production as they can minimize key fodder challenges experienced during the dry season and substitute for inorganic N-fertilizer inputs, thereby mitigating and facilitating adaptation to climate change (Lüscher *et al*., 2014).

1.4 Aim and objective of the study

1.4.1 Aim

The aim of this study was to evaluate the production potential and nutritive value of different summer annual forage legumes and their impact on succeeding winter grass.

1.4.2 Objectives

The objectives of this study were to:

I. Determine the biomass production and nutritive value of sunnhemp, forage cowpea, lablab, and forage pigeon pea at two diverse agroecological zones.
II. Determine the plants' leaf gaseous exchange parameters, nitrogen uptake, and nodule production of sunnhemp, forage cowpea, lablab, and forage pigeon pea at two diverse agroecological zones.
III. Assess the contribution of the preceding summer forage legumes (sunnhemp,forage cowpea, lablab, forage pigeon pea) to biomass production and nutritional value of a succeeding winter stooling rye (*Secale cereale*) grass.

1.5 Hypotheses

The hypotheses of the study were:

I. The biomass production and nutritive value of sunnhemp, forage cowpea, lablab, and forage pigeon pea at two diverse agroecological zones are not different from each other.

II. Plants leaf gaseous exchange, nitrogen status, and symbiotic activities of the selected summer annual forage legumes do not differ from each other.

III. The biomass production and nutritional value of a following winter stooling ryegrass are not affected by the preceding winter forage legumes.

CHAPTER 2: LITERATURE REVIEW

2.1 Description of selected summer forage legumes.

2.1.1 Sunnhemp

Sunnhemp (*Crotalaria juncea*) is a tropical annual legume mostly grown in India, Pakistan, Bangladesh, and Brazil. It is grown for green manure, fibre, and animal fodder crop (Lepcha *et al*., 2019). Sunnhemp is a legume adapted to a wide range of environmental conditions and soil types. The crop produces a high biomass yield, fixes N, and is resistant to several nematodes (Mosjidis *et al*., 2013). It consists of a strong taproot system with nodules on the root surface. This plant can grow to a height of up to 2.5-3.0m. Leaves are simple, stipulated, entire, and elliptical to oblong in shape. Sunnhemp bears terminal raceme inflorescence within determinate growth habit. Flowers are typical of standard type with a broad ovate standard petal with a strong midrib at the back of the petal (Bhandari *et al*., 2016)

2.1.2 Cowpea

Cowpea (*Vigna unguiculata*) is a grain and fodder pulse crop grown around the world. It serves as a dual-purpose grain legume crop, providing food for livestock fodder and human consumption (Mfeka *et al*., 2019). Cowpea is important for nutritious fodder for livestock. The nutritive value of cowpea grain, leaves, and haulms is also very high. The crude protein content ranges from 22 % to 30 % in the grain and leaves on a dry weight basis and from 13 % to 17 % in the haulms and stems with high digestibility and low fiber level (Rathore *et al*., 2015).

2.1.3 Pigeon pea

Pigeon pea (*Cajanus cajan*) is one of the extensively common tropical and subtropical legumes cultivated for its edible seeds. Pigeon pea is a fast-growing, hardy, widely adaptable, and drought tolerant. At the end of the dry season, pigeon pea gives green forage of outstanding value when other forages have disappeared (Heuzé *et al*., 2016). As an animal feed, pigeon pea leaves are used as dry or green fodder and the seed by-products from the split seed mills are used as animal feed (Beyero and Kassu 2015).

2.1.4 Lablab

Lablab *(Lablab purpureus)* commonly known as lablab or hyacinth bean is extremely diverse and remarkably adaptable with its various genotypes thriving in different areas and under diverse conditions including arid, semi-arid, and humid regions. It is insufficiently being utilized but a multipurpose crop used for food, forage, soil improvement, soil protection, and weed control (Ewansiha, 2016). Lablab forage is a good source of metabolizable protein for ruminants. However, its protein is degradable in the rumen, resulting in a fairly low contribution to by-pass protein (Heuzé *et al*., 2016).

2.1.5 Stooling rye

Stooling rye (*Secale cereal*) is a tufted annual grass species that normally grow up to1.5 m tall. Their fibrous root system can go as deep as 1.5 m. Stooling rye is regarded as valuable fodder for pasture, hay, or silage and a cover crop during winter. During late winter to spring it provides valuable forage to animals going into summer (Truter *et al.*, 2015). Among all the cereal crops, stooling rye is reported as being the tallest and the hardiest annual species. There are many cultivars of *Secale cereale* and research has shown that diploid cultivars are more drought-hardy than tetraploid cultivars (Casey, 2012). Cereal rye can absorb residual N in soil from previously grown row crops. It typically assimilates 56,04 $kg.ha^{-1}$ N but can retain as much as 112.09 $kg.ha^{-1}$ N (Casey, 2012).

2.2 Description of Biological Nitrogen Fixation by forage legumes.

Most legumes (belong to the *Fabaceae f*amily) and can develop a symbiotic relationship with varieties of bacteria which are collectively called rhizobia (Sprent and Sprent, 1990). The rhizobia bacteria through the process of biological nitrogen fixation can convert atmospheric nitrogen (N) into the biological functional form of N inside the root nodules (Sprent, 2009). This symbiotic relationship is a form of N cycle to maintain natural systems (Vitousek *et al*., 2002). Additionally, biological nitrogen fixation is very important because it provides pools of soil with N for crops planted after forage legumes and grain legumes for strategic rotation of sequence for benefiting non-N-fixing planted in intercropping systems (Iannetta *et al*, 2016). Biological Nitrogen Fixation is regarded as one of the mechanisms for maintaining the production of agriculture and healthy ecosystem functioning (Muhmud *et al*, 2020). BNF is defined as the process where N from the atmosphere can be converted into ammonia which can be available for plant absorption. Crops can also benefit from the dyeing decomposing bacteria in soil because they release nitrogen compounds which can be available for enhanced plant growth and development. This form of incorporating forage legumes and grain legumes into cropping systems reduces the reliance on inorganic fertilizers needed for growing ryegrass.

The following group of prokaryotes can achieve BNF: bacteria and archaea.

Different groups of bacteria are involved, this includes free-living bacteria from genera such as Azospirillum, Azotobacter, Clostridium, or Bacillus; symbiotic bacteria such as Rhizobium are related to legumes; actinorhizal plants associated with Frankia; and cycads associated with cyanobacteria. For archaea, nitrogen fixation is still restricted to groups that produce methane, called methanogens (Santi et al., 2013).

In many countries around the world, it has been reported that cereals that are grown after the leguminous crops had higher yields when it was compared with the yield of cereal planted after non-leguminous crops. It has been reported that ploughing in of leguminous pastures or crop residues, will not need any N fertilization in the first year to produce reasonable yield or biomass, as only 50 $kg.N.ha^{-1}$ will be needed in the following year to maintain N demand in that year. The report that was published in Northern New South Wales in Australia, has also shown that crops that will be planted after the lucerne or clover pastures will not need organic fertilization for at least one or two following years (Kumar and Goh, 2000).

2.3 Availability of soil nitrogen

Soils with insufficient amounts of nitrogen or medium doses of N have been shown to stimulate N-fixation and growth of seedlings (Coskan and Dogan, 2011). Legumes that are planted under a low amount of N need application of inorganic N fertilizers to stimulate their nodule formation, root, and shoot growth before the process of N-fixation can take place (Dabessa *et al*., 2018). Dabessa *et al*., (2018) stated that too much N in the soils can also reduce nitrogenase activities in the soil. The initial stage of vegetative development needs a moderate concentration of N in the soil to support the development of nodules and encourage the process of symbiotic nitrogen fixation. This finding is also supported by a study which was done by Weisany *et al*, (2013) where it was stated that, a high level of N in the soil will discourage biological nitrogen fixation by legumes.

2.4 Available Soil Phosphorus

Soils with low phosphorus (P) availability are a major constrain to forage legume production, hence, a sufficient amount of P is needed in the soil for stimulating legume crops nitrogen fixation and low P availability, however, remain problematic for forage legumes in most soils (Saad and Lam-Son, 2017).

2.5 Salinity

Abd-Alla (2019) reported that nodule development on Rhizobium-legumes is eventually reduced by noxious outcomes of soil salt stress, causing poor development of infection threads and root hair curling. Nodule productivity is impeded under salt stress, even if the signs of stress on the plant might not be visible. The permeability of nodules is also decreased by the presence of salt in the soil (Mohammadi *et al*., 2012). This decrease is related to the high presence of abscisic content and contraction of the inner-cortex of the nodule. Insufficient carbon for soil bacteria which are related to the sucrose reduction and hence, reduced the number of nodules under saline conditions are caused by constraints of sucrose synthesis by the different types of enzymes involved in sucrose hydrolysis (Mohammadi *et al*., 2012).

2.6 Soil Rhizobia Detection

Rhizobia inoculants encounter many challenges. Rhizobia that are originally found in the soil can out-compete the manually introduced rhizobia inoculants, which will then inhibit the nodule (Geetha and Joshi., 2013). This will result in poor performance of introduced inoculum at field level variability as they will be altered by the native strain that was originally in the soil. Therefore, before introducing rhizobia inoculants, the population of indigenous rhizobia must be determined as well as nodule occupancy so to allow desired results by manually introducing rhizobia inoculants on a given field. The successful process of nodulation is determined by the number of active rhizobia that are present to infect the root of the legume. Pesticides, desiccation, seed coat toxicity, and temperature are factors that can cause loss of viability on seed-applied rhizobia (Thilakarathna and Raizada., 2018).

2.7 Available Soil Micronutrients

The nutrient status of soil affects the growth of the crop and symbiosis and survival of this two depend on the availability of nutrients. Maturity of legume crop results in the decrease in fixation, mainly rising N in the soil (Thilakarathna and Raizada., 2018). Micronutrients especially molybdenum and boron are important for the legume rhizobial symbiosis (Moisaco-factor for nitrogenise), and consequently, an insufficient concentration of micronutrients will decrease N from symbiotic fixation. In developing countries where the limitation of resources is most common, the laboratories testing of soil micronutrients is unaffordable. Developing countries are also experiencing a similar problem because of the high cost of micronutrient analysis. Soil mapping (e.g. B and Mo) is currently being done by soil sample chemical analysis, which is efficient enough to become a foundation of precision agriculture-based on diagnostics (Brear et al., 2013).

2.8 Effects of climate change on physiological responses of annual forage legumes.

The stomatal control and physiological performance of the plant are mostly affected by carbon dioxide (CO_2) and temperature (Wang *et al.,* 2012). Now it is important to understand the influence of CO_2 and the warming of the plant particularly on stomatal functioning and also to understand how climate change impacts the production of pasture crops, especially in subtropical and tropical regions. Atmospheric concentrations made up of different greenhouse gases (GHGs) and climate change are more likely to impact the production of pastures. This impact can be through soil mineral availability for plant uptake or the physiological impact of the plant (Habermann *et al.,* 2019). There is mounting evidence from researches showing that from 1850, the global mean temperature has been increasing by 0.8 °C. This impact of warming is found in three independent records of temperature in ocean surface water, seas, and overland. The atmospheric CO_2 since 1832 has increased from 284 $mg.kg^{-1}$ to 391 $mg.kg^{-1}$ until 2012 (Tans and Keeling, 2012). These changes are mainly caused by fossil fuel burning, with the lowest changes coming from land use (IPCC, 2007).

2.9 Effects of elevated carbon dioxide

Photosynthesis stimulation can be caused by increasing atmospheric CO_2 concentration, which will lead to nutrient cycle, modified water, and improved plant production (Körner *et al*., 2006). Experimental research which was performed under optimum conditions revealed that atmospheric CO_2 concentration which was doubled can improve plant leaf photosynthesis from 30% to 50 in C3 plants and C4 plant species, the increase is from 10% to 25% (Tubiello et al., 2007). It was reported that global mean temperature has been increasing by 0.8°C from the 1850s to 2012 (IPCC, 2007). In the next century according to models predictions, it is expected that there will be a 2 °C to 4 °C increase in temperature (Tadross et al. 2007). The rate of crop growth and development, grain production, and survival of plants can be affected by the warming of temperature. The period from planting to flowering and harvest of the crop depends on day length and mean temperature (Craufurd and Wheeler, 2009). As the climate temperature increases, the period to harvest decreases, at least until the optimal temperature is surpassed.

2.10 Effects of CO_2 and warming temperature of stomata functioning

The stomata on the plant leaf are responsible for the movement of the gases between the atmosphere and plants, i.e., entering of CO_2 from the atmosphere and releasing of water vapour from the plant into the atmosphere. Plant hormones, guard cell turgor, and calcium concentration are regarded as the main factors that allow stomatal opening and closing (Lawson and Blatt*,* 2014; Assmann, 1999). The concentration of CO_2, temperature, soil water deficiency, light, and vapour pressure deficit are regarded as factors that will affect stomatal behaviour either alone or/and in combination (Šigut et al., 2015; Laanemetsetal., 2013; Hubbartetal., 2013; Perez-Martinet et al., 2009; Lee *et al.,* 2008). Moreover, the development of long-term (stomatal density and size) and short-term (stomatal opening), depending on genotype and plant species reaction to environmental fluctuations might occur together.

2.11 Lack of feeds and quality feeds

The quality of feeds and shortage of fodder has been identified as a major constraint in livestock production, especially in developing countries. These countries experience a shortage of conventional animal feed from time to time (Bhat *et al*., 2013). The unavailability of adequate and quality feed is one of the critical challenges that most smallholder's livestock owners are faced with especially in tropics regions (Tangka and Jabbar, 2005). The high fibre content and low nitrogen (N) content of native grasses and crop residues are the major constraints that limit the productivity of the animals created by imbalances of feeds (Aliyu, 2018). This problem is exacerbated by the seasonal availability of feed resources. Furthermore, when ruminants are feed with low quality and highly fibrous forages, they end up with enteric methane production instead of better quality forages, which represents a 5 to 15% loss in gross energy intake depending on the type of carbohydrate, the addition of dietary fat, the quantity of feed ingested, and processing of forages (Halmemies-Beauchet-Filleau *et al*., 2018).

2.12 Nutritive value

Nutritive value is defined as measuring of availability of nutrients that are present in the feeds, required by animals and to access production input from the animal which was fed (Coleman and Henry, 2002). Factors such as water availability, climatic variation, and soil types are factors that influence forage nutritive value (Amary, 2016).

2.13 Nutrient content of herbage.

Determining the nutritional value of forages is important in livestock nutrition because effective livestock production is related to the number of nutrients in the forage.

2.13.1 Proteins

Proteins are the most important nutrients for livestock. Approximately 60-80% of the total nitrogen of the plant is made by these proteins. Crude protein (CP) is the term used to represent the amount of nitrogen present as protein, as well as nitrogen that is in the form of non-protein nitrogen which includes nitrates, ammonia, urea, and single amino acids. The Kjeldahl method is used to determine nitrogen concentration which is in feeds samples (Fulgueira *et al*. 2007). With the assumption that true protein has 16% of nitrogen, 6.25 is used as a conversion factor and multiplied by the total amount of nitrogen present in the feed analysed to obtain crude protein (Fulgueira *et al*., 2007).

2.13.2 Fibre

Acid detergent fibre (ADF) and neutral detergent fibre (NDF) are the most important forage indicator tested for fibre analysis. ADF represents lignin and cellulose, whereas NDF represents constituents of the total cell wall including hemicellulose (Ball *et al*., 2001). ADF is used to calculate digestibility and feeds intakes are predicted by the levels of NDF. Feed intake decrease in proportion to an increase in the level of NDF and this can have a negative impact on animal production (Fulgueira *et al*., 2007). A high NDF content shows the presence of higher fibre content in the forage. Therefore, the lower the NDF value of the forage sample, the better (Trammell and Walker, 2019).

2.13.3 Mineral elements

Productivity in animals and physiological functions of the plants are controlled by the presence of essential minerals for optimal growth and minerals are also important for health, reproduction, and livestock growth. The health and performance of livestock depend strictly on the availability and adequacy of essential mineral elements that are provided by the pasture and soils (Mokolopi, 2019). Ash is the amount of total mineral content in a forage, which includes soil contaminants and inorganic compounds in the plant. A high ash content indicates significant contamination by soil, which can inflate NDF. Mineral nutrients essential for metabolic functions mostly considered for analysed forage feed report, include Ca, P, Mg, K, Na, Fe, Zn, Cu, Mn, Mo, S, and Cl (Chamberlain, 2016).

2.13.4 Sodium (Na) and Potassium (K)

One of the reasons for production losses in animal production is high potassium forage and low sodium chlorides feed diet. Deficiency of other minerals and immune suppression is induced by excessive potassium in forages. There appears to be a causal relationship between high potassium forages and the high content of Na in the ration is important in determining the adequacy of the minerals (Mirzaei, 2012). Forage should at least contain more than 0.15% sodium to meet the requirement of highly productive animals. Tropical pastures species are the ones more likely to be affected by Na deficiency. Compare to temperate pastures species, tropical species generally accumulate less Na. Natural forages low in Na has been reported in numerous tropical countries throughout the world. Opportunistic diseases of livestock (Mirzaei, 2012).

CHAPTER 3: METHODOLOGY ANALYTICAL PROCEDURE

3.1 Study sites

The experiment was conducted at two locations in the Limpopo province, namely: University of Limpopo experimental farm at Syferkuil, and at Itemeleng Ba Makhutjwa Farming Cooperative known at Ofcolaco during January 2019 to September 2020 growing season. Ofcolaco is situated 43 km southeast of the town of Tzaneen with a geographical coordinate of 24º4´60´S and 30º22´0´E and Syferkuil (coordinates: 23º 50º´S; 29º 0´E) is situated about 35 km southeast of Polokwane City.

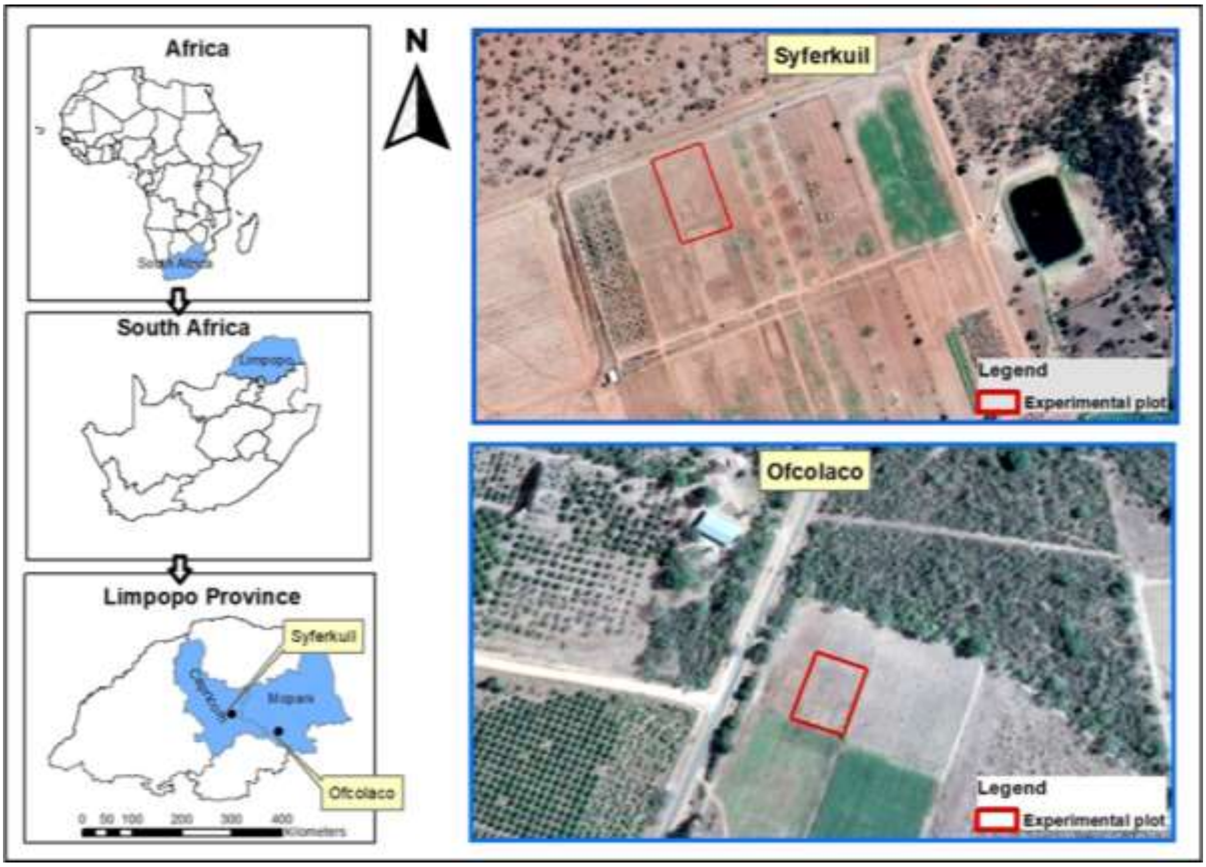


Figure 3.1: Map of study locations (adopted from Mante, 2019)

3.2 Weather at the study locations

The climatic condition of Syferkuil is classified as semi-arid with annual precipitation of ±495 mm. The minimum annual temperature is at an average of 15 ºC and a maximum of 28.1 ºC, with an average of 170 frost-free days from late October to mid-April annually. The soils at this location are categorized as sandy loam Hutton. Ofcolaco receives an annual rainfall of about 650 to 700mm during the summer months (October to March) and climate conditions are classified as humid. The minimum annual temperature is at an average of 15 ºC and the maximum annual temperature is at 28.1 ºC with no frost in the area. The soils at this location are classified as clay loam of Hutton.

3.3 Forage legumes research procedure

3.3.1 Soil sampling

Prior to the study, soil samples at the two selected locations were randomly collected at the depth of 0-30 cm using an augur to determine soil chemical and physical properties at pre-plant. Table 3.1 below shows the initial soil analysis results at both locations namely Syferkuil and Ofcolaco. The pH of the soils were 7.57 and 5.5 at Syferkuil and Ofcolaco, respectively. Soil pH >7.0 are classified as alkaline or basic and soil pH range between 5.2-6.0 are classified as moderately acidic (Horneck *et al*., 2011). Thus, the soil at Syferkuil was alkaline and that of Ofcolcao was moderately acidic before the establishment of the trials. The available topsoil N (%) was 0.06 and 0.53 at Syferkuil and Ofcolaco, respectively, which is relatively low at Syferkuil and high at Ofcolaco. The P concentration was 6.75 mg.kg^{-1} at Syferkuil and 4.75 mg.kg^{-1} at Ofcolaco. These values were very low, requiring external P to meet the crop need. At Syferkuil, the concentration of K was 498.50 mg.kg^{-1} and at Ofcolaco, was 75.25 mg.kg^{-1}. These were adequate for crops. The calcium concentrations were 852.00 mg kg^{-1} and 496.50 mg.kg^{-1} at Syferkuil and Ofcolaco, respectively, whereas the concentrations of magnesium were 483.25 mg.kg^{-1} 160.75 mg.kg^{-1} at the two locations respectively.

Table 3.1 Soil nutrients status at Syferkuil and Ofcolaco farms in the 2019 planting season.

Soil PH and nutrients	Syferkuil	Ofcolaco
pH (KCl)	7.57	5.5
N (%)	0.06	0.53
P (mg kg^{-1})	6.75	4.75
K (mg kg^{-1})	498.50	75.25
Ca (mg kg $^{-1}$)	852.00	496.50
Mg (mg kg^{-1})	483.25	160.75

N=available nitrogen, P=phosphorus, K=potassium, Ca=calcium, Mg=magnesium

3.3.2 Land preparations

At Syferkuil, the demarcated plots for the experiment were planted with cowpea in the 2016/2017 planting season, under tilled condition. The experimental site at Syferkuil was left fallow one year prior to our experiment establishment. Glyphosate herbicide was applied at least two weeks before planting annual summer forage legumes to control weeds. At Ofcolaco, the experimental site was left fallow for 5year prior to the experimental establishment. Herbicide was applied to the experimental plots and was left unploughed, but a hand hoe was used to open the rows for planting the seeds. Aphox (carbonate) was used to control aphids on cowpea only and irrigation was applied during the experiment using a sprinkler irrigation system. The experiment took place under a no-till system at both locations.

3.3 3 Experimental design and treatments

Two experiments were established at both locations during (summer and winter) in 2019. The experiments were laid out as a randomized complete block design (RCBD) with the four selected summer annual forage legumes as the treatments, replicated six times at the two locations. The forage legumes and their recommended row spacing are presented in Table 3.2.

Table 3.2: Summer forage legumes species, cultivars, and planting spacing.

Species	Cultivar	Spacing
Sunnhemp (*Crotalaria juncea*)	Benares hemp	30 cm by 30cm
Lablab (*Lablab purpureus*)	High worth	30cm by 10 cm
Forage cowpea (*Vigna unguiculata*)	Dr Saunders	30 cm by 15 cm
Pigeon Pea (*Cajanus cajans*)	Pulses red gram	30 cm by 30cm

3.3.4 Planting procedure

The four legumes were planted on 22 January 2019 and 29 February 2019 at Syferkuil and Ofcolaco, respectively, according to their respective spacing shown in Table 3.2

under no-till condition. All seeds were inoculated with their recommended commercial *Bradyrhizobium* inoculum before planting. Each plot was 10 m × 5 m, which is equal to an area of 50 m². Phosphorus was applied at planting up to a rate of 20 kg.P ha^{-1} based on pre-plant soil analysis. The seeds were planted at their recommended densities on 70 $kg.ha^{-1}$ at both locations. All experiments at both locations were established under irrigation using a sprinkler irrigation system. The forage legume crops were watered at the rate of 4 hours per application, resulting in total application amounts of 400mm and 300 mm at Syferkuil and Ofcolaco respectively during the growing season.

3.3.5 Data collection

Below and aboveground biomass was taken at 3 weeks interval, from a selected area of 1m × 1m of the plots. The final above groundbiomass was collected from the centre of the plots at an area of 2m × 2m. Sickle was used to cut the grass. Sampled biomass of the individual forage legume species were oven-dried at 65 °C for up to 72 hours to obtain constant weight and then weighed to determine dry biomass weight. Thereafter, the samples were ground to pass through a 2 mm sieve and 10 g of a fine fraction was used to determine their chemical composition. The evaluated parameters were crude protein (CP) and mineral nutrients which include Ca, P, Mg, K, Na, Fe, Zn, Cu, Mn, Mo, S, and Cl. The Green Seeker handheld crop sensor was used remotely to assess the nitrogen and vigour of a crop from 30 cm above the canopy. The CCM-200 Plus, Opti-Science series of Leaf Chlorophyll Content Meters measured the chlorophyll content of leaves of the legumes. Leaf gaseous exchange including photosynthesis, sub-stomatal CO_2, stomatal conductance, and transpiration was collected using an LCi-SD ultra-compact photosynthesis measurement system (ADC BioScientific, UK). Data collection was done at three-week intervals between 11.00 and 13.00 hr under clear or uniform sky conditions. The symbiotic activity of legumes was assessed through nodule count, nodule mass, nodule colour.

3.4 Stooling rye grass methodology

Stooling ryegrass (*Secale cereal*, cv. Stoelrog-Echo-1) was planted in the winter season after harvesting the summer forage legumes. The stooling ryegrass was planted on 15 June 2020 at Syferkuil and on 22 June 2020 at Ofcolaco as a subsequent crop on the same plots where the summer legumes were previously planted at both locations..

3.4.1 Soil sampling

Prior to planting stooling ryegrass, soil samples at the two selected locations were randomly collected within each plot at the depth of 0-30cm to determine soil chemical and physical properties at pre-plant and post-harvest

3.4.2 Land preparations

Both plots at Syferkuil and Ofcolaco did not have weeds because the legumes planted before stooling ryegrass had suppressed the emergence of weeds. Hand hoes were used to open the rows for placing the seeds. No pesticide was used in the study and irrigation was applied at the rate of 4 hours per application, resulting in total application amounts of 420mm and 310 at Syferkuil and Ofcolaco, respectively.

3.4.3 Planting procedure

The seeds of stooling ryegrass were hand planted at the row spacing of 30 cm apart and 20 $kg.ha^{-1}$ sowing rate.

3.4.4 Data collection

Aboveground biomass was collected once at maturity, from an area of 2 m × 2 m. The samples were oven-dried at 65ºc to obtain dry weight and ground to pass through a 2 mm sieve. Ten grams of a fine fraction was used was analysed to determine the chemical composition and mineral nutrients which include Ca, P, Mg, K, Na, Fe, Zn, Cu, Mn, Mo, S, and Cl . CP = %N × 6.25 was used to determine crude protein and expressed in percentage. Crude protein yield (CPY) was determined by multiplying the dry matter yield with crude protein percentage.

3.5 Data analysis

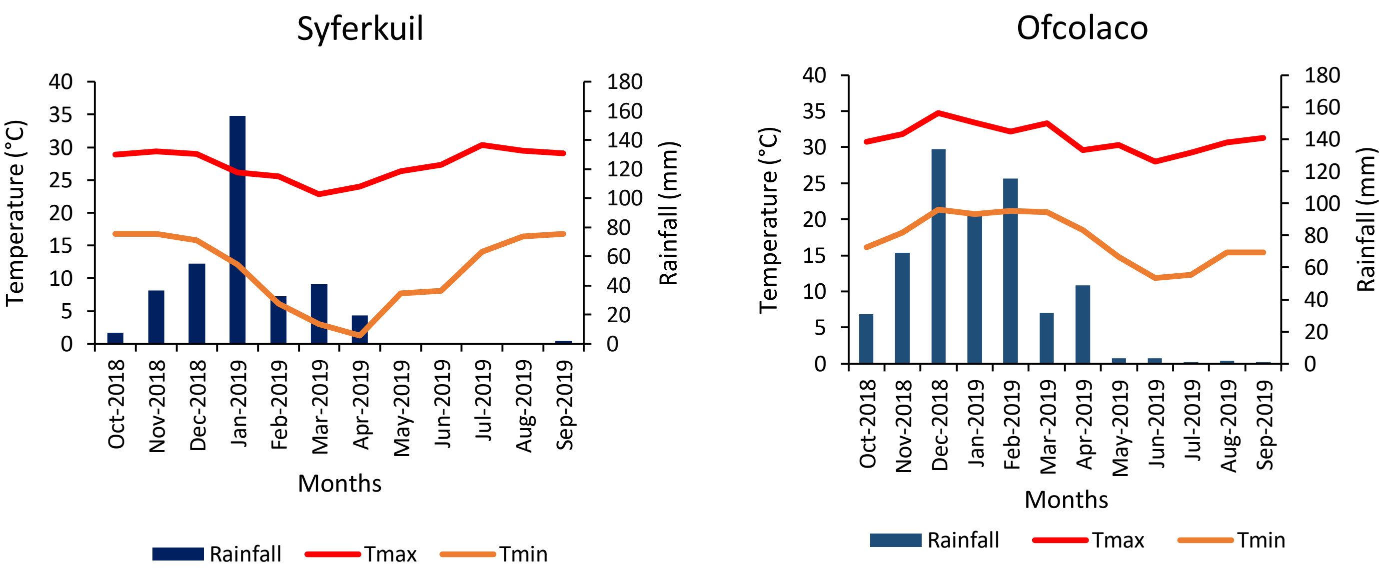
Data was entered into a Microsoft Excel spreadsheet and then subjected to analysis of variance (ANOVA), using the Statistical Analysis System (SAS institute, North Carolina State University), Enterprise Version 9.4. Microsoft Excel was used to generate graphs. Means were compared at the probability level of 5% using Duncan's Multiple Range Test**.**

CHAPTER 4: RESULTS

4.1 Weather conditions during the growing season

Figure 4.1 provides the ambient temperatures and rainfall at Syferkuil and Ofcolaco during the 2018-2019 planting season. Sunnhemp, forage cowpea, lablab, and pigeon pea were planted in January 2019 at both locations and in July 2019, the stooling ryegrass followed as a winter crop in the same year at both locations. The minimum temperature at Syferkuil ranged from 1 ˚C to 16 ˚C and the maximum temperature, from 22 ˚C to 30 ˚C. The highest rainfall of 156 mm was observed in January at Syferkuil. At Ofcolaco, the minimum temperature ranged from 11 ˚C to 21 ˚C and the maximum temperature, from 27 ˚C to 35 ˚C. The highest rainfall was observed between December 2018 and February 2018, where the highest of 133 mm occurred in December 2018.

Figure



4.1: Monthly temperature and rainfall experienced at Syferkuil and Ofcolaco farms during the 2018-2019 planting season.

4.2 Forage legumes

4.2 1 Biomass accumulation of forage legumes

Biomass yield of the legume species varied across the season and species, ranging from 173.8 to 3142.4 kg.ha^{-1} at Syferkuil and from 90.0 to 8970.0 kg.ha^{-1} at Ofcolaco (Figures 4.1 and 4.2). The changes in biomass accumulation were significant over time and among species within sampling dates, especially at the later sampling dates. The measured changes in biomass accumulation over time among the species revealed a strongly fit model using a second-order polynomial function. The value of R^2 for all the graphs was above 90% indicating that over 90% of the variation in seasonal biomass accumulation of the legume species observed at the two locations could be explained by a quadratic relationship. At Syferkuil, there was not much difference in biomass production of the forage species at 24 DAE except that of lablab which was 119.42% higher compared to pigeon biomass production. From 44 DAE onwards, the biomass accumulation of sunnhemp, cowpea, and lablab were similar, whereas that of pigeon pea was consistently lower than the others. Lablab, cowpea, and sunnhemp at 87 DAE were statistically similar, pigeon pea biomass accumulation was 57% lower than the average of the three other legumes.

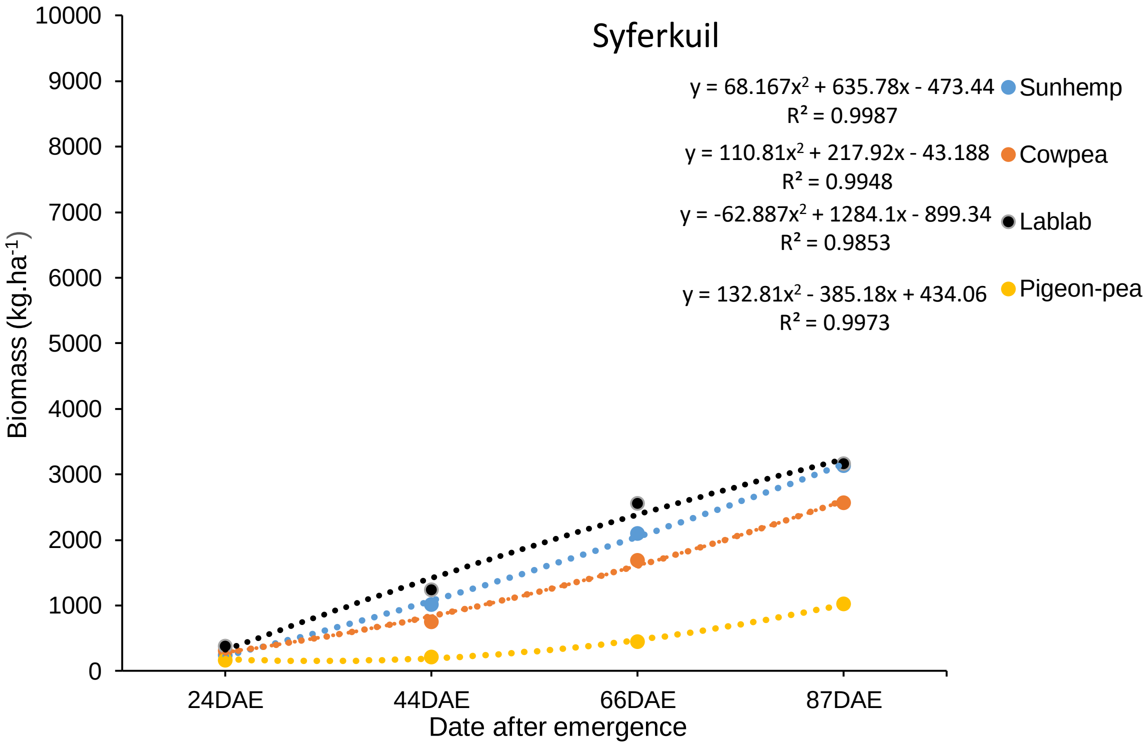


Figure 4.2: Biomass accumulation of four different forage legumes evaluated in the 2019 growing season at Syferkuil in Limpopo Province.

Similar to Syferkuil, there were no differences in biomass production among species during the early stage of growth at Ofcolaco. At this location, sunnhemp produced superior biomass compared to the three other species at 42 DAE, reaching a peak yield of 8970.8 kg.ha^{-1} at 65 DAE. The biomass yields of sunnhemp, however, was similar to that of pigeon pea at 65 DAE but the yields of pigeon pea, cowpea, and lablab were similar. At 78 DAE, the biomass yield of all species was reduced relative to biomass yield at 65 DAE, with sunnhemp producing 98.9% higher biomass than the average biomass yield of cowpea and lablab. The biomass yield of sunnhemp was again similar to that of pigeon pea at this stage of growth. Pigeon pea biomass yields were also similar to that of cowpea and lablab at 78 DAE.

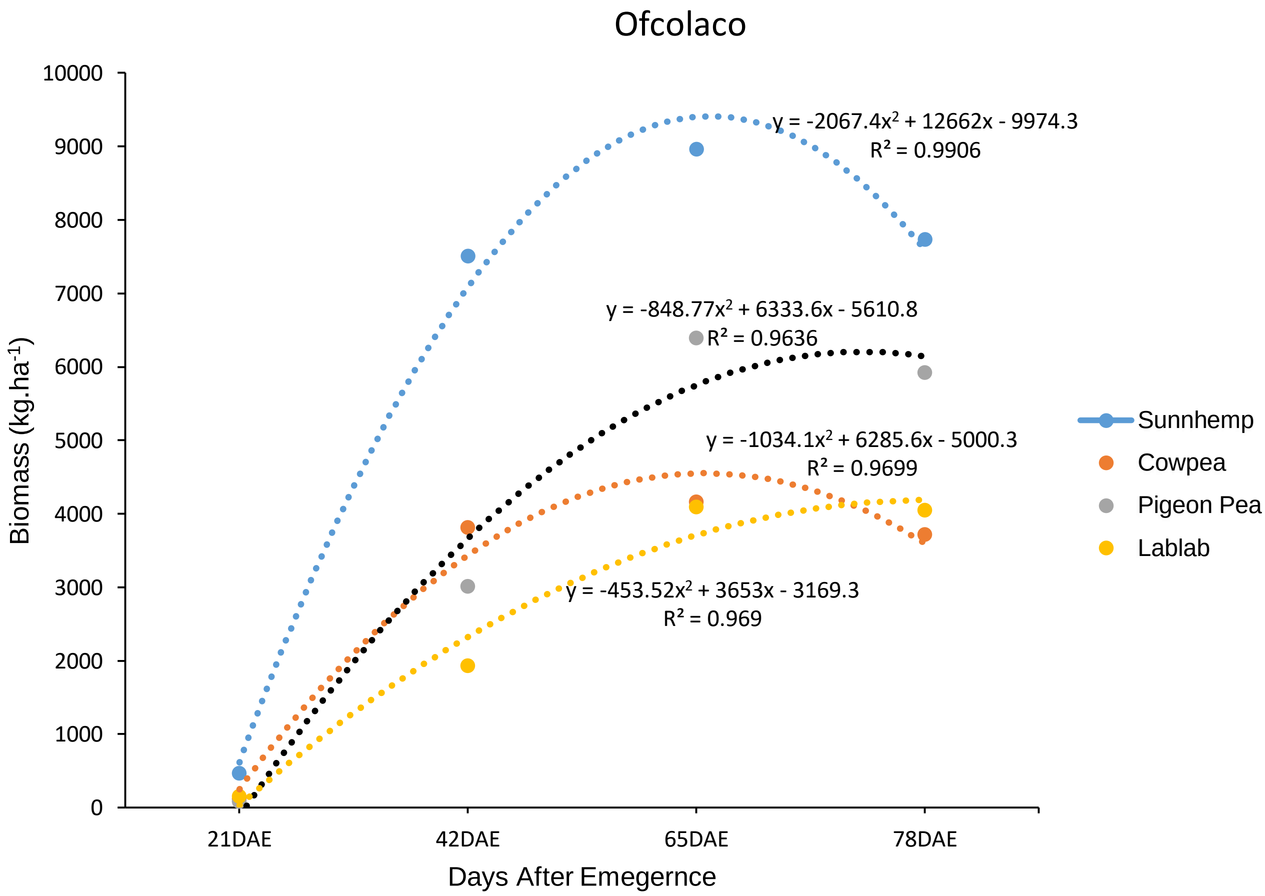


Figure 4.3: Biomass accumulation of four different forage legumes evaluated in the 2019 growing season at Ofcolaco in Limpopo Province.

4.2.2 Leaf chlorophyll content

At Syferkuil, all the forage species tested differed in leaf chlorophyll content at every stage of growth sampled but with a much-reduced R^2 fit, especially in cowpea. However, the chlorophyll content of cowpea tended to be superior to all the other forage legume species at all stages of growth except at 66 DAE where its chlorophyll content was similar to that of sunnhemp. At 87 DAE at Syferkuil, cowpea's chlorophyll content was 55.83% higher compared to other forage species.

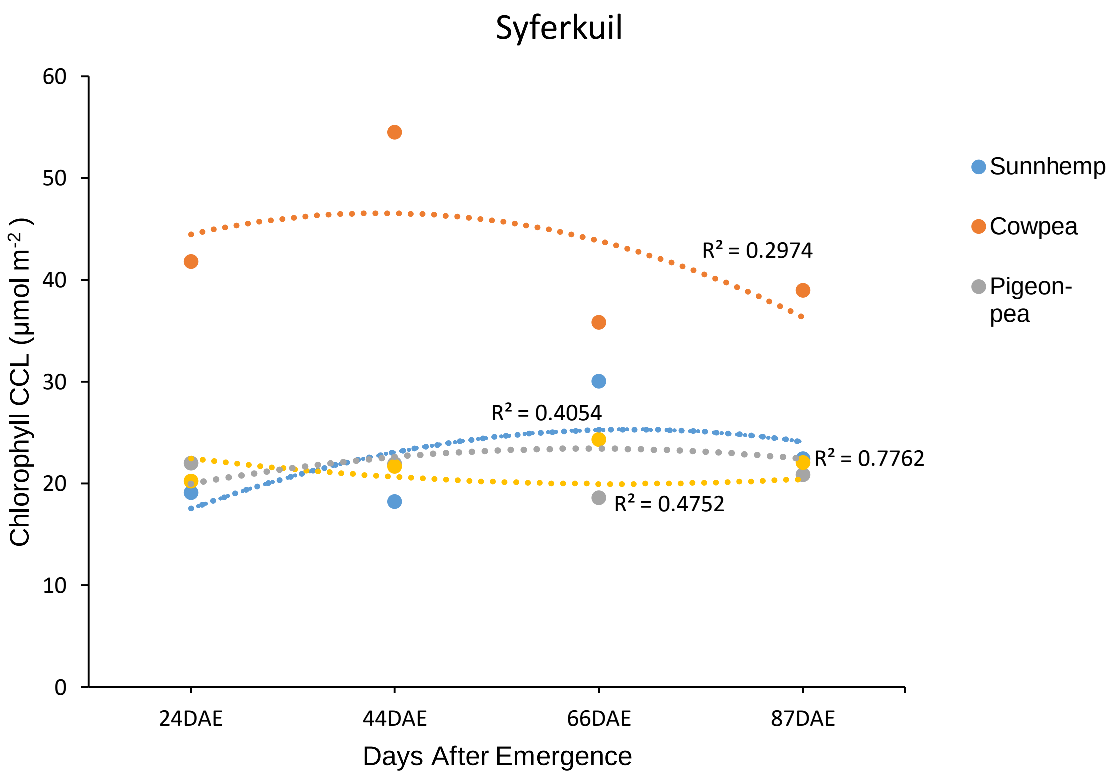


Figure 4.4*:* Chlorophyll content on four selected forage legumes planted at Syferkuil.

At Ofcolaco, the chlorophyll content differed significantly among the species (Fig. 4.5). At 24 DAE the chlorophyll content of pigeon pea, cowpea, and sunnhemp was similar but higher than that of lablab. Pigeon pea was relatively higher at 44 DAE and 65 DAE, resulting in a 50.91% increase at 66 DAE compared to other forage species. Pigeon pea was again superior in leaf chlorophyll content with a 41.92% increase compared with the other three forage legumes at 87 DAE. The leaf chlorophyll content of sunnhemp increased steadily over the season whereas that of cowpea showed a steady decline over the season at this location.

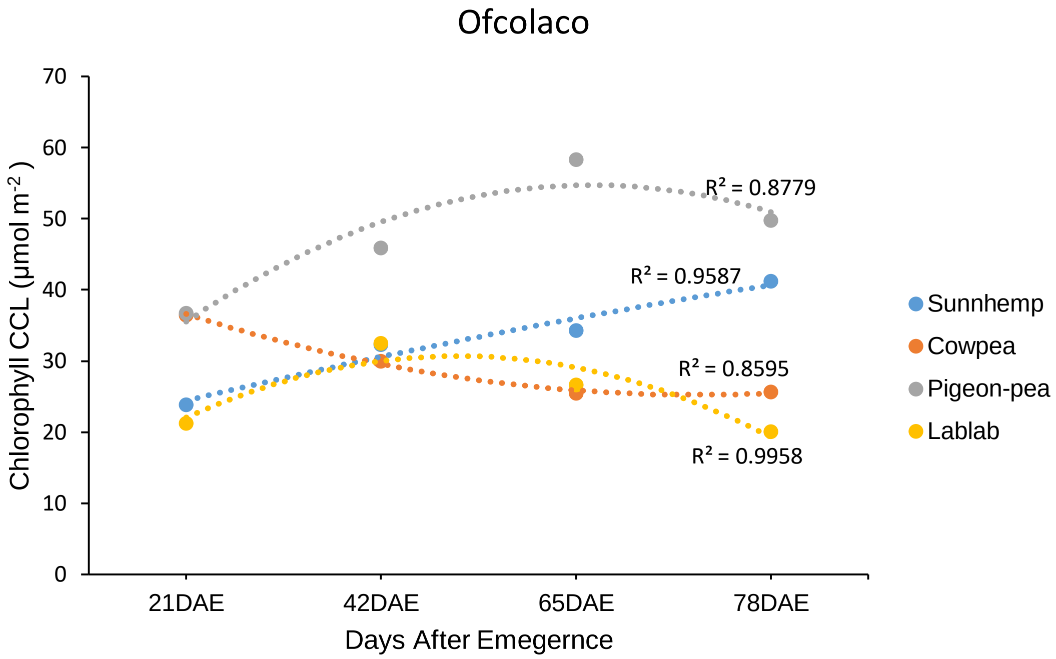


Figure 4.5: Chlorophyll content on four selected forage legumes evaluated in the 2019 growing season at Ofcolaco.

4.2.3 Normalized difference vegetation index (NDVI)

The R^2 value was above 76% in all the forage legume species. Lablab, sunnhemp, and cowpea were above 93%, which is an indication of a strongly fit model using the second-order polynomial graph. The NDVI indicates the health condition of a plant, with higher values signifying healthier plants. At Syferkuil, pigeon pea was generally found to be unhealthy based on the relatively lower NDVI values recorded from 24 DAE onwards. At 24 DAE, pigeon pea was however similar to that of lablab but was 47.8% lower than the average NDVI of sunnhemp and cowpea. Generally, the three species sunnhemp, cowpea, and lablab were rated as very healthy plants from 24 DAE until 87 DAE except for Lablab at 24 DAE that resulted in 0.21 NDVI. The NDVI values of all the species peaked at 66 DAE and declined as the crop matures, towards the last days of sampling.

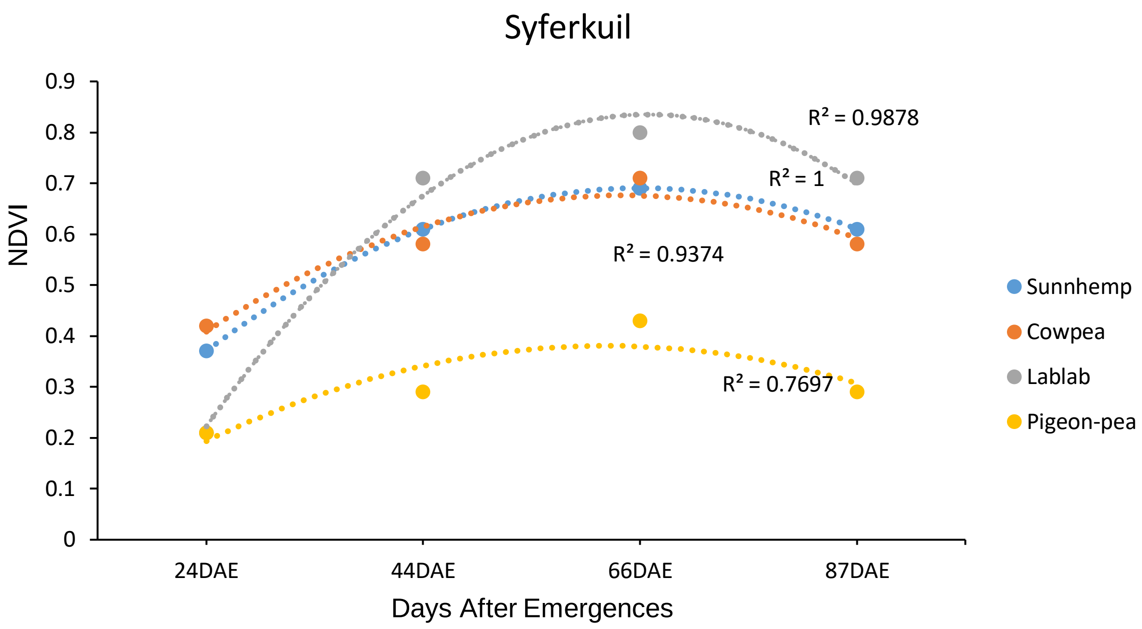


Figure 4.6: Normalized Difference Vegetation Index of different forage legumes evaluated at Syferkuil in the 2019 growing season in Limpopo province.

At Ofcolaco, all the treatments significantly influenced NDVI readings throughout the growth stages. Contrary to Syferkuil, the highest NDVI values of the four species were recorded at 24 DAE, ranging from 0.68 to 0.84. The NDVI values of pigeon pea, sunnhemp, and cowpea were similar but that of lablab was lower than sunnhemp at this stage of growth. From 44DAE, the NDVI of the forage species were similar except pigeon pea, which recorded higher values. At 66 and 88 DAE, pigeon pea and lablab were generally superior even though sunnhemp was also found to have high NDVI values at 87DAE. Cowpea was consistently lower than pigeon pea from 44 DAE until 87DAE.

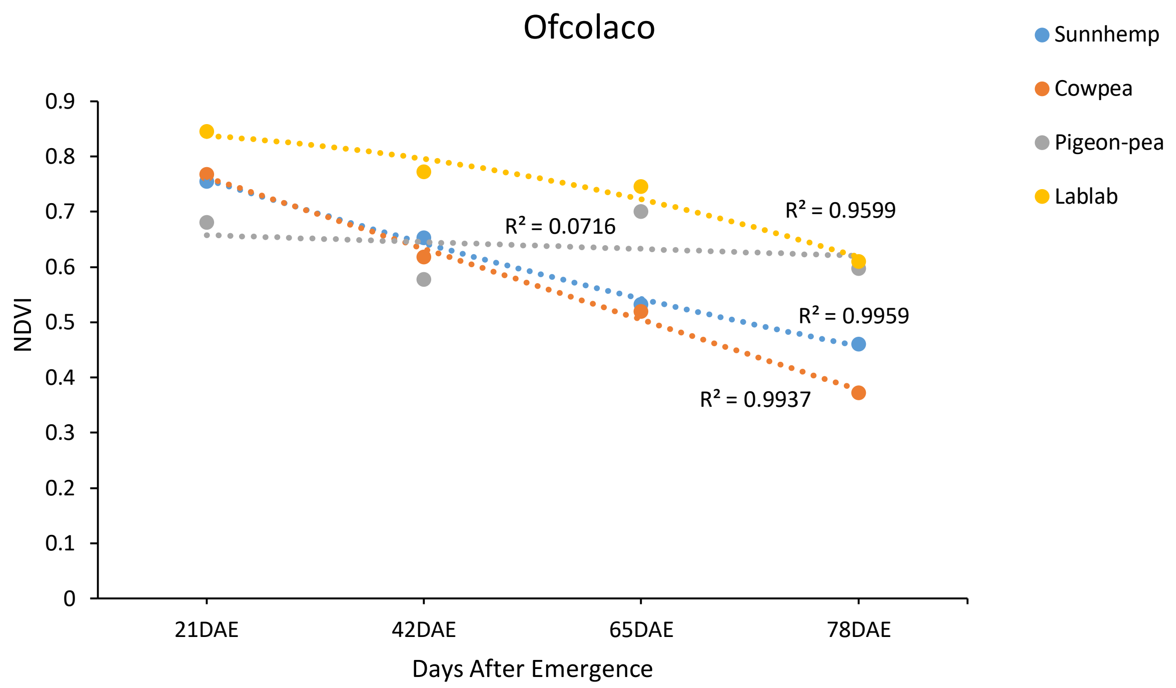


Figure 4.7. NDVI of different forage legumes evaluated at Ofcolaco in the 2019 growing season in Limpopo province.

4.2.4 Leaf gaseous exchange

Photosynthetic rate

The photosynthetic rate measured in the study varied across sampling dates and species, ranging from as low as 6.67 $\mu molm^{-2}\ s^{-1}$ to 79.7 $\mu molm^{-2}\ s^{-1}$ at Syferkuil (Table 4.1). During the first sampling (24 DAE) measurements at Syferkuil, the photosynthesis rate was relatively low across all the species with no significant difference among them (Table 4.1). However, from 44 DAE onwards, differences in leaf photosynthetic rates were observed among the species. The photosynthetic rates at 44 DAE were similar in sunnhemp, cowpea, and pigeon pea but cowpea had a higher photosynthetic rate than lablab. From 66 DAE onwards, pigeon pea significantly increased photosynthetic rate when compared to the other species, but at 87 DAE, the photosynthetic rate of pigeon pea was similar to that of cowpea and was 64.11% higher when compared to sunnhemp and lablab. At Ofcolaco, there was no significant species effect on photosynthesis rate throughout the season. Photosynthetic ranged at this location from 4.88 to 46.35 $\mu molm^{-2}\ s^{-1}$ across sampling date and species. There was however a tendency of higher photosynthetic in sunnhemp up to 65 DAE followed by cowpea which was high at 21 DAE and 42 DAE but declined afterwards.

Table 4.1: Photosynthesis rate of summer forages at two distinct locations

	Photosynthetic rate (A) (*µmolm*$^{-1}$ *s*$^{-1}$)			
		Syerkuil		
	24DAE	**44DAE**	**66DAE**	**87DAE**
Sunnhemp	6.67	65.81ab	31.46bc	34.32b
Cowpea	12.02	75.73a	25.35c	37.59ab
Lablab	8.90	35.14b	43.89b	29.39b
Pigeon pea	8.99	54.52ab	79.70a	52.27a
P≤0.05	ns	*	***	*
		Ofcolaco		
	21DAE	**42DAE**	**65DAE**	**78DAE**
Sunmhemp	39.61	41.64	46.35	8.81
Cowpea	26.81	24.62	17.73	11.66
Lablab	24.67	31.92	7.81	5.22
Pigeon pea	24.86	42.33	18.98	4.88
P≤0.05	ns	ns	ns	ns

*Means in the same column followed by the same letter are not significantly different from each other at the 5% probability level, *Significant at p≤0.05, **significant at 0.01, ***significant at 0.001, ns = Not significant.*

Transpiration rate

Transpiration rate at Syferkuil was influenced by forage species except at 24 DAE where no variation was observed amongst the forage species (Table 4.2). However, after the initial growth stage, differences in transpiration rates among the species were observed. At 44 DAE, the transpiration rate in lablab, cowpea, and pigeon pea was similar but that of lablab was greater than sunnhemp at this sampling date. At 66 DAE, the transpiration rate in cowpea, lablab, and pigeon pea was similar, although lablab was superior to sunnhemp at this growth stage. At 87 DAE, a relatively lower transpiration rate was recorded in sunnhemp compared to lablab and pigeon pea. Table 4.2 shows that the transpiration rate at Ofcolaco did not differ among the forage species across all the sampling dates. The mean transpiration rates ranged from 3.61 *mmol* $m^{-2}s^{-1}$ to 17.68 *mmol* $m^{-2}s^{-1}$ across both locations and species. Lablab constantly transpired more from 21 DAE until 65 DAE and declined rapidly at 78 DAE, followed by pigeon-pea at 42 DAE and 65 DAE.

Table 4.2: Transpiration rate of forage summer legumes at Syferkuil and Ofcolaco

	Transpiration rate (E) (*mmol* $m^{-2}\ s^{-1}$)			
	Syferkuil			
Treatment	**24DAE**	**44DAE**	**66DAE**	**87DAE**
Sunnhemp	6.24	11.44b	13.99b	10.55b
Cowpea	7.50	11.72ab	17.56ab	12.25ab
Lablab	7.55	12.79a	19.18ab	13.16a
Pigeon pea	7.01	12.05ab	23.71a	14.25a
P (≤ 0.05)	ns	**	***	***
	Ofcolaco			
	21DAE	**42DAE**	**65DAE**	**78DAE**
Sunnhemp	16.93	14.86	15.34	6.86
Cowpea	14.67	12.87	12.43	7.10
Lablab	15.80	18.00	18.48	3.61
Pigeon pea	16.04	17.68	15.78	4.65
(P≤0.05)	ns	ns	ns	ns

*Means in the same column followed by the same letter are not significantly different from each other at the 5% probability level, *Significant at p≤0.05, **significant at 0.01, ***significant at 0.001, ns = Not significant.*

Stomatal conductance

The forage legume species did not influence stomatal conductance at all growth stages at both Syferkuil and Ofcolaco (Table 4.3). The mean values at Syferkuil ranged from 0.06 *mol* $m^{-2}\ s^{-1}$ *to* 5.59 *mol* $m^{-2}\ s^{-1}$, with lablab having the tendency of higher stomatal conductance from 44 DAE until 87 DAE, followed by pigeon pea from 24 DAE until 87 DAE. The means at Ofcolaco ranged from 0.1 *mol* $m^{-2}\ s^{-1}$ to 5.15 *mol* $m^{-2}\ s^{-1}$ across all sampling dates of the species. Lablab had higher *gs* at 42 DAE and 65 DAE, followed by pigeon pea on similar dates.

Table 4.3: Stomatal conductance of different forage legumes at two locations namely Syferkuil and Ofcolaco.

	Stomatal conductance (gs) (*mol m-^{2} s-^{1}*)			
	Syferkuil			
	24DAE	**44DAE**	**66DAE**	**87DAE**
Sunnhemp	0.26	2.64	0.68	1.19
Cowpea	0.26	4.58	0.06	1.96
Lablab	0.28	6.80	1.83	2.97
Pigeon pea	0.37	5.59	1.26	2.40
P≤0.05	ns	ns	ns	ns
	Ofcolaco			
	21DAE	**42DAE**	**65DAE**	**78DAE**
Sunnhemp	5.15	0.83	1.04	0.19
Cowpea	2.98	0.58	0.63	0.2
Lablab	3.43	0.97	3.19	0.08
Pigeon pea	3.39	0.93	2.81	0.10
P≤0.05	ns	ns	ns	ns

*Means in the same column followed by the same letter are not significantly different from each other at the 5% probability level, *Significant at p≤0.05, **significant at 0.01, ***significant at 0.001, ns = Not significant.*

Sub-stomatal carbon dioxide

The sub-stomatal CO_2 of the summer forage legumes did not differ at the two locations except at 24 DAE at Syferkuil where a lower concentration of 266.50 *mol m-^{2}s-^{1}* was recorded in cowpea relative to sunnhemp (Table 4.4). There was, however, a tendency of higher sub-stomatal CO_2 concentration in lablab throughout the season at Syferkuil. Sub-stomatal CO_2 concentration at this location ranged from 129.75 *mol m^{-2}s-^{1} to 374 mol m-^{2}s-^{1}* across sampling dates and species. At Ofcolaco, differences in sub-stomatal CO_2 concentration were not observed among the legume species. Sub-stomatal CO_2 concentration ranged from *185 mol m^{-2}s^{-1}* to 390.25 *mol m^{-2}s^{-1}* across all the species and sampling time at this location. There was, however, a tendency of higher sub-stomatal CO_2 concentration in lablab throughout the season followed by pigeon pea. Sub-stomatal CO_2 concentration ranged from 255.75 to 317.00 in lablab and from 267.25 to 382.25 *mol m-^{2}s-^{1}* in pigeon pea at Ofcolaco.

Table 4.4: Sub-stomatal carbon dioxide concentration of the summer forage legumes at Syferkuil and Ofcolaco.

	Sub-stomatal CO_2 (ci) (*mol $m^{-2}s^{-1}$*)			
	Syferkuil			
Treatment	**24DAE**	**44DAE**	**66DAE**	**87DAE**
Sunnhemp	374.25a	232.25	189.00	264.75
Cowpea	266.50b	216.75	254.75	245.75
Lablab	317.00ab	273.75	255.75	282.00
Pigeon pea	326.50ab	219.00	129.75	224.75
P≤0.05	*	ns	ns	ns
	Ofcolaco			
	21DAE	**42DAE**	**65DAE**	**78DAE**
Sunnhemp	344.50	195.00	216.00	318.50
Cowpea	323.75	258.25	313.25	297.75
Lablab	380.50	185.00	390.75	310.50
Pigeon pea	286.65	267.25	382.25	308.75
P≤0.05	ns	ns	ns	ns

*Means in the same column followed by the same letter are not significantly different from each other at the 5% probability level, *Significant at p≤0.05, **significant at 0.01, ***significant at 0.001, ns = Not significant.*

4.2.5 Root nodule production

The forage legumes studied did not produce any nodules from day 24 DAE to the last day of sampling at Syferkuil**.** At Ofcolaco, during the early stage of growth (21 DAE), there was no significant difference in the number of nodules produced by the legumes species. However, at 42 DAE, cowpea produced a significantly higher number of nodules when compared with the other species. The nodules produced by cowpea were 92.32% higher than the average of that of sunnhemp and lablab. Pigeon pea had zero nodules. No difference in nodule weight was observed at the two sampling dates. Furthermore, no nodules were observed at 65 DAE and this might be because the crops had already reached their maturity growth stage.

Table 4.5: Effects of summer forage legumes on the production of nodules at Ofcolaco.

	Ofcolaco			
	Number of Nodules		Nodule weight (g)	
	21DAE	42DAE	65DAE	78DAE
Sunnhemp	0.74	3.70	1.16	0.73
Cowpea	4.25	94.5	2.7	4.25
Lablab	0.95	18	3.84	0.95
Pigeon pea	0	0	0	0
P≤0.05	Ns	**	ns	ns

*Means in the same column followed by the same letter are not significantly different from each other at the 5% probability level, *Significant at p≤0.05, **significant at 0.01, ***significant at 0.001, ns = Not significant.*

4.2.6 Plant nutritive value

The macro and micro-nutrient concentrations of the forage legumes are presented in table 4.5 and table 4.6.

4.2.6.1 Macronutrient at Syferkuil

In terms of macronutrient concentration, a range of 3.67- 4.29% nitrogen,1.16-2.01% in calcium, 0.58-1.01% in magnesium; 1.88-3.79% in potassium, 0.34-55% in phosphorous and 598-1165 mg kg^{-1} in sodium was recorded in the legume species at Syferkuil. Although significant variation was not observed among the forage legume species, cowpea tended to measure high concentrations in most of the macronutrients compared to the others. For instance, the concentration of potassium in cowpea was 3.79% compared to the average concentration of 2.70% of sunnhemp, lablab, and pigeon pea. The concentrations of calcium, magnesium, and phosphorous in cowpea were respectively 0.26, 0.26, and 0.14 percentage points higher than the averages in the other three legumes at Syferkuil. A high concentration of sodium was also recorded in cowpea, 1165 mg.kg^{-1} which was 436 percentage points higher than the average of sunnhemp, lablab, and pigeon pea. Besides cowpea, lablab appeared to accumulate higher concentrations of calcium and potassium compared to sunnhemp and pigeon pea, whereas sunnhemp had a relatively higher magnesium concentration compared to lablab and pigeon pea.

Table 4.6: Plant nutritive value of the forage legumes at Syferkuil in the 2019 planting.

SYFERKUL					
	Units	Sunnhemp	Cowpea	Lablab	Pigeon-pea
Macronutrients					
N	%	4.29±0.63	4.00±067	3.67±0.33	3.93±0.11
Ca	%	1.57±0.15	1.84±0.31	2.01±0.22	1.16±0.15
Mg	%	0.92±0.09	1.01±0.18	0.71±0.18	0.58±0.10
K	%	2.88±0.96	3.79±0.47	3.35±0.52	1.88±0.27
P	%	0.47±0.15	0.55±0.08	0.43±0.05	0.34±0.05
Na	%	0.09±0.02	0.12±0.04	0.06±0.03	0.07±0.01
Micronutrients					
Zn	mg kg^{-1}	36.5±28	37.5±24	28.6±14	25.8±12
Cu	mg kg^{-1}	5.82±0.3	6.96±1.8	6.28±0.5	5.64±0.5
Mn	mg kg^{-1}	100±27	108±36	94±25	113±18
Fe	mg kg^{-1}	1125±405	853±549	656±277	1506±1040

P = phosphorus, K = potassium, Ca = calcium, Mg = magnesium, Na = sodium

4.2.6.2 Micronutrient at Syferkuil

The concentrations of zinc, copper, and manganese tendered to be higher in cowpea compared to the other species, even though statistical significance was not observed. However, a high concentration of iron, 1125 and 1506 mg.kg^{-1}, was observed in sunnhemp and pigeon pea respectively. The concentration of zinc and manganese were relatively low in lablab whereas pigeon pea was found to be low in zinc and copper compared to the other species.

4.2.6.3 Macronutrient at Ofcolco

Similarly, to Syferkuil, no statistical variation in macro and micro-nutrient concentrations among the forage legume species was observed at Ofcolaco. However, cowpea and sunnhemp generally revealed the tendency of higher macronutrient concentrations relative to lablab and pigeon pea except in pigeon pea where the calcium concentration was 0.38% point higher than the average concentration of the other three legumes. The macronutrient concentration range at this location was 2.73-3.81 in nitrogen, 1.16-1.75% in calcium, 0.58-1.01% in magnesium; 1.88-3.79% in potassium, 0.34-55% in phosphorous and 83.52-420.12mg kg^{-1} in sodium.

4.2.6.4 Micronutrient Ofcolcao

At Ofcolaco, the range of micronutrient concentration was 20.23 – 30.97% in zinc, 5.86 - 7.20% in copper, 64.77 – 97.09% in manganese, and 205.40 – 379.15 $mg.kg^{-1}$ in iron (Table 4.6). The distribution of micronutrients among the legume species indicated a relatively higher accumulation of zinc in cowpea, copper, and magnesium in lablab and pigeon pea, and iron in sunnhemp. The zinc was exceptionally high in sunnhemp 379.15 $mg.kg^{-1}$ which was 57.4% higher than the average of the cowpea, lablab, and sunnhemp.

Table 4.7: Plant nutritive value of four forage legumes planted at Ofcolaco in 2019 planting plating season.

		OFCOLACO			
	Units	Sunnhemp	Cowpea	Lablab	Pigeon-pea
Macronutrient					
N	%	3.49±1.00	2.73±0.36	3.81±0.20	2.90±0.27
Ca	%	1.51±0.48	1.43±0.94	1.16±0.09	1.75±0.03
Mg	%	0.65±0.26	0.62±0.20	0.42±0.03	0.47±0.03
K	%	1.83±0.23	2.22±0.61	1.53±0.25	1.87±0.16
P	%	0.32±0.09	0.32±0.05	0.28±0.04	0.34±0.04
Na	%	0.02±0.00	0.04±0.02	0.01±0.00	0.01±0.00
Micronutrient					
Zn	mg kg^{-1}	20.84±50	30.97±1.00	20.23±60	21.10±4.00
Cu	mg kg^{-1}	6.69±0.80	5.86±0.48	7.20±1.10	7.02±0.5.00
Mn	mg kg^{-1}	67.52±17	64.77±39	97.09±4.00	83.18±6.00
Fe	mg kg^{-1}	379.15±250	249.39±163	267.99±39	205.40±20

P = phosphorus, K = potassium, Ca = calcium, Mg = magnesium, Na = sodium

4.2.6.5 Crude protein concentration

Crude protein concentration was calculated from the formula CP= %N ^{x} 6.25. Table (4.8) revealed a higher crude protein concentration of 26.82% in sunnhemp at Syferkuil followed by cowpea and pigeon pea. Unlike Syferkuil, the highest mean of protein at Ofcolaco was recorded in lablab at 23.84% followed by sunnhemp with a concentration of 21.8%. Cowpea and pigeon were relatively lower in crude protein concentrations, 17.0% and 18.1% respectively.

Table 4.8. Crude protein concentration of four legumes planted at two different locations in Limpopo Province.

Treatment	Syferkuil CP (%)	Ofcolaco CP (%)
Sunnhemp	26.82±3.91	21.81±6.28
Cowpea	24.99±4.20	17.03±2.24
Lablab	22.91±2.04	23.84±1.24
Pigeon Pea	24.53±0.70	18.13±1.68

CP = Crude Protein

4.2.6.6 Crude Protein yield

The value of protein yield of the individual forage species was obtained by multiplying biomass of relevant forage legume by its crude protein concentration. The lowest protein yield of 675 kg.ha^{-1} was found in pigeon pea at Syferkuil. The protein yield of sunnhemp, cowpea and lablab were similar at Syferkuil, ranging from 1146 kg.ha^{-1} to 1715 kg.ha^{-1} across the three species (Fig. 4.8). This is an indication that these three species produced a higher protein per unity area compared to pigeon pea at this location. Similarly, sunnhemp produced the highest protein yield of 1946 kg.ha^{-1} at Ofcolaco, but this was statistically similar to that of pigeon pea which produced a yield of 1530 kg.ha^{-1}. The protein yield of cowpea and sunnhemp at Ofcolaco were similar, producing 834.6 kg.ha^{-1} and 800.3 kg.ha^{-1} respectively which were lower than that of sunnhemp and pigeon pea.

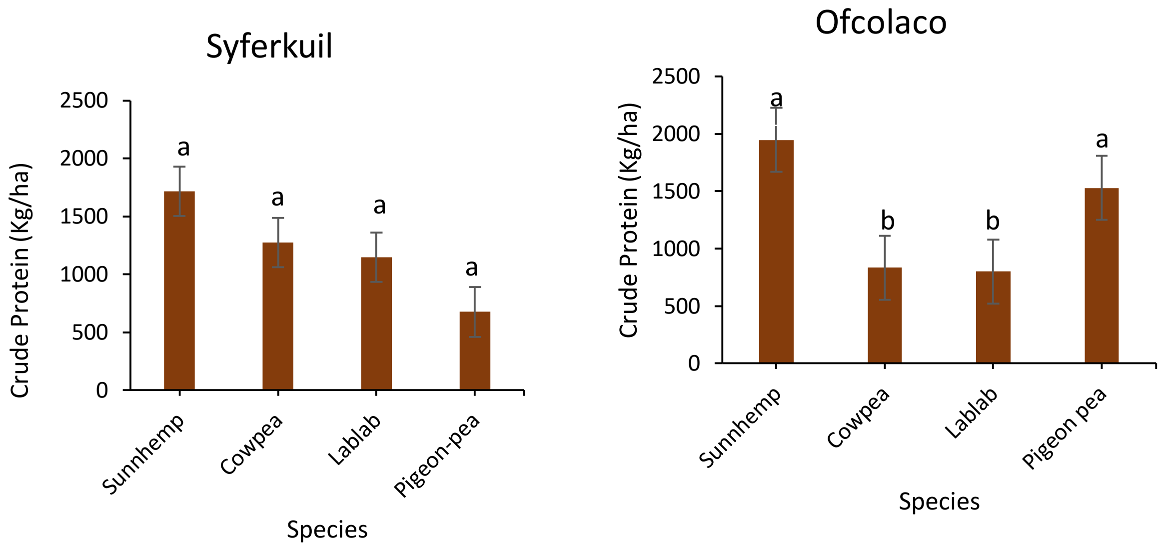


FIG: 4.8. Crude protein yield of forage summer legumes evaluated at Syferkuil and Ofcolaco in the 2019 growing season.

4.2.7 The relationship between biomass and crude protein, protein yield, and leaf gaseous parameters at Syferkuil.

The relationship between measured variables was done using Spearman's correlation procedure for each species at different days after emergence and those with a level of meaningful relationships are presented in the following section:

Sunnhemp 24 DAE

Sunnhemp biomass had a strong positive correlation with the following parameters: chlorophyll content, NDVI, and sub-stomatal conductance (Table 4.9). The biomass had a moderately strong correlation with crude protein and a weaker correlation with crude protein yield. Other strong positive correlation relationships in sunnhemp were observed between crude protein and crude protein yield, crude protein and sub-stomatal conductance and stomatal conductance. Chlorophyll also had a strong positive correlation with NDVI.

Table 4.9. Correlations between biomass, crude protein, crude protein yield, and other parameters at 24 DAE of Sunnhemp planted at Syferkuil in 2019 planting season.

	BIO	*CP*	*CPY*	*CHLOR*	*NDVI*	*ci*	*E*	*gs*	*A*
BIO	1								
CP	0.61	1							
CPY	0.22	0.80*	1						
CHLOR	0.82*	0.58	0.53	1					
NDVI	0.93*	0.36	0.11	0.89*	1				
ci	0.83*	0.94*	0.60	0.69	0.61	1			
E	-0.85	-0.93	-0.57	-0.70	-0.63	-1.00	1		
gs	-0.28	0.08*	-0.23	-0.71	-0.59	0.02	-0.01	1	
A	-0.28	-0.49	0.03	0.21	0.07	-0.53	0.53	-0.84	1

BIO = Biomass, CP = Crude protein, CPY = Crude protein yield, ci = Sub-stomatal CO_2*, E = Transpiration rate, gs = Stomatal conductance, A = Photosynthetic rate, CHLOR = leaf chlorophyll content, and NDVI = Normalised difference vegetation index*.

Cowpea 66 DAE

Cowpea biomass had a strong positive correlation relationship with normalized difference vegetation index (NDVI) and, a fairly strong relationship between biomass and chlorophyll. Another strong correlation relationship was observed between transpiration and photosynthesis rate. Furthermore, an increase in chlorophyll increased in leaf chlorophyll content, increased the stomatal conductance and photosynthesis rate of cowpea. A strong negative correlation was observed between crude protein, chlorophyll content, stomatal conductance, and photosynthesis rate.

Table 4.10. Correlations between biomass, crude protein, crude protein yield, and other parameters at 66 DAE of Cowpea planted at Syferkuil in 2019 planting season.

	BIO	*CP*	*CPY*	*CHLOR*	*NDVI*	*ci*	*E*	*gs*	*A*
BIO	1								
CP	-0.60	1							
CPY	0.54	-0.09	1.00						
CHLOR	0.60	-1.00	0.08*	1					
NDVI	0.98*	-0.73	0.53	0.72	1				
ci	0.21	0.61	0.66	-0.61	0.07	1			
E	0.22	-0.70	0.44	0.70	0.38	-0.37	1		
gs	-0.03	-0.78	-0.33	0.79	0.14	-0.93	0.68	1	
A	0.47	-0.79	0.55	0.79	0.61	-0.27	0.96*	0.61	1

BIO=Biomass, CP=Crude protein, CPY=Crude protein yield, c ci=Sub-stomatal CO_2 *E= Transpiration rate, gs=Stomatal conductance, A=Photosynthetic rate, CHLOR=leaf chlorophyll content, and NDVI-Normalised difference vegetation index*.

Lablab 88 DAE

Lablab biomass had a strong positive correlation relationship with crude protein and photosynthesis rate. There was a moderately strong correlation between sub-stomatal, transpiration rate and stomatal conductance. An increase in transpiration rate showed an increase in the stomatal conductance of lablab. A strong negative correlation was observed between crude protein and chlorophyll content.

Table 4.11. Correlations between biomass, crude protein, crude protein yield, and other parameters at 88 DAE of Lablab planted at Syferkuil in 2019 planting season.

	BIO	*CP*	*CPY*	*CHLOR*	*NDVI*	*ci*	*E*	*gs*	*A*
BIO	1								
CP	0.06	1							
CPY	0.97*	0.31	1						
CHLOR	0.39	-0.86	0.14	1					
NDVI	0.26	-0.20	0.22	0.06	1				
ci	-0.93	-0.23	-0.94	-0.28	0.10	1			
E	-0.36	-0.74	-0.51	0.35	0.62	0.65	1		
gs	-0.29	-0.21	-0.30	-0.17	0.85*	0.60	0.80*	1	
A	0.81*	0.41	0.89*	-0.12	0.57	-0.67	-0.27	0.14	1

BIO=Biomass, CP=Crude protein, CPY=Crude protein yield, c ci=Sub-stomatal CO_2 *E= Transpiration rate, gs=Stomatal conductance, A=Photosynthetic rate, CHLOR=leaf chlorophyll content, and NDVI-Normalised difference vegetation index*.

Pigeon pea 44 DAE

Pigeon pea biomass correlated positively strong with chlorophyll content and NDVI, meanwhile a strong negative correlation was found between biomass versus crude protein and transpiration rate. Pigeon pea crude protein had a strong negative correlation between chlorophyll content, NDVI, sub-stomatal conductance, and stomatal conductance. Both crude protein yield and chlorophyll content had a strong positive correlation relationship with sub-stomatal conductance.

Table 4.12. Correlations between biomass, crude protein, crude protein yield, and other parameters at 44 DAE of pigeon pea planted at Syferkuil in 2019 planting season.

	BIO	*CP*	*CPY*	*CHLO*	*NDVI*	*ci*	*E*	*gs*	*A*
BIO	1								
CP	-0.86	1							
CPY	0.52	-0.74	1						
CHLO	0.86*	-1.00	0.73*	1					
NDVI	0.98*	-0.87	0.43	0.87*	1				
ci	0.67	-0.89*	0.96*	0.88*	0.62	1			
E	-0.87	0.73	-0.74*	-0.71	-0.77	-0.76	1		
gs	0.62	-0.87	0.45	0.89*	0.71	0.67	-0.31	1	
A	-0.69	0.23	0.02	-0.22	-0.63	-0.04	0.65	0.08*	1

**BIO=Biomass, CP=Crude protein, CPY=Crude protein yield, ci=Sub-stomatal* CO_2 *E= Transpiration rate, gs=Stomatal conductance, A=Photosynthetic rate, CHLOR=leaf chlorophyll content, and NDVI-Normalised difference vegetation index*

4.2.7 The relationship between biomass and crude protein, protein yield, and leaf gaseous parameters at Ofcolaco.

Sunnhemp 65 DAE

Sunnhemp at Ofcolaco had a strong negative relationship between biomass versus crude protein, crude protein yield, chlorophyll content, and stomatal conductance. Then strong positive correlation was found when the crude protein of sunnhemp was correlated with crude protein, chlorophyll content, and sub-stomatal conductance. Another strong positive correlation relationship was observed between chlorophyll content, transpiration rate, and stomatal conductance. Transpiration rate increased with an increase in stomatal conductance and photosynthesis rate.

Table 4.13: Correlations between biomass, crude protein, crude protein yield, and other parameters at 65 DAE of Sunnhemp planted at Ofcolaco in 2019 planting season.

	BIO	*CP*	*CPY*	*CHLOR*	*NDVI*	*ci*	*E*	*gs*	*A*
BIO	1								
CP	-0.92	1							
CPY	-0.84	0.82*	1						
CHLOR	-0.88	0.98*	0.90*	1					
NDVI	0.26	-0.23	0.30	-0.04	1				
ci	-0.33	0.50	-0.09	0.35	-0.86	1			
E	-0.72	0.66	0.97*	0.77*	0.49	-0.32	1		
gs	-0.86	0.87*	0.99*	0.94*	0.23	0.01	0.94*	1	
A	-0.35	0.25	0.76	0.41	0.80	-0.71	0.89*	0.69	1

BIO=Biomass, CP=Crude protein, CPY=Crude protein yield, ci=Sub-stomatal CO_2 *E= Transpiration rate, gs=Stomatal conductance, A=Photosynthetic rate, CHLOR=leaf chlorophyll content, and NDVI-Normalised difference vegetation index*.

Cowpea 65 DAE

Cowpea biomass at Ofcolaco strongly correlated positively with crude protein, crude protein yield, and NDVI. Crude protein strongly correlated positively with chlorophyll content and a strong negative correlation was observed between crude protein versus NDVI and sub-stomatal conductance. Chlorophyll content of cowpea showed a strong correlation with NDVI, while NDVI has correlated positively strong with transpiration rate stomatal conductance. Another strong positive correlation was found between transpiration rate and stomatal conductance.

Table 4.14: Correlations between biomass, crude protein, crude protein yield, and other parameters at 65 DAE of Cowpea planted at Ofcolaco in 2019 planting season.

	BIO	*CP*	*CPY*	*CHLOR*	*NDVI*	*ci*	*E*	*gs*	*A*
BIO	1								
CP	0.20	1							
CPY	0.94*	0.52	1						
CHLOR	0.19	0.89*	0.46	1					
NDVI	0.91*	-0.08	0.76	0.08*	1				
ci	0.29	-0.82	-0.04	-0.58	0.60	1			
E	0.93*	0.01	0.82*	-0.12	0.81*	0.33	1		
gs	0.77*	-0.44	0.52	-0.46	0.82*	0.71*	0.89*	1	
A	0.29	0.66	0.50	0.27	-0.13	-0.72	0.38	-0.03	1

BIO=Biomass, CP=Crude protein, CPY=Crude protein yield, ci=Sub-stomatal CO_2 E= Transpiration rate, gs=Stomatal conductance, A=Photosynthetic rate, CHLOR=leaf chlorophyll content, and NDVI-Normalised difference vegetation index.

Pigeon pea 45 DAE

Pigeon pea biomass correlated strongly with crude protein and it had a fairly strong correlation relationship with photosynthesis rate. Crude protein yield had a strong correlation with photosynthesis rate, meanwhile, sub-stomatal CO_2 concentration increased with an increase in chlorophyll content of pigeon pea.

Table 4.15: Correlations between biomass, crude protein, crude protein yield, and other parameters at 45 DAE of pigeon pea planted at Ofcolaco in 2019 planting season.

	BIO	*CP*	*CPY*	*CHLOR*	*NDVI*	*ci*	*E*	*gs*	*A*
BIO	1								
CP	0.91*	1							
CPY	0.29	0.22	1						
CHLOR	-0.63	-0.29	-0.59	1					
NDVI	-0.79	-0.96	-0.30	0.13	1				
ci	-0.57	-0.39	-0.93	0.84*	0.37	1			
E	-0.16	0.18	-0.71	0.85	-0.26	0.79	1		
gs	-0.75	-0.47	0.13	0.68	0.22	0.25	0.26	1	
A	0.66	0.59	0.91*	-0.70	-0.61	-0.96	-0.58	-0.18	1

BIO=Biomass, CP=Crude protein, CPY=Crude protein yield, ci=Sub-stomatal CO_2 *E= Transpiration rate, gs=Stomatal conductance, A=Photosynthetic rate, CHLOR=leaf chlorophyll content, and NDVI-Normalised difference vegetation index*.

Lablab 45 DAE

Lablab biomass at Ofcolaco correlated positively with crude protein and a weaker positive correlation was observed between biomass and crude protein yield. Transpiration rate correlated positively strong with photosynthesis rate at 96%.

Table 4.16: Correlations between biomass, crude protein, crude protein yield, and other parameters at 45 DAE of Lablab planted at Ofcolaco in 2019 planting season

	BIO	*CP*	*CPY*	*CHLOR*	*NDVI*	*ci*	*E*	*Gs*	*A*
BIO	1								
CP	0.74*	1							
CPY	0.28*	0.71	1						
CHLOR	0.03*	0.50	0.96	1					
NDVI	0.51	-0.15	-0.22	-0.28	1				
ci	0.54	0.29	-0.46	-0.68	0.09	1			
E	-0.68	-0.75	-0.08	0.17	0.17	-0.84	1		
gs	-0.19	-0.58	-0.98	-0.98	0.13	0.61	-0.11	1	
A	-0.45	-0.70	-0.11	0.10	0.45	-0.75	0.96*	-0.09	1

BIO=Biomass, CP=Crude protein, CPY=Crude protein yield, ci=Sub-stomatal CO_2 E= Transpiration rate, gs=Stomatal conductance, A=Photosynthetic rate, CHLOR=leaf chlorophyll content, and NDVI-Normalised difference vegetation index.

4.3 Stooling ryegrass

The data on stooling ryegrass presented below is only for Syferkuil since the crop was destroyed by wild animals at Ofcolaco and hence, the data could not be collected.

4.3.1 Biomass accumulation

Not much treatment effect was observed in the biomass of stooling rye planted after the summer forage legumes. There was a tendency for higher biomass of the grass species grown after pigeon pea and lablab compared to those following sunnhemp and cowpea. On average, the stooling ryegrass following pigeon pea and lablab was 30.3% higher than the average biomass accumulation of the grass following sunnhemp and cowpea (Fig 4.9). The grass biomass accumulation ranged from 912.6 to 1394.2 kg ha^{-1}.

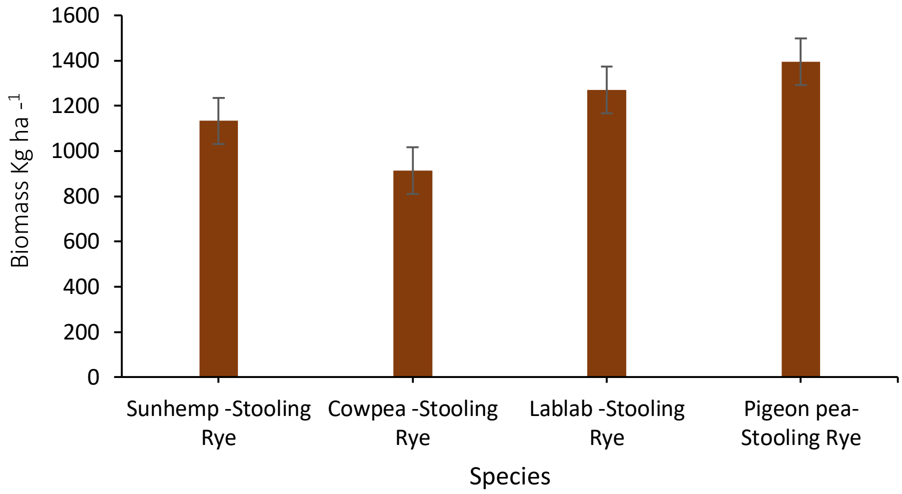


Fig 4.9**:** Biomass accumulation of stooling rye at Syferkuil during 2019 winter planting season.

4.3.2 Nutritive value

4.3.2.1 Macro and micronutrients

Nutrient ion concentration of stooling ryegrass was not responsive to the preceding summer forage legumes at Syferkuil. This could probably be due to the fact that there were not enough plant residues left on the plots after harvest to support stooling ryegrass growth and its nutritive value. However, under sunnhemp residues, stooling ryegrass tended to have the highest mean values of Ca (0.34%), Mg (0.34%), K (2.01%) when compared with other forage legumes. When comparing means values of plant nutritive elements of stooling rye with that of forage legumes, stooling rye values were drastically low in almost all the elements.

Table 4.17: Plant nutritive elements of stooling rye (*Secale cereale*) at Syferkuil.

	UNITS	Sunnhemp-Stooling rye	Cowpea-Stooling rye	Lablab- Stooling rye	Pigeon-pea-Stooling rye
Macronutrients					
N	%	1.85±0.22	1.75±0.12	1.73±0.09	1.75±0.13
Ca	%	0.34±0.08	0.30±0.04	0.31±0.03	0.32±0.03
Mg	%	0.34±0.06	0.32±0.04	0.33±0.03	0.33±0.03
K	%	2.01±0.27	1.89±0.12	1.19±0.13	1.95±0.11
Na	%	0.04±0.02	0.04±0.00	0.03±0.01	0.03±0.01
P	mg/kg	0.24±0.01	0.24±0.03	0.24±0.02	0.25 ±0.02
Micronutrients					
Zn	mg/kg	29.07±6.20	26.19±3.71	29.13±4.67	29.48±3.72
Cu	mg/kg	3.07±0.66	2.62±0.10	2.81±0.42	3.00±0.43
Mn	mg/kg	99.15±9.68	91.85±11.28	104.89±9.63	102.86±14.63
Fe	mg/kg	893.07±631.80	834.09±457.14	1279.11±598.59	1340.92±681.81
P	mg/kg	0.24±0.01	0.24±0.03	0.24±0.02	0.25 ±0.02

P=phosphorus, K=potassium, Ca=calcium, Mg=magnesium, Na sodium

4.3.2.2 Crude protein

The protein yield of stooling ryegrass at Syferkuil did not differ among the different forage crop treatments. The protein yield of the grass was also not responsive to the proceeding stooling ryegrass. This data revealed that not much of the plant residues material was left on the soil by predecessor crops. Crude protein % values ranged from 10.86% and 11.55% and crude protein yield ranges from 99.07 $kg.ha^{-1}$ to 15.75 $kgha^{-1}$.

Table 4.18: Crude protein (CP) of stooling rye at Syferkuil under different plant residues treatment.

Treatment	CP (%)
Sunnhemp- Stooling rye	11.5±1.38
Cowpea-rye- Stooling rye	10.94±0.77
Lablab-rye- Stooling rye	10.86±0.56
Pigeon pea- Stooling rye	10.92±0.81

4.3.2.3 Crude protein yield

Even though the biomass of stooling rye was enough for grazing, but the crude protein yield was lower, it ranged from 99.07 to 152.75 kg.ha^{-1}. This may be due to the lower crude protein concentration of stooling rye.

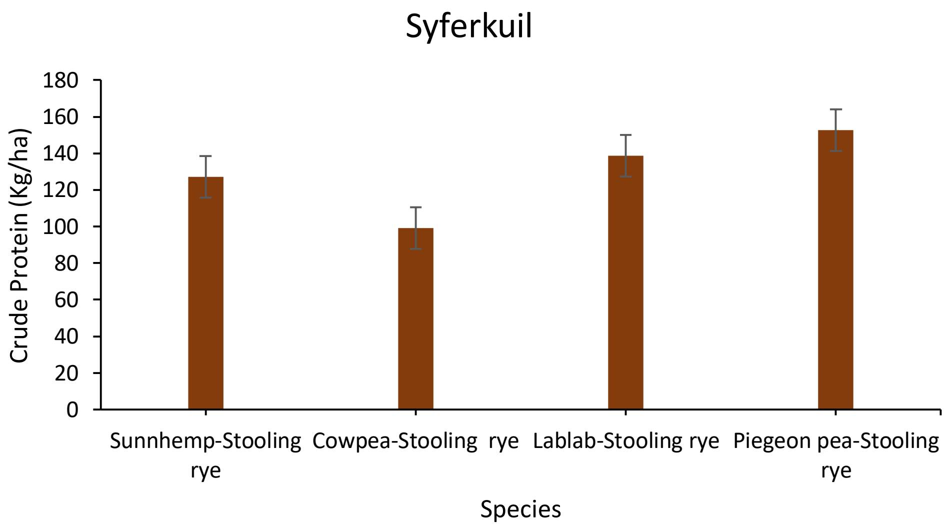


Fig 4.10: Crude Protein yield of forage stooling rye evaluated at Syferkuil in 2019 growing season.

CHAPTER 5: DISCUSSION

Assessment of biomass productivity across the two locations revealed that high biomass production among the species occurred at 87 DAE at Syferkuil, whereas at Ofcolaco, the highest productivity was observed at 65 days after emergence. According to Craufurd and Wheeler, (2009) higher temperatures hasten the phonological rate of the crops, thus shortening the harvesting period of the crop. These results concur with our current study as an average maximum temperature at Ofcolaco was higher than that of Syferkuil. The data further revealed that the biomass yield of Sunnhemp was consistently high at both locations whereas, that of pigeon pea was low at Syferkuil but relatively high at Ofcolaco. Lablab yield was similar to that of Sunnhemp at Syferkuil but relatively lower at Ofcolaco. Similarly, cowpea biomass production was similar to Sunnhemp at Syferkuil but lower at Ofcolaco. The biomass accumulation of sunnhemp, cowpea, and lablab was constantly similar from 44 DAE until 87 DAE, whereas pigeon pea continued to produce lower biomass as compared to the other forage summer legume species at Syferkuil. At 87 DAE, Pigeon pea biomass accumulation was 57% lower than the average of the three other legumes.

La Guardia Nave and Corbin, (2018) reported that in a study involving the productivity of sunnhemp, cowpea, corn (*Zea may*), and crabgrass (*Digitaria sanguinalis*), sunnhemp produced higher biomass compared to the other crops. This study is in agreement with our current study where sunnhemp biomass was constantly higher at both locations. In the same study, La Guardia Nave *et al.,* (2018) found out that cowpea produced biomass of 2446 kg.ha^{-1} on average, even though the biomass production was lower than sunnhemp but it was still enough for grazing. This is similar to the observation in our study where cowpea biomass was 2573.3 kg.ha^{-1} and 4057.1 kg.ha^{-1} at Syferkuil and Ofcolaco, respectively. Foster *et al.,* (2017) in a study where they compared nine warm-season legumes including sunnhemp, forage cowpea, lablab, and pigeon pea in a semi-arid environment, the authors reported high biomass production in sunnhemp compared to the other forage legumes. The average biomass yield of sunnhemp was 8650 kg.ha^{-1} followed by cowpea and lablab, producing 4700 kg.ha^{-1} and 4000 kg.ha^{-1} respectively. In terms of chlorophyll production, the forage legume species significantly differed at almost every stage of sampling. Cowpea tended to be superior to the other forage legumes in almost every sampling stage. Odhiambo (2011) conducted a study in Limpopo Province where annual summer forage legumes were screened for the potential use of green manure, sunnhemp produced 13586 kg.ha, cowpea produced 4044 kg.ha and lablab produced 8701 kg.ha of biomass.

The chlorophyll content of the forage legume species at Ofcolaco was different, with pigeon pea producing the greatest chlorophyll content when compared with the other forage legume species. The chlorophyll meter has been used many times in research studies to predict the N status of the plant and a generally positive correlation between chlorophyll content and N is found (Wang *et al*., 2014). In our study, we recorded higher chlorophyll content, higher crude protein, and higher micronutrient content in cowpea at Syferkuil indicating strong relationships among these parameters. These results are supported by the study done by Van Heerden *et al*. (2007) where it was reported that chlorophyll content values among the tested treatment did not differ significantly from each other. These results indirectly show that there was no difference in the N status of the plants (Van Heerden *et al*., 2007). Bugbee *et al.,* (2015) found a positive relationship between chlorophyll content and leaf nitrogen status. At Ofcolaco sunnhemp and cowpea had a strong positive correlation of 0.98 and 0.89%, respectively with nitrogen. According to Dordas et al, (2012), the chlorophyll meter has been used extensively to quickly analyse the N status of the crop and the degree of the greenness of the crop has been found to be positively correlated to good quality forage with high crude protein quality. In a study done by Hughes et al, (2014) where the use of non-destructive foliar chlorophyll meter measurements was used to predict the amount of crude protein in signal grass (*Bracharia decumbens*), the authors reported a coefficient of determination value of R^2=0.71, when leaf chlorophyll content was regressed with crude protein.

Not much treatment effects were observed on stooling rye biomass which was planted after the legumes in the winter month. The grass biomass accumulation ranged from 912.6 to an1394.2 kg.ha^{-1}. In a study conducted by Balliu *et al.* (2017), where the influence of legume crops on subsequent vegetable crops was evaluated, it was found that the legume crop, faba bean (*Vicia faba*) influenced biomass yield of selected vegetable crops. The biomass yield of the vegetable from legume pre-crop was higher compared to those in non-legume pre-crop. Xing *et al*, (2017), also reported the benefits of incorporating grain legumes into a cereal-based cropping system. These findings are contrary to our results in this study, this could be possibly due to insufficient forage legumes residues left on the soil in our study.

At Syerkuil, higher NDVI values were generally recorded in sunnhemp, cowpea, and lablab. The values of NDVI ranged from 0.21 to 0.80 among the three species, whereas in pigeon pea, the range was from 0.21 to 0.43. At Ofcolaco NDVI values ranged from 0.37 to 0.84 among all four summer forage legumes which indicate that at this location, all the crops had a healthy growth. The concept of the Normalized Difference Vegetation Index has been used by many researchers for monitoring plant health, growth and development, and plant stress (Zhao *et al*., 2020). NDVI at Syferkuil correlated positively with biomass production and chlorophyll content, and according to Naser et al., (2020), increasing the greenness of the crop results in increasing biomass production whereas a decrease in greenness results in decreasing biomass production. The positive correlation between NDVI and chlorophyll content is also supported by the study of Caruso *et al.,* (2017).

During the first sampling date (24 DAE) at Syferkuil, the photosynthesis rate was low in all the species. Cowpea measured the highest photosynthesis rate at 44 DAE, then consistently dropped from 66 DAE onwards. Across the four sampling dates, pigeon pea had a higher photosynthesis rate compared to the other species. At Ofcolaco there were no treatment effects in relation to photosynthesis across the whole season. However, sunnhemp showed the tendency of high photosynthesis rate from 21 DAE to 65 DAE, then dropped drastically at 78 DAE to 8.8 mol $m^{-2}s^{-1}$. Suárez (2010) reported similar trends of photosynthesis rate, where the rate of photosynthesis increased steadily towards crop physiological maturity and then decreased sharply when the crop reached its senescence. In principle, photosynthesis is enhanced by an increase in stomatal conductance, which governs water and gas exchange CO_2 (Kusumi *et al,*. 2012).

The transpiration rate at Syferkuil did not differ that much among the species. All the species started with a low transpiration rate from 24 days after planting then reached its peak at 66 DAE in all the species. Overall, pigeon pea had the highest mean transpiration rate compared to the other species. Wang *et al*., (2013) stated that the transpiration rate increased slightly a few days before the anthesis stage and then decreased a few days after reaching that stage. At Ofcolaco the forage legume treatment did not have any significant influence on transpiration rate. The means ranged 0.1 *mol* m^{-2} *s-1* to 5.15 *mol* m^{-2} s^{-1} across all sampling dates of the forage species. The transpiration rate of all forage summer legumes was consistently higher on all the species from 21 DAE until 66 DAE it then dropped at 78 DAE at Ofcolaco. Stomatal conductance was also higher from 21 DAE and 66 DAE and then dropped at 87 DAE. Zhang *et al*., (2020) stated that the higher photosynthesis rate and transpiration are influenced by higher stomatal conductance. Low stomatal conductance results in a low photosynthesis rate by not allowing the flow of CO_2 into the leaf.

Summer forage legume treatment did not affect stomatal conductance at both Syferkuil and Ofcolaco, stomatal conductance ranged from 0.06 *to 5.59 mol* $m\text{-}^{2}$ $s\text{-}^{1}$, and 0.1 to 5.15 *mol* $m\text{-}^{2}$ $s\text{-}^{1}$ at Syferkuil and Ofcolaco respectively, across all sampling dates and species. Halbritter *et al.,* (2020) stated that stomatal conductance functioning influences both transpiration rate and photosynthesis rate. The more the stomatal opens, the more transpiration will occur. This observation is well supported by the findings from our current study, where a higher transpiration rate was influenced by higher stomatal conductance.

The summer forage legumes treatments did not have any effect on sub-stomatal conductance at the two locations namely, Syferkuil and Ofcolaco. At Syferkuil, the mean of stomatal conductance values of the forage species ranged from 129.75 mol $m^{-2}s^{-1}$ to 374 mol $m^{-2}s^{-1}$ across the sampling date and species. As for Ofcolaco, the means ranged from 185 mol $m^{-2}s^{-1}$ to 390.25 mol $m^{-2}s^{-1}$ across all the species. There were behavioural differences in terms of stomatal conductance and sub-stomatal conductance at both locations. At Syferkuil when stomatal conductance was lower, the CO_2 concentration was higher. However, at Ofcolaco when gs was higher, the CO_2 concentration was also higher. The trends at Ofcolaco are supported by Marino *et al*., (2018) where the authors stated that lower values of stomatal conductance corresponded to a parallel decrease in the sub-stomatal CO_2 concentration, which directly affected the photosynthetic rate.

At Syferkuil, no nodules were observed among all the forage species from day 24 after emergence until 87DAE. At Ofcolaco, Cowpea produced significantly higher nodules at 45 DAE accounting for 92.32% higher than the average of sunnhemp, lablab, and pigeon-pea. At this location pigeon pea did not nodulate. No difference in nodule weight was observed at the two sampling dates. Furthermore, no nodules were observed at 65 DAE and this might be because the crops had already reached their mature growth stage. Historically at Syferkuil, the demarcated plot before this experiment was established, herbicides were used throughout the study to control weeds. Application of these herbicides was done before planting and after planting. Raghavendra and Gundappagol, (2017) stated that the application of herbicides can reduce or disturb the development of nodules by affecting sensitive microorganisms which are responsible for soil nutrients. In a similar study Raghavendra and Gundappagol, (2017) found that microbial population decreased with increased treatment of herbicides and increased its population on non-herbicides application.

Minerals are very important in animals' health, growth, and reproduction. Forage minerals are categorized into two groups namely macro minerals and trace elements. Macronutrients are needed in high quantity as compared to trace minerals which are only needed in small quantities. These macronutrients include phosphorus, potassium, calcium, magnesium, and sodium (Lemus, 2013). The four forage legumes treatments did not differ in the nutrient concentration at both Syferkuil and Ofcolaco.

The Na concentration ranged from 0.06 to 0.12% and 0.01 to 0.04% at Syferkuil and Ofcolaco, respectively. According to Dambe *et al*., (2009), adequate ruminants' requirement for Na ranges from 0.19 to 0.82%. Thus, the concentrations of Na are in the forage legume species were lower than the reported range at both Syferkuil and Ofcolaco. The concentration of Na in Stooling ryegrass was also low ranging from 0.03 to 0.04%.

At Syferkuil, Ca concentration among the forage species ranged from 1.16 to 2.01% and at Ofcolaco, it ranged from 1.16 to 1.75%. Stooling ryegrass accumulated the lowest Ca concentration when compared with the concentration accumulated by different summer forage legumes. It is not clear yet about the amount of Ca required by grazing ruminants as it is reported to be influenced by many factors such as animal type, weight, and age, and production level (Khan *et al*. 2006).

The P concentration at Syferkuil ranged from 0.34 to 0.55%, and at Ofcolaco, it ranged between 0.28 to 0.34% among the species. Stooling rye recorded 0.24 to 0.25% range. The recommended amount of phosphorus in a ruminant animal range between 0.09% to 0.3 (Xin *et al*., 2011). Thus, the amount of P in the forage species including stooling ryegrass is adequate at both locations.

The K concentration of the legume species ranged from 1.88 to 3.79% and 1.53 to 2.22% at Syferkuil and Ofcolaco, whereas that of stooling ryegrass was from 1.19 to 2.01%. The recommended level amount of K for grazing animals ranges between 0.19 to 0.82% Dambe *et al*., (2015) indicating K concentration in both the legume and stooling ryegrass were, above the recommended level.

Mg ranged from 0.58 to 1.01%, 0.42 to 0.65 % at Syferkuil and Ofcolaco respectively. Stooling ryegrass Mg ranged from 0.32 to 0.34%. Recommended Mg ranged from 0.12 to 0.20% Dambe *et al*., (2015). Looking at the results from the summer forage legumes together with stooling ryegrass, the forage legumes generally managed to accumulate minimum amounts of minerals recommended by different research.

At Syferkuil, the recorded crude protein concentration ranged from 22.91 to 26.82% and at Ofcolaco, the range was 17.03 and 23.84%. Overall, the legumes planted at Syferkuil had higher crude protein as compared to those from Ofcolaco. Sabetha *et al*.,(2014) when comparing crude protein of cowpea at different planting systems at different N application rates, found that crude protein ranged from 18 to 25% under a mono-cropping system. These results are within the same range as found in our study.

At Syferkuil, cowpea had 24.99% crude protein and Ofcolaco recorded 17.03%. Srisaikham and Lounglawan, (2020), in a study where the effects of sunnhemp (*Crotalaria juncea*) in feed were evaluated, the authors reported a crude protein range of 18.82 to 25.69%. This study is in agreement with our current study where Sunnhemp had crude protein of 26.82 and 21.81% at Syferkuil and Ofcolaco respectively. According to Kabaija *et al*., (2013), the minimum crude protein content in feed required by ruminant is 7.5%. Thus, the crude protein content of the forage legumes evaluated is adequate for ruminants. In terms of stooling ryegrass, the data revealed that not much of the plant residues material was left on the soil by the predecessor crops. The crude protein values range of stooling ryegrass ranged from 10.86 and 11.55%. Rakau *et al*., (2018) reported similar results of 12.37% crude protein of stooling rye, where different winter forages were evaluated. Even though the nutritional value of forage declined with maturity, it was still enough to maintain animal weight (Rakau *et al.*, 2018). This study is also in agreement with Backer *et al.*, (2008), where 10.8% of stooling rye crude protein has been reported.

CHAPTER 6: CONCLUSION AND RECOMMENDATION

The data revealed that the biomass yield of sunnhemp was consistently high at both locations compared with the other forage legumes whereas, that of pigeon pea was low at Syferkuil but high at Ofcolaco. Based on consistent higher accumulation of biomass and relatively satisfactory nutrient profile of sunnhemp at both locations, it makes it a better choice to be recommended for farmers. Although cowpea, lablab, and pigeon pea were lower in biomass production than sunnhemp, these species managed to produce enough biomass for grazing and had similar nutritive value which was above minimum recommendations. Generally, biomass production in the summer forage legumes differed significantly at both locations. Therefore, the hypothesis of no difference in biomass and nutritive value of the summer forage legumes failed to be accepted.

The summer forage legumes influenced photosynthesis and transpiration but not stomatal conductance and substomatal conductance at Syferkuil and Ofcolaco. Thus, the hypothesis of no difference in photosynthesis and transpiration among the forage species is rejected whereas that of stomatal conductance and substomatal conductance is accepted.

At Syferkuil, forage cowpea had superior chlorophyll content as compared to the other summer forage legumes and at Ofcolaco, pigeon pea had superior chlorophyll content compared to the other species. Furthermore, forage Cowpea produced superior nodules as compared to other summer forage legumes. Therefore, the hypothesis of no difference is rejected as there were differences in nitrogen status and symbiotic activities amongst the summer forage legumes.

There were no treatment effects observed on stooling rye biomass yield. However, there was a tendency for higher biomass of the grass species grown after pigeon pea and lablab compared to those following sunnhemp and cowpea. The grass biomass accumulation ranged from 912.6 to 1394.2 kg ha^{-1}. This means forage legume residues were not enough for supporting the biomass accumulation of stooling rye. Even though no treatment effect observed on stooling ry biomass, but the nutritional value was still enough at maturity, to maintain animal weight. Due to the lack of statistical difference in the biomass production of the stooling grass following the forage legumes, we fail to reject the hypothesis that the biomass production and nutritional value of a following winter stooling ryegrass are not affected by the preceding winter forage legumes.

The overall assessment of the species revealed the forage summer legumes species studied managed to produce enough biomass for grazing and had similar nutritive value which was above minimum recommendations. They can therefore be cultivated in the province to meet the constraint of feed gap, though sunnhemp will be a preferred species based on its characteristics outlined above. A continuous experiment on these is required in different locations besides the selected two in our study to understand biomass production, symbiotic activities, leaf gaseous exchange of these four different legumes at different growing stages, and their nutritive value in Limpopo province. Further research must also be done to understand the residue production of these forage legumes left on the soil to understand the benefits of residual nitrogen for subsequent winter grasses.

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LIST OF APPENDICES

Analysis of variance (ANOVA) tables for forage legumes at Syferkuil

Appendix 4.1: 24DAE Biomass

Source	DF	SS	MS	F	P
REP	3	237.69	79.229		
SPECIES	3	987.19	329.063	4.85	0.0282
Error	9	610.06	67.785		
Total	15	1834.94			

Appendix 4.2: 44DAE Biomass

Source	DF	SS	MS	F	P
REP	3	26273	8757.5		
SPECIES	3	78433	26144.3	24.89	0.0001
Error	9	9454	1050.4		
Total	15	114159			

Appendix 4.3: 66DAE Biomass

Source	DF	SS	MS	F	P
REP	3	29305	9768		
SPECIES	3	485612	161871	21.53	0.0002
Error	9	67673	7519		
Total	15	582589			

Appendix 4.4: 87DAE Biomass

Source	DF	SS	MS	F	P
REP	3	861025	287008		
SPECIES	3	4185097	1395032	2.85	0.0976
Error	9	4410662	490074		
Total	15	9456783			

Appendix 4.5: 24DAE Chlorophyll content

Source	DF	SS	MS	F	P
REP	3	81.55	27.182		
SPECIES	3	1384.74	461.579	17.55	0.0004
Error	9	236.77	26.308		
Total	15	1703.06			

Appendix 4.6: 44DAE Chlorophyll content

Source	DF	SS	MS	F	P
REP	3	484.89	161.63		
SPECIES	3	3473.37	1157.79	6.98	0.01
Error	9	1491.82	165.76		
Total	15	5450.08			

Appendix 4.7: 66DAE Chlorophyll content

Source	DF	SS	MS	F	P
REP	3	8.993	2.998		
SPECIES	3	347.078	115.693	12.91	0.0013
Error	9	80.651	8.961		
Total	15	436.723			

Appendix 4.8: 87DAE Chlorophyll content

Source	DF	SS	MS	F	P
REP	3	55.5	18.501		
SPECIES	3	1227.35	409.117	22.94	
Error	9	160.51	17.834		0.0159
Total	15	1443.36			

Appendix 4.9: 24DAE NDVI

Source	DF	SS	MS	F	P
REP	3	0.05857	0.01952		
SPECIES	3	0.08532	0.02844	5.98	0
Error	9	0.04281	0.00476		
Total	15	0.18669			

Appendix 4.10: 44DAE NDVI

Source	DF	SS	MS	F	P
REP	3	0.03022	0.01007		
SPECIES	3	0.39267	0.13089	42.06	0
Error	9	0.02801	0.00311		
Total	15	0.45089			

Appendix 4.11: 66DAE NDVI

Source	DF	SS	MS	F	P
REP	3	0.03677	0.01226		
SPECIES	3	0.29722	0.09907	34.15	0
Error	9	0.02611	0.0029		
Total	15	0.36009			

Appendix 4.12: 87DAE NDVI

Source	DF	SS	MS	F	P
REP	3	0.04072	0.01357		
SPECIES	3	0.22773	0.07591	90.79	0.064
Error	9	0.00753	0.00084		
Total	15	0.27598			

Appendix 4.13: 24DAE Sub-stomatal CO_2

Source	DF	SS	MS	F	P
REP	3	18596.7	6198.9		
SPECIES	3	23408.2	7802.73	3.47	0.3363
Error	9	20224.1	2247.12		
Total	15	62228.9			

Appendix 4.14: 44DAE Sub-stomatal CO_2

Source	DF	SS	MS	F	P
REP	3	20269.7	6756.56		
SPECIES	3	8389.7	2796.56	1.29	
Error	9	19526.6	2169.62		0.1406
Total	15	48185.9			

Appendix 4.15: 66DAE Sub-stomatal CO_2

Source	DF	SS	MS	F	P
REP	3	6877	2292.2		
SPECIES	3	43791	14597.1	2.35	0.0749
Error	9	55928	6214.2		
Total	15	106595			

Appendix 4.16: 87DAE Sub-stomatal CO_2

Source	DF	SS	MS	F	P
REP	3	3149.2	1049.73		
SPECIES	3	7291.2	2430.4	3.23	0.3797
Error	9	6767.1	751.9		
Total	15	17207.4			

Appendix 4.17: 24DAE Transpiration rate

Source	DF	SS	MS	F	P
REP	3	4.569	1.52301		
SPECIES	3	4.4293	1.47644	1.15	0.0192
Error	9	11.5234	1.28038		
Total	15	20.5218			

Appendix 4.18: 44DAE Transpiration rate

Source	DF	SS	MS	F	P
REP	3	17.9831	5.99436		
SPECIES	3	4.0643	1.35476	5.59	0.0087
Error	9	2.1823	0.24248		
Total	15	24.2296			

Appendix 4.19: 66DAE Transpiration rate

Source	DF	SS	MS	F	P
REP	3	29.399	9.7997		
SPECIES	3	195.023	65.0078	7.33	0.0023
Error	9	79.823	8.8692		
Total	15	304.246			

Appendix 4.20: 87DAE Transpiration rate

Source	DF	SS	MS	F	P
REP	3	8.9244	2.97481		
SPECIES	3	29.4545	9.81817	11.02	0.4825
Error	9	8.0153	0.89059		
Total	15	46.3942			

Appendix 4.21: 24DAE Stomatal conductance

Source	DF	SS	MS	F	P
REP	3	0.02835	0.00945		
SPECIES	3	0.03095	0.01032	0.89	0.0889
Error	9	0.1043	0.01159		
Total	15	0.1636			

Appendix 4.22: 44DAE Stomatal conductance

Source	DF	SS	MS	F	P
REP	3	2.8848	0.9616		
SPECIES	3	37.174	12.3913	2.98	0.2441
Error	9	37.4198	4.1578		
Total	15	77.4786			

Appendix 4.23: 66DAE Stomatal conductance

Source	DF	SS	MS	F	P
REP	3	1.62627	0.54209		
SPECIES	3	2.77062	0.92354	1.66	0.0715
Error	9	5.00768	0.55641		
Total	15	9.40457			

Appendix 4.24: 87DAE Stomatal conductance

Source	DF	SS	MS	F	P
REP	3	0.3584	0.11948		
SPECIES	3	6.766	2.25534	3.3	0.3925
Error	9	6.1453	0.68281		
Total	15	13.2698			

Appendix 4.25: 24DAE Photosynthesis rate

Source	DF	SS	MS	F	P
REP	3	44.256	14.7521		
SPECIES	3	57.914	19.3047	1.12	0.0158
Error	9	155.624	17.2915		
Total	15	257.794			

Appendix 4.26: 44DAE Photosynthesis rate

Source	DF	SS	MS	F	P
REP	3	786.07	262.02		
SPECIES	3	3638.54	1212.85	5.98	0
Error	9	1824.14	202.68		
Total	15	6248.74			

Appendix 4.27: 66DAE Photosynthesis rate

Source	DF	SS	MS	F	P
REP	3	321.25	107.08		
SPECIES	3	7099.41	2366.47	37.72	0.0162
Error	9	564.57	62.73		
Total	15	7985.23			

Appendix 4.28: 87DAE Photosynthesis rate

Source	DF	SS	MS	F	P
REP	3	63.68	21.226		
SPECIES	3	1163.47	387.823	5.94	0,0006
Error	9	587.83	65.314		
Total	15	1814.97			

Appendix 29: plant Nitrogen

Source	DF	SS	MS	F	P
Rep	3	0,67457	0,22486		
Species	3	0,79872	0,26624	1,08	0,4056
Error	9	2,21841	0,24649		
Total	15	3,69169			

Appendix 4.30: 87DAE Calcium

Source	DF	SS	MS	F	P
Rep	3	0,25354	0,08451		
Species	3	1,64961	0,54987	15,91	0,0015
Error	9	0,31109	0,03457		
Total	15	2,21424			

Appendix 4.31: 87DAE Magnesium

Source	DF	SS	MS	F	P
Rep	3	0,14525	0,04842		
Species	3	0,45848	0,15283	12,51	0,0100
Error	9	0,10990	0,01221		
Total	15	0,71363			

Appendix 4.32: 87DAE Potassium

Source	DF	SS	MS	F	P
Rep	3	1,0331	0,34438		
Species	3	8,0617	2,68723	7,00	0,0483
Error	9	3,4555	0,38395		
Total	15	12,5503			

Appendix 4.33: 87DAE Sodium

Source	DF	SS	MS	F	P
Rep	3	371516	123839		
Species	3	749065	249688	3,92	0,6936
Error	9	573303	63700		
Total	15	1693884			

Appendix 4.34: 87DAE Zinc

Source	DF	SS	MS	F	P
Rep	3	2510,46	836,819		
Species	3	405,11	135,035	0,50	0,3159
Error	9	2446,97	271,886		
Total	15	5362,54			

Appendix 4.35: 87DAE Copper

Source	DF	SS	MS	F	P
Rep	3	2,1479	0,71597		
Species	3	4,1487	138 289	1,36	0,7228
Error	9	9,1513	1,01681		
Total	15	15,4479			

Appendix 4.36: 87DAE Manganese

Source	DF	SS	MS	F	P
Rep	3	3376,48	1125,49		
Species	3	846,61	282,20	0,45	0,1892
Error	9	5631,01	625,67		
Total	15	9854,10			

Appendix 4.37: 87DAE Iron

Source	DF	SS	MS	F	P
Rep	3	2404560	801520		
Species	3	1623389	541130	1,97	0,0810
Error	9	2472614	274735		
Total	15	6500563			

Appendix 4.38: 87DAE Phosphorus

Source	DF	SS	MS	F	P
Rep	3	0,01088	0,00363		
Species	3	0,09411	0,03137	3,12	0.081
Error	9	0,09059	0,01007		
Total	15	0,19558			

Appendix 4.39: Crude Protein

Source	DF	SS	MS	F	P
Rep	3	26,155	8,7184		
Species	3	31,040	10,3467	1,08	0,4073
Error	9	86,575	9,6194		
Total	15	143,770			

Appendix 4.40: Crude protein yield for forage legumes

Source	DF	SS	MS	F	P
Rep	3	291114	97038		
Species	3	2196440	732147	2,83	0,0989
Error	9	2329218	258802		
Total	15	4816772			

Appendix 4.41: Stooling ryegrass biomass

Source	DF	SS	MS	F	P
REP	3	2906277	968759		
Species	3	511152	170384	3,33	0,0704
Error	9	461046	51227		
Total	15	3878475			

Appendix 4.42: plant ryegrass Nitrogen

Source	DF	SS	MS	F	P
Rep	3	0,07487	0,02496		
Species	3	0,03549	0,01183	0,56	0,6546
Error	9	0,19007	0,02112		
Total	15	0,30043			

Appendix 4.43: Stooling ryegrass Calcium

Source	DF	SS	MS	F	P
Rep	3	0,00292	9,738E-04		
Species	3	0,01974	6,581E-03	8,08	0,0064
Error	9	0,00733	8,142E-04		
Total	15	0,02999			

Appendix 4.44: Stooling ryegrass Magnesium

Source	DF	SS	MS	F	P
Rep	3	0,00158	5,282E-04		
Species	3	0,01513	5,043E-03	14,18	0,0009
Error	9	0,00320	3,557E-04		
Total	15	0,01992			

Appendix 4.45: Stooling ryegrass Potassium

Source	DF	SS	MS	F	P
Rep	3	0,02905	0,00968		
Species	3	0,19790	0,06597	4,17	0,0416
Error	9	0,14249	0,01583		
Total	15	0,36945			

Appendix 4.46: Stooling ryegrass Sodium

Source	DF	SS	MS	F	P
Rep	3	29980	9993,3		
Species	3	37505	12501,8	1,26	0,3438
Error	9	89008	9889,7		
Total	15	156493			

Appendix 4.47: Stooling ryegrass Zinc

Source	DF	SS	MS	F	P
Rep	3	28,055	9,3516		
Species	3	241,544	80,5147	32,83	0,0000
Error	9	22,070	2,4523		
Total	15	291,669			

Appendix 4.48: Stooling ryegrass Copper

Source	DF	SS	MS	F	P
Rep	3	0,50334	0,16778		
Species	3	1,52148	0,50716	5,04	0,0255
Error	9	0,90549	0,10061		
Total	15	2,93031			

Appendix 4.49: Stooling ryegrass Manganese

Source	DF	SS	MS	F	P
Rep	3	396,13	132,044		
Species	3	1201,59	400,530	9,45	0,0038
Error	9	381,34	42,371		
Total	15	1979,06			

Appendix 4.50: Stooling ryegrass Iron

Source	DF	SS	MS	F	P
Rep	3	811810	270603		
Species	3	3335881	1111960	10,45	0,0027
Error	9	958081	106453		
Total	15	5105772			

Appendix 4.51: Stooling ryegrass Phosphorus

Source	DF	SS	MS	F	P
Rep	3	5,432E-04	1,811E-04		
Species	3	1,224E-03	4,081E-04	1,04	0,4209
Error	9	3,534E-03	3,927E-04		
Total	15	5,302E-03			

Appendix 4.52: Stooling ryegrass Crude Protein

Source	DF	SS	MS	F	P
REP	3	2,9244	0,97481		
Treat	3	1,3863	0,46209	0,56	0,6546
Error	9	7,4248	0,82498		
Total	15	11,7355			

Appendix 4.53: Stooling ryegrass Crude Protein Yield

Source	DF	SS	MS	F	P
REP	3	26996,0	8998,67		
Treat	3	4284,0	1428,01	4,17	0,0416
Error	9	3083,2	342,58		
Total	15	34363,2			

Analysis of variance (ANOVA) tables for forage legumes at Ofcolaco

Appendix 4.54: 21DAE Biomass

Source	DF	SS	MS	F	P
REP	3	2590	863		
SPECIES	3	342631	114210	64.35	0
Error	9	15975	1775		
Total	15	361196			

Appendix 4.55: 42DAE Biomass

Source	DF	SS	MS	F	P
REP	3	6774	2258		
SPECIES	3	1263151	421050	21.18	0.0002
Error	9	178936	19882		
Total	15	1448861			

Appendix 4.56: 65DAE Biomass

Source	DF	SS	MS	F	P
REP	3	199599	66533		
SPECIES	3	1671335	557112	17.45	0.0004
Error	9	287301	31922		
Total	15	2158235			

Appendix 4.57: 78DAE Biomass

Source	DF	SS	MS	F	P
REP	3	114732	38244		
SPECIES	3	1623901	541300	3.11	0.0812
Error	9	1564805	173867		
Total	15	3303437			

Appendix 4.58: 21DAE Chlorophyll content

Source	DF	SS	MS	F	P
REP	3	60.53	20.175		
SPECIES	3	805.76	268.588	6.75	0.0111
Error	9	358.04	39.782		
Total	15	1224.33			

Appendix 4.59: 42DAE Chlorophyll content

Source	DF	SS	MS	F	P
REP	3	665.09	221.698		
SPECIES	3	631.37	210.456	2.78	0.1021
Error	9	680.52	75.613		
Total	15	1976.98			

Appendix 4.60: 65DAE Chlorophyll content

Source	DF	SS	MS	F	P
REP	3	138.81	46.271		
SPECIES	3	2801.14	933.713	19.77	0.0003
Error	9	425	47.222		
Total	15	3364.95			

Appendix 4.61: 78DAE Chlorophyll content

Source	DF	SS	MS	F	P
REP	3	115.34	38.448		
SPECIES	3	2255.73	751.909	12.38	0.0015
Error	9	546.72	60.747		
Total	15	2917.79			

Appendix 4.62: 21DAE NDVI

Source	DF	SS	MS	F	P
REP	3	0.04237	0.01412		
SPECIES	3	0.05477	0.01826	5.72	0.018
Error	9	0.02871	0.00319		
Total	15	0.12584			

Appendix 4.63: 42DAE NDVI

Source	DF	SS	MS	F	P
REP	3	0.04445	0.01482		
SPECIES	3	0.0849	0.0283	10.5	0.0027
Error	9	0.02425	0.00269		
Total	15	0.1536			

Appendix 4.64: 65DAE NDVI

Source	DF	SS	MS	F	P
REP	3	0.02762	0.00921		
SPECIES	3	0.15842	0.05281	5.8	0.0173
Error	9	0.08196	0.00911		
Total	15	0.26799			

Appendix 4.65 78DAE NDVI

Source	DF	SS	MS	F	P
REP	3	0.0209	0.00697		
SPECIES	3	0.15625	0.05208	4.67	0.0313
Error	9	0.10045	0.01116		
Total	15	0.2776			

Appendix 4.66: 21DAE Sub-stomatal CO_2

Source	DF	SS	MS	F	P
REP	3	36925	12308.3		
SPECIES	3	18478	6159.3	1.08	0.405
Error	9	51243	5693.6		
Total	15	106645			

Appendix 4.67: 44DAE Sub-stomatal CO_2

Source	DF	SS	MS	F	P
REP	3	18359.2	6119.75		
SPECIES	3	21532.2	7177.42	1.89	0.2026
Error	9	34268.3	3807.58		
Total	15	74159.7			

Appendix 4.68: 65DAE Sub-stomatal CO_2

Source	DF	SS	MS	F	P
REP	3	8178.2	2726.1		
SPECIES	3	45106.2	15035.4	3.57	0.0601
Error	9	37928.1	4214.2		
Total	15	91212.4			

Appendix 4.69: 78DAE Sub-stomatal CO_2

Source	DF	SS	MS	F	P
REP	3	40399.2	13466.4		
SPECIES	3	876.2	292.1	0.05	0.983
Error	9	49852.2	5539.1		
Total	15	91127.7			

Appendix 4.70: 21DAE Transpiration

Source	DF	SS	MS	F	P
REP	3	349.542	116.514		
SPECIES	3	10.167	3.389	0.2	0.8938
Error	9	152.566	16.952		
Total	15	512.275			

Appendix 4.71: 42DAE Transpiration

Source	DF	SS	MS	F	P
REP	3	93.075	31.0248		
SPECIES	3	71.304	23.7681	0.95	0.4564
Error	9	225.006	25.0006		
Total	15	389.385			

Appendix 4.72: 65DAE Transpiration

Source	DF	SS	MS	F	P
REP	3	132.506	44.1686		
SPECIES	3	73.765	24.5883	1.36	0.3154
Error	9	162.489	18.0543		
Total	15	368.759			

Appendix 4.73: 78DAE Transpiration

Source	DF	SS	MS	F	p
REP	3	25.42	8.4733		
SPECIES	3	34.699	11.5662	2.54	0.122
Error	9	41.017	4.5575		
Total	15	101.136			

Appendix 4.74: 21DAE Stomatal conductance

Source	DF	SS	MS	F	P
REP	3	43.786	14.5953		
SPECIES	3	11.16	3.72	0.55	0.6582
Error	9	60.417	6.713		
Total	15	115.363			

Appendix 4.75: 45DAE Stomatal conductance

Source	DF	SS	MS	F	P
REP	3	0.26272	0.08757		
SPECIES	3	0.38032	0.12677	0.46	0.7158
Error	9	2.47046	0.2745		
Total	15	3.11349			

Appendix 4.76: 65DAE Stomatal conductance

Source	DF	SS	MS	F	P
REP	3	11.4196	3.80652		
SPECIES	3	19.3482	6.44939	2.66	0.1119
Error	9	21.8551	2.42835		
Total	15	52.6228			

Appendix 4.77: 78DAE Stomatal conductance

Source	DF	SS	MS	F	P
REP	3	0.01732	0.00577		
SPECIES	3	0.04627	0.01542	2.44	0.1309
Error	9	0.05681	0.00631		
Total	15	0.12039			

Appendix 4.78: 24DAE Photosynthesis rate

Source	DF	SS	MS	F	P
REP	3	1003.95	334.651		
SPECIES	3	612.86	204.285	1.39	0.3072
Error	9	1320.73	146.747		
Total	15	2937.54			

Appendix 4.79: 45DAE Photosynthesis rate

Source	DF	SS	MS	F	P
REP	3	280.33	93.442		
SPECIES	3	859.45	286.484	0.97	0.4478
Error	9	2653.81	294.867		
Total	15	3793.58			

Appendix 4.80: 65DAE Photosynthesis rate

Source	DF	SS	MS	F	P
REP	3	2622.68	874.23		
SPECIES	3	3277.35	1092.45	3.2	0.0767
Error	9	3076.4	341.82		
Total	15	8976.42			

Appendix 4.81: 78DAE Photosynthesis rate

Source	DF	SS	MS	F	P
REP	3	123.164	41.0548		
SPECIES	3	123.975	41.3248	2.22	0.1552
Error	9	167.538	18.6153		
Total	15	414.677			

Appendix 4.82: Forage legumes Nitrogen

Source	DF	SS	MS	F	P
Rep	3	1,76680	0,58893		
Species	3	3,09490	1,03163	4,70	0,0307
Error	9	1,97700	0,21967		
Total	15	6,83870			

Appendix 4.83:Forage legumes Calcium

Source	DF	SS	MS	F	P
Rep	3	1,25039	0,41680		
Species	3	0,69764	0,23255	0,99	0,4409
Error	9	2,11719	0,23524		
Total	15	4,06522			

Appendix 4.84: Forage legumes Magnesium

Source	DF	SS	MS	F	P
Rep	3	0,08544	0,02848		
Species	3	0,14930	0,04977	1,75	0,2260
Error	9	0,25564	0,02840		
Total	15	0,49038			

Appendix 4.85: Forage legumes Potassium

Source	DF	SS	MS	F	P
Rep	3	0,35457	0,11819		
Species	3	0,95143	0,31714	2,41	0,1347
Error	9	1,18666	0,13185		
Total	15	2,49266			

Appendix 4.86: Forage legumes sodium

Source	DF	SS	MS	F	P
Rep	3	47457	15819,1		
Species	3	289498	96499,2	7,21	0,0091
Error	9	120520	13391,1		
Total	15	457475			

Appendix 4.87: Forage legumes Zinc

Source	DF	SS	MS	F	P
Rep	3	56,235	18,745		
Species	3	316,657	105,552	1,89	0,2023
Error	9	503,586	55,954		
Total	15	876,478			

Appendix 4.88: Forage legumes copper

Source	DF	SS	MS	F	P
Rep	3	3,0565	1,01882		
Species	3	4,2388	1,41293	1,24	0,3518
Error	9	10,2681	1,14090		
Total	15	17,5634			

Appendix 4.89: Forage legumes Manganese

Source	DF	SS	MS	F	P
Rep	3	1481,91	493,969		
Species	3	2703,28	901,093	2,04	0,1791
Error	9	3979,80	442,200		
Total	15	8164,99			

Appendix 4.90: Forage legumes Iron

Source	DF	SS	MS	F	P
Rep	3	80228	26742,7		
Species	3	65581	21860,3	1,02	0,4267
Error	9	192055	21339,4		
Total	15	337864			

Appendix 4.91: Forage legumes Phosphorus

Source	DF	SS	MS	F	P
Rep	3	0,02132	7,106E-03		
Species	3	0,00793	2,644E-03	1,18	0,3716
Error	9	0,02022	2,246E-03		
Total	15	0,04946			

Appendix 4.92: Crude protein for forage legumes

Source	DF	SS	MS	F	P
Rep	3	69,016	23,0052		
Species	3	120,895	40,2982	4,70	0,0307
Error	9	77,227	8,5807		
Total	15	267,137			

Appendix 4.91: Crude protein yield for forage legumes

Source	DF	SS	MS	F	P
Rep	3	8537,5	2845,8		
Species	3	69553,6	23184,5	19,32	0,0003
Error	9	10798,6	1199,8		
Total	15	88889,8			