Regional Communication

Establishment yield and nutrient composition of four legumes as influenced by age of growth in a cool tropical climate at Jos, Plateau State, Nigeria

Rendimiento y composición de nutrientes de cuatro leguminosas influenciadas por las etapas de crecimiento en un clima tropical fresco en Jos, estado de Plateau, Nigeria

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Abstract

An experiment was conducted with 2 temperate (Trifolium pratense and T. repens) and 2 tropical (Stylosanthes guianensis and Centrosema molle syn. C. pubescens) forage legumes in an elevated tropical environment of Jos, Nigeria to determine the influence of age of growth on forage yield and nutrient concentrations in the establishment year. The experiment was a 4 harvest times (9, 13, 17 and 21 weeks after sowing; WAS) × 4 legume species (2 temperate and 2 tropical) factorial treatment arrangement in a randomized complete block design with 4 replications, conducted in the growing seasons of 2015 and again 2016. In 2015, S. guianensis produced highest (P<0.05) dry matter yield (8.2 t DM/ha), while T. pratense produced the highest yield (3.6 t DM/ha) in 2016. In both years leaf:stem ratio decreased significantly with age. In 2016 crude protein (CP) concentration declined in all species as age at harvest increased (P < 0.05), while at any given age highest CP concentration occurred in T. repens and lowest in S. guianensis (P<0.05). At any age, concentration of calcium followed the pattern T. pratense>T. repens>C. molle>S. guianensis (P<0.05), while phosphorus concentration in forage declined with age at harvest with significant (P<0.05) differences only for tropical legumes. The detergent fiber concentrations (NDF and ADF) were higher in S. guianensis (P<0.05) at any harvest stage. Non-linear regression analysis suggested that these forage legumes, when planted in early June in this environment, could be harvested at the optimum stages of 15, 16, 18 and 21 WAS for T. pratense, T. repens, S. guianensis and C. molle, respectively. However, more studies, especially with earlier planting dates, need to be conducted on the temperate legumes to determine their full yield potential in this environment, especially over a wider range of seasonal conditions.

Keywords: Centrosema molle, crude protein, mineral composition, stage of maturity, *Stylosanthes guianensis*, *Trifolium pratense*, *Trifolium repens*.

Resumen

Se realizó un experimento con 2 leguminosas forrajeras de clima templado (*Trifolium pratense* y *T. repens*) y 2 tropicales (*Stylosanthes guianensis* y *Centrosema molle* sin. *C. pubescens*) en un ambiente de trópico de altura en Jos, Nigeria, para determinar la influencia de la edad de crecimiento en el rendimiento de forraje y las concentraciones de nutrientes. El experimento consistió en un arreglo de tratamiento factorial de 4 tiempos de cosecha (9, 13, 17 y 21 semanas después de la siembra; WAS) × 4 especies de leguminosas (2 templadas y 2 tropicales) en un diseño de bloques completos al azar con 4 repeticiones. Los cultivos se establecieron a principios de junio de las temporadas de

Correspondence: T.T. Akpensuen, Department of Animal Production, Faculty of Agriculture, University of Jos, Nigeria. Email: <u>theophilusa@unijos.edu.ng</u> crecimiento de 2015 y 2016. En 2015, el *S. guianensis* produjo el rendimiento más alto (P<0.05) de materia seca (8.2 t MS/ha), mientras que *T. pratense* produjo el rendimiento más alto (3.6 t MS/ha) en 2016. En ambos años, la relación hoja:tallo disminuyó significativamente con edad. En 2016, la concentración de proteína bruta (PB) disminuyó en todas las especies a medida que aumentaba la edad de cosecha (P<0.05), mientras que a cualquier edad la concentración de PB más alta se registró en *T. repens* y la más baja en *S. guianensis* (P<0.05). A cualquier edad, la concentración de Ca siguió el patrón *T. pratense>T. repens>C. molle>S. guianensis* (P<0.05), mientras que la concentración de fósforo en el forraje disminuyó con la edad de cosecha y las diferencias fueron significativas (P<0.05) solo para las leguminosas tropicales. Los contenidos de fibra detergente (FDN y FDA) fueron mayores en *S. guianensis* (p<0.05) en cualquier etapa de cosecha. El análisis de regresión no lineal sugirió que estas leguminosas forrajeras sembradas a principios de junio en este ambiente podrían cosecharse en las etapas óptimas de 15, 16, 18 y 21 WAS para *T. pratense, T. repens, S. guianensis* y *C. molle.* respectivamente. Sin embargo, es necesario realizar más estudios con la leguminosas de clima templado, especialmente con fechas de siembra más tempranas, para determinar su potencial de rendimiento completo en este entorno, especialmente en una gama más amplia de condiciones estacionales.

Palabras clave: Centrosema molle, composición mineral, etapa de crecimiento, proteína cruda, Stylosanthes guianensis, Trifolium pratense, Trifolium repens.

Introduction

Legumes play vital roles for sustainable agricultural production. Søegaard et al. (2007) noted that legumes play an important role in grassland management through their ability to contribute to the nitrogen (N) economy of the sward via N fixation by associated rhizobial bacteria. According to Nulik et al. (2013) levels of fixed N can range from less than 50 kg/ha/yr (equivalent to approximately 100 kg urea/ha) to more than 200 kg/ha/yr (>400 kg urea/ha). Legumes can also act as a cover crop to control weeds and conserve soil moisture during dry periods (Kabirizi et al. 2013), as well as control pests and protect soil from erosion, including loss of soil organic matter by water and wind erosion (Rao et al. 2016). Legumes can increase dry matter yields when sown in mixtures with grasses and improve protein concentrations in associated grasses (Zemenchik et al. 2002; Johnson et al. 2007).

The temperate legumes selected for the study, red clover (*Trifolium pratense*) and white clover (*T. repens*), are widely grown for livestock feeding in temperate regions of the world, as well as in subtropical areas of Australia. The crops provide forages high in protein and digestibility, which facilitates high feed intake by livestock (Black et al. 2009). The tropical legumes, Cook stylo (*Stylosanthes guianensis* cultivar 'Cook') and centro (*Centrosema molle* syn. *C. pubescens*), provide similar benefits in subhumid tropical and subtropical zones (Heuzé et al. 2016).

Many factors limit full utilization of tropical forages by livestock. Tropical legumes and grasses are more difficult to physically breakdown by livestock, and their N concentration can be lower than those in C3 grasses and cold-climate legumes (<u>Chapman et al. 2014</u>). Humphreys (<u>1996</u>) stated that tropical forage legumes are about 4 units less digestible than their temperate counterparts and have a lower bulk density of succulent green leaf, so grazing animals have difficulty in taking sufficient bites to provide energy required for high production levels.

Locations like Jos-Plateau, Mambilla and Obudu in Plateau, Taraba and Cross-Rivers States in Nigeria are at elevations of 1,200–1,600 masl, which are suited to the growth of temperate crop varieties. Thus, at Jos-Plateau examples of temperate crops are Irish potato (*Solanum tuberosum*) and strawberry (*Fragaria* × *ananassa*) and Holstein-Friesian cattle are commonly kept. Temperatures in these locations are in the range of 15–25 °C during the rainy season, which is similar to the 15.5–27 °C required for optimum growth of temperate species (<u>Karki 2013</u>).

Clovers have the potential to provide high quality forage and complement the existing tropical forages to improve animal productivity and food security in this environment. Temperate legumes can be grown in association with grasses and/or other tropical legumes for livestock feeding. The crops could also be grown as pure stands for hay making to be used as supplementary feeds during the dry season. Presently, sowing of forage legumes in grassland management is not a common practice in Nigeria, as farmers mostly depend on planted and natural grasses only. Therefore there is a paucity of literature on the production and utilization of forage legumes in Nigerian grassland systems.

Age of growth at harvest is an important factor affecting forage quality, which decreases with advancing maturity (<u>Ball et al. 2001</u>), especially from vegetative to reproductive stage (<u>Karki 2013</u>). As forage crops mature, dry matter yield increases, but digestibility of

neutral detergent fiber (NDF), starch, sugar and crude protein declines (<u>Kilcer et al. 2003</u>). Decisions on when to utilize a forage are based on stage of maturity to obtain a compromise between dry matter yield and quality. This might vary in different environments, so forages should be evaluated in different regions to determine the optimum growth stage that should translate to highest animal production. Optimal time of harvest could also vary with the particular species being used.

The aim of this study was to evaluate the effects of age of growth on dry matter yield and nutrient composition of 2 temperate (*Trifolium pratense* and *T. repens*) and 2 tropical (*Stylosanthes guianensis* and *Centrosema molle*) forage legumes in a cool tropical climate of Jos, Plateau State, Nigeria. The legumes were established in 2015 and again in 2016 as the temperate species did not produce seeds in 2015 to build up a soil seed bank for seedling recruitment in 2016 and also failed to regenerate from the crowns.

Materials and Methods

Location of the study

The experiment was carried out at the Nigerian Institute for Trypanosomiasis Research (NITR), Vom, Jos, Nigeria (9°44'15" N and 8°48'31" E; 1,263 masl) (<u>Google Earth Engine, 2022</u>). Meteorological data for the study area during the 2015 and 2016 cropping seasons were obtained from the National Root Crops Research Institute, Vom, Jos (1.5 km from the experimental site) and are shown in Table 1. The area is characterized by 2 major seasons: the rainy season extending from late May to early October, and the dry season lasting from late October to early May. Peak rainfall normally occurs in the months of July and August. Temperatures range from 15–27 °C during the rainy season, while 11–32 °C is normally observed during the dry season. Grasses found on these highlands are shorter and trees are fewer than at lower elevations (Aregheore 2009). Soil samples (0-15 cm depth) from the experimental area were collected at different random sites with a soil auger for evaluation of physical and chemical properties. The soil was low in N (0.33 %), organic matter (2.91 %) and P (7.53 ppm) and fair in K (247 ppm). The soil was classified as infertile clay-loam, with sticky soil surface when wet, and quickly becoming dry and hard and cracking easily (Aregheore 2009), when there was no rain for 5-7 days.

Land preparation and experimental design

In each year the land was ploughed and harrowed twice using a tractor mounted with appropriate implements. The field was leveled with locally made hoes and all debris was removed to provide clean seedbeds. The trial design was a factorial arrangement of 4 forage legumes (*T. pratense*, *T. repens*, *S. guianensis* and *C. molle*) and 4 ages at harvest (9, 13, 17 and 21 weeks after sowing; WAS) making 16 treatment combinations in a randomized complete block design replicated 4 times. Sixty-four plots of 5×3 m were used for the study with 1 m spacing between blocks and 0.5 m spacing between plots.

Table 1. Monthly mean maximum and minimum temperatures and rainfall for 2015 and 2016 plus medium-term mean rainfall(2004–2014) for Vom, Jos, Nigeria.

Month	Maximum temperature (°C)			Minimum temperature (°C)			Rainfall (mm)		
-	2015	2016	2004–2014	2015	2016	2004–2014	2015	2016	2004–2014
January	29	30	29	12	11	11	0	8	9
February	32	31	30	15	13	15	10	2	2
March	32	34	32	18	19	19	28	19	18
April	32	32	30	17	15	16	10	83	82
May	30	29	28	19	20	18	217	138	145
June	29	28	27	18	17	17	15	173	192
July	27	29	25	20	18	18	225	384	207
August	26	27	25	18	17	17	301	401	290
September	27	25	26	17	16	17	209	181	205
October	29	25	27	17	17	16	60	170	182
November	30	27	29	13	12	13	0	0	0
December	27	30	28	12	10	11	0	3	3

Source: National Root Crop Research Institute, Vom, Jos, Nigeria

Sources of planting materials

Seed of the temperate forage legumes *T. pratense* (AberClaret variety) and *T. repens* (AberHerald variety) was obtained from the Institute of Biological, Environmental and Rural Sciences (IBERS), University of Aberystwyth, United Kingdom, while seed of the tropical forage legumes *S. guianensis* and *C. molle* (cultivar 'Cook' and accession ILRI 152, respectively) was obtained from the Feeds and Nutrition Research Programme of the National Animal Production Research Institute (NAPRI), Ahmadu Bello University, Shika, Zaria, Nigeria. The particular varieties of the temperate legumes were bred for high performance in a temperate environment and not specifically for high performance in a subtropical environment.

Establishment of the forage legumes

Trials were conducted when rains were well established in the month of June during 2015 and 2016 rainy seasons. While only 15 mm was received in June in 2015, 217 mm had been recorded in May. Seeding rates of 15.2 and 6.6 kg/ha for T. pratense and T. repens, and 8.8 and 11.6 kg/ha for S. guianensis and C. molle, respectively, were used. These seeding rates were determined using a Pure Live Seed (PLS) index [(% Germination \times % Purity) \div 10,000)] described by Karki (2013). To overcome hardseededness of the tropical legumes and improve germination and establishment, seeds of S. guianensis were scarified by immersing in hot water at 90 °C for 1 min (Amodu 2004) prior to sowing, while seeds of C. molle were immersed in hot water at 80 °C for 4 min (Crowder and Chedda 1982). Based on previous observations at the experimental site, the two tropical legumes were known to nodulate with native rhizobia and subsequent nodulation was observed on all species, including the two clovers. Therefore, all the seeds were not inoculated. Seeds of T. pratense and T. repens were planted in a continuous flow of seeds at the recommended distance of 0.3 m between rows within a plot (Frater 2013), while those of S. guianensis and C. molle were planted at spacings of 0.3 and 0.5 m between rows, respectively (Crowder and Chedda 1982). The recommended sowing depth of 0.5 cm was used for Trifolium species (Frater 2013), while the depth for S. guianensis and C. molle was 1 and 2.5 cm, respectively (Crowder and Chedda 1982).

Yield measurements

Five (5) plants in the middle row of each plot were tagged in each treatment combination and used to determine morphological variables of plant height, number of leaves and number of branches per plant for the different stages of growth. Plant heights were measured from ground level to the tips of the plants using a graduated rule for *T. pratense* and *S. guianensis*. For *C. molle* and *T. repens*, the main stem was traced and the length of the stem was measured using a graduated meter rule. Numbers of leaves and branches were counted for each of the marked plants. The same 5 plants were used to determine leaf:stem ratio by harvesting and separating leaves from stems at each stage of growth on the appropriate plots. Leaves and stems were weighed immediately in the field after separation, then oven-dried at 65 °C until constant weight was attained. Thereafter, leaf dry weight was divided by stem dry weight to determine leaf:stem ratio. Forage DM yields were determined using 0.25 m² quadrats (1 quadrat/plot due to uniformity of growth within the plot). Plants within quadrats placed in the middle rows of the plots were cut at 5 cm above ground level with a sickle to determine forage yield. Harvested forages were weighed immediately to determine fresh weight, after which they were oven-dried at a temperature of 65 °C until constant weight was achieved to determine forage dry matter yields.

Sample analyses

Dried samples were ground with a Thomas Willey Laboratory Mill using a 1 mm sieve. N concentration was determined by the Kjeldahl method using digestion, distillation and titration, and crude protein (CP) percentage was determined as N $\% \times 6.25$ (AOAC 1990). CP yield was obtained as CP % × DM yield. Neutral and acid detergent fiber (NDF and ADF) concentrations were determined by the Refluxing method of Van Soest et al. (1991) and as specified in AFIA (2011) Forage Laboratory Manual. Approximately 2.0 g ground forage was burned in a box-type muffle furnace at 550 °C for 12 h, and the residue was weighed to determine ash concentration. CP, ash and fiber analyses were carried out at the Biochemistry and Animal Nutrition Laboratories, Departments of Animal Science and Animal Nutrition of Ahmadu Bello University (ABU), Zaria and University of Agriculture, Makurdi, Nigeria,

respectively. Mineral elements were determined using atomic absorption spectrophotometry (AOAC 1990) at National Animal Production Research Institute, Shika, ABU and Department of Science, ABU, Zaria, Nigeria.

Statistical analysis

All data generated were subjected to Analysis of Variance (ANOVA). The general linear model of SAS (2001) statistical software was used for the analyses and means were separated using the Tukey test. Non-linear regression was used to assess relationships between age at harvest, DM yield, number of leaves per plant and plant height to determine optimum stages at which legumes could be harvested to achieve different goals.

$$\begin{split} & Y_{ijkl} = \mu + A_i + B_j + Y_k + A_i \times B_j + e_{ijkl}, \\ \text{where:} \\ & \mu = \text{population mean;} \end{split}$$

A =ith effect of legume species;

 B_{i}^{1} = jth effect of growth stage;

 Y_{L} =kth effect of year;

 $A_i \times B_j$ =interaction between legume species and growth stage; and

e_{iikl}=random error.

Results

Rainfall and temperature during the study

Mean monthly precipitation, plus minimum and maximum temperatures are presented in Table 1 above. Precipitation was higher in 2016 than in 2015, especially in June, when only 15 mm was recorded in 2015, which was well below the medium-term mean of 192 mm. Such a low amount of rainfall could affect seed germination and establishment of the crop in this area. Peak rainfall was recorded in July and August each year, in line with the medium-term means. Temperature during the study in each year ranged between a minimum of 16 °C and a maximum of 29 °C, which was suitable for the growth of all legume species involved.

Effects of species, stage of growth and year on morphological characters and yield

Interaction effects of growth stage and legume species on plant height, number of branches/plant and forage dry matter yield (DMY) in 2015 and 2016 seasons are presented in Table 2. While there were significant interactions between growth stage and species for plant height in 2015, plant height increased with age for all species except *T. repens*, which did not establish effectively, and no records were taken. Tropical legumes were taller/longer (P<0.05) than *T. pratense*. In 2016 all species increased in height with age, while *C. molle* was taller/longer than other legumes (P<0.05).

For number of branches per plant in 2015, all species showed increases with age and *S. guianensis* displayed more branches than other species (P<0.05). In 2016 all species except *T. pratense* displayed more branches with increasing age (P<0.05) and in contrast to 2015, *T. repens* had more branches than other species (P<0.05).

Highest forage dry matter yields in 2015 ranged from 2.0 to 8.2 t DM/ha for the different species with no yield recorded for *T. repens*, which failed to establish effectively, and highest yield for *S. guianensis* (P<0.05). In 2016 highest DM yields ranged from 1.8 to 3.6 t/ha, with few consistent patterns in differences with age and species. Temperate legumes did not manifest their full DM yield potential because the combination of limited rain in October in 2015 and increasing temperature led to wilting and loss of leaves, when fewer than 2 % of plants started flowering, unlike the tropical species. Non-linear regression analysis showed highest estimated DMY at 15, 16, 18 and 21 WAS for *T. pratense*, *T. repens*, *S. guianensis* and *C. molle*, respectively (Figure 1).

Main effects of legume species and stage of growth on leaf number per plant and leaf:stem ratio for 2015 and 2016 cropping seasons are presented in Table 3. In 2015 number of leaves per plant was greater (P<0.01) in *S. guianensis* (85.6) than in other species, with *T. repens* having no record. In contrast, *T. repens* had highest (P<0.01) number of leaves per plant (156.9) in 2016. In both years, leaf numbers were affected by age (P<0.01), increasing until 17 weeks after sowing and then plateauing or declining. Leaf:stem ratio in *C. molle* was greater (P<0.01) than in other legumes in both years but decreased significantly with time since sowing (P<0.01). No data were available for *T. repens* in 2015.

Table 4 shows interaction effects of growth stage and legume species on CP, ash, NDF and ADF concentrations and CP yield for 2016. CP concentration declined in all species as age at harvest increased (P<0.05), while at any given age highest CP concentration occurred in *T. repens* and lowest in *S. guianensis* (P<0.05). In all species CP

Legume	WAS	2015			2016		
		Plant height	Branches/plant	DMY	Plant height	Branches/plant	DMY
T. pratense	9	28.6 ^g	2.7 ^{gh}	1.0 ^{hi}	26.2 ^{ef}	4.0 ^g	1.6 ^{cdef}
	13	35.0 ^{fg}	4.2^{fgh}	1.6 ^g	42.0 ^{de}	5.0^{fg}	2.8 ^{ab}
	17	41.9 ^f	6.3 ^{ef}	2.7 ^d	63.1°	6.5^{efg}	3.6ª
	21	42.1 ^f	6.5 ^{de}	1.6 ^g	64.6°	$7.0^{\rm efg}$	1.9 ^{bcd}
T. repens	9	na	na	na	20.7 ^e	13.2 ^{def}	1.1^{def}
	13	na	na	na	40.7^{def}	24.5 ^b	1.8^{bcde}
	17	na	na	na	62.2 ^{cd}	37.7ª	1.8^{bcde}
	21	na	na	na	62.5°	37.5ª	1.3^{def}
S. guianensis	9	29.7 ^g	5.3 ^{efg}	1.1 ^h	26.0 ^{ef}	2.0 ^g	$0.7^{\rm ef}$
	13	64.4 ^d	22.0 ^b	5.6°	25.3 ^{ef}	9.5^{efg}	1.9 ^{bcd}
	17	99.2 ^b	34.3ª	8.2ª	67.7°	14.7 ^{cde}	1.9 ^{bcd}
	21	101.1 ^b	36.3ª	6.3 ^b	64.7°	22.7 ^{bc}	2.6^{abc}
C. molle	9	52.6°	2.0 ^h	0.4 ^j	70.3°	4.2^{fg}	0.6 ^f
	13	83.0°	4.0^{gh}	0.9^{i}	99.0 ^b	8.2^{efg}	1.4^{def}
	17	102.5 ^b	8.7 ^{cd}	2.0 ^e	148.4ª	17.2 ^{cd}	3.4ª
	21	111.7ª	9.5°	1.8^{f}	148.5ª	19.3 ^{bc}	2.9 ^{ab}
s.e.m.		3.5	1.4	0.1	10.2	4.6	0.6

Table 2. Interaction effects of species and growth stage (WAS) on plant height (cm), number of branches and forage dry matter yield (DMY; t/ha) of four legumes in 2015 and 2016 seasons.

Means with different letters within columns are significantly different (P<0.05). na=data not available.

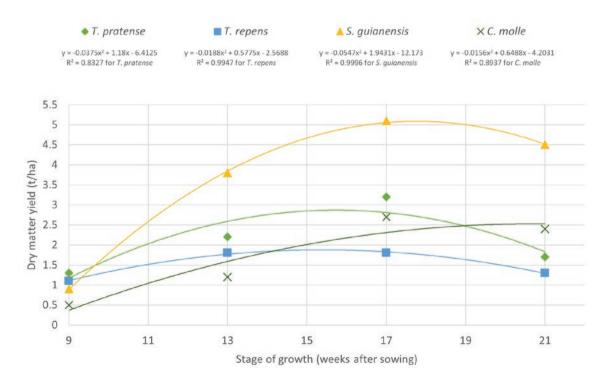


Figure 1. Correlations between dry matter yield and growth stage for 2 temperate and 2 tropical forage legumes. Data for *T. pratense, S. guianensis* and *C. molle* are averages for 2 years (2015 and 2016), while *T. repens* data are for 1 year (2016).

Parameter	Leaf no	o./plant	Leaf:ste	em ratio
	2015	2016	2015	2016
Species				
T. pratense	27.8°	53.4 ^b	0.6 ^b	0.5 ^b
T. repens	na	156.9ª	na	0.4°
S. guianensis	85.6ª	46.8 ^b	0.6 ^b	0.3°
C. molle	31.7 ^b	57.0 ^b	0.8^{a}	0.9^{a}
s.e.m.	1.4	10.5	0.02	0.08
P value	< 0.01	< 0.01	< 0.01	< 0.01
Stage of growth (weeks after sowing)				
9	15.9d	23.7°	1.1ª	1.0ª
13	33.7°	57.5 ^b	0.7 ^b	0.6 ^b
17	71.5ª	115.6ª	0.5°	0.3°
21	65.2 ^b	117.2ª	0.3d	0.2°
s.e.m.	2.2	11.89	0.02	0.06
P value	< 0.01	< 0.01	< 0.01	< 0.01

Table 3. Main effects of species and growth stage on yield components of four forage legumes for 2015 and 2016 rainy season.

Means with different letters within columns and parameters are significantly different (P<0.05). na=data not available.

Table 4. Interaction effects of species and growth stage on crude protein (CP) concentration, crude protein yield (CPY) and ash, acid detergent fiber (ADF) and neutral detergent fiber (NDF) concentrations of four legumes for the year 2016 (g/kg).

Legume	WAS	СР	CPY	Ash	ADF	NDF
T. pratense	9	208.8 ^d	275.0 ^{efgh}	118.0 ^{bc}	234.7 ^{gh}	341.2 ⁱ
	13	198.2°	308.7^{defgh}	113.7 ^{cd}	247.0 ^{fg}	368.3 ^g
	17	188.3^{fgh}	608.7 ^{abc}	100.6^{f}	262.3^{f}	399.0°
	21	180.3^{hi}	453.7 ^{bcde}	96.0^{fg}	282.4°	435.2°
T. repens	9	256.2ª	292.7 ^{efgh}	132.5ª	198.8 ⁱ	278.3 ^k
	13	246.5 ^b	452.9 ^{bcde}	129.9ª	221.1 ^h	308.7 ^j
	17	240.4 ^b	440.4^{bcde}	120.6 ^b	238.9 ^g	348.4^{hi}
	21	222.2°	280.2^{efgh}	111.2 ^{de}	261.4^{f}	377.7 ^{fg}
S. guianensis	9	189.9 ^{efg}	167.5 ^{gh}	97.9 ^f	304.2 ^d	402.8 ^{de}
	13	176.7 ^{ij}	572.5 ^{bc}	80.1 ^{hi}	339.5°	434.5°
	17	162.2 ^{jk}	816.3ª	76.9 ^{ij}	361.2 ^ь	470.5 ^b
	21	143.6 ¹	640.0 ^{ab}	71.2 ^{ij}	391.3ª	494.1ª
C. molle	9	195.1 ^{ef}	106.3 ^h	108.3 ^{bc}	275.4°	361.1 ^{gh}
	13	188.5^{fgh}	219.0 ^{fgh}	96.9^{fg}	301.2 ^d	363.3 ^{gh}
	17	181.9 ^{hi}	494.7 ^{bcde}	91.0 ^g	332.2°	430.9 ^e
	21	169.2 ^{jk}	384.7^{defgh}	84.6 ^h	357.3ь	471.5 ^b
s.e.m.		4.2	113.7	3.1	8.1	9.4

Means with different letters within columns are significantly different (P<0.05).

yield increased with harvesting age to 17 weeks (P < 0.05) and then declined but not significantly so (Table 4). Highest CP yields were recorded for *S. guianensis* and *T. pratense* at 17 WAS, despite having lower CP % at that age than at younger ages.

In all species, ash concentration declined with age at harvest (P<0.05) and at any given age followed the order *T. repens>T. pratense>C. molle> S. guianensis* (P<0.05). NDF and ADF concentrations increased significantly in all species as age at harvest increased (P<0.05) and followed the pattern of *S. guianensis>C. molle>T. pratense>T. repens* (P<0.05). Generally, fiber concentrations were higher in tropical species than in temperate species.

Figure 2 shows a negative linear correlation ($R^2=0.85$) between CP and NDF concentrations.

Interaction effects of growth stage and legume species on macro-mineral concentrations in forage for the year 2016 are presented in Table 5. Calcium concentration in forage changed little with age (P>0.05), except for *S. guianensis*, where significant decline in Ca concentration occurred with age. At any age, concentration of Ca followed the pattern *T. pratense>T. repens>C. molle> S. guianensis* (P<0.05). While phosphorus concentration in forage declined with age at harvest, differences were significant (P<0.05) only for tropical legumes. At any age, there was a trend of highest values for *T. repens* and lowest for *C. molle*. Concentrations of magnesium, potassium and sodium in forage also declined with age at harvest (P<0.05). At any age at harvest, Mg concentration followed the pattern *T. pratense>T. repens>S.* guianensis>*C. molle* (P<0.05), while potassium concentration followed the pattern *T. repens>T. pratense>S.* guianensis>*C. molle* (P<0.05). Sodium concentrations in forage at 9 weeks of age were highest for *T. repens* and lowest for *C. molle* (P<0.05) but differences declined with age at harvest with few significant differences past 13 weeks of age.

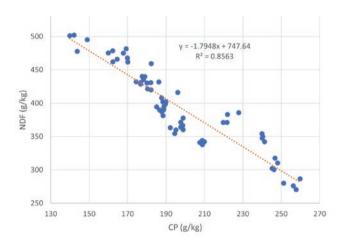


Figure 2. Correlation between neutral detergent fiber and crude protein concentrations for 2 temperate (*T. pratense* and *T. repens*) and 2 tropical (*S. guianensis* and *C. molle*) legumes.

Legume	WAS	Calcium	Phosphorus	Magnesium	Potassium	Sodium
T. pratense	9	18.3ª	3.6 ^{abc}	5.1ª	20.1°	1.6 ^b
	13	18.1ª	3.2^{abc}	4.5 ^b	19.1°	1.0°
	17	17.8 ^{ab}	2.4 ^{cd}	3.9°	18.6 ^f	0.8^{de}
	21	17.7 ^{ab}	2.4 ^{cd}	3.5 ^d	18.0 ^{gh}	0.2^{f}
T. repens	9	17.2 ^{bc}	4.7ª	3.8°	25.2ª	2.6ª
	13	16.6 ^{cd}	4.1 ^{ab}	3.2^{fg}	24.4 ^b	2.1 ^{ab}
	17	16.3 ^d	3.7 ^{abc}	2.6 ⁱ	24.1 ^b	1.8 ^b
	21	16.2 ^d	3.4^{abc}	3.3^{def}	23.9°	0.8^{de}
S. guianensis	9	12.4 ^{gh}	4.1 ^{ab}	3.4 ^{de}	19.5 ^d	0.7^{def}
	13	11.8 ^h	3.1 ^{bc}	3.0 ^{gh}	18.2 ^g	0.5^{def}
	17	10.1 ⁱ	2.7^{bcd}	2.1 ^j	17.7 ^h	0.4^{ef}
	21	9.8 ⁱ	2.3 ^{cd}	1.5 ^k	17.0 ⁱ	0.2^{f}
C. molle	9	14.8°	3.3 ^{abc}	3.1 ^{gh}	15.4 ^j	0.3 ^{ef}
	13	14.8 ^e	2.3 ^{cd}	2.5 ⁱ	14.7 ^k	0.2^{f}
	17	14.1 ^{ef}	1.5 ^d	2.1 ^j	14.2 ¹	0.4^{ef}
	21	13.6 ^f	1.2 ^d	1.0^{1}	13.2m	0.2^{f}
s.e.m.		0.4	0.8	0.1	0.2	0.3

Table 5. Interaction effects of species and growth stage on macro-mineral concentrations of four legumes for 2016 (g/kg).

Means with different letters within columns are significantly different (P<0.05).

Discussion

Morphological characters and DM yield

This study has provided information which should prove useful in assessing the likely performance of temperate and tropical legumes in elevated areas of Nigeria. Morphological characteristics provide information on traits influencing crop improvement and productivity. While climatic conditions during the study were quite similar to the medium-term means for the area, except for very low rainfall in June 2015, the performance of the different species varied markedly between years, especially in the case of T. repens and S. guianensis. Our data from the first year of the study suggested that T. repens was particularly susceptible to low rainfall in the month of June when planted, which led to poor plant establishment. Only few isolated stands of plants of T. repens survived in 2015, and most of them were not in the sampling area, resulting in no record of morphological characters and DM yields. This could be attributed to low rainfall (15 mm) in the month of June 2015 during plant establishment. Nichols et al. (2014) and Brock and Hay (1993) noted that, once soil moisture falls towards wilting point in hot weather, leaves of T. repens wither, die and disappear quickly, leaving stolons and soil surface exposed to direct radiation. Rainfall is one of the major environmental factors affecting performance of T. repens (Brock et al. 2003) and crop survival is reduced with less than 35 mm rain and temperatures above 20 °C (Rattray 2005). This was in marked contrast to results in the second year when rainfall during June was similar to the mean figure and yields were quite significant (1.1-1.8 t DM/ha). Dry matter yields of S. guianensis on the other hand were much higher in 2015 than in 2016 (5.3 vs. 1.8 t DM/ha) as a result of infestations of Botrytis cinerea and Colletotrichum gloeosporioides in 2016, which caused head blight and anthracnose diseases, respectively, at the peak of the rainy season (months of July and August) in 2016. Yields of T. pratense (1.7 vs. 2.5 t DM/ha) and C. molle (1.3 vs. 2.1 t DM/ha) were lower in 2015 than in 2016, responding to better rainfall during establishment in 2016.

The higher number of leaves and branches in *S. guianensis* in 2015 were indicative of the plant's ability to accumulate DM yield. However, the morphological components of temperate species in 2016 resulted in promising DM yields in this cool tropical environment. As reported by Ramírez de la Ribera et al. (2008) leaf:stem ratio declined as plants aged, reflecting the

increase in stem weight as plants grow. The higher DMY of the tropical forages in 2015 compared with temperate legumes agrees with Buxton (1996) that tropical forages can accumulate more dry matter than temperate species, especially if heat and moisture stress occur. This may be due to a more efficient process of photosynthesis by tropical species to accumulate DM (Crowder and Chedda 1982; Humphreys 1999). However, DM yields of the temperate legumes in 2016 offer the prospect that the crops can be grown successfully in the cool tropical climate of Jos, as these legumes produced acceptable vields outside the environment to which they are known to be well adapted. The dry matter yields obtained in this study in 2015 for the legumes at 9 WAS were similar to the findings of Akpensuen (2022) at the primary harvest at 9 WAS in the same location in another experiment. The non-linear regression (Figure 1) clearly demonstrated that the tropical species could withstand moisture stress combined with high temperatures better than the temperate species, especially C. molle, which is known to remain relatively green for 2-3 months in the early dry season. This could be due to the deep tap root system and the better adaptation of tropical legumes to these conditions. Improved forage yields displayed by temperate species in 2016 cropping season could be attributed to better plant establishment as a result of a well-distributed rainfall pattern in the establishment month of June (173 mm) compared with 15 mm in the same month of 2015, followed by above-average rainfall in July and August.

Nutrient composition

As was to be expected (Marković et al. 2008), all legume species showed a decline in nutritive value as stage of growth at harvest increased, as reflected in declining CP concentration and increasing fiber concentration. The negative correlation between CP and NDF concentrations corroborated the statement of Matias et al. (2016), that a negative correlation between CP and fiber concentrations exists in forage species. Reduction in CP concentration with age of forage would also be due to increase in proportion of stem and decrease in proportion of leaves (declining leaf:stem ratio) as stems contain lower amounts of CP than leaves (Humphreys 1999; Solati et al. 2017). Lower fiber and higher CP concentrations in T. repens forage compared with those of the other legumes highlighted the value of this species as forage for livestock in agreement with the findings of Woodfield and Caradus (1996) and INRA (2010), as the crop lacks

structural components such as stems and sheaths. The higher CP concentrations in temperate legumes than in tropical legumes was in agreement with the findings of Archimède et al. (2011) and Lowe et al. (2017). CP concentrations in forage from T. repens, T. pratense and C. molle as a sole diet would meet the CP requirements of 15-17 % and 18-19 % for growth and reproduction, respectively, in rabbits (Lebas 2004), while that of S. guianensis would meet requirements for growth only. Similarly, these legumes would meet the CP requirements of 7-16 % required by small ruminants for growth and all productive/physiological functions (Rashid 2008), as well as the 7–14 % CP generally required by beef cows and 10.5-14 % CP for replacement heifers and steers in the tropics (NRC 2000). Temperate legumes could also meet the 17-18.5 % CP required by lactating dairy cows (NRC 2001), while animals consuming the tropical legumes would need to be supplemented with cereal and legume seed concentrates. All legumes would be most useful for feeding as a supplement to low quality roughage for ruminants. Van Soest (1994) stated that NDF concentration in forages is the most important factor limiting intake, and concentrations in excess of 60 % could reduce forage consumption. In this study NDF concentrations in the legumes were well below 60 %, indicating that at the stages when harvested these legumes are valuable forage resources for improving livestock production.

The general decrease in mineral composition of the forage legumes from 9 to 21 weeks after sowing agreed with findings of Kellems and Church (2002), that concentrations of macro-minerals and most trace minerals decline as forage matures. All forages can meet Ca (0.3-0.8 %) and Mg (0.18-0.4 %) concentrations required for growth and all productive/physiological functions of small ruminants (Rashid 2008). The legumes can also supply 0.53-0.67 % Ca, 0.22-0.44 % P, 0.18-0.21 % Mg and 0.11 % K required for lactating cows (NRC 2001).

Conclusions

This study has shown that both tropical and temperate forage legumes can be grown satisfactorily in elevated regions of Nigeria when planted in June, but limited rainfall during the establishment phase could have a marked impact on obtaining acceptable establishment and growth, especially in the case of *T. repens*. With the onset of the dry season in October, temperate legumes can suffer moisture stress resulting in leaf fall and a decline in both quantity and quality of harvestable forage. However, while rainfall during May is traditionally adequate to ensure satisfactory establishment of forages, it is suggested that investigating earlier planting, especially of temperate species, is warranted to take advantage of earlier rainfall and avoid moisture stress as plants mature. If planting in June, our results suggest harvesting should be at the optimum stages of 15, 16, 18 and 21 weeks of age for *T. pratense*, *T. repens*, *S. guianensis* and *C. molle*, respectively, to minimize leaf fall and achieve a balance between quantity and quality of forage DM harvested. Similar studies should be conducted over a wider range of seasonal conditions than examined in this study.

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References

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- AFIA (Australian Fodder Industry Association). 2011. AFIA -Laboratory Methods manual. Publication No. 03/001. New Generation Print and Copy, Melbourne, VIC, Australia. <u>bit.ly/3H1ANQm</u>
- Akpensuen TT. 2022. Defoliation frequencies of forage legumes: Effects on yield and nutritive value for beef cattle production. Sumerianz Journal of Agriculture and Veterinary 5(1):6–13. doi: <u>10.47752/sjav.51.14.19</u>
- Amodu JT. 2004. Stylosanthes: A promising legume for Africa. In: Chakraborty S, ed. 2004. High-yielding anthracnose-resistant *Stylosanthes* for agricultural systems. ACIAR Monograph No. 111. Australian Centre for International Agricultural Research (ACIAR), Canberra, ACT, Australia. p. 225–234. <u>bit.ly/3wkzAyJ</u>
- AOAC. 1990. Official Methods of Analysis. 15th Edn. Association of Official Analytical Chemists, Washington DC, USA. p. 200–210.
- Archimède H; Eugène M; Marie-Magdeleine C; Boval M; Martin C; Morgavi DP; Lecomte P; Doreau M. 2011. Comparison of methane production between C3 and C4 grasses and legumes. Animal Feed Science and Technology 166–167:59–64. doi: <u>10.1016/j.anifeedsci.2011.04.003</u>
- Aregheore EM. 2009. Country pasture/forage resource profiles: Nigeria. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.
- Ball DM; Collins M; Lacefield GD; Martin NP; Mertens DA; Olson KE; Putnam DH; Undersander DJ; Wolf MW. 2001. Understanding Forage Quality. American Farm Bureau

Federation Publication 1-01, Park Ridge, IL. bit.ly/3J9ZPzs

- Black AD; Laidlaw AS; Moot DJ; O'Kiely P. 2009. Comparative growth and management of white and red clovers. Irish Journal of Agricultural and Food Research 48:149–166. <u>hdl.handle.net/11019/644</u>
- Brock JL; Hay MJM. 1993. An ecological approach to forage management. In: Proceedings of the XVII International Grassland Congress, Palmerston North, New Zealand. p. 837–842.
- Brock JL; Hyslop MG; Widdup KH. 2003. A review of red and white clovers in the dryland environment. In: Moot DJ, ed. Legumes for dryland pastures. Grassland Research and Practice Series No. 11. New Zealand Grassland Association, Wellington, New Zealand. p. 101–107.
- Buxton DR. 1996. Quality-related characteristics of forages as influenced by plant environment and agronomic factors. Animal Feed Science and Technology 59:37–49. doi: 10.1016/0377-8401(95)00885-3
- Chapman DJA; Lee B; Waghorn GC. 2014. Interaction between plant physiology and pasture feeding value: A review. Crop and Pasture Science 65(8):721–734. doi: 10.1071/CP13379
- Crowder LV; Chedda HR. 1982. Tropical grassland husbandry. Wiley, Hoboken, NJ, USA.
- Frater P. 2013. Managing clover for better returns. Beef and sheep BRP manual 4. EBLEX Better Returns Programme, Huntingdon, Cambridgeshire, UK. <u>bit.ly/3XS8WZD</u>
- Google Earth Engine. 2022. Google earth Engine imagery and data. <u>bit.ly/3Ht9gsv.</u> Accessed 02 September 2022
- Heuzé V; Tran G; Bastianelli D; Boudon A; Lebas F. 2016. Stylo (*Stylosanthes guianensis*). Feedipedia.org. A programme by INRA, CIRAD, AFZ and FAO. <u>feedipedia.org</u>
- Humphreys LR. 1999. Tropical pastures and fodder crops. Longman Group, London, UK.
- INRA. 2010. Alimentation des bovins, ovins et caprins. Besoins des animaux - valeurs des aliments. Tables Inra 2007. Éditions Quae, Versailles, France. <u>bit.ly/3H0mCes</u>
- Johnson KD; Rhykerd CL; Hertel JM; Hendrix KS. 2007. Improving pastures by renovation. Purdue University Extension, West Lafayette, IN, USA. <u>bit.ly/3iWAluL</u>
- Kabirizi JM; Ziiwa E; Mugerwa S; Ndikumana J; Nanyennya W. 2013. Dry season forages for improving dairy production in smallholder systems in Uganda. Tropical Grasslands-Forrajes Tropicales 1:212–214. doi: <u>10.17138/tgft(1)212-214</u>
- Karki U. 2013. Forage definition and classification. In: Karki U, ed. Sustainable year-round forage production and grazing/browsing management for goats in the Southern Region. Handbook for Training Field Extension and Technical Assistance Personnel. Tuskegee University Extension Programme, Alabama, USA. p. 6–12. <u>bit.</u> ly/3DcQoLL
- Kellems OR; Church DC. 2002. Roughages. In: Kellems OR; Church DC, eds. Livestock feeds and feeding. Pearson, Upper Saddle River, NJ, USA. p. 145–159.

- Kilcer TF; Ketterings QM; Cerosaletti P; Barney P; Cherney JP. 2003. Cutting management for brown mid-rib sorghum × sudangrass. What's cropping up? 13:4–6. <u>bit.</u> <u>ly/3wolsC3</u>
- Lebas F. 2004. Reflections on rabbit nutrition with a special emphasis on feed ingredients utilization. Invited Paper. Proceedings of the 8th World Rabbit Congress, Puebla, Mexico, 7–10 September 2004. p. 686–736. <u>bit.</u> <u>ly/3XWeqTl</u>
- Lowe KF; Hume DE; Fulkerson WJ. 2017. Forages and Pastures: Perennial Forage and Pasture Crops – Species and Varieties. Encyclopedia of Dairy Sciences. p. 576– 585. doi: 10.1016/B978-0-12-374407-4.00195-3
- Marković J; Vasić T; Terzić D; Đokić D; Štrbanović R. 2008.
 Cell wall composition of red clover (*Trifolium pratense* L.) stems and leaves differing in maturity. Proceedings of 13th International Conference on forage conservation, Nitra, Slovakia, 3–5 September 2008. p. 70–71.
- Matias FI; Barrios SCL; Valle CB do; Mateus RG; Martins LB; Moro GV. 2016. Estimate of genetic parameters in *Brachiaria decumbens* hybrids. Crop Breeding and Applied Biotechnology 16:115–122. doi: 10.1590/1984-70332016v16n2a18
- Nichols SN; Hofmann RW; Williams WM. 2014. Drought resistance of *Trifolium repens* × *Trifolium uniflorum* interspecific hybrids. Crop and Pasture Science 65:911– 921. doi: 10.1071/CP14067
- NRC (National Research Council). 2000. Nutrient Requirements of Beef Cattle. National Research Council, Seventh Revised Edn. doi: 10.17226/9791
- NRC (National Research Council). 2001. Nutrient requirements of dairy cattle. Seventh Revised Edn, 2001. The National Academies Press, Washington, DC, USA. doi: <u>10.17226/9825</u>
- Nulik J; Dalgliesh N; Cox K; Gabb S. 2013. Integrating herbaceous legumes into crop and livestock systems in eastern Indonesia. ACIAR Monograph No. 154. Australian Centre for International Agricultural Research, Canberra, ACT, Australia. <u>bit.ly/3XP4oTS</u>
- Ramírez de la Ribera JL; Kijora C; Acosta IL; Cisneros López M; Tamayo Soza W. 2008. Effect of age and growing season on DM yield and leaf to stem ratio of different grass species and varieties growing in Cuba. Livestock Research for Rural Development 20(9):148. <u>bit.ly/400FYsw</u>
- Rao I; Peters M; Castro A; Schultze-Kraft R; White D; Fisher M; Miles J; Lascano C; Blümmel M; Bungenstab D; Tapasco J; Hyman G; Bolliger A; Paul B; Van Der Hoek R; Maass B; Tiemann T; Cuchillo M; Douxchamps S; Villanueva C; Rincón Á; Ayarza M; Rosenstock NT; Subbarao G; Arango J; Cardoso J; Worthington M; Chirinda N; Notenbaert A; Jenet A; Schmidt A; Vivas N; Lefroy R; Fahrney K; Guimarães E; Tohme J; Cook S; Herrero M; Chacón M; Searchinger T; Rudel T. 2016. LivestockPlus – The sustainable intensification of forage-based agricultural systems to improve livelihoods and ecosystem services

in the tropics. Tropical Grasslands-Forrajes Tropicales 3(2):59-82. doi: <u>10.17138/TGFT(3)59-82</u>

- Rashid M. 2008. Goats and their nutrition. Manitoba Goat Association, Winnipeg, Manitoba, Canada. <u>bit.</u> <u>ly/3XAPLDO</u>
- Rattray PV. 2005. Clover Management, Research, Development and Extension in the New Zealand Pastoral Industries. Ministry for Primary Industries, Wellington, New Zealand. <u>bit.ly/3Jf951N</u>
- SAS. 2001. Statistical Analysis System, Users Guide. 7th Edn. SAS, North Carolina, USA.
- Søegaard K; Gierus M; Hopkins A; Halling M. 2007. Temporary grassland – challenges in the future. In: Vligher A; Carlier L, eds. Proceedings of the 14th Symposium of the European Grassland Federation, Ghent, Belgium, 3–5 September 2007. p. 28–38.
- Solati Z; Jørgensen U; Eriksen J; Søegaard K. 2016. Dry matter yield, chemical composition and estimated

extractable protein of legume and grass species during the spring growth. Journal of Science, Food and Agriculture 97:3958–3966. doi: <u>10.1002/jsfa.8258</u>

- Van Soest PJ. 1994. Nutritional ecology of the ruminant. 2nd Edn. Cornell University Press, Ithaca, NY, USA.
- Van Soest PJ; Robert JB; Lewis BA. 1991. Method for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74:3583–3597. doi: <u>10.3168/jds.S0022-0302(91)78551-2</u>
- Woodfield DR; Caradus JR. 1996. Factors affecting white clover persistence in New Zealand pastures. Proceedings of the New Zealand Grassland Association 58:229–235. doi: 10.33584/jnzg.1996.58.2196
- Zemenchik RA; Albrecht KA; Shaver RD. 2002. Improved nutritive value of kura clover- and birdsfoot trefoil-grass mixtures compared with grass monocultures. Agronomy Journal 94:1131–1138. doi: <u>10.2134/agronj2002.1131</u>

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