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Peter Kerridge
(1935–2022)



Richard (Dick) Date
(1934-2022)

This issue of the journal is dedicated jointly to the memory of Peter Kerridge (1935-2022) and Richard (Dick) Date (1934-2022), both distinguished Australian scientists who made significant contributions to global research on forage productivity through studies on forages and soils. Peter worked on inorganic fertilizers and plant nutrient status, while Dick worked on the biotic component of rhizobial diversity and ecology of forages in tropical soils.

Both worked with CSIRO in Brisbane during their long careers and their contributions to tropical pasture research were recognized through the award of fellowships by the Tropical Grassland Society of Australia. They also contributed to global forage research through their associations with national and international institutions outside Australia and both have many research publications to their credit. Their friends and colleagues in the tropical forages community will miss and remember them as dedicated, rigorous and hardworking scientists.

Este fascículo está dedicado conjuntamente a la memoria de Peter Kerridge (1935-2022) y Richard (Dick) Date (1934-2022), ambos destacados científicos australianos que hicieron contribuciones significativas a la investigación global sobre la productividad de forrajes a través de estudios en forrajes y suelos Peter trabajó con fertilizantes inorgánicos y el estado nutricional de las plantas, mientras que Dick trabajó en el componente biótico de la diversidad de rizobios y la ecología de forrajes en suelos tropicales.

Ambos trabajaron con CSIRO en Brisbane durante sus largas carreras y sus contribuciones a la investigación de pastos tropicales fueron reconocidas mediante la concesión de becas por parte de la Tropical Grassland Society of Australia. También contribuyeron a la investigación global de forrajes a través de sus asociaciones con instituciones nacionales e internacionales fuera de Australia y ambos tienen muchas publicaciones científicas en su haber. Sus amigos y colegas en la comunidad de forrajes tropicales los extrañarán y los recordarán como científicos dedicados, rigurosos y trabajadores.

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Artículo científico

Dinámica de tallos e índice de estabilidad en cinco cultivares de *Urochloa* en condiciones semiáridas

Stem dynamics and stability index in five Urochloa cultivars in semi-arid conditions

FERNANDO LUCIO RUIZ¹, SANTIAGO JOAQUÍN CANCINO¹, JONATHAN RAÚL GARAY MARTÍNEZ², YURIDIA BAUTISTA MARTÍNEZ³, BENIGNO ESTRADA DROUILLET¹ AND ANDRÉS GILBERTO LIMAS MARTÍNEZ¹

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Resumen

El objetivo de esta investigación fue evaluar los efectos de la temperatura y precipitación sobre la dinámica de tallos y el índice de estabilidad en cinco cultivares del género *Urochloa* en condiciones semiáridas. Los cultivares evaluados fueron Camello I, Camello II, Mulato II, Convert 330 y Cobra, en un diseño completamente al azar con seis repeticiones. La temperatura y precipitación presentes durante el periodo de evaluación estimularon el comportamiento de la dinámica de tallos ($P < 0.05$). Los cultivares Cobra y Mulato II presentaron las mayores densidades de tallos ($> 4,000$ tallos/m² en promedio), como respuesta al mayor número de hijuelos (769 y 718/m², respectivamente), aún con una mortalidad de tallos relativamente alta (336 y 449 tallos/m², respectivamente). El tener los valores máximos de unidades calor acumulados en periodos con poca o nula precipitación, comprometió el índice de estabilidad de la pradera (con valores menores a 1). El corte en el periodo de heladas (noviembre) provocó el incremento del 20 % en la tasa de mortalidad y, por consiguiente, una disminución en el índice de estabilidad de hasta 0.83. Las condiciones ambientales y el crecimiento ininterrumpido en los cultivares de *Urochloa*, provoca la pérdida en la estabilidad de la pradera, debido a la menor tasa de ahijamiento con respecto a la tasa de mortalidad de tallos.

Palabras claves: Condiciones ambientales, forraje, sobrevivencia, unidades calor.

Abstract

The objective of this research was to evaluate the effects of temperature and rainfall on stem dynamics and the stability index in 5 cultivars of the genus *Urochloa* under semi-arid conditions. The cultivars evaluated were Camello I, Camello II, Mulato II, Convert 330 and Cobra, in a completely randomized design with 6 replicates. The temperature and precipitation during the evaluation period stimulated stem dynamics ($P < 0.05$). Cobra and Mulato II cultivars presented densities higher than 4,000 stems/m² on average, as a response to the higher number of tillers (769 and 718/m², respectively) even with a relatively high stem mortality (336 and 449 stems/m², respectively). Having the maximum values of accumulated heat units during periods of little or no precipitation compromised the sward stability index (values < 1.0). Cuts during the frost period (November) caused a 20 % increase in the mortality rate and, consequently, a

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decrease in the stability index of up to 0.83. The prevalent environmental conditions along with the continuous growth resulted in losses of stability of *Urochloa* cultivars, due to a lower tillering rate with respect to stems' mortality rate.

Keywords: Environmental conditions, forage, heat units, plant survival.

Introducción

Las praderas de pastos perennes representan la fuente más económica para la alimentación de los rumiantes, ya que los animales cosechan su propio alimento y evitan gastos de procesamiento y transporte; además, disminuye los costos de sembrar nuevamente la pradera al tener la capacidad de regenerarse ([Merchant-Fuentes y Solano-Vergara 2016](#)). Esta característica de regeneración se da mediante la capacidad de producir nuevos tallos para sustituir los tallos muertos y así mantener estable la densidad poblacional e incrementar la permanencia de la pradera con los mismos índices de producción ([Ramírez-Reynoso et al. 2011](#)); en este sentido, el tallo es considerado como la unidad principal de crecimiento de los pastos ([Castro et al. 2013](#)). Para lograr esta estabilidad, es importante considerar factores tales como el manejo ([Hernández-Garay et al. 2012](#)) y las condiciones ambientales en las cuales se desarrolla la pradera ([Rueda et al. 2018](#)). Entre estas últimas se encuentran la temperatura y la precipitación; así, cuando se presentan temperaturas cercanas al óptimo se favorece el crecimiento de las plantas, mientras que, temperaturas por debajo del umbral mínimo limitan el crecimiento y temperaturas por encima del rango óptimo pueden ser perjudiciales, aún más si no se tiene humedad suficiente en el suelo ([Orozco et al. 2012](#)).

Por otra parte, las especies del género *Urochloa* han tenido aceptación por parte de los ganaderos, principalmente, debido a su capacidad de adaptarse a diversas condiciones edafoclimáticas, como suelos ácidos y fertilidad baja e irregularidades en la distribución de las lluvias, además, varias de ellas presentan menor susceptibilidad al ataque de nemátodos y al salivazo (Homoptera: Cercopidae) ([Villalobos-Villalobos y Montiel-Longhi 2015](#)). En cuanto a las temperaturas, se ha determinado que en especies de pastos tropicales como las del género *Urochloa*, la temperatura base por encima de la cual no presentan problemas de crecimiento es de 15 °C ([Moreno et al. 2014](#)).

Antes de exponer especies forrajeras a diferentes condiciones de manejo práctico, es recomendable conocer cómo es la dinámica de los tallos a través del tiempo,

para incrementar la eficiencia de utilización y asegurar la persistencia de estas en respuesta a condiciones ambientales tales como temperatura y precipitación cambiantes. Por ello, el objetivo de este estudio fue evaluar el efecto de las variaciones estacionales en temperatura y precipitación sobre la dinámica de tallos y el índice de estabilidad en cinco cultivares del género *Urochloa* manejadas en condiciones semiáridas.

Materiales y Métodos

El estudio se realizó de febrero a diciembre de 2018 en Güémez, Tamaulipas, México (23°56' 17.55" N, 99°06' 2.45" O), a 167 msnm. El clima del lugar es de tipo BS1 (h')hw ([Vargas et al. 2007](#)). El suelo es de textura arcillosa (11.3; 23.3 y 65.4 % de arena, limo y arcilla, respectivamente), con pH de 8.3, la relación de adsorción de sodio es de 0.19, materia orgánica de 4.2 %, 0.25 % de N y 7.4; 288.6; 1.4 y 0.46 mg/kg de P, K, Fe y Zn, respectivamente ([Garay-Martínez et al. 2018](#)).

Se evaluaron cinco cultivares híbridos del género *Urochloa*, Camello I (GP3207-Papalotla), Camello II (GP3025-Papalotla), Mulato II (CIAT 36087), Convert 330 (Dow Agrosiences) y Cobra (CIAT BR02/1794), que contaban con 18 meses de establecidos, sembrados en hileras con separación de 30 cm, con una densidad de 5 kg/ha⁻¹ de semilla gámica con 90 % de germinación. Los cultivares estaban distribuidos aleatoriamente en parcelas de 9 m² (3 × 3 m). Para iniciar y evaluar lo que ocurre con la dinámica de tallos posterior al corte, se realizó un corte de uniformidad el 24 febrero del 2018 con una intensidad de 10 cm sobre el suelo y en las parcelas se colocó de manera aleatoria cuatro cuadros fijos de alambre de 100 cm² (10 × 10 cm) ([Rojas-García et al. 2017](#)). Posteriormente, en el mes de septiembre se realizó nuevamente un corte de uniformidad. Estos cortes se efectuaron para ver en qué medida estos podrían incidir en cambios morfológicos en las plantas que estaban sufriendo efectos de competencia por luz como producto de la acumulación de biomasa aérea.

Dentro de cada cuadro (100 cm²), se marcaron los tallos vivos presentes con anillos de alambre del mismo color; luego, cada cuatro semanas, los hijuelos se marcaron

con un nuevo color y se eliminaron los tallos muertos, llevando registro de los conteos en cada muestreo. Estos datos se utilizaron para calcular los cambios en la densidad poblacional de tallos (DPTa), hijuelos nuevos y tallos muertos por metro cuadrado, considerando el porcentaje de cobertura de la pradera. Además, se calcularon la tasa de ahijamiento (TA) y la tasa de mortalidad de tallos (TM) de la siguiente manera; donde:

DPTa= Número total de tallos vivos existentes en cada muestreo

TA= (Número de hijuelos / DPT del muestreo anterior) * 100

TM= (Número de tallos muertos / DPTa del muestreo anterior) * 100

La tasa de supervivencia de tallos (TSTa) se obtuvo de manera indirecta por la diferencia de 100 menos la tasa de mortalidad, y el índice de estabilidad de la población de tallos (Pf / Pi) se obtuvo de la siguiente manera ([Rojas-García et al. 2017](#)):

$$Pf / Pi = TSTa (1 + TA)$$

Donde: Pf / Pi muestra la relación entre la población final o actual de tallos respecto a la población anterior observada, considerando la tasa de supervivencia de tallos (TSTa) y la tasa de ahijamiento (TA). Este valor indica el efecto del ahijamiento y la muerte de los tallos con relación a la densidad total, para un periodo determinado, donde: valores próximos o iguales a 1 (0.95 a 1.05), indican una población de tallos estable, y una estabilidad negativa y positiva presenta valores menores a 0.95 y mayores a 1.05, respectivamente.

Las temperaturas máximas y mínimas fueron registradas en la estación meteorológica de la Facultad de Ingeniería y Ciencias ubicada en el mismo lugar donde se realizó el experimento. Para determinar la influencia de la temperatura sobre la dinámica de los tallos, se estimó la suma térmica (unidades calor) entre fechas de muestreo, mediante la siguiente fórmula propuesta por Ferri ([2011](#)):

$$\text{Unidades calor} = \sum_{i=1}^n (T_m - T_b)$$

Donde Tm es la temperatura media diaria, Tb es la temperatura base de los pastos tropicales (15 °C) y n es el número de días transcurrido entre las fechas de muestreo.

Los datos se analizaron mediante el procedimiento PROC GLM del paquete estadístico de SAS (SAS, 2002), en un diseño completamente aleatorizado con seis repeticiones y un arreglo de parcelas divididas en el tiempo, en el que los cultivares constituyeron la parcela principal y la fecha de muestreo las subparcelas. Para la comparación de medias se aplicó la prueba de Tukey (P=0.05).

Resultados

La dinámica de tallos se vio afectada por las condiciones ambientales y la expresión genotípica de cada cultivar. La mayor densidad de tallos se presentó para los cultivares Cobra y Mulato II (4,086 y 4,195 tallos/m², respectivamente), mientras que, los cultivares Camello I, II y Convert 330, tuvieron densidades menores pero similares entre ellas (promedio 3,300 tallos/m²; P>0.05) (Cuadro 1).

En mayo se observó la mayor densidad de tallos para todos los cultivares, sin diferencias entre ellos (P>0.05), mientras que, la menor densidad ocurrió en diciembre, con 4,729 y 1,516 tallos/m², para mayo y diciembre, respectivamente (P<0.05); sin embargo, en el mes de diciembre, Camello II y Cobra mostraron densidades superiores a los demás cultivares. La menor densidad de tallos en diciembre se atribuye al efecto de la presencia de temperaturas menores a los 15 °C (Figura 1), las cuales tienen efecto sobre el número de hijuelos y el incremento en el número de tallos muertos (Cuadro 1).

Los cultivares Cobra y Mulato II presentaron el mayor número de hijuelos (769 y 718 tallos/m², respectivamente), seguidos por Convert 330 (638 tallos/m²); de manera similar, los cultivares Cobra y Mulato II presentaron la mayor cantidad de tallos muertos (436 y 449 tallos/m², respectivamente). La mayor aparición de hijuelos entre fechas de muestreo se presentó en el mes de mayo (995 tallos/m²) y el mayor número de tallos muertos se presentó en julio y noviembre (818 tallos/m², promedio). En el muestreo de mayo, el cultivar Camello II resultó el menos afectado al sumar un total de pérdidas de 626 tallos/m², mientras que, durante el mes de noviembre el cultivar Convert 330 fue quien presentó la menor cantidad de tallos muertos (240 tallos/m²).

Se registraron diferencias en la tasa de ahijamiento y la tasa de mortalidad entre cultivares y entre los promedios de las fechas de muestreo (P<0.05; Cuadro 2). En todos los cultivares se observó una disminución en la tasa de ahijamiento conforme avanzó el tiempo a partir de junio, derivado de la estimulación por el corte de uniformidad realizado al inicio del experimento, el cual produjo incrementos entre 25 y 29 % en los meses de marzo a mayo, mostrando el mismo comportamiento para todos los cultivares (p>0.05). No obstante, posterior al segundo corte de uniformidad realizado en el mes de septiembre, no se logró detectar ese mismo comportamiento.

Independientemente de los cultivares, en los meses de julio, septiembre, noviembre y diciembre se presentó la

Cuadro 1. Cambios en la densidad poblacional, ahijamiento y muerte de tallos en cultivares de *Urochloa* en intervalo de muestreo de cuatro semanas, en condiciones semiáridas.

Fecha de muestreo	Cultivar					Promedio
	Camello I	Camello II	Cobra	Convert 330	Mulato II	
Densidad poblacional de tallos (Tallos/m ²)						
24-feb	2,091 ^a	2,003 ^a	2,717 ^a	2,891 ^a	2,952 ^a	2,531 ^E
24-mar	2,617 ^a	2,527 ^a	3,448 ^a	3,455 ^a	3,580 ^a	3,125 ^{DE}
21-abr	3,229 ^a	3,150 ^a	4,423 ^a	4,416 ^a	4,521 ^a	3,948 ^{ABC}
19-may	3,858 ^a	4,099 ^a	4,947 ^a	5,186 ^a	5,555 ^a	4,729 ^A
18-jun	3,823 ^a	3,918 ^a	4,952 ^a	4,729 ^a	5,488 ^a	4,582 ^{AB}
13-jul	3,617 ^a	4,071 ^a	5,135 ^a	3,610 ^a	4,839 ^a	4,254 ^{AB}
11-ago	3,706 ^a	3,933 ^a	4,451 ^a	3,607 ^a	4,918 ^a	4,123 ^{ABC}
08-sep	3,337 ^a	3,631 ^a	4,351 ^a	3,142 ^a	4,686 ^a	3,829 ^{BCD}
06-oct	3,648 ^a	3,838 ^a	4,472 ^a	3,277 ^a	4,936 ^a	4,034 ^{ABC}
03-nov	2,989 ^a	3,157 ^a	4,007 ^a	3,172 ^a	3,416 ^a	3,348 ^{DC}
01-dic	1,234 ^b	1,802 ^a	2,040 ^a	1,242 ^b	1,260 ^b	1,516 ^F
Promedio	3,104 ^b	3,284 ^b	4,086 ^a	3,521 ^b	4,195 ^a	
Hijuelos (Tallos/m ²)						
24-feb	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^G
24-mar	602 ^a	672 ^a	730 ^a	609 ^a	723 ^a	667 ^{CD}
21-abr	762 ^a	670 ^a	1,005 ^a	1,005 ^a	1,020 ^a	892 ^{BC}
19-may	701 ^a	1,073 ^a	1,008 ^a	1,079 ^a	1,112 ^a	995 ^A
18-jun	294 ^{ab}	108 ^b	503 ^a	243 ^{ab}	439 ^a	317 ^{EF}
13-jul	309 ^c	579 ^{ab}	795 ^a	289 ^c	394 ^{bc}	473 ^{DE}
11-ago	399 ^a	258 ^a	388 ^a	272 ^a	441 ^a	351 ^{DEF}
08-sep	89 ^a	107 ^a	286 ^a	177 ^a	268 ^a	185 ^{EF}
06-oct	417 ^a	566 ^a	503 ^a	301 ^a	361 ^a	429 ^{DEF}
03-nov	179 ^a	92 ^a	156 ^a	135 ^a	173 ^a	147 ^F
01-dic	61 ^a	181 ^a	373 ^a	15 ^a	16 ^a	129 ^F
Promedio	536 ^c	573 ^{bc}	769 ^a	638 ^{abc}	718 ^{ab}	
Tallos muertos (Tallos/m ²)						
24-feb	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^F
24-mar	76 ^b	149 ^a	0 ^c	4 ^{bc}	95 ^{ab}	73 ^D
21-abr	150 ^a	47 ^b	29 ^b	45 ^b	74 ^b	69 ^E
19-may	72 ^b	124 ^b	484 ^a	310 ^{ab}	78 ^b	213 ^D
18-jun	328 ^b	289 ^b	498 ^{ab}	700 ^a	507 ^{ab}	464 ^{BC}
13-jul	516 ^{cd}	426 ^d	626 ^c	1,408 ^a	1,042 ^b	803 ^A
11-ago	309 ^b	396 ^b	1,073 ^a	275 ^b	412 ^b	493 ^B
08-sep	458 ^b	408 ^b	386 ^b	642 ^a	500 ^{ab}	479 ^{BC}
06-oct	106 ^b	359 ^b	383 ^b	166 ^a	110 ^a	225 ^D
03-nov	838 ^b	773 ^b	621 ^b	240 ^c	1,693 ^a	833 ^A
01-dic	355 ^b	290 ^b	694 ^a	376 ^b	425 ^b	428 ^C
Promedio	291 ^c	296 ^c	436 ^a	382 ^b	449 ^a	

Literales diferentes entre cultivares (a, b, c) y entre fechas de muestreo (A, B, C, D, E, F), indican diferencia estadística significativa (Tukey P=0.05)

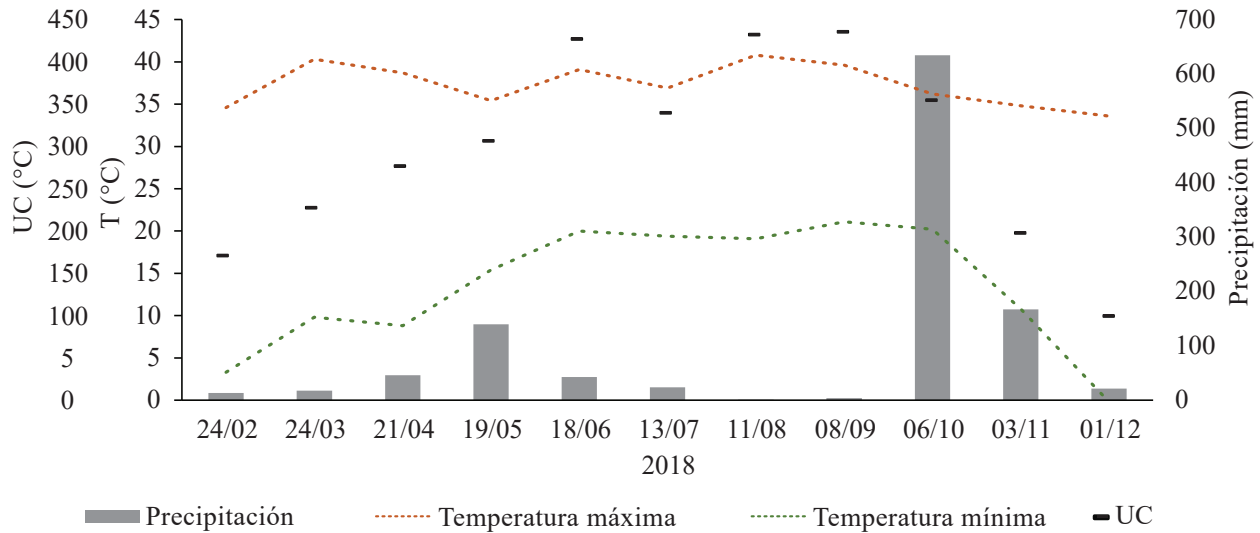


Figura 1. Temperaturas máxima y mínima, precipitación y unidades calor (UC) acumulada para cada fecha de muestreo.

Cuadro 2. Tasa de ahijamiento y mortalidad de tallos en cultivares de *Urochloa*, a intervalo de muestreo de cuatro semanas, en condiciones semiáridas.

Fecha de muestreo	Cultivar					Promedio
	Camello I	Camello II	Cobra	Convert 330	Mulato II	
Tasa de ahijamiento (%)						
24-mar	34.60 ^a	36.53 ^a	27.94 ^a	22.40 ^a	28.33 ^a	27.88 ^A
21-abr	34.28 ^a	29.59 ^a	29.25 ^a	30.25 ^a	27.59 ^a	29.55 ^A
19-may	23.19 ^a	34.56 ^a	22.62 ^a	24.21 ^a	24.54 ^a	25.77 ^A
18-jun	8.26 ^a	3.00 ^a	9.92 ^a	4.51 ^a	7.80 ^a	6.80 ^{BC}
13-jul	7.95 ^b	15.36 ^a	16.45 ^a	5.66 ^b	6.95 ^b	10.44 ^{BC}
11-ago	11.61 ^a	6.44 ^a	7.68 ^a	9.32 ^a	11.04 ^a	8.58 ^{BC}
08-sep	2.77 ^c	2.85 ^{bc}	6.57 ^a	4.95 ^{abc}	5.13 ^{ab}	4.38 ^{BC}
06-oct	13.59 ^a	15.63 ^a	13.24 ^a	9.88 ^a	10.09 ^a	11.70 ^B
03-nov	4.65 ^a	2.45 ^a	3.44 ^a	4.22 ^a	3.17 ^a	3.72 ^C
01-dic	1.87 ^a	7.23 ^a	9.60 ^a	0.76 ^a	0.69 ^a	3.56 ^C
Tasa de mortalidad (%)						
24-mar	4.39 ^a	9.25 ^a	0.00 ^a	1.56 ^a	2.97 ^a	3.33 ^{EF}
21-abr	6.02 ^a	2.08 ^a	0.81 ^a	1.45 ^a	1.90 ^a	2.45 ^F
19-may	2.23 ^a	4.54 ^a	10.93 ^a	8.11 ^a	1.96 ^a	5.18 ^{DEF}
18-jun	8.89 ^a	7.21 ^a	9.90 ^a	13.20 ^a	10.69 ^a	9.71 ^{CDEF}
13-jul	14.55 ^a	10.53 ^a	12.47 ^a	32.77 ^a	20.41 ^a	17.31 ^{AB}
11-ago	8.30 ^b	9.72 ^b	22.24 ^a	7.02 ^b	7.72 ^b	11.23 ^{BCDE}
08-sep	14.02 ^a	9.88 ^a	10.18 ^a	16.36 ^a	12.79 ^a	12.10 ^{ABCD}
06-oct	3.70 ^a	9.71 ^a	7.91 ^a	5.83 ^a	2.34 ^a	5.99 ^{DEF}
03-nov	23.43 ^{ab}	21.08 ^{ab}	14.17 ^{ab}	7.40 ^b	31.55 ^a	19.99 ^A
01-dic	12.19 ^a	12.71 ^a	17.76 ^a	12.40 ^a	13.36 ^a	12.69 ^{ABC}

Literales diferentes entre cultivares (a, b, c) y entre fechas de muestreo (A, B, C, D, E, F), indican diferencia estadística significativa (Tukey P=0.05)

mayor mortalidad con valores promedio de 17.31; 12.10; 19.99 y 12.69 %, respectivamente (Cuadro 2), lo cual puede estar asociado a la competencia por espacio y al efecto del estrés por temperaturas altas, la poca o nula disponibilidad de humedad y la exposición a temperaturas menores a 10 °C en las etapas finales del año (Figura 1).

La tasa de sobrevivencia de tallos tendió a disminuir con el tiempo (Cuadro 3), teniendo porcentajes superiores al 90 % en la mayor parte de muestreos ($P < 0.05$), sin presentar diferencias entre cultivares ($P > 0.05$); sin embargo, en el mes de noviembre se detectaron diferencias entre cultivares, donde el híbrido Convert 330 resultó menos afectado con una permanencia de la población de tallos superior al 92 % respecto a la fecha anterior, mientras que Mulato II mostró la mayor afectación de la densidad de tallos con pérdidas del 32 %.

Al evaluar de manera conjunta el efecto del ahijamiento y la mortalidad sobre la densidad total de tallos, se mostró una disminución en el índice de estabilidad conforme avanzó el tiempo (Cuadro 3); no obstante, fue en julio, septiembre, noviembre y diciembre cuando se comprometió la estabilidad de la población, con índices inferiores a 0.95, como resultado de las mayores tasas de mortalidad respecto a las tasas de ahijamiento. Cabe resaltar, que en los meses mencionados los cultivares presentaron el mismo comportamiento, excepto en el mes de noviembre, donde el cultivar Mulato II resultó más afectado, con un índice de estabilidad de 0.70. Este comportamiento puede deberse también a la falta de defoliación, dado que cuando se tiene intervalos largos entre cortes, se estimula la disminución en la densidad de tallos por la competencia

Cuadro 3. Tasa de sobrevivencia e índice de estabilidad de tallos en cultivares de *Urochloa*, a intervalo de muestreo de cuatro semanas, en condiciones semiáridas.

Fecha de muestreo	Cultivar					Promedio
	Camello I	Camello II	Cobra	Convert 330	Mulato II	
Tasa de sobrevivencia (%)						
24-mar	95.60 ^a	90.75 ^a	100.00 ^a	98.31 ^a	97.03 ^a	96.34 ^{AB}
21-abr	93.98 ^a	97.92 ^a	99.19 ^a	98.55 ^a	98.10 ^a	97.54 ^A
19-may	97.77 ^a	95.46 ^a	89.07 ^a	91.89 ^a	98.04 ^a	94.44 ^{ABC}
18-jun	91.11 ^a	92.79 ^a	90.10 ^a	86.80 ^a	89.31 ^a	90.02 ^{ABCD}
13-jul	85.45 ^a	89.47 ^a	87.53 ^a	67.23 ^a	79.59 ^a	81.85 ^{EF}
11-ago	91.70 ^a	90.28 ^a	77.76 ^b	92.98 ^a	92.28 ^a	89.00 ^{BCDE}
08-sep	85.98 ^a	90.12 ^a	89.82 ^a	83.64 ^a	87.21 ^a	87.35 ^{CDEF}
06-oct	96.30 ^a	90.29 ^a	92.09 ^a	94.17 ^a	97.66 ^a	94.10 ^{ABC}
03-nov	76.57 ^{ab}	78.92 ^{ab}	85.83 ^{ab}	92.60 ^a	68.45 ^b	80.47 ^F
01-dic	87.81 ^a	87.29 ^a	82.24 ^a	87.60 ^a	86.64 ^a	86.31 ^{DEF}
Índice de estabilidad (%)						
24-mar	1.29 ^a	1.22 ^a	1.28 ^a	1.21 ^a	1.25 ^a	1.25 ^A
21-abr	1.26 ^a	1.27 ^a	1.28 ^a	1.28 ^a	1.25 ^a	1.27 ^A
19-may	1.20 ^a	1.28 ^a	1.09 ^a	1.15 ^a	1.22 ^a	1.19 ^A
18-jun	0.99 ^a	0.95 ^a	0.99 ^a	0.90 ^a	0.96 ^a	0.96 ^{BC}
13-jul	0.92 ^a	1.03 ^a	1.02 ^a	0.71 ^a	0.85 ^a	0.91 ^{CD}
11-ago	1.03 ^a	0.96 ^{ab}	0.84 ^b	1.01 ^a	1.02 ^a	0.97 ^{BC}
08-sep	0.88 ^a	0.93 ^a	0.96 ^a	0.88 ^a	0.92 ^a	0.91 ^{CD}
06-oct	1.09 ^a	1.05 ^a	1.04 ^a	1.03 ^a	1.07 ^a	1.06 ^B
03-nov	0.80 ^{ab}	0.80 ^{ab}	0.88 ^{ab}	0.96 ^a	0.70 ^b	0.83 ^D
01-dic	0.90 ^a	0.92 ^a	0.90 ^a	0.88 ^a	0.87 ^a	0.89 ^{CD}

Literales diferentes entre cultivares (a, b, c) y entre fechas de muestreo (A, B, C, D, E, F), indican diferencia estadística significativa (Tukey $P = 0.05$)

por luz, dado que se presentan tallos de mayor altura y hojas más grandes (Cruz-Hernández et al. 2017b).

Respecto a la acumulación de la temperatura media diaria por encima de la temperatura base (15 °C) o unidades calor, los valores más altos se detectaron para los muestreos realizados en los meses de junio a octubre, acumulando más de 300 °C entre cada muestreo (Figura 1), factor determinante en el comportamiento del índice de estabilidad de tallos. No obstante, en el muestreo realizado en agosto, la precipitación tuvo la mayor acumulación con más de 600 mm, y esto sumado al corte de uniformidad realizado después del muestreo de julio, propició un incremento en la estabilidad de la pradera. Al respecto, Cruz-Hernández et al. (2017a) mencionan que, aunque se tengan las temperaturas óptimas para el desarrollo de las praderas, el déficit hídrico en períodos de sequía limita el desarrollo y producción de forraje.

Discusión

La distribución de la precipitación en el área de estudio siguió un patrón estacional (Figura 1), lo cual influyó en las actividades fisiológicas determinantes de la dinámica de tallos (Cruz-Hernández et al. 2017b); así mismo, la temperatura es un factor importante en el desarrollo fotosintético (Sage y Kubien 2007), aún más relevante cuando la estacionalidad de las precipitaciones no es muy marcada (Rodrigues et al. 2013). En el caso particular de este estudio hubo lluvias todos los meses excepto en agosto-setiembre, pero los niveles más altos de precipitación se presentaron en octubre-noviembre, cuando las temperaturas tendieron a ser más bajas. De igual manera, la ausencia de cortes frecuentes durante las etapas de desarrollo repercutió en la dinámica poblacional de tallos, ya que la interacción entre cortes y épocas del año produce cambios en los procesos morfogénicos (Ramírez-Reynoso et al. 2010).

En general, el comportamiento de la densidad de tallos (Cuadro 1) corresponde a lo ocurrido en los pastos manejados bajo corte, dado que después de realizada la defoliación, se estimuló la regeneración y se mantuvo por un tiempo la densidad en el espacio ocupado, pero luego ocurrió muerte de tallos como resultado de la competencia por espacio, tal como ha sido reportado por Ramírez-Reynoso et al. (2020).

En este estudio, se observó un incremento en el número de tallos en la primera fase hasta el muestreo realizado en mayo (Cuadro 2), como producto de una tasa de ahijamiento superior a la tasa de mortalidad; pero, posteriormente ocurrió una disminución en la densidad

de tallos debido a que las tasas de ahijamiento fueron muy bajas o nulas, y de esa manera fueron incapaces de compensar el incremento de las tasas de mortalidad. Lo mismo sucedió para el conteo realizado en el mes de octubre, donde la defoliación estimuló una mayor tasa de ahijamiento en relación con la tasa de mortalidad, sin embargo, este efecto fue pasajero, debido probablemente a la menor disponibilidad de unidades calor necesarias para estimular el crecimiento, más aún que en los meses posteriores se presentaron no solo temperaturas más bajas que afectan negativamente la tasa fotosintética (Sage y Kubien 2007), sino también una menor cantidad y calidad de radiación fotosintéticamente activa, lo cual inhibe la emergencia de hijuelos (Flores-Santiago et al. 2018).

El incremento en las tasas de ahijamiento observado en los cultivares de *Urochloa* (Cuadro 2) en las fases iniciales del estudio se debieron a la presencia de condiciones ambientales adecuadas y al corte de uniformidad realizado al inicio, lo cual estimuló la activación de yemas axilares y basales mediante la recepción de luz en la base de los tallos (Difante et al. 2008). Comúnmente esto sucede en las épocas de primavera y verano, cuando se tiene niveles adecuados de temperatura y precipitación, pero puede suceder en otras épocas si se dan condiciones ambientales adecuadas para el crecimiento de los pastos (Caminha et al. 2010). En el caso particular de este estudio, en los últimos meses del año se produjo afectación en la densidad de tallos por las mayores tasas de mortalidad, en comparación con las de ahijamiento, debido a las bajas temperaturas y menor luminosidad, pese a que había suficiente humedad en el suelo. Resultados similares fueron reportados por Maldonado-Peralta et al. (2019), quienes encontraron una disminución en la densidad de tallos en pasto Cuba OM-22, atribuido a la disminución en la presencia de la luz solar directa en los estratos inferiores donde se encuentran los nuevos tallos, como producto del incremento en el área foliar, lo cual resultó en la muerte de esos tallos (Cruz-Hernández et al. 2017b). Por otro lado, Rojas-García et al. (2016) mencionan que en pasturas de pasto ovido (*Dactylis glomerata* L.) solo y asociado con ryegrass perenne (*Lolium perenne* L.) y trébol blanco (*Trifolium repens*) una densidad de 30 plantas/m² favorece el crecimiento, y que la densidad de tallos puede ser afectada por la manipulación de la defoliación, en términos de la intensidad y frecuencia de corte. Sin embargo, ese tipo de información no está disponible para los cultivares de *Urochloa* incluidos en este estudio.

Un aspecto importante para considerar cuando se analiza la estabilidad de las pasturas (Cuadro 3) es el

balance entre el número de tallos muertos y la tasa de aparición de hijuelos (Cuadro 2), pues si esta última es alta podrían compensar las pérdidas por mortalidad. Al respecto, Ramírez-Reynoso et al. (2011) y Caminha et al. (2010) encontraron mayores tasas de sobrevivencia en la época de sequía en comparación a la época de lluvias para *Megathyrus maximus* cv. Mombasa y *Urochloa brizantha*, por lo que, el recambio de tallos se constituye en un mecanismo de adaptación para mantener estable la población y ayudar en la persistencia de la especie cuando enfrenta condiciones críticas.

El índice de estabilidad de la población de tallos mostró que las praderas de los cultivares de *Urochloa* en los primeros meses de evaluación incrementaron y mantuvieron el número de tallos, pero disminuyeron posteriormente, resultando en índices de estabilidad inferiores a 1. Este mismo comportamiento fue reportado por Ramírez-Reynoso et al. (2020) con el pasto Llanero (*Andropogon gayanus* Kunth) que con intervalos de corte de hasta 28 días no se comprometió la estabilidad de la especie, mientras que, intervalos de más de 42 días resultaron en índices de estabilidad menores a 1.0. En el caso de este estudio, es posible que el mantener las plantas de *Urochloa* sin defoliación hasta el mes de mayo (lo que representó 84 días de rebrote) no comprometió la persistencia de la pradera, pero la respuesta para los cortes de octubre fue diferente, sugiriendo que en ese período de año no se debería realizar defoliaciones tan tardías, y con alturas de corte a los 10 cm sobre el suelo, ya que las temperaturas bajas y la falta de tolerancia de estas especies a intensidades severas compromete la estabilidad de la pradera (Habermann et al. 2019).

En cuanto a la acumulación de unidades calor, se ha reportado que a medida que esta se incrementa, aumenta de manera lineal la materia seca total, con una mayor proporción de tallos que de hojas, estas últimas esenciales como determinantes de la calidad nutritiva del forraje. En un estudio con *Megathyrus coloratum* L., cultivar Verde, Ferri (2011) observó que el porcentaje de proteína disminuyó en 8 unidades en por ciento a medida que se incrementó la acumulación térmica o unidades calor hasta valores cercanos a los 1,800 °C. En este estudio, la acumulación de unidades calor entre el corte de uniformidad inicial y el realizado en el mes de septiembre fue mayor a los 2,600 °C, habría que preguntarse cuánto puede haber afectado esto la calidad nutritiva, pero con seguridad debería efectuarse un aprovechamiento más temprano mediante la defoliación, para no afectar la calidad ni la persistencia de los cultivares estudiados.

Este estudio fue desarrollado en parcelas pequeñas manejadas bajo corte, pero el siguiente paso es evaluar bajo pastoreo el efecto del manejo y los factores ambientales asociados a las épocas del año sobre la dinámica y estabilidad de tallos en algunos de estos cultivares, de manera similar a los estudios realizados en el pasto *Megathyrus maximus* cv. Mombasa (Montagner et al. 2012) y *Urochloa* híbrido cv. Mulato (Silva et al. 2017).

Conclusiones

El incremento en la temperatura medida mediante la acumulación de unidades calor y la distribución de la precipitación, aunado a intervalos largos entre cortes, resultan en una disminución en el índice de estabilidad, debido a la menor tasa de ahijamiento respecto a la tasa de mortalidad de tallos en los cinco cultivares de *Urochloa* (Camello I, Camello II, Mulato II, Convert 330 y Cobra).

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Research Paper

Nutritional and biomass evaluation of a *Megathyrsus maximus* collection in a dry tropical climate in Colombia

Evaluación nutricional y de biomasa de una colección de Megathyrsus maximus en un clima tropical seco en Colombia

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Abstract

Agronomic and nutritional parameters of a set of 28 accessions of *Megathyrsus maximus* established in Colombia during the rainy season were evaluated to identify accessions with differences in nutritional quality and characterize germplasm of *M. maximus*. ANOVA and multivariate analysis showed differences among accessions. Agronomic variables such as plant height, dry matter yield and green fresh weight were not correlated with nutritional variables. Flowering affected nutritional quality (neutral detergent fiber, acid detergent fiber and relative feed value). Flowering, fiber concentration, digestibility and crude protein concentration had the most influence on forage quality of *M. maximus*. The integral evaluation of biomass and nutritional parameters showed that the set of 28 *M. maximus* accessions contained 2 accessions with high nutritional quality and competitive biomass production. Heterogeneity of the collection in nutritional and agronomic characteristics indicates opportunities for plant breeding to produce additional accessions for improving cattle production in the tropics.

Keywords: Digestibility, grassland, quality, Relative feed value (RFV), yield.

Resumen

En una colección de 28 accesiones de *Megathyrsus maximus* establecida durante periodo de lluvias en trópico colombiano se evaluaron parámetros nutricionales y agronómicos con el objetivo de identificar accesiones con diferente calidad y caracterizar material forrajero de *M. maximus*. Los análisis de varianza y multivariado mostraron diferencias entre accesiones. Variables agronómicas como tales como altura, materia seca y forraje verde no presentaron correlación con las variables nutricionales. La floración afectó la calidad nutricional (fibra detergente neutro, fibra detergente ácido y el valor relativo del alimento). La floración, la concentración de fibra, la digestibilidad y la concentración de proteína cruda fueron los que más influyeron en la calidad del forraje de *M. maximus*. La evaluación integral de biomasa y parámetros nutricionales mostró que el conjunto de 28 accesiones de *M. maximus* había dos materiales promisorios con alta calidad nutricional y producción de biomasa competitiva. La heterogeneidad de la colección en las características nutricionales y agronómicas indica oportunidades para el trabajo en fitomejoramiento de producir accesiones adicionales que mejoren la producción ganadera en los trópicos.

Palabras clave: Calidad, digestibilidad, pastizal, rendimiento, Valor Relativo de forrajes (VRF).

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Introduction

Forages are the main feed for cattle production systems in the tropics (Gerber et al. 2015). Yield and nutritional quality are important variables for understanding genotype-environment-management relationships (Lemaire and Belanger 2020). These parameters can be used for identification of forages that significantly contribute to increased production efficiency in agricultural production systems (Khan et al. 2020) and sustainable cattle intensification (Cardoso et al. 2020; Mwendia et al. 2022).

The eco-efficient use of grasslands as animal feed can contribute to food security and to sustainable meat and milk production. In a livestock production and climate change context, several strategies are promoted for sustainable production, including interventions in feeding and nutrition of ruminants (Bhatta et al. 2017). Nutritional composition and digestibility of forages influence the productivity of grazing animals (Bezabih et al. 2014) and their methane emissions (Barahona-Rosales et al. 2014).

Integration of biomass yield and forage quality is economically important for production (Schaub et al. 2020) and adoption and use of forage by farmers (Keba et al. 2013; Garcia et al. 2020). These characteristics contribute to identifying suitable grassland management practices for adapting to climate change (Perotti et al. 2021) and are of high interest to forage breeders, researchers and cattle producers (Jank et al. 2011; Carvajal-Tapia et al. 2022).

Megathyrsus maximus is a grass commonly used in tropical livestock production systems with outstanding agronomic, nutritional and environmental characteristics under dry tropical conditions (Carvajal-Tapia et al. 2021b). This grass has potential for inclusion in silvopastoral forage systems (Paciullo et al. 2017) and forage mixtures in grassland (Matínez-Mamian et al. 2020).

The present study evaluated agronomic parameters, including plant height, flowering, dry matter yield (DMY) and green fresh weight (GFW) together with nutritional parameters, including gas production (GP), neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), organic matter (OM), organic matter digestibility (DOM), metabolizable energy (ME), in vitro dry matter digestibility (IVDMD), non-fiber carbohydrate (NFC), total digestible nutrients (TDN) and relative feeding value (RFV) of 28 accessions of *M. maximus* established in a dry tropical agroecosystem in Colombia. The aim was to determine biomass and nutritional quality of a selection of accessions of *M. maximus* by quantifying a range of variables to identify those accessions with

potential for use in plant breeding for enhancing cattle production in the tropics.

Materials and Methods

Climatic characteristics

This study was conducted in a dry tropical forest agroecosystem located in the Patía Valley, Cauca Department, southwestern Colombia, at 625 masl with annual average temperature of 27.9 °C, annual precipitation of 1,414 mm and two rainy seasons per year. Specific environmental conditions for the trial period were on average 27.4 °C, 77 % and 172 mm for temperature, relative humidity and cumulative precipitation, respectively (Figure 1).

Agronomic evaluation

Soils at the experimental site were Mollisols, suborder Ustolls and group Haplustolls. The chemical analysis of samples collected from 0–20 cm depth had the following values: pH=6.26, C_{ox}=18.14 g/kg, total N=0.22 %, organic matter=4.50 %, P=6.3 ppm, Ca=14.58 cmol/kg, Mg=6.91 cmol/kg, K=0.59 cmol/kg, Na=0.10 cmol/kg, cation exchange capacity=27.10 cmol/kg and B=83 ppm or mg/kg.

A collection of 130 *Megathyrsus maximus* accessions provided by the germplasm bank of the Alliance of Bioversity International and CIAT were planted as tillers in 4 m² plots separated by 1m wide pathways in a randomized complete block design with 3 replicates in 2015. In 2017, agronomic data were recorded for 28 accessions selected as displaying above average in green forage weight (GFW), dry matter yield (DMY) and plant height in previous experiments (Table 1).

Agronomic evaluation was carried out in the Patía Valley, Colombia during the rainy season from 24 March to 4 May 2017. A standardization cut at 30 cm above the ground was carried out 23 March 2017. After a regrowth period of 41 days, forage on plots was harvested for evaluation. Variables measured were plant height (cm) (Toledo and Schultze-Kraft 1982) and flowering (%) (estimated flowering percentage of full plot). Using a 1 m² quadrat, each plot received a mechanical cut at a height of 30 cm from ground level and the resulting green forage mean was weighed from each of the quadrats. The production of green forage per ha (GFW) of each accession was calculated. Subsamples of about 200 g were taken from each plot,

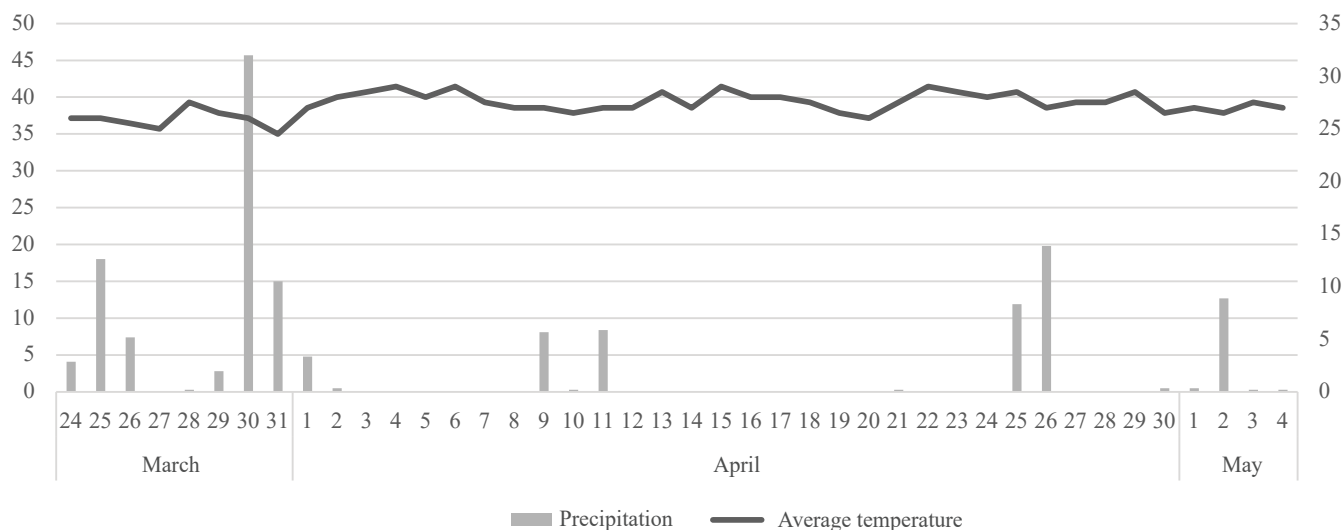


Figure 1. Average temperature and daily precipitation during the experimental period in 2017. Data from the NUTRIFACA meteorological station, Patía Valley of Colombia.

Table 1. CIAT number, origin, and code of other institutions of the evaluated 28 accessions of *Megathyrsus maximus*.

Origin	CIAT and other genebank accession numbers
Kenya	693 (RWS-025, CPI-059903), 6536 (K-741299-303, FAO-01682, FAO-01686), 6900 (K-70, BRA-006351), 6901 (BRA-006360, K-71) and 16004 (KK-15, BRA-007641).
Tanzania	6949 (BRA-005797, K-172), 6955 (K-180, BRA-005851), 6963 (BRA-006653, K-190B), 16011 (BRA-007013, T-3), 16034 (BRA-005029, T-64), 16035 (BRA-007269, T-65), 16038 (BRA-005037, T-69), 16039 (BRA-007293, T-71), 16044 (BRA-007343, T-80), 16058 (T-99) and 16059 (BRA-007471, T-102).
Unknown	673 (ILRI-16553, 3622) ^a , 6171 ^b , 6461 (K-6331), 6497 (K-74895-96), 6836 (G-95, BRA-004839), 6839 (BRA-004863, G-98), 6840 (BRA-004871, G-99), 26723 (CPAC-3273), 26925 (BRA-005576, ORSTOM-K146) and 26944 (BRA-003638, ORSTOM-63).
Commercial	16031 Tanzania (BRA-007218, ILCA-16554, T-58) and 6299 Tobiatá (CPI-089251, ILRI-07160, CNPGC-132/78, K-00187).

^aPromising accessions in Colombia and Vietnam

^bPromising accessions in Colombia (Carimagua)

weighed and oven-dried under controlled ventilation at temperatures from 60 to 70 °C until they reached constant weight (48 to 72 hours) to calculate dry matter yield (DMY). The dried subsamples were processed and sent to the laboratory of Animal Nutrition and Rangeland Management in the Tropics and Subtropics of the University of Hohenheim in Stuttgart, Germany for the nutritional evaluation.

Nutritional evaluation

The dried samples were ground in a Retsch SM 100 mill (Retsch GmbH, Haan, Germany) to pass through a 1 mm sieve. Gas production (mL/200 mg DM 24 h) was determined using a completely randomized design with six replicates using the Hohenheim gas test (GP). Based on GP, ruminal degradability of organic matter (g/kg DM)

and metabolizable energy (MJ/kg DM) were estimated (Close and Menke 1986). Rumen fluid was collected from 2 cannulated Jersey cows that were fed a diet composed of concentrate mixture (251 g/kg), maize silage (243 g/kg), grass silage (243 g/kg), grass hay (170 g/kg), rapeseed meal (52 g/kg), barley straw (22 g/kg) and a mineral-amino-acid-vitamin mixture (19 g/kg). The forage:concentrate ratio was 68:32 [Net energy for lactation (NEL)=6.2 MJ/kg DM and crude protein (CP)=134 g/kg DM] as-fed basis. Samples were also analyzed using the Dumas combustion procedure for total N determination (AOAC 2005) and values multiplied by 6.25 to estimate crude protein concentration (CP; method 4.1.2) and crude ash (CA; method 8.1) using the official methods of the Verband Deutscher (VDLUF 2007). NDF and ADF were determined using the ANKOM fiber analyzer (Van Soest et al. 1991). DOM and ME (Close and Menke 1986) were

calculated from GP (g/kg DM), crude protein, ash and crude lipids (EE, ether extract) by the equations:

$$\% \text{ DOM} = 14.88 + 0.889 \text{ GP} + 0.045 \text{ CP} + 0.065 \text{ ash}; \text{ and} \\ \text{ME} = 1.242 + 0.146 \text{ GP} + 0.007 \text{ CP} + 0.0224 \text{ EE}$$

A value of 16.4 g/kg EE was used for all accessions.

In vitro dry matter digestibility (IVDMD) was determined with the near infrared spectroscopy (NIRS) equipment model Foss 6500 and MINISIS software (IS-2250) version 2.71 as reported by Mazabel et al. (2020) in the Alliance of Bioversity International and CIAT forage and animal nutrition quality laboratory.

Quality indices were calculated based on the nutritional data. Relative feed value (RFV) was calculated and classified on the scale: RFV > 151 = excellent; 125–151 = first quality; 103–124 = second quality; 87–102 = third quality; and 75–86 = fourth quality (FEDNA 2014) using the equation:

$$\text{RFV} = (\text{DDM} \times \text{DMI}) / 1.29$$

where:

DDM = digestible dry matter (88.9 - (0.779 × % ADF));

DMI (kg/d) = dry matter intake (% of BW) (120/% NDF); and

BW = body weight (kg).

Total digestible nutrients (TDN) were estimated according to the method of Jayanegara et al. (2019):

$$\text{TDN} = 0.479 \text{ NDF} + 0.704 \text{ NFC} + 1.594 \text{ EE} + 0.714 \text{ CP}$$

where:

NDF = neutral detergent fiber; and

NFC (non-fiber carbohydrate) = OM - (NDF + EE + CP).

Statistical analysis

Agronomic variables (plant height, flowering, DMY and GFW) were used for analysis. Nutritional variables were: NDF, ADF, CP, OM, DOM, GP, ME, IVDMD, RFV, NFC and TDN. ANOVA and Tukey's multiple range test were carried out using statistical package SAS version 9.2.1 to determine statistical differences in plant height, flowering, GFW, DMY and GP among the accessions. The multivariate analysis was carried out with software R version 4.1.2 for the variables plant height, flowering, DMY, GFW, NDF, ADF, CP, OM, DOM, ME, IVDMD, NFC, RFV and TDN. Pearson correlation was obtained with ggcorrplot in R (Kassambara 2019) using the Bonferroni test for hypothesis testing on non-zero

correlation. To classify the accessions, the data were standardized with Z-score. The multivariate analysis with principal components and clustering was performed using the package FactoMineR (Lê et al. 2008). Based on the variables evaluated, hierarchical clustering was carried out using the agglomerative algorithm of Ward and Euclidean distance. Visualization was performed using the functions fviz_pca_biplot and fviz_cluster (factoextra library). Figures were created using the package 'ggplot2' (Wickham 2016).

Results

Analysis of variance indicated significant difference ($P < 0.05$) between accessions in the variables flowering, plant height and GP (Table 2). Accessions 16059, 16031 (Tanzania), 26723, 6497, 6840 and 16035 had the highest GP, which placed them in the first 10 accessions with the highest DOM and ME values. Accessions 673, 6171, 6461, 6497, 6536, 6836, 6839, 6900, 6949, 6955, 16004, 16011, 16058, 16059, 26944 and 6840 displayed full flowering at harvest, while accessions 6963, 26723, 26925, 16035, 6299 Tobiata and 16044 displayed ≤ 5 % flowering at harvest (Table 2). The commercial variety Tobiata and accession 693 were the tallest but not significantly different from most accessions ($P > 0.05$). The analysis of variance showed no significant differences between accessions for dry biomass yield (Table 2).

All accessions contained high concentrations of NDF (range 655–733 g/kg) and OM (range 810–872 g/kg) and IVDMD ranged from 50.7 to 63.7 % with DOM ranging from 44.1 to 55.7 % (Table 3). CP concentration ranged from 45.8 to 108.5 g/kg. Relative feed value of the accessions ranged from 67.2 to 86.6, indicating that forage was in the fourth quality category. TDN values ranged from 43.8 to 47.0 (Table 3).

There was a moderate to high positive correlation between flowering and NDF and ADF concentrations, and a negative correlation with IVDMD and RFV. Structural carbohydrates represented by the concentrations of NDF and ADF were related negatively and moderately to CP, DOM and IVDMD, and positively to OM only for NDF. CP concentration, IVDMD and DOM presented positive relationships with RFV, while RFV was negatively correlated with NDF and ADF (Figure 2).

Principal component analysis (PCA) identified components 1 and 2 representing up to 63.1 % of the variation (Figure 3). Accessions 26723 and 693 in Cluster 1 were tall and high-yielding plus late-flowering (Figure 4). They have low concentrations of ADF and NDF and high

concentrations of DOM and ME, with an RFV of 78.1. Cluster 2 grouped 10 accessions that included commercial varieties, characterized by low concentrations of ADF

and NDF, high CP concentrations, adequate IVDMD, late flowering, average DMY of 5,196 kg/ha and a high value of RFV (Table 4; Figure 4).

Table 2. Mean values of agronomic characteristics and gas production of 28 accessions of *M. maximus* grown in a Colombian dry tropical agroecosystem.

CIAT accession no.	Height (cm)	Flowering (%)	DMY (kg/ha)	GFW (kg/ha)	GP (mL/mg DM 24 h)
673	120.0 ^{bc}	100 ^a	5,997 ^a	21,190 ^{ab}	26.10 ^{hij}
693	160.3 ^a	33.3 ^{cd}	8,720 ^a	32,733 ^{ab}	29.75 ^{cdef}
6171	136.0 ^{abc}	100 ^a	8,198 ^a	25,933 ^{ab}	27.26 ^{efghi}
6299 Tobiata	160.7 ^a	0 ^d	4,856 ^a	18,627 ^{ab}	26.50 ^{ghi}
6461	144.0 ^{abc}	100 ^a	6,594 ^a	22,680 ^{ab}	29.17 ^{defg}
6497	131.5 ^{abc}	100 ^a	5,742 ^a	19,980 ^{ab}	30.83 ^{abcd}
6536	130.7 ^{abc}	100 ^a	6,553 ^a	23,613 ^{ab}	29.20 ^{defg}
6836	142.0 ^{abc}	100 ^a	6,496 ^a	26,773 ^{ab}	26.00 ^{hij}
6839	139.7 ^{abc}	100 ^a	6,047 ^a	25,027 ^{ab}	28.20 ^{defgh}
6840	148.3 ^{ab}	93.3 ^{abc}	6,808 ^a	25,393 ^{ab}	30.30 ^{abcd}
6900	141.3 ^{abc}	100 ^a	5,312 ^a	21,107 ^{ab}	23.70 ^{ijkl}
6901	147.0 ^{ab}	50.0 ^{abc}	4,948 ^a	18,193 ^{ab}	24.90 ^{ijk}
6949	123.7 ^{abc}	100 ^a	7,375 ^a	26,800 ^{ab}	24.70 ^{ijkl}
6955	138.0 ^{abc}	100 ^a	5,998 ^a	19,653 ^{ab}	26.30 ^{hij}
6963	157.3 ^{ab}	5.0 ^d	6,645 ^a	28,593 ^{ab}	22.10 ^l
16004	135.3 ^{abc}	100 ^a	8,622 ^a	30,177 ^{ab}	26.80 ^{ghi}
16011	143.3 ^{abc}	100 ^a	6,099 ^a	23,760 ^{ab}	22.50 ^{kl}
16031 Tanzania	133.3 ^{abc}	50.0 ^{abc}	4,028 ^a	17,960 ^{ab}	32.60 ^{ab}
16034	145.7 ^{ab}	30.0 ^{cd}	4,134 ^a	16,543 ^{ab}	25.80 ^{hij}
16035	157.0 ^{ab}	0 ^d	5,666 ^a	20,067 ^{ab}	30.30 ^{abcd}
16038	152.0 ^{ab}	33.3 ^{cd}	7,098 ^a	22,267 ^{ab}	29.86 ^{bcde}
16039	147.3 ^{ab}	35.0 ^{abc}	4,319 ^a	16,013 ^{ab}	27.20 ^{efghi}
16044	141.3 ^{abc}	0 ^d	6,123 ^a	21,573 ^{ab}	26.20 ^l
16058	142.3 ^{abc}	100 ^a	7,041 ^a	21,023 ^{ab}	25.80 ^{hij}
16059	128.7 ^{abc}	100 ^a	6,069 ^a	20,467 ^{ab}	32.80 ^a
26723	154.7 ^{ab}	2.0 ^d	11,056 ^a	46,080 ^a	32.10 ^{abc}
26925	106.0 ^c	1.0 ^d	4,145 ^a	15,093 ^{ab}	29.58 ^{cdef}
26944	136.0 ^{abc}	100 ^a	4,279 ^a	15,520 ^{ab}	27.06 ^{efghi}
Means	141.95	65.06	6,255	22,994	27,636
Root MSE	11.78	20.08	2.41	8.8	1,273
CV	8.35	30.86	38.68	38.29	4.6
Pr > F	0.0001	<0.0001	0.2116	0.1162	<0.0001

GP=gas production with Hohenheim gas test; DMY=dry matter yield; GFW=green forage weight.

Values within columns followed by different letters are significantly different according to the Tukey HSD (honestly significant difference) test (P<0.005).

Table 3. Nutritional characteristics of 28 accessions of *M. maximus* grown in a Colombian dry tropical agroecosystem.

CIAT accession no.	NDF (g/kg)	ADF (g/kg)	CP (g/kg)	OM (%)	DOM (MJ/kg)	ME (%)	IVDMD (%)	NFC (%)	RFV	TDN (%)
673	710	431	63.2	856	47.0	6.1	50.9	6.6	72.5	45.8
693	683	385	75.0	838	51.4	6.7	57.9	6.3	80.3	45.1
6171	724	439	67.8	864	47.9	6.4	53.0	5.7	70.3	46.1
6299 Tobiata	684	397	74.9	824	50.2	6.2	56.3	4.8	78.9	44.1
6461	686	389	73.0	839	50.8	6.6	58.4	6.4	79.5	45.2
6497	716	424	60.0	855	50.7	6.7	58.8	6.3	72.6	45.6
6536	711	418	62.1	867	49.4	6.5	53.8	7.8	73.7	46.6
6836	724	442	59.4	861	46.5	6.1	50.7	6.1	70.0	45.8
6839	673	381	86.7	843	51.3	6.5	58.6	6.7	81.9	45.8
6840	709	413	69.7	872	50.5	6.7	52.2	7.7	74.4	47.0
6900	719	433	68.1	851	45.3	5.8	52.1	4.7	71.4	45.2
6901	719	431	71.8	840	46.8	6.0	53.3	3.3	71.6	44.5
6949	733	462	45.8	864	44.1	5.8	53.9	6.9	67.2	45.8
6955	727	446	66.9	864	47.3	6.2	54.5	5.5	69.3	46.0
6963	690	393	69.5	838	44.9	5.6	56.1	6.2	78.6	45.0
16004	702	414	73.9	856	48.4	6.3	57.8	6.3	75.1	46.0
16011	725	428	61.6	837	44.6	5.6	51.9	3.5	71.3	44.2
16031 Tanzania	675	370	108.5	831	55.7	7.3	60.8	3.1	82.9	44.9
16034	670	375	76.3	833	48.8	6.1	59.7	7.0	82.9	45.1
16035	682	369	88.7	858	51.3	6.8	63.7	7.1	82.1	46.6
16038	714	395	72.2	868	49.8	6.7	57.2	6.5	75.7	46.6
16039	690	404	74.4	847	48.9	6.3	58.7	6.6	77.5	45.6
16044	680	369	88.7	846	49.0	6.3	60.9	6.1	82.3	45.8
16058	693	401	84.4	830	49.3	6.2	52.0	3.6	77.4	44.4
16059	698	416	76.7	861	53.4	7.1	57.5	7.0	75.3	46.5
26723	697	411	70.2	841	53.3	7.0	58.7	5.8	75.9	45.1
26925	654	359	76.6	810	53.2	6.7	59.7	6.2	86.6	43.8
26944	714	427	81.0	854	49.0	6.3	54.3	4.3	72.5	45.6

NDF=neutral detergent fiber; ADF=acid detergent fiber; CP=crude protein; OM=organic matter; DOM=organic matter digestibility; ME=metabolizable energy; IVDMD=in vitro dry matter digestibility; NFC=non-fiber carbohydrate; RFV=relative feed value; and TDN=total digestible nutrients.

Table 4. Mean characteristics for the three clusters identified in the *M. maximus* collection.

Cluster	Plant height (cm)	Flowering (%)	DMY (kg/ha)	GFW (kg/ha)	NDF (g/kg)	ADF (g/kg)	CP (g/kg)	OM (g/kg)	DOM (%)	ME (MJ/kg)	IVDMD (%)	NFC (%)	RFV	TDN (%)
1	157.5	17.6	9,888	39,407	690	398	72.6	839	52.3	6.8	58.3	6.1	78.1	45.1
2	144.8	20.4	5,196	19,493	686	386	80.2	839	49.9	6.4	58.6	5.7	79.9	45.1
3	136.3	99.5	6,452	23,069	710	423	68.8	855	48.5	6.3	54.4	5.9	73.4	45.7

DMY=dry matter yield; GFW=green forage weight; NDF=neutral detergent fiber; ADF=acid detergent fiber; CP=crude protein; OM=organic matter; DOM=organic matter digestibility; ME=metabolizable energy; IVDMD=in vitro dry matter digestibility; NFC=non-fiber carbohydrate; RFV=relative feed value; TDN=total digestible nutrients.

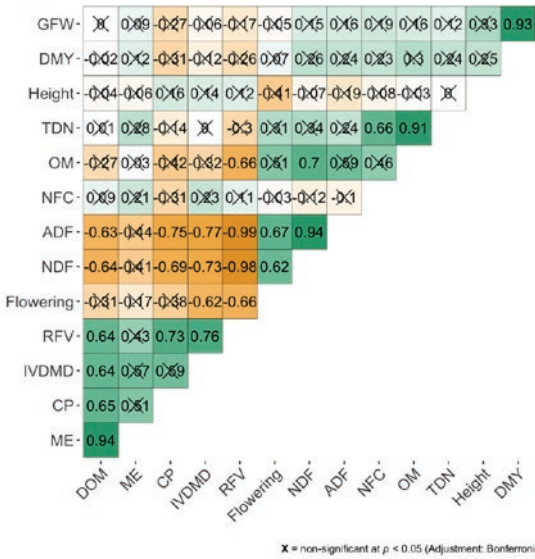


Figure 2. Pearson correlation coefficients among agronomic, nutritional, and quality index variables in a collection of *M. maximus* established in the tropics of Colombia.

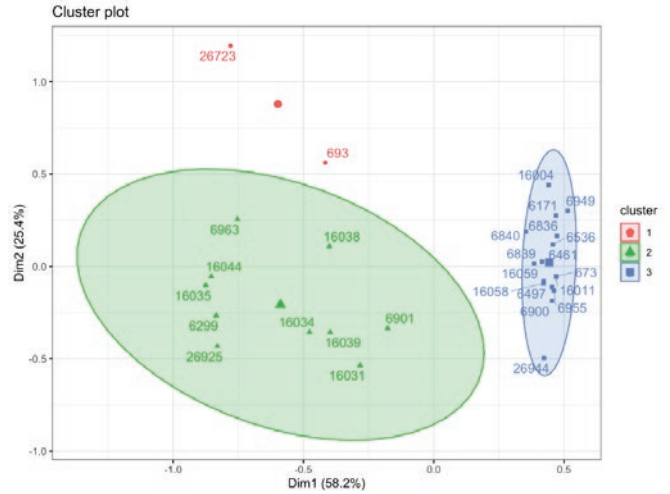


Figure 4. Cluster analysis based on principal components of a set of accessions of *M. maximus* established in the Colombian tropics.

Discussion

Yield, quality and cutting interval of tropical grasses (Mwendia et al. 2022) are fundamental parameters for evaluating the performance of forage accessions for sustainable intensification of cattle production. Results from the evaluation suggested good agronomic adaptation for Accessions 6497, 6840, 16031 (Tanzania), 16035, 16059 and 26723 with adequate DMY and good forage energy value.

The flowering composition of grasslands is modulated by their phenological stage and rainfall (Ferner et al. 2015). In the Patía Valley, flowering is not seasonal and occurs from 40 to 60 days after grazing depending on climatic conditions, being faster in dry periods than in rainy periods (Carvajal-Tapia et al. 2021a). The relationships among the variables and their distribution demonstrate the negative influence of structural carbohydrates and flowering on nutritional quality. Flowering was positively correlated with NDF and ADF concentrations and negatively associated with IVDMD and RFV, reducing grass nutritional value (Seepaul et al. 2016), possibly by reduction in metabolism (Costa et al. 2017) and association with physiological aspects related to the maturation process of forage (Vranić et al. 2009). Accessions with early flowering had a lower index of RFV, similar to that found in *Bulbous barley* (Uzun 2010). This suggests that late-flowering accessions

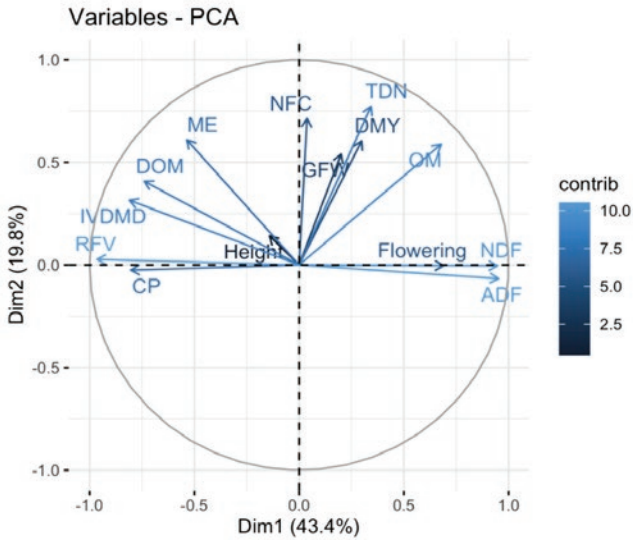


Figure 3. Biplot PCA of agronomic, nutritional, and quality variables in the classification of *M. maximus* accessions established in the Colombian tropics. DMY=dry matter yield; GFW=green forage weight; NDF=neutral detergent fiber; ADF=acid detergent fiber; CP=crude protein; OM=organic matter; DOM=organic matter digestibility; ME=metabolizable energy; IVDMD=in vitro dry matter digestibility; NFC=non-fiber carbohydrate; RFV=relative feed value; and TDN=total digestible nutrients.

have higher quality than earlier-flowering accessions after 41 days of regrowth. Twelve of the 28 accessions studied were late flowering (Clusters 1 and 2) and 16 accessions were early flowering (Cluster 3). RFV is an indicator of forage quality and is related to digestibility parameters (IVDMD) (Akdeniz et al. 2019) and fiber concentration (Escobar et al. 2020) and is obviously related to stage of maturity (Jeranyama and Garcia 2004; Seydosoglu and Bengisu 2019), the percentage of flowering at harvest and fodder intake. The range observed for RFV (67.2–86.6) shows values higher than those reported by Keba et al. (2013) and for most tropical grasses (Mwendia et al. 2017) and are similar to those reported for temperate *Festuca* species (Akdeniz et al. 2019). This indicates that *M. maximus*, under the edaphoclimatic conditions of the Patía Valley agroecosystem, is an outstanding species for its quality indices.

Plant height has high heritability and is a good in situ indicator of biomass components (green fresh weight and dry matter yield) of *M. maximus* (Carvajal-Tapia et al. 2022). This is a morphological characteristic associated with tolerance of shade (Malaviya et al. 2020), which is related to grass adaptation and growth under silvopastoral arrangements or grass-legume associations. Plant height ranged from 106.0 to 160.7 cm, similar to those presented by Malaviya et al. (2020) (94.3–153.3 cm), which suggests that the set of accessions evaluated have adequate yield for use in silvopastoral systems. Forage yield is important for farmers and Accessions 693 and 26723 were identified as promising by Carvajal-Tapia et al. (2022), during evaluations over 2 years in different harvests under contrasting rainfall conditions. In addition to their high production, these accessions have adequate nutritional quality with low values of NDF and ADF and high IVDMD, ME and RFV. A similar result was reported by Carvajal-Tapia et al. (2021a), from the nutritional classification of 129 accessions of *M. maximus*.

CP concentration had a positive correlation with RFV and a negative correlation with NDF and ADF, similar to results from other research on tropical forages (Musco et al. 2016), perennial temperate grasses (Uzun 2010) and legumes (Barahona-Rosales 1999). Protein and fiber concentrations are important for reducing enteric emissions (Barahona-Rosales and Sánchez-Pinzón 2005; Rivera-Herrera et al. 2017). Greater metabolizable energy availability from forage (Pell and Schofield 1993), higher digestibility and superior quality are characteristics associated with greater feed efficiency (Akdeniz et al. 2019) and late or limited flowering (Espinoza-Canales et al. 2017). Therefore,

accessions of Clusters 1 and 2 can be considered as having potential for improving animal diets and meeting ruminant dietary requirements. Accessions CIAT 693, 6299, 16031, 16034, 16038 and 16044 from Clusters 1 and 2 are accessions were classified as promising for productive, nutritional and environmental parameters (Carvajal-Tapia et al. 2021b).

Commercial varieties such as Tanzania and Tobiata grouped in Cluster 2 and showed similar nutritional quality to but different yields from those reported by Villegas et al. (2020) and Carvajal-Tapia et al. (2021a), when evaluated under greenhouse and field conditions. This indicates that edaphoclimatic conditions have more influence on agronomic characters than on nutritional characters in this species. Clusters 1 and 2 included *M. maximus* accessions with promising nutritional quality under tropical dry forest edaphoclimatic conditions, while Cluster 3 included accessions with high NDF and ADF contents and early flowering.

Conclusions

Flowering, fiber concentrations, digestibility and crude protein concentrations are variables that have marked influence on the classification of *M. maximus* accessions evaluated, including commercial varieties with high nutritional quality (693 and 26723) and biomass yields (>4,000 kg/ha). Accessions with low quality and high average DMY of 6,452 kg/ha were also identified. Heterogeneity in nutritional and agronomic characters will facilitate their use in plant-breeding to develop elite genotypes that promote development of eco-efficient livestock production systems. Among the agronomic variables, flowering behavior is equal to or more important than forage production because of its influence on nutritional quality. Researchers are encouraged to study the physiological behavior of accessions under tropical environmental conditions during grass evaluation.

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Research Paper

Effect of irrigation on biomass production and components of dallis grass (*Paspalum dilatatum*) and Bahia grass (*P. notatum*) in Uruguay

Efecto del riego sobre la producción de biomasa y componentes del pasto dallis (*Paspalum dilatatum*) y pasto Bahía (*P. notatum*) en Uruguay

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Abstract

Use of irrigation in forage production systems based on perennial C4 grasses has been limited because of inconsistent responses of biomass production to water. The effects of three different levels of irrigation on growth of *Paspalum notatum* (Bahia grass) and *P. dilatatum* (dallis grass) over two consecutive growing seasons were studied. The phenological state of the plants was inferred by the proportion of lamina and non-lamina components. While irrigation failed to increase annual dry matter yield of forage, phenological composition of forage was affected by irrigation with both species showing changes in distribution of dry matter accumulation among different above-ground plant parts. Future research on these species should study the effects of moisture deficit on phenology to better understand the effects of irrigation.

Keywords: Growth rate, phenology, tropical forage, water management, warm-season grasses.

Resumen

El uso de riego en sistemas de producción de forraje basados en gramíneas perennes C4 se ha visto limitado debido a las respuestas erráticas de la producción de biomasa a la irrigación. Se estudió el efecto de tres niveles diferentes de riego sobre *Paspalum notatum* y *P. dilatatum* durante dos temporadas de crecimiento consecutivas. El estado fenológico de las plantas se infirió por la proporción de componentes lámina y no lámina. Los resultados mostraron que la composición fenológica del forraje se vio afectada por el riego, aunque no resultó en un aumento en el rendimiento anual, ambas especies mostraron cambios en la distribución de la acumulación de materia seca entre las diferentes partes aéreas de la planta. Futuras investigaciones sobre estas especies deberían estudiar el efecto del déficit de humedad sobre la fenología, para comprender mejor los efectos del riego.

Palabras clave: Especies herbáceas tropicales, fenología, forraje, manejo del agua, tasa de crecimiento.

Introduction

Adoption of C4 grass species is an efficient alternative for producing feed for livestock ([Biran et al. 1981](#)) in regions like Argentina, southern Brazil, Uruguay, South Africa and New Zealand. Limitations to the use of highly productive C4 perennial grasses ([Cunha et](#)

[al. 2007](#)), including difficulties in crop establishment and uncertainty in production associated with irregular weather conditions, have prevented wide adoption of perennial warm season grasses by commercial producers ([McCormick et al. 2009](#)). Appropriate species for these areas require high water use efficiency to produce high yield and should be adapted to the

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environment (Neal et al. 2011). The unpredictable nature of rainfall and evapotranspiration during the warm season limits potential growth rates (Giménez and Lanfranco 2012) and results in a high year-to-year variability in herbage production (Royo Pallarés et al. 2005) with many producers opting for C3 species with simpler management and higher resilience but lower productivity in such environments. The use of irrigation in forage production can be a potentially valuable strategy to reduce pasture seasonality (Giménez and García Petillo 2011). However, it is necessary to improve the understanding of why highly productive perennial species do not always respond to irrigation (Forde et al. 1976; Marais et al. 2006).

Water management is intended to overcome water deficits allowing pastures to achieve high growth rates and increase forage production and quality during critical periods (Beaty et al. 1974). Adjustments to the quantity and timing of irrigation are needed to achieve efficient water use, which involves maintaining soil moisture at levels sufficient for plants to reach their potential transpiration, while minimizing losses to the environment. Understanding root depth, minimum water content in the soil to avoid stress and the soil water holding capacity are important for determining irrigation frequency (Snyman 1994). Recently, a model to estimate water use efficiency was developed for bioenergy perennial grasses (Kiniry and Kim 2020). In many cases, effects of irrigation are not consistent and it may either increase production (Harris and Lazenby 1974) or even depress growth at high water volumes (Marais et al. 2006; Stone et al. 2012).

Bahia grass (*Paspalum notatum* Flügge) and dallis grass (*Paspalum dilatatum* Poir.) produce early forage in spring and have a high level of adaptation to frost (Costa and Scheffer-Basso 2003). Both species show high variability in the summer, associated primarily with climate and its effects on the phenological stage of the plant (Pereira et al. 1978). Positive responses to irrigation in these species may be expected in areas with high levels of radiation and temperature and soils with non-limiting nitrogen availability (Stone et al. 2012). The irrigation regime modulates partitioning of photoassimilates by producing a larger and deeper root system under reduced irrigation (Jordan et al. 2003), which in turn affects the phenological responses of the plant. Dallis grass exhibits a considerable degree of phenological synchronization among tillers (González-Barríos et al. 2016), reflected in the proportions of lamina and non-lamina components. Increased growth rates of the whole plant during both

vegetative and reproductive stages have been reported as a response to rainfall during the summer (Speranza 2017).

Both Bahia grass and dallis grass are adapted and productive species with potential to improve forage availability, reduce pasture variability over time and avoid negative impacts on the cool season component of the pasture mixture (Costa and Scheffer-Basso 2003; Tejera et al. 2015). Irrigation is expected to improve warm season forage production. Most published results for warm season grasses report only cumulative seasonal values for whole plants and responses of different plant components to different levels of water availability are more limited. The aim of this research was to evaluate the responses of different production components under cutting of two C4 grasses subjected to different levels of water availability.

Materials and Methods

Pasture treatments, management and measurements

The study was carried out at the Mario A. Cassinoni Experimental Station of the Facultad de Agronomía, Universidad de la República, in Paysandú, Uruguay (32°23' S, 58°02' W). The experiment was located on a mild slope on soils classified as Cambisols, with a composition of 35.1 % sand, 29.7 % silt and 35.2 % clay (Altaminino et al. 1976). Rainfall shows an isohygric pattern throughout the year; average annual rainfall is 1,238 mm and mean annual temperature is 18.4 °C (Castaño et al. 2011). The experimental site had been planted previously with a mixture of *Dactylis glomerata* L. and *Medicago sativa* L. for 4 years. Plots were cleared and sown in February 2010 to dallis grass (*P. dilatatum* var. *dilatatum* Australian commercial seed) and Bahia grass (*P. notatum* var. *saurae* cultivar 'Pensacola') at a rate of 12 kg/ha. All plots were fertilized with 43.6 kg P/ha at seeding and received 5 applications per year of 50 kg N/ha, applied after each harvest during the experimental period.

The trial was established as a Randomized Complete Block Design with a split-plot treatment arrangement with 3 replications. The entire plot was irrigation level and sub-plots were the grasses (Bahia grass and dallis grass). Each individual experimental unit was 10 m². Treatments consisted of three levels of water availability: no irrigation (NIR), irrigation to 50 % of reference evapotranspiration (50 % ET₀) and irrigation to 100 % of reference evapotranspiration (100 % ET₀). Irrigation was applied with fixed sprinklers with a flow of 70 l/h

during the night to avoid drift. The Penman-Monteith equation ([Allen et al. 2006](#)) was used to estimate evapotranspiration (ET), while values of temperature, humidity, radiation and wind speed were collected from an automatic weather station every 30 minutes. The time for irrigation was estimated by calculating water balance ([Thornthwaite and Mather 1955](#)), the desired level of soil moisture and intended ET for each treatment by providing differing numbers of irrigation events, applying 48 mm of water at each event. Data published by Jia et al. ([2009](#)) for Bahia grass were used to obtain the crop coefficient (kc) and applied to both species due to the lack of specific information for dallis grass and because no differences were expected between warm season grasses. Water balance was calculated ([Thornthwaite and Mather 1955](#)) showing the crop ET (ETC: potential without nutrition or disease limitations, kc=0.9). An approximation of ET (average kc=0.75) was used for the irrigation treatments to account for foliar recovery after harvests ([Jia et al. 2009](#)).

Samples were taken during the warm season from December 2010 to April 2011 (year 1) and November 2011 to April 2012 (year 2). Subperiods between harvests varied between 25 and 37 days to allow the pasture to reach a minimum height of 15 cm. Prior to the first evaluation, pastures were harvested at 5 cm stubble to homogenize the plots. Biomass was sampled from three 0.2 m × 0.5 m quadrats/plot cut to 5 cm stubble. Following sampling, remaining forage on each experimental unit was mowed to 5 cm stubble and removed. Samples were sorted into green lamina (lamina) and other components, including stems, inflorescences, dead material and leaf sheaths (pseudostem), in the laboratory, dried at 60 °C for 72 h and weighed. At the end of each experimental period, tiller density was determined using a 0.2 m × 0.5 m quadrat and tiller weight was measured by sampling 20 tillers/plot cut at ground level and dried at 60 °C until constant weight.

Statistical analysis

The response variables analyzed were total production, lamina and pseudostem production, tiller density and tiller weight by species. These variables were analyzed individually using ANOVA. By adjusting the split plot design per year for each subperiod and species, the accumulation rates of lamina and pseudostem were analyzed to determine the interaction between irrigation level and the corresponding subperiod effect. Cumulative yield for the 2 years or comparison between

species was not considered. Data were analyzed using GLM and means were compared by a Tukey test using a P-value 0.05 significance level. Prior to analysis, model assumptions (normal distribution, independence of errors and homogeneity of variances) were validated. Subperiods (repeated measurements in time on GLM) were also studied by an autocorrelation matrix of the 1st order to improve model fitness. The model used was:

$$Y_{ijk} = \mu + \tau_i + \beta_j + \delta_{ij} + (\tau\beta)_{ij} + P_k + (\tau P)_{ik} + \xi_{ijk}$$

where:

Y is the response variable;

μ is the general experimental mean;

τ is the effect of ith treatment;

β_j is the effect of jth block;

δ_{ij} is the random effect associated with observation Y_{ij} (error A);

$(\tau\beta)_{ij}$ is the effect of interaction between ith treatment and jth block;

P_k is effect of kth subperiod;

$(\tau P)_{ik}$ is the effect of interaction between ith treatment and kth subperiod; and

ξ_{ijk} is the random error associated with observation Y_{ijk} .

Statistical analysis was done using InfoStat ([Di Rienzo et al. 2018](#)).

Results

The analysis of rainfall and ET data by period shows potentially critical subperiods with lower rainfall and ET, as well as subperiods with a higher level of precipitation and lower ET (Figure 1). Subperiods 1 and 2 in 2010–2011 and 2011–2012 required more frequent irrigation to allow a greater ET rate in irrigated treatments associated with higher temperature (Figure 1). Comparing precipitation during the trial period with the historical trend (1980–2009) of average monthly precipitation ([Castaño et al. 2011](#)) (106 mm January, 125 mm February, 138 mm March, 159 mm April, 102 mm May, 68 mm June, 56 mm Jul, 55 mm August, 71 mm September, 121 mm October, 123 mm November, 113 mm December), the monthly distribution of rainfall was similar to the historical trends during both years under study and lower rainfall was observed only in the first half of the growing season (Figure 1).

Yields of lamina, pseudostem and lamina + pseudostem were not significantly different between irrigation treatments or species, except for Bahia grass in 2011–2012 with only the 50 % ETO producing significantly higher yields than NIR, both for lamina +

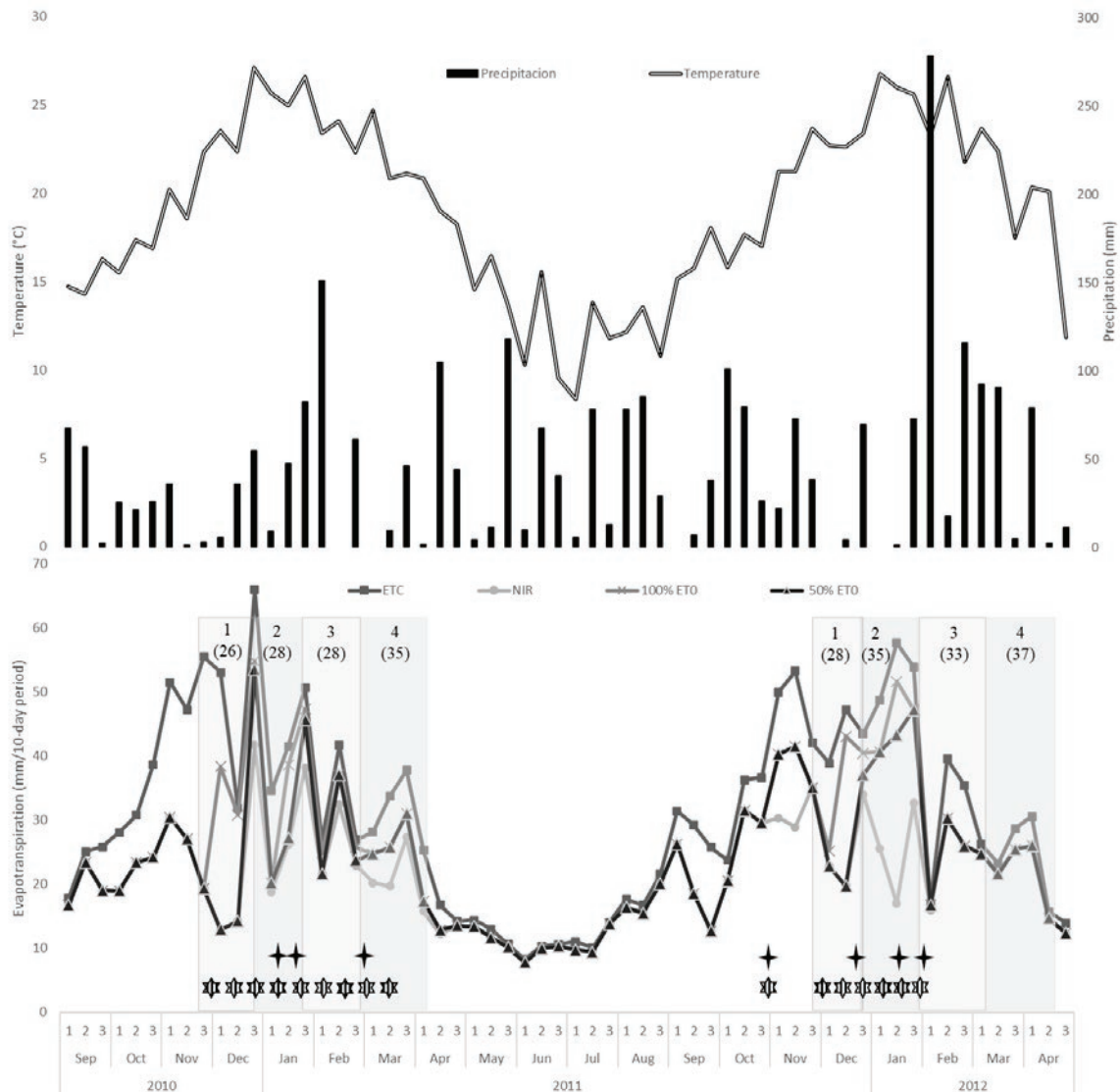


Figure 1. Precipitation, temperature, crop evapotranspiration (ETC) and actual evapotranspiration (ET) in the three irrigation treatments (100 % ET0, 50 % ET0 and NIR) from September 2010 until April 2012. 100 % ET0=irrigation to 100 % of evapotranspiration; 50 % ET0=irrigation to 50 % of evapotranspiration; NIR=no irrigation; ★=time of irrigation in 100 % ET0; ◆=time of irrigation in 50 % ET0. Four grey bands with numbers 1–4 and parenthesized values show subperiods 1–4 (length of regrowth periods following harvesting, days). Numbers 1, 2 and 3 above the months indicate the first, second and third 10-day periods (8 d in February and 11 d in October, December, January and March).

pseudostem and pseudostem (Table 1). For dallis grass, treatments were not significantly different in any season (Table 2). Both species were almost unresponsive to the irrigation treatments, showing a similar behavior in response to supplemental water. Production in 2011–2012 was much lower than in 2010–2011 due to a reduction in tiller population ($P < 0.001$), but no significant differences ($P > 0.05$) were found among treatments (Tables 1 and 2).

Production by subperiods showed significant differences in above-ground production for both species (Figures 2 and 3). For Bahia grass, differences in lamina production were detected among treatments in subperiod

2 in 2010–2011 (Figure 2). For dallis grass, differences in pseudostem production were detected for subperiod 2 in the first summer only (Figure 3). Irrigation increased production of both species ($P < 0.01$) in periods of high ET and radiation when water deficits took place (subperiod 2; Figure 1). However, there was no response to irrigation treatments towards the end of the summer (Figures 2 and 3). Components of aboveground production showed significant differences in dallis grass for the pseudostem component in 2010–2011 and the lamina fraction in 2011–2012 (Figure 3). Alternation in growth rate was observed with an earlier significant increase in the pseudostem

Table 1. Yields of lamina + pseudostem, lamina, pseudostem and density and weight of tillers in *Paspalum notatum* (Bahia grass) under different irrigation treatments (100 % ET0=irrigation to 100 % of evapotranspiration; 50 % ET0=irrigation to 50 % of evapotranspiration; NIR=no irrigation) in 2010–2011 and 2011–2012.

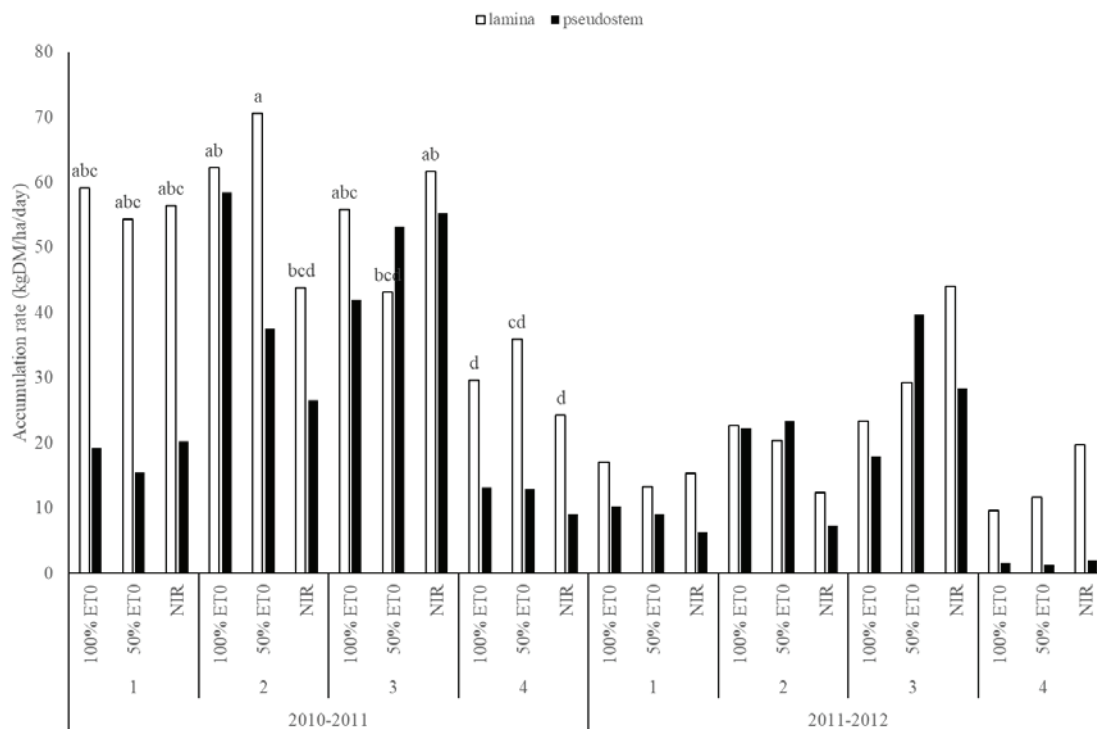
Year	2010–2011			2011–2012		
	100 % ET0	50 % ET0	NIR	100 % ET0	50 % ET0	NIR
Lamina + pseudostem (kg DM/ha)	9,653 ±390	9,253 ±662	8,402 ±251	4,968ab ±368	5,881a ±439	4,229b ±297
Lamina (kg DM/ha)	5,878 ±451	5,858 ±589	5,268 ±225	3,413 ±248	3,609 ±329	2,834 ±292
Pseudostem (kg DM/ha)	3,774 ±70	3,395 ±400	3,134 ±466	1,555ab ±188	2,236a ±234	1,395b ±51
Tiller density (number/m ²)	2,760 ±205	2,813 ±167	2,253 ±114	1,397 ±87	1,597 ±117	1,440 ±179
Tiller weight (g)	0.37 ±0.06	0.32 ±0.01	0.35 ±0.03	0.41 ±0.02	0.4 ±0.14	0.4 ±0.07

Values are mean ± s.e. Values with different letters within each variable and each year differ significantly (P < 0.05).

Table 2. Yields of lamina + pseudostem, lamina, pseudostem and density and weight of tillers in *Paspalum dilatatum* (dallis grass) under different irrigation treatments (100 % ET0=irrigation to 100 % of evapotranspiration; 50 % ET0=irrigation to 50 % of evapotranspiration; NIR=no irrigation) in 2010–2011 and 2011–2012.

Year	2010–2011			2011–2012		
	100 % ET0	50 % ET0	NIR	100 % ET0	50 % ET0	NIR
Lamina + pseudostem (kg DM/ha)	11,091 ±768	8,288 ±661	9,175 ±5,126	4,024 ±131	4,058 ±849	5,019 ±1,506
Lamina (kg DM/ha)	6,106 ±145	4,812 ±459	4,683 ±445	2,173 ±105	2,262 ±200	2,933 ±850
Pseudostem (kg DM/ha)	4,985 ±636	3,476 ±205	4,492 ±280	1,851 ±155	1,670 ±347	2,086 ±665
Tiller density (number/m ²)	2,720 ±144	2,813 ±127	2,613 ±127	743 ±140	990 ±201	1,020 ±140
Tiller weight (g)	0.33 ±0.06	0.32 ±0.05	1.08 ±0.73	0.62 ±0.06	0.48 ±0.17	0.62 ±0.06

Values are mean ± s.e.

**Figure 2.** Rates of lamina and pseudostem accumulation in *Paspalum notatum* (Bahia grass) in irrigation treatments (100 % ET0=irrigation to 100 % of evapotranspiration; 50 % ET0=irrigation to 50 % of evapotranspiration; NIR=no irrigation) and regrowth periods (subperiods 1–4) in 2010–2011 and 2011–2012. Values with different letters within each component and year differ significantly (P < 0.05).

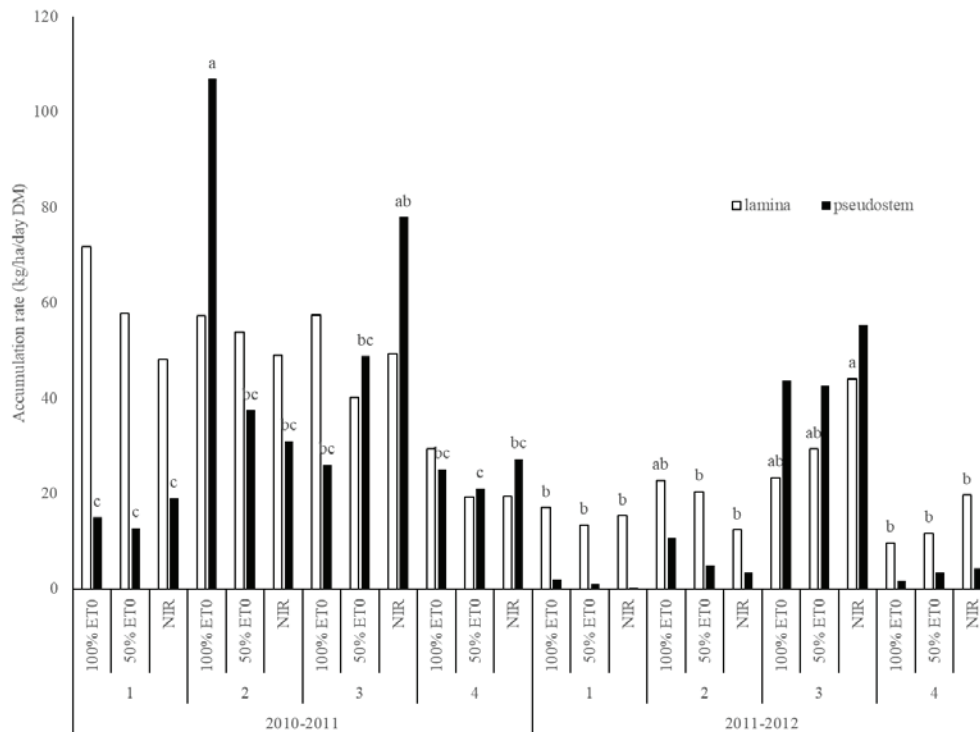


Figure 3. Rates of lamina and pseudostem accumulation in *Paspalum dilatatum* (dallis grass) in irrigation treatments (100 % ETO=irrigation to 100 % of evapotranspiration; 50 % ETO=irrigation to 50 % of evapotranspiration; NIR=no irrigation) and regrowth periods (subperiods 1–4) in 2010–2011 and 2011–2012. Values with different letters within each component and year differ significantly ($P < 0.05$).

component for the 100 % ETO (subperiod 2). For Bahia grass in 2010-2011, the lamina component was significantly lower than for 50 % ETO in subperiod 2 for NIR. The pseudostem component was not significantly reduced, with an amount proportional to the lamina accumulation rate, with increasing growth rate for NIR in subperiod 3.

Discussion

The addition of supplementary water did not produce a consistent and significant increase in forage production for the species studied. One of the possible reasons may be that both grasses are drought-tolerant and the distribution and volume of precipitation did not result in frequent or extended periods of moisture stress (Pezzopane et al. 2017). Total forage production of dallis grass was not affected by supplementary water at any time during the growing season, while the effects on Bahia grass were significant in the second growing season. The same absence of overall response has been reported before for several warm season perennials. Rodrigues et al. (2005) did not find a response when evaluating *Cynodon* sp. under 3 water management conditions in the Brazilian tropical zone and Forde et al. (1976) found no response in dallis grass during 2 summers with below-average precipitation

under similar climatic conditions. Owensby et al. (1970) found no responses in forage production when comparing addition of nitrogen + moisture with nitrogen only to a number of warm season grasses in Manhattan, Kansas. Mislevy and Everett (1981) even reported a negative response to supplementary irrigation on warm season grasses and found that non-irrigated plants accumulated nutrients more efficiently. Bahia grass and dallis grass maintained effective nutrient and water uptake under low soil moisture through the development and penetration of roots into the soil (Ogata et al. 1985), although Pezzopane et al. (2017) reported these species can exhibit different strategies of response and potential adaptation during water stress conditions.

One factor that is not always considered when measuring the response of perennial warm season grasses to irrigation is the phenological state of the plant when moisture stress occurs. A comparison between Bahia grass and *P. urvillei*, a member of the Dilatata group and a close relative of dallis grass (Vaio et al. 2019), showed that these species were very different in terms of phenological synchronization among tillers through the growing season. *P. urvillei* showed concentrated flowering earlier in the season (Lopes and Franke 2011) consistent with the curves reported for dallis grass (González-Barríos et al.

2016). Synchronization in the phenological stage of the tillers can be approximated by recording the development of lamina and non-lamina components (including pseudostem and inflorescence) because only tillers induced to the reproductive stage will elongate stems and internodes, for both dallis grass (Costa and Scheffer-Basso 2003) and Bahia grass (Beaty et al. 1968).

A considerable degree of phenological synchronicity among tillers is expected in dallis grass (González-Barrios et al. 2016). Plants of dallis grass clipped at different phenological stages can vary substantially in forage production and levels of reserves (Pereira et al. 1978). Under field conditions, the availability of moisture when the leaf/sheath ratio is low (reproductive stage) may not be translated into a higher growth rate, while for plants which received the same precipitation in a vegetative stage, production increased more than twofold in dallis grass (Speranza 2017). At least for some warm-season species which show several relatively synchronic reproductive phases during the growing season, the efficiency of using supplementary water for forage production may be highly dependent on the general phenological stage of the plants.

For Bahia grass, differences in yield among treatments affected both lamina and pseudostem in relatively similar ways. The peaks of pseudostem production observed in dallis grass were not found in Bahia grass (Adjei et al. 1992), which is explained by the different phenological behavior of the two species. Owing to differences in plant architecture (Lopes and Franke 2011), variations in the non-lamina components in Bahia grass cannot be attributed to flowering as in dallis grass. The most noteworthy result obtained with Bahia grass was the observed trend of intermediate irrigation levels outperforming higher irrigation, which deserves further study. Lack of response to different moisture levels in root development of Bahia grass has been also reported, although moderate deficits may even promote underground growth (Sinclair et al. 2011).

The data show that the phenological composition was affected by irrigation. However, it is necessary to understand how moisture deficit is related to the onset of each flowering cycle. Our results also suggest that there may be negative effects of the highest irrigation treatments. In the second growing season, when tiller densities were lower, subperiods were observed in which the addition of water at 100 % ET₀ tended to depress growth rates for both species. In the case of Bahia grass, plots under intermediate levels of irrigation tended to outperform those at higher irrigation levels. This detrimental effect of higher irrigation levels has been observed before and has been attributed to leaching or changes in the use of

nutrients (Mislevy and Everett 1981; Marais et al. 2006). These species have been shown to maintain effective nutrient and water uptake at low soil moisture levels through the development and penetration of roots in plants under water stress (Ogata et al. 1985), which may also explain the lack of response to higher irrigation. This trend should be specifically addressed in future research by specifically recording performance using intermediate levels of water replenishment.

Conclusions

This research confirmed that phenological composition of forage from Bahia grass and dallis grass was indeed affected by irrigation, although not directly reflected in an increase in annual dry matter yield. Instead, effects of irrigation depended on the species and were mostly observed during specific periods of the growth cycle. Forage production of Bahia grass and dallis grass during summer may not be as directly related to the availability of water as expected, even under significant moisture stress. Although different in growth habit and morphology, both species showed changes in the distribution of dry matter accumulation among the different aboveground structures in response to watering, not always resulting in an increase in yield. Further research to examine moisture deficit in these species should take into account plant phenology as well as the compensating mechanisms of each species and responses to intermediate levels of irrigation.

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(Note of the editors: All hyperlinks were verified 5 January 2023).

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Artículo Científico

Efecto de diferentes niveles de cobertura vegetal en la germinación, sobrevivencia y establecimiento de *Tithonia diversifolia* (Hemsl.) A. Gray. vc ICACUBA Oc-10 sembrada con semilla gámica

Effect of different mulch levels on germination, survival, and establishment of Tithonia diversifolia (Hemsl.) A. Gray. vc ICACUBA Oc-10, planted with gamic seeds under field conditions

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Resumen

Se estudiaron niveles de cobertura vegetal en la germinación, sobrevivencia y establecimiento por semilla gámica de tithonia. Se usó un diseño de bloques al azar con 5 réplicas. Los tratamientos consistieron en cubrir las semillas con 0, 0.250, 0.338 y 0.500 kg/m² de cobertura vegetal. A los 12, 34, 54 y 78 días después de la siembra el porcentaje de germinación, número de hijos y hojas/planta, altura, diámetro del tallo fueron significativamente superiores cuando se aplicó cobertura vegetal con relación al control. La germinación se incrementó hasta un 29 % con el nivel de 0.500 kg/m² de cobertura vegetal y fue menor ($p < 0.05$) en el control. El rendimiento 6.43–10.87 t MS/ha en el primer corte se incrementó significativamente con los niveles de cobertura vegetal. En el tercero, los mejores ($p < 0.05$) rendimientos 5.6–6.0 t MS/ha se lograron con 0.338 y 0.500 kg/m² de cobertura vegetal. Se concluye que es necesario cubrir la semilla con cobertura vegetal de residuos vegetales a razón de 0.338–0.500 kg/m² para asegurar la germinación, supervivencia y desarrollo de las plantas e incrementar la producción de biomasa.

Palabras clave: Arbustos, niveles de residuos vegetales, siembra, rendimientos.

Abstract

Different mulch levels were studied for establishing tithonia using gamic seeds. A randomized complete block design with 5 replications was used. The treatments included covering the seeds with 0, 0.250, 0.338 and 0.500 kg/m² of African star grass dry residues. The germination percentage was lower ($P < 0.05$) for the control treatment, at 12 and 30 days after sowing and increased up to 29 % when 0.500 kg/m² of mulch was applied. In the first stages after sowing (12, 34, 54 and 78 days) the percentage of germination, number of tillers and leaves/plant, height and stem diameter were significantly higher for the mulch treatments. Forage yield in the first harvest increased significantly ($P < 0.05$) with mulch levels, from 6.43 to 10.87 t DM/ha. In the third harvest, the best ($P < 0.05$) yields (5.6-6.0 t DM/ha) were obtained with 0.338 and 0.500 kg of mulch/m². It is concluded that it is necessary to cover tithonia seeds with mulch of vegetable residues at a rate of 0.338–0.500 kg/m², to ensure proper germination, survival and development of the plants, and higher biomass yields.

Keywords: Levels of plant residues, shrubs, sowing, yields.

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Introducción

Observaciones de campo han evidenciado que la propagación de *T. diversifolia* ocurre principalmente por vía asexual (Ramírez 2008), mediante el uso de estacas, que son fáciles de adquirir de otras áreas de tithonia, pero cabe resaltar que este tipo de propagación presenta algunas dificultades como el transporte y almacenamiento que solo se puede realizar durante periodos cortos, sin afectar la calidad de las estacas (Sánchez et al. 2014). Entre las principales limitantes para la expansión del botón de oro como arbusto para ramoneo y forraje (Ríos Kato 1999; Romero et al. 2014) se destacan la baja oferta de material asexual (estacas) y el elevado costo de las mismas, para iniciar la plantación.

Por otra parte, la reproducción sexual sigue siendo escasa o casi nula (Ríos Kato 1999; Romero et al. 2014), aunque en los últimos años algunos productores e investigadores han hecho observaciones casuales de la reproducción del botón de oro a partir de semilla sexual, principalmente para evaluar la posibilidad de obtener una mejor germinación de la semilla gámica (Saavedra 2016; Padilla et al. 2018; Mattar et al. 2019; Rodríguez et al. 2019). En cuanto a las siembras por la vía gámica, los principales logros se limitan fundamentalmente a esparcir inflorescencias maduras en sustratos orgánicos y después de germinadas las semillas, las plántulas se trasplantan a bolsas o directamente al campo (Gallego-Castro 2016; Gallego-Castro et al. 2017). Esta tecnología exige cumplir las más estrictas medidas de protección de las plántulas germinadas hasta su trasplante (Solarte et al. 2013). A ello se une la ausencia de una tecnología comercial de producción de semilla científicamente avalada desde el punto de vista técnico económico que propicie su disponibilidad en el mercado.

A lo anterior se une que el productor primario aún no dispone de una metodología documentada científicamente, que se integre en un paquete tecnológico para la producción de semilla, siembra y establecimiento por vía gámica de la tithonia. Por otra parte, es casi nula la disponibilidad de resultados de investigación acerca del cómo lograr siembra directa de esta especie usando semilla botánica. Trabajos recientes de Padilla et al. (2020a) mostraron avances sobre la posibilidad real de lograr resultados satisfactorios en el establecimiento cuando se emplearon diferentes prácticas de protección de la semilla gámica de tithonia en un suelo Ferralítico Rojo Éutrico.

Teniendo en cuenta lo anterior el objetivo de este trabajo fue la evaluación de diferentes volúmenes

de rastrojos vegetales (cobertura vegetal) en la germinación, sobrevivencia y establecimiento por semilla gámica de *Tithonia diversifolia* vc ICACUBA Oc-10 en condiciones de campo.

Materiales y Métodos

La investigación se realizó en el Centro Experimental de Pastos y Forrajes 'Miguel Sistachs Naya' del Instituto de Ciencia Animal, ubicada en el municipio de San José de las Lajas, provincia de Mayabeque, Cuba, situada a los 23°55' LN y a los 82°0' LW a 92 msnm. El tipo de suelo es el Ferralítico Rojo Éutrico, de rápida desecación, arcilloso y profundo sobre calizas (Hernández et al. 2015). Los datos climáticos de la región aparecen en la Figura 1.

Se usó un diseño de bloques al azar con 5 réplicas. La unidad experimental fue de 5 × 4 m. Los tratamientos consistieron: T1=Control, sin cubrir la semilla, T2=Cubrir las semillas 0.250 kg/m², T3=Cubrir las semillas con 0.338 kg/m², T4=Cubrir las semillas con 0.500 kg/m² de cobertura vegetal. Para lo cual se utilizó residuos secos (75 % MS) de forraje de pasto estrella (*Cynodon nlemfluensis*) que no produce semilla fértil.

La semilla utilizada se recolectó en el mismo Centro Experimental, según el mejor momento de cosecha determinado por Padilla et al. (2018) y Padilla et al. (2020b), para esta especie. Posterior a la cosecha, las semillas llenas fueron separadas por un equipo de aire forzado (Modelo CB2001 Marca OEM, Inc) y la germinación del lote en condiciones de laboratorio fue 50 %.

Procedimiento experimental

La labranza se realizó con preparación convencional del suelo, consistente en aradura y cruce con pase de grada media alterna. La siembra se realizó por semilla gámica el 15 de junio de 2018, en surcos de 15 cm de profundidad separados a 80 cm entre hileras. Las semillas se depositaron en el fondo del surco, separados a 1.0 m entre golpes y se apisonaron con el pie para garantizar su contacto con el suelo y evitar que se arrastren con la lluvia. Se sembraron 10 semillas/golpe. Después de colocadas las semillas en el fondo de surco y asentadas con el pie, se cubrieron con una cobertura vegetal 0.250, 0.338 y 0.500 kg/m² de heno de pasto estrella (*Cynodon nlemfluensis*) que contenía un 75 % de MS.

Las parcelas no se regaron, pero se aplicó fertilizante a razón de 250 kg/ha un mes después de la siembra y 350 kg/ha en el segundo corte, con fórmula 13-9-17 para N, P y K, respectivamente. La fertilización se realizó de

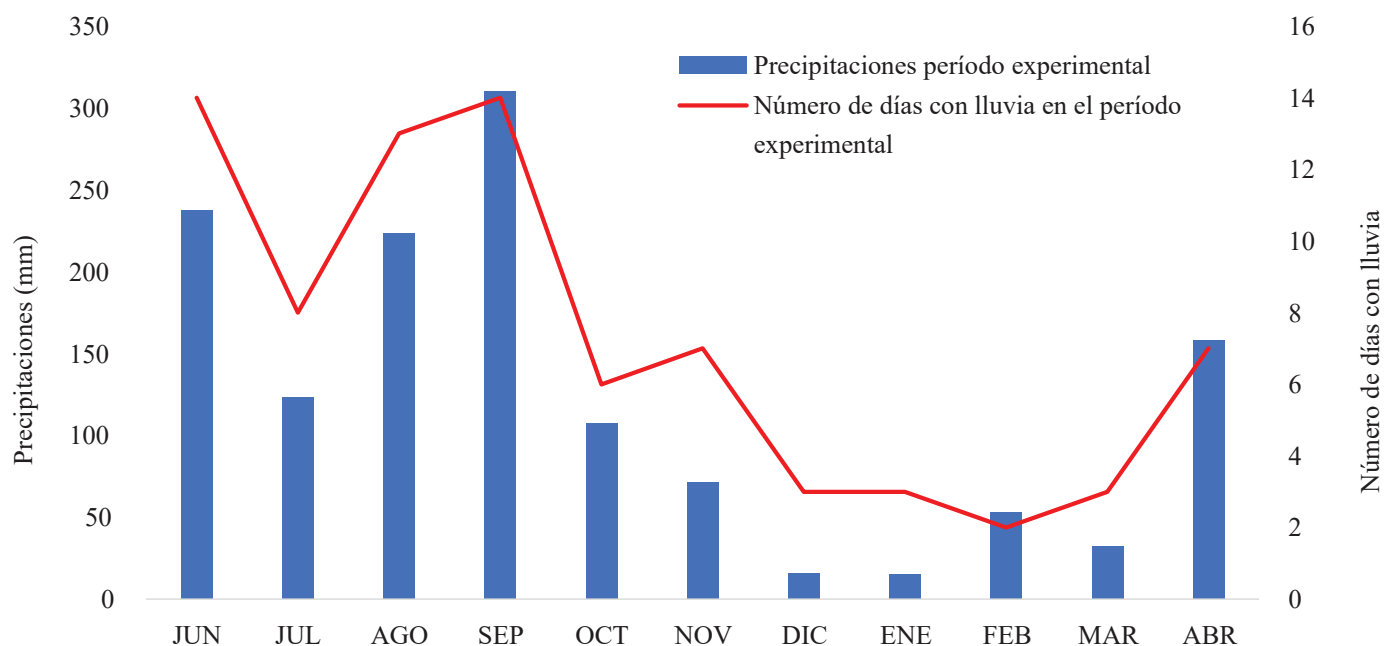


Figura 1. Datos climáticos de la región experimental (2018-2019).

forma localizada evitando el contacto con las hojas. En el tercer corte no se aplicó fertilizante. La germinación de las semillas se midió a los 12 y 34 días después de realizada la siembra. El porcentaje de germinación se calculó a partir de 250 semillas que se sembraron por parcela, replicadas cinco veces por tratamiento.

Se realizaron muestreos a los 12, 34, 51 y 78 días posteriores a la siembra, las variables evaluadas fueron el número de plantas/m², diámetro del tallo (cm), altura (cm) y número de hojas/tallo en 10 plantas por parcela. El rendimiento de MV y MS en t/ha se determinó en los tres cortes evaluados cortando el área total de las parcelas (20 m²). El primer corte de establecimiento se realizó a los 120 días después de la siembra, el segundo a 120 días del primero y el tercero a 90 días del segundo. Los dos últimos ocurrieron en el período poco lluvioso.

Se probaron los supuestos teóricos del análisis de varianza para las variables originales número de hijos/plantas, número de hojas/plantas, población (plantas/m²) y número de tallos/m² de *T. diversifolia*. Para la normalidad de los errores, se utilizó la dócima de Shapiro-Wilk (1965), y la homogeneidad de varianza, por la dócima de Levene (1960). Posteriormente, se aplicó la transformación \sqrt{x} , para las variables anteriormente analizadas, sin embargo, esta no mejoró el cumplimiento de dichos supuestos, por lo que se realizó análisis de varianza no paramétrico, de dos clasificaciones por rango de Friedman. Para la comparación de los rangos medios se utilizó la dócima de Conover (1999) para $p < 0.05$.

Las variables, porcentaje de germinación, altura de la planta, diámetro del tallo y rendimiento, se analizaron con el empleo de un análisis de varianza, según diseño de bloques al azar. Para la comparación de medias se aplicó la dócima de Duncan (1955) para $p < 0.05$ en los casos necesarios. Todos los datos se procesaron en el paquete estadístico INFOSTAT versión 2012 (Di Rienzo et al. 2012).

Resultados

El porcentaje de germinación a los 12 y 34 días después de la siembra fue menor ($p < 0.05$) para el tratamiento de siembra sin cobertura de la semilla. En general hubo un incremento lineal del porcentaje de germinación en el campo con el aumento de los niveles de cobertura vegetal (Figura 2 y 3).

Los niveles de cobertura vegetal utilizados a los 51 días después de la siembra no afectaron el número de hijos/planta, el cual osciló entre 4.36 y 5.80. A los 78 días, el mayor número de hijos/planta ($p < 0.05$) fue cuando se aplicó 0.500 kg/m² de cobertura vegetal, sin diferir del tratamiento de 0.338 kg/m², y se obtuvo el menor resultado en el control. La variable número de hojas/planta a los 51 y 78 días, fue superior ($p < 0.05$) en todos los niveles de cobertura vegetal utilizados respecto al control, sin diferencias entre estos (Cuadro 1).

A los 12 días después de la siembra la menor población (No. de plantas/m²) fue en el control ($p < 0.05$) y similar entre los tratamientos que se cubrieron con diferentes niveles de cobertura vegetal. No se detectaron diferencias

significativas entre los tratamientos para este indicador en los muestreos realizados a los 34, 51 y 78 días (Cuadro 2).

La menor altura de las plantas, después de la siembra, se obtuvo en el control, con respecto a los tratamientos en que se aplicó cobertura vegetal (Figura 4). A los 78 días, en todos los tratamientos la altura fue superior respecto a los 34 y 51 días. El diámetro del tallo fue significativamente mejor ($p<0.05$) cuando se utilizó cobertura vegetal a los 51 días después de la siembra con relación al control; sin embargo, no se detectaron diferencias entre tratamientos al aplicar esta labor a los 78 días.

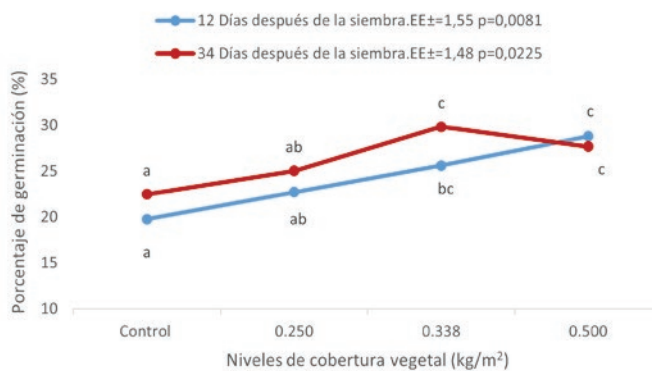


Figura 2. Efecto de los niveles de cobertura vegetal en el porcentaje de germinación de las semillas gámica de *T. diversifolia* establecida directamente en el campo. ^{abc}=medias con letras distintas difieren a $P<0.05$ (Duncan 1955)

El número de tallos/m² en el primer corte fue similar en todos los tratamientos, en el segundo fue menor ($p<0.05$) en el control y las mayores poblaciones se lograron cuando se aplicaron 0.338 y 0.500 kg/m² de cobertura vegetal (Cuadro 3). En el tercero, el mayor número de tallos/m² ($p<0.05$) fue cuando se aplicó 0.338 kg/m². Sin embargo, en la variable número de hojas/planta se determinó un comportamiento significativo ($P=0.0320$) en el primer corte, con una mayor respuesta con los niveles de cobertura vegetal de 0.250 y 0.338 kg/m², sin diferir del tratamiento 0.500 kg/m² (Cuadro 3).



Figura 3. Vista de la utilización de cobertura vegetal en la siembra directa de semilla gámica.

Cuadro 1. Efecto del nivel de cobertura vegetal utilizado en el establecimiento de *T. diversifolia* en el número de hijos y hojas/planta a los 51 y 78 días después de la siembra.

Momento después de la siembra (días)	Control	Niveles de cobertura vegetal (kg/m ²)			EE± P
		0.250	0.338	0.500	
Número de hijos/plantas					
51	2.16 (4.36) DE=2.46 1.76 a	2.42 (4.68) DE=1.93 2.42 b	2.86 (5.80) DE=2.93 2.72 bc	2.56 (5.00) DE=2.08 3.10 c	0.2489
78	(2.64) DE=2.56	(5.28) DE=3.70	(5.60) DE=2.57	(6.72) DE=3.22	0.0010
Número de hojas/plantas					
51	1.24 a (7.08) DE=1.49	2.90 b (11.32) DE=2.33	2.74 b (11.38) DE=2.37	3.12 b (12.51) DE=2.79	<0.0001
78	1.66 a (12.52) DE=1.71	2.60 b (14.24) DE=2.63	2.98 b (15.08) DE=2.78	2.76 b (14.40) DE=3.24	0.0004

^{a,b,c}=Rangos medios con letra distintas por filas difieren para $P<0.05$, Conover (1999).

Datos entre paréntesis indica la mediana.

DE=Desviación estándar.

La altura de la planta fue mayor ($p<0.05$) con el nivel de cobertura vegetal de 0.500 kg/m^2 en el primer corte, en el segundo fue similar en todos los tratamientos y en el tercero fue mejor en el nivel 0.338 kg/m^2 , sin diferencias con el control y 0.250 kg/m^2 . La menor altura se produjo con 0.500 kg/m^2 que, a la vez, no difirió significativamente de cuando se aplicó 0.250 kg/m^2 de cobertura vegetal (Figura 5).

El diámetro del tallo en el primer corte (Figura 5) fue mayor ($p<0.05$) cuando se cubrió la semilla 0.500 kg/m^2 , sin diferencias con el nivel de 0.338 kg/m^2 ; a la

vez este último fue similar al resto de los tratamientos. El diámetro del tallo en el segundo y tercer corte fue similar en todos los tratamientos.

El rendimiento t MS/ha (Figura 6) en la fase de establecimiento en el primer corte se incrementó significativamente ($p<0.05$) en la medida que se elevaron los niveles de cobertura vegetal. En el tercero, los mejores ($p<0.05$) rendimientos se lograron cuando se aplicaron 0.338 y 0.500 kg/m^2 de cobertura vegetal. En el segundo corte los rendimientos fueron similares.

Cuadro 2. Comportamiento de la población (plantas/m²) de *T. diversifolia*, en cuatro momentos después de la siembra, con la utilización de diferentes niveles de cobertura vegetal.

Momento después de la siembra (días)	Control	Niveles de cobertura vegetal (kg/m ²)			EE± P
		0.250	0.338	0.500	
12	1.82 ^a (9.88) DE=2.92	2.68 ^b (12.84) DE=4.73	2.44 ^{ab} (11.36) DE=4.19	3.06 ^b (14.40) DE=4.29	0.0042
34	1.92 (11.52) DE=4.33	2.72 (14.92) DE=4.38	2.72 (13.56) DE=5.16	2.64 (14.32) DE=4.25	0.0700
51	2.04 (11.56) DE=4.70	2.76 (15.48) DE=4.82	2.56 (13.08) DE=4.67	2.64 (14.44) DE=4.28	0.2037
78	2.14 (11.52) DE=4.98	2.64 (13.40) DE=3.69	2.38 (12.04) DE=4.00	2.84 (13.16) DE=3.54	0.2336

^{a,b}=Rangos medios con letra distintas por filas difieren para $P<0.05$, Conover (1999). Datos entre paréntesis indica la mediana. DE=Desviación estándar.

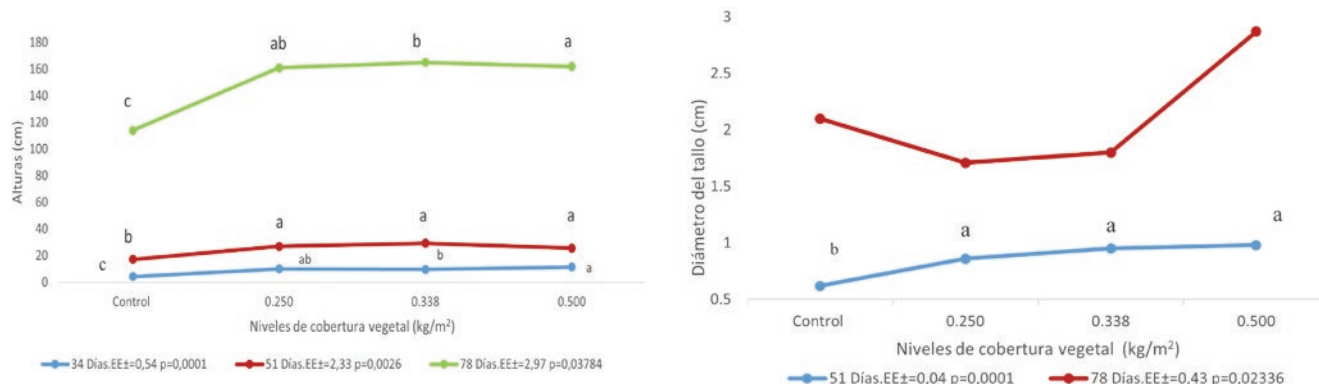


Figura 4. Comportamiento de la altura y el diámetro del tallo de las plantas de *T. diversifolia* en diferentes momentos después de la siembra con la aplicación de niveles de cobertura vegetal. ^{abc}: medias con letras distintas difieren a $P<0.05$ (Duncan 1955)

Cuadro 3. Comportamiento de la población (tallos/m²) y el número de hojas/plantas en los diferentes cortes.

Cortes	Control	Niveles de cobertura vegetal (kg/m ²)			EE± P	
		0.250	0.338	0.500		
Población de tallos/m ²	1	8.40	7.80	7.20	7.60	0.30 0.0905
	2	12.40 ^a	14.40 ^b	15.20 ^c	15.20 ^c	0.23 <0.0001
	3 [#]	2.20 ^{ab} (19.60) DE=0.89	1.80 ^a (19.20) DE=0.45	4.00 ^c (21.60) DE=0.89	2.00 ^{ab} (19.40) DE=0.55	0.0004
Número de hojas/planta	1	28.40 ^b	35.80 ^a	34.40 ^a	33.20 ^{ab}	1.59 0.0320
	2	19.60	16.20	16.80	18.80	0.99 0.0964
	3 [#]	3.40 (17.00) DE=2.12	2.60 (15.20) DE=1.64	1.60 (14.00) DE=1.22	2.00 ^{ab} (19.40) DE=0.55	0.1300

^{a,b,c}=Rangos medios con letra distintas por filas difieren para P<0.05, Conover (1999). Datos entre paréntesis indica la mediana. DE=Desviación estándar; [#]=No cumplió con los supuestos del ANAVA, por lo que fue necesario transformar

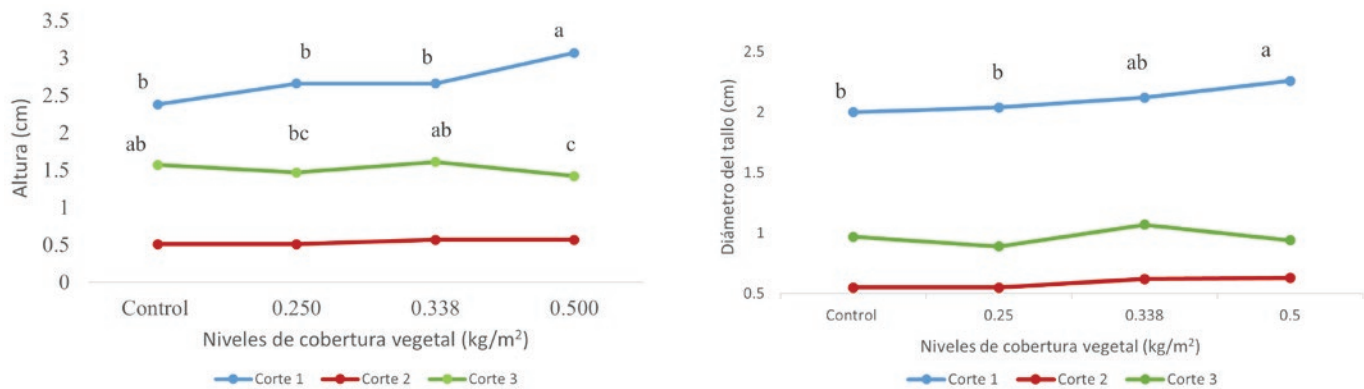


Figura 5. Comportamiento de la altura y el diámetro del tallo de las plantas de *T. diversifolia* en los diferentes cortes con la aplicación o no de niveles de cobertura vegetal. ^{abc}=medias con letras distintas difieren a P<0.05 (Duncan 1955).

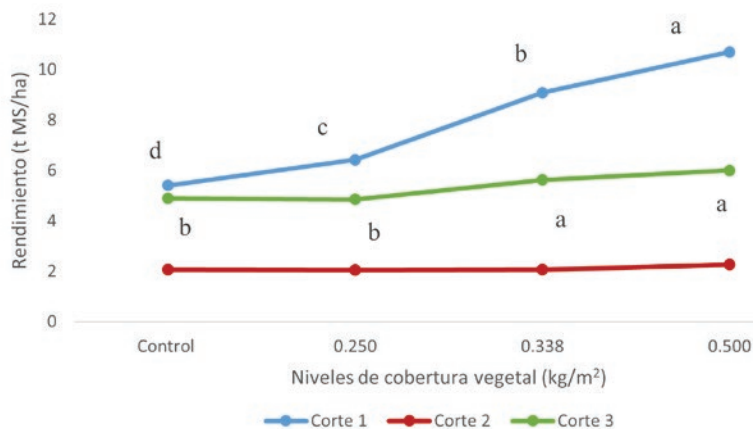


Figura 6. Rendimiento de *T. diversifolia* establecida con la aplicación o no de niveles de cobertura vegetal en el momento de la siembra. ^{abc}=medias con letras distintas difieren a P<0.05 (Duncan 1955).

Discusión

El hecho que con el empleo de mayores niveles de cobertura vegetal las semillas logran los mayores valores de germinación: 28,8 % (con 0.500 kg/m²) y 29,84 % (con 0.388 kg/m²) a los 12 días y 34 días después de germinación, respectivamente, sin diferencias significativas entre ambos tratamientos, avala los beneficios de esta práctica para el establecimiento de la tithonia. El hecho que el 58 % de las semillas sembradas en campo lograron germinar en solo 12 días, comparado con el potencial de estas en el laboratorio, es un buen indicador que la cobertura vegetal vigoriza la hipótesis que es posible establecer el botón de oro usando semilla gámica.

En este sentido Obukohwo y Olayinka (2015) al estudiar el porcentaje de germinación en placas de Petri con sustratos de algodón y macetas con suelo encontraron que la semillas germinaron mejor cuando fueron sembradas en un sustrato de tierra, además en ese mismo estudio informaron que hay diferencias entre tipos de suelos, logrando los mejores resultados para la altura, área foliar y número de hojas/planta en 12 semanas en un suelo sobre piedra caliza, intermedios en arcillosos y peores en el suelo arenoso. Lo cual demuestra el requerimiento de humedad en el suelo en la fase de establecimiento de la tithonia, y en lo cual contribuye el uso de la cobertura vegetal.

Como se demuestra en este estudio (Figura 1) la mayor germinación se produjo en los primeros 12 días, lo que lleva a inferir que la no repuesta a los niveles de cobertura vegetal en la etapa posterior se debe a que a partir de este momento la germinación es mínima, además que en este periodo comienza a producirse pérdidas de población por muertes de plántulas, por carencia de humedad en el suelo, sobre todo en aquellas semillas que no se cubrieron con una mínima capa de suelo y probablemente, por ataque de plagas y enfermedades que se producen en la fase inicial de establecimiento. Todo lo anterior puede ser debido a que se logró un equilibrio de la población, pues las plantas que sobrevivieron ya estaban en una mejor capacidad para competir en el agroecosistema que fueron introducidas.

Esta repuesta a la aplicación de cobertura vegetal hasta los 12 días después de la siembra puede estar dada por la capacidad que tiene las semillas de esta planta a lograr su mayor germinación en los primeros 10 días (Padilla et al. 2018; Padilla et al. 2020a; Rodríguez et al. 2019; Santos-Gally et al. 2019). Esto puede documentarse por el reconocido efecto beneficioso que provoca el empleo de cobertura vegetal en la germinación y sobrevivencia

de las plántulas en los primeros estudios de desarrollo cuando se realiza la siembra directa de las semillas en campo (Padilla et al. 2020a). La importancia de la protección de las siembras en condiciones de vivero para la obtención de plántulas para el trasplante es considerada como una vía necesaria para la propagación de esta especie por semilla agámica (Gallego-Castro 2015; Gallego-Castro 2016; Mahecha y Angulo 2017).

El éxito para un buen establecimiento de los pastos y forrajes en las áreas ganaderas del trópico va a depender en gran medida de la germinación y el desarrollo integral de las plantas en el periodo comprendido desde la emergencia al primer corte o pastoreo, así como garantizar precipitaciones abundantes y bien distribuidas, que unidas a las exigencias de temperatura y luz contribuyan a asegurar el mejor desarrollo de la especie sembrada para poder competir con las arvenses nativas por el nicho ecológico.

Los resultados de este estudio indican que cubrir las semillas con residuos vegetales secos propicia un mejor desarrollo de la planta íntegramente. Ello se materializó en un mejor comportamiento en los primeros estadios de las plántulas en los indicadores porcentaje de germinación, número de hijos y hojas/planta, altura, diámetro del tallo y población (Cuadro 1 y 2, Figura 2 y 3).

El comportamiento exitoso de los anteriores componentes del rendimiento estuvo favorecido por la protección que producen la cobertura vegetal en semillas pequeñas de escasas reservas y las plántulas, resguardando a éstas de la deshidratación por las altas intensidades lumínicas, temperaturas y el viento, así como el arrastre provocado por la escorrentía del agua de lluvias. Estos factores que en condiciones extremas pueden provocar la muerte de la especie que queremos establecer en los agroecosistemas ganaderos tropicales, por ello es aún más importante esta práctica pues favorece crear un mejor nicho ecológico que beneficia a la especie sembrada a rivalizar con las arvenses por agua, luz y nutriente y que esta competencia no sea excluyente para la especie deseada.

En los tres cortes realizados en la fase de establecimiento, el comportamiento de los indicadores población, altura, diámetro del tallo y el número de hojas e hijos/planta también evidenció el efecto beneficioso de la aplicación de cobertura vegetal en el mejor comportamiento de estos componentes del rendimiento, perdurando las mejoras producidas en los primeros estadios de desarrollo antes de someterse al corte. Estos resultados reafirman los beneficios del empleo de cobertura vegetal en el establecimiento de esta planta reportado por Padilla

et al. (2020b) en condiciones de suelo y clima similares, cuando las semillas son sembradas directamente en el suelo en condiciones de campo.

La no respuesta de algunos indicadores como número de hojas e hijos/planta, diámetro del tallo y altura de la planta en el segundo corte fue motivada porque el crecimiento de la planta ocurrió en el periodo diciembre-febrero que es cuando el período poco lluvioso es más crítico en el área experimental (Figura 1). En esta fecha es donde ocurren las menores precipitaciones y número de días con lluvias. Según Herrera et al. (2016) y Herrera et al. (2018) en este periodo climático, en esta misma región, es donde se producen las menores intensidades de luz y temperatura, lo cual pudo provocar que las plantas no pudieran expresar su potencial a los beneficios que induce el empleo de cobertura vegetal (Figura 5).

Rendimientos alcanzados del orden de 6-11 t MS / ha en el primer corte y de 4.86-6 t MS /ha en el tercero, en siembras directas de tithonia usando semilla gámica, resultan altos y novedosos, lo cual justifica que el momento óptimo de siembra debe coincidir con el inicio del período lluvioso de modo que se garantice precipitaciones abundantes y bien distribuidas, además que en ese período ocurren intensidades lumínicas, horas luz y temperatura que favorecen a que las plantas tropicales expresen su máximo potencial en la producción de biomasa (Herrera et al. 2016; Herrera et al. 2018). La correcta fertilización con fórmula completa y amplios intervalos entre cortes, en esta fase de establecimiento, también ayudaron a los altos rendimientos logrados. Este manejo agronómico resulta primordial para que las siembras realizadas alcancen satisfactorios establecimientos y sobre todo por la influencia que tiene este manejo en la vida útil y productiva de los pastizales.

La tithonia es reconocida y estudiada como una planta invasora por algunos investigadores (Muoghalu y Chuba 2005; Agboola et al. 2014). Sin embargo, resulta paradójico que no se había encontrado métodos de propagación por la vía gámica sembrando las semillas directamente en el suelo, pues las plantas ocupantes son capaces de desplazar otras especies por su capacidad de imponerse en los agroecosistemas. Si bien es cierto que no se disponía de reportes anteriores a Padilla et al. (2020b) sobre el establecimiento de botón de oro usando esta vía de propagación, los resultados de estos dos estudios indican que es posible alcanzar altos volúmenes de biomasa en la etapa de establecimiento, superiores a los reportados por Ruíz et al. (2012) en condiciones de clima y suelo similares y con semilla vegetativa, quienes lograron rendimientos entre 1.70–0.85 t MS/ha/corte

en el periodo lluvioso y poco lluvioso respectivamente, cuando se cortó a 15 cm de altura a los 80 días siendo éste un período inferior a nuestra evaluación. En otros trabajos de González et al. (2013) lograron rendimientos del orden de 10.0–13.0 t MS ha/año cuando plantó la sección basal y media de tallos de botón de oro. Aunque estas diferencias de producción de biomasa pudieran estar dadas porque dichos investigadores no emplearon fertilizantes mientras que en este estudio se aplicaron niveles de fertilización adecuados e intervalos de corte mayores, que unido al vigor juvenil que se ocasiona en la etapa de establecimiento, la tithonia logró expresar mejor su potencial forrajero.

Los resultados de este estudio sobre el establecimiento de tithonia y los reportados por Padilla et al. (2020a) confirman la posibilidad técnica de propagar el botón de oro por semilla gámica realizando la siembra directa de la semilla en campo. Si a esto se une otras investigaciones realizadas en la producción de semilla realizada por este grupo de trabajo en el Departamento de Pastos del Instituto de Ciencia Animal, algunos ya publicados (Padilla et al. 2018; Ruíz et al. 2018; Rodríguez et al. 2019; Padilla et al. 2020b) y otros en proceso de publicación, darán una solución a la producción y siembra por semilla gámica de tithonia en condiciones de campo. Ello pondrá a disposición de productores, técnicos y directivos una nueva opción para el establecimiento de esta especie por vía gámica, que es más factible desde el punto de vista técnico-económico.

Se concluye que es necesario cubrir la semilla de *Tithonia diversifolia* con residuos vegetales a razón de 0.338–0.500 kg/m² para asegurar la germinación, supervivencia y desarrollo de las plantas e incrementar la producción de biomasa. Se recomienda integrar en una guía técnica sobre producción de semilla de tithonia, estos resultados y otros obtenidos previamente, para extenderlos a una mayor escala productiva.

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Research Paper

Effects of grazing intensity and pasture type on soil organic carbon stock in the semi-arid tropics of India

Efectos de la intensidad del pastoreo y el tipo de pastura en las reservas de carbono orgánico del suelo en los trópicos semiáridos de la India

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Abstract

Pastures may act as carbon sources and sinks depending on grazing pressure and management practices. Soil organic carbon (SOC) stock and its fractions were quantified under 3 different grazing intensities using 5, 10 and 15 sheep/ha under sown, improved and natural pastures in the semi-arid tropics of India. Results revealed that after 3 years, improved pasture had significantly higher particulate organic carbon (POC~4.5 g/kg), SOC (~0.53 %), total organic carbon (TOC~7 g/kg) and SOC stock (~15 mg/ha) as compared with sown and natural pastures. Labile carbon (LC ~185 mg/kg) and soil microbial biomass carbon (SMBC ~378 µg/g soil) were higher under natural pasture. A moderate grazing intensity of 10 sheep/ha resulted in significantly greater carbon fractions, TOC and SOC stock. SOC stock and its fractions were significantly higher in the topsoil layers as compared with the subsoil layers. These results indicate that improved pasture management practices with moderate grazing intensity can be recommended for improving SOC stock and its fractions in semi-arid tropical pastures.

Keywords: Carbon fractions, grazing pressure, improved pasture.

Resumen

Los pastos pueden actuar como fuentes y sumideros de carbono según la presión del pastoreo y las prácticas de manejo. El stock de carbono orgánico del suelo (SOC) y sus fracciones se cuantificaron bajo 3 intensidades de pastoreo diferentes utilizando 5, 10 y 15 ovejas/ha bajo pasturas sembradas, mejoradas y naturales, en los trópicos semiáridos de la India. Los resultados revelaron que después de 3 años, el suelo bajo pasturas mejoradas tenía partículas significativamente más altas de carbono orgánico (POC~4.5 g/kg), SOC (~0.53 %), carbono orgánico total (TOC~7 g/kg) y existencias de SOC (~15 mg/ha) en comparación con pasturas sembradas y pastos naturales. El carbono lábil (LC ~185 mg/kg) y el carbono de la biomasa microbiana del suelo (SMBC ~378 µg/g suelo) fueron más altos en pastos naturales. Una intensidad de pastoreo moderada de 10 ovejas/ha dio como resultado fracciones de carbono, existencias de TOC y SOC significativamente mayores. El stock de SOC y sus fracciones fueron significativamente más altos en las capas superiores del suelo en comparación con las capas del subsuelo. Estos resultados indican que se recomiendan prácticas de manejo de pasturas mejoradas con intensidad de pastoreo moderada para mejorar el stock de COS y sus fracciones en pastizales tropicales semiáridos.

Palabras clave: Fracciones de carbono, presión de pastoreo, pastura mejorada.

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Introduction

Over 40 % of the earth's land is used for grazing livestock. Grazing lands are reported to store more than 10 % of terrestrial biomass carbon and around 30 % of global soil organic carbon (SOC) stock ([Scurlock and Hall 1998](#)). Grazing lands are also known to provide ecosystem services, including regulating water flow and storage, nutrient cycling and carbon (C) storage ([Schlesinger et al. 2000](#); [Havstad et al. 2007](#); [Morgan et al. 2016](#)). Tropical pastures are constrained in their capacity to sequester SOC due to nitrogen (N) and phosphorus deficiencies and frequent overgrazing, which result in low biomass production and SOC losses ([Saraiva et al. 2014](#)). Recent studies suggested that adoption of improved pasture management practices increase soil C stock at a rate of 0.28 mg C/ha/yr ([Conant et al. 2017](#)). Ogle et al. (2004) conducted a meta-analysis using a global dataset and found 17 % increase in SOC stocks of improved grazing lands in tropical regions. Similarly, Maia et al. (2009) also found 19 % increase of SOC in improved grazing lands in the Amazon and Cerrado regions of Brazil. These studies indicated that overgrazing and inadequate pasture management were the main factors for SOC losses. High temperature and evapotranspiration throughout the year and low and erratic rainfall were associated with reduction in SOC stock in the semi-arid regions ([Oliveira et al. 2015](#)).

In India, grazing is practised on 40 % of the country's geographical area ([National Remote Sensing Centre 2010](#)). When total livestock, expressed as Adult Cattle Unit (ACU), and grazing pressure were calculated for the 80.54 million ha total grazing land available, India had 2.95 ACU/ha, which is much higher than carrying capacity ([NITI Aayog 2018](#)). High livestock grazing pressure resulted in grazing land deterioration and desertification, making grazing lands more susceptible to climate change and targets for restoration ([Shinde and Mahanta 2020](#)). Grazing lands in India remain a neglected natural resource with less than 1 % of Indian grasslands designated as protected areas, making them a poorly addressed and abused ecosystem. Despite the vast area of arid and semi-arid grazing lands in India, meagre information is available on the impacts of pasture and grazing management strategies on SOC stock and its fractions, which is a prerequisite for improving, as well as sustaining, soil health and biomass production. We hypothesized that higher grazing intensity would affect SOC stocks and its fractions differently in natural, improved and sown pastures. An experiment was initiated

in 2015–16 with the aim to understand the impact of grazing intensity on SOC stock and its fractions in natural, improved and sown pastures in the semi-arid tropics of India. It is intended that information generated will be used for developing sustainable grazing and livestock production, while providing enhanced ecosystem services in arid and semi-arid climates, enabling policy decisions on SOC stock management strategies.

Materials and Methods

Description of the study area

A field experiment was conducted during the rainy (kharif) season (June–July) of 2015 at the Central Research Farm, ICAR-Indian Grassland and Fodder Research Institute (IGFRI), Jhansi (25°26'08" N, 78°30'21" E; 216 masl), in the semi-arid tropics of India. The region is characterized with erratic and uncertain rainfall with a long-term average annual rainfall of 908 mm, received mostly from the southwest monsoon during July to September. Drought is a recurring feature and occurs once in 4 years. The average maximum and minimum temperatures are 32.7 °C and 25.1 °C, respectively ([Rai et al. 2018](#)).

Pasture description and experimental design

Improved and sown pastures of 1.5 ha each were developed in June 2015 from natural pasture. Botanical composition prior to the start of the study was determined in 9 randomly selected 1 m² quadrats in each of the natural, improved and sown pastures in the months of September and December. Number of species present in the 1 m² area was counted in each of the 9 quadrats in natural, improved and sown pastures. Species were further classified into grasses, legumes and shrubs. The soil of the experimental site was sandy loam hyperthermic typic haplustepts, with acidic to slightly neutral pH (5.84–6.80). The soil was low in SOC (4.5 g/kg), available N (180 kg/ha) and medium in available P (13.5 kg/ha) and available K (170 kg/ha) in the topsoil layers before the start of the experiment.

The experiment was laid out as a 3-factor nested design to determine effects of grazing intensities on pasture types. Three different grazing intensities of 1 ACU (adult cattle unit)/ha (I₁), 2 ACU/ha (I₂) and 3 ACU/ha (I₃) were achieved by using 5, 10 and 15 adult Jalauni female sheep/ha with an average body weight of 35 kg. The 3 grazing intensities were imposed on a fenced area of 0.5 ha for each grazing intensity within the natural, improved and sown pasture

areas. A total of 90 sheep were divided into 3 equal groups of 30 for each pasture and were allowed to graze following local rotational practices under different grazing intensities (I_1 , I_2 and I_3) during the growing and post-growing seasons of herbage (August to February). Numbers of sheep per ACU were selected based on body weight and assigned to different grazing intensities (Table 1).

Table 1. Descriptions of design of experimental grazing of *Jalauni* sheep.

Pasture type	Grazing intensity*	Grazing paddock (ha)	Animal numbers per paddock
Natural pasture	I_1 (1 ACU/ ha)	0.5	5
	I_2 (2 ACU/ ha)	0.5	10
	I_3 (3 ACU/ ha)	0.5	15
Improved pasture	I_1 (1 ACU/ ha)	0.5	5
	I_2 (2 ACU/ ha)	0.5	10
	I_3 (3 ACU/ ha)	0.5	15
Sown pasture	I_1 (1 ACU/ ha)	0.5	5
	I_2 (2 ACU/ ha)	0.5	10
	I_3 (3 ACU/ ha)	0.5	15

*1 Adult Cattle Unit (ACU) = 10 adult *Jalauni* female sheep.

Biomass yield and quality

Experimental grazing using different grazing intensities was continued in the third year on the natural, improved and sown pastures to assess biomass yield and quality of pastures after grazing. Vegetation samples were taken when biomass had reached its maximum height from 9 randomly placed 1 m² quadrats in each pasture (3 quadrats in each grazing intensity). Weight of vegetation from each quadrat was recorded. For dry matter yield (DMY), harvested fresh biomass samples were sun dried first and then oven dried at 72 °C for 3 days to constant weight. After weighing, dry matter percentage was determined. Quadrat average dry matter percentage was converted to biomass yield in t DM/ha. Dry biomass samples were ground in a Wiley mill having a 1 mm mesh screen. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and crude protein (CP) were determined following the AOAC (1995) procedure.

Soil sampling and analysis

Soil sampling was done in April 2018 after completion of the third grazing cycle. A total of 28 quadrats (0.25 m²) per treatment (a combination of grazing intensity and pasture type) were sampled. Each grazing intensity treatment for

each pasture was divided into 3 equal blocks of 40 m × 40 m and 9–10 subsamples were randomly collected from each block using quadrats at least 0.5 m from the margin of the block to avoid any edge effects. Soil samples were taken from 4 soil depths (0–20, 20–40, 40–60 and 60–80 cm) by using a soil auger (diameter 5 cm). Subsamples were combined from grazing treatments within pasture types at each depth to make 4 representative replicate soil samples. Collected soil samples were analysed for SOC stock and carbon fractions.

SOC was estimated by the wet digestion method of Walkley and Black (1934). Total SOC concentration of soils was calculated using a multiplication factor of 1.28 applied for arid and semi-arid regions of the Indo-Gangetic Plain (Bhattacharyya et al. 2015). Hot-water-soluble carbon (HWSC) was extracted by the hot extraction method of McGill et al. (1986). Particulate organic carbon (POC) was analysed by a mechanical dispersion and separation method (Cambardella and Elliott 1992). Labile carbon (LC, KMnO₄ extractable-C) in soil samples was analyzed using 333 mM KMnO₄ following the procedure of Blair et al. (1995). Soil microbial biomass carbon (SMBC) was analysed by the fumigation method (Vance et al. 1987). Soil organic carbon (SOC) stock was calculated by the following formula:

$$\text{SOC stock (mg/ha)} = \text{SOC (g/kg)} \times \text{bulk density (mg/m}^3\text{)} \times \text{soil depth (m)} \times 10$$

Statistical analysis

Impacts of pasture, grazing intensity and soil depth and their interactions on SOC stock, carbon fractions (HWSC, POC, LC, SMBC) and total organic carbon (TOC) were analysed using SAS version 9.1 mixed model procedures (SAS Institute 2011). Differences in all response parameters were evaluated by treating pasture type, grazing intensity and soil depth as fixed effects and replication as a random effect using a 3-factor nested design. Mean comparisons were made using Tukey's test ($P < 0.05$). Sigmaplot (version 10; Systat Software, Inc., San Jose, California, USA) was used to make graphics.

Results

Botanical composition, biomass yield and quality

Botanical composition in each pasture type (Table 2) affected yield with maximum biomass yield reported in the sown pasture (6.57 t DM/ha), followed by improved

(5.87 t DM/ha) and natural (5.70 t DM/ha) pastures. CP content of the biomass was also higher in sown pasture (6.74 %) as compared with improved (6.41 %) and natural (6.34 %) pastures. NDF and ADF contents were relatively lower in sown pasture (Table 2).

Soil organic carbon, total organic carbon and SOC stock

Pasture type and soil depth had significant ($P < 0.05$) effects on all the studied parameters. Interactions of grazing intensity \times pasture were significant ($P < 0.05$) for SOC, HWSC, POM, LC, SMBC, TOC and SOC stock. Interactions of pasture \times soil depth were significant ($P < 0.05$) for SOC, POC, LC, SMBC, TOC and SOC stock. Interactions of soil depth \times grazing intensity (pasture) and grazing intensity \times soil depth (pasture) were significant ($P < 0.05$) only for POC, LC and SMBC (Table 3).

SOC, TOC and SOC stock were significantly ($P < 0.05$) different by pasture type, grazing intensity and soil depth (Table 4). Among the pasture types, improved pasture resulted in a greater accumulation of SOC (15–20 %), TOC (16–39 %) and SOC stock (15–23 %) as compared with natural and sown pastures. No significant ($P > 0.05$) differences between natural and sown pastures were recorded in the SOC, TOC and SOC stock. In different grazing intensities under studied pastures, I_2 grazing intensity in improved pasture resulted in significantly ($P < 0.05$) higher SOC (0.59 %), TOC (7.89 g/kg) and SOC stock (16.20 mg/ha), which were at par with I_3 grazing intensity in improved pasture. In sown and natural pastures, no significant ($P > 0.05$) changes were recorded in SOC, TOC and SOC stock under different grazing intensities. Topsoil layers (0–40 cm) had significantly ($P < 0.05$) higher SOC, TOC and SOC stock than subsoil layers (40–80 cm), decreasing with increased soil depth in all studied pastures.

Table 2. Botanical composition, biomass yield and quality of different experimental pastures.

Pasture	Botanical composition	DMY (t DM/ha)	Quality (%)		
			CP	NDF	ADF
Natural pasture	Grasses: <i>Heteropogon-Dichanthium-Sehima</i> dominated natural grassland associated with different annual and perennial grasses, including <i>Chrysopogon fulvus</i> , <i>Cynodon dactylon</i> , <i>Digitaria eriantha</i> , <i>Themeda</i> sp., <i>Bothriochloa bladhii</i> , <i>Cenchrus ciliaris</i> , <i>Chloris gayana</i> , <i>Pennisetum pedicellatum</i> , <i>Paspalum notatum</i> , <i>Setaria sphacelata</i> . Legumes: <i>Cajanus scarabaeoides</i> , <i>Indigofera hirsuta</i> , <i>Clitoria ternatea</i> (Blue & white flowered), <i>Alysicarpus rugosus</i> , <i>Aeschynomene indica</i> , <i>Vigna aconitifolia</i> , <i>Tephrosia</i> spp. Shrub species: <i>Zizyphus nummularia</i>	5.70	6.34	75.59	54.81
Improved pasture	Grasses: <i>Heteropogon-Dichanthium-Sehima</i> natural grassland reseeded with <i>Cenchrus ciliaris</i> with <i>Chrysopogon fulvus</i> , <i>Cynodon dactylon</i> , <i>Paspalum notatum</i> , <i>Pennisetum pedicellatum</i> , <i>Digitaria eriantha</i> . Legumes: <i>Stylosanthes hamata</i> , <i>Cajanus scarabaeoides</i> , <i>Indigofera hirsuta</i> , <i>Clitoria ternatea</i> , <i>Tephrosia</i> spp. Shrub species: <i>Zizyphus nummularia</i> , <i>Ailanthus excelsus</i> , <i>Leuceaena leucocephala</i> and Thornless cactus (<i>Opuntia</i> species)	5.87	6.41	76.92	53.72
Sown pasture	Grass: <i>Cenchrus setigerus</i> . Legume: <i>Stylosanthes hamata</i>	6.57	6.74	74.84	51.71

Table 3. Analysis of variance (ANOVA) for soil organic carbon (SOC), carbon fractions (HWSC = hot-water-soluble carbon; POC = particulate organic carbon; LC = KMnO_4 oxidizable carbon; SMBC = soil microbial biomass carbon; TOC = total organic carbon and SOC stock) under different pasture, grazing intensities and soil depth.

Source of variation	df	SOC (%)	HWSC (mg/kg)	POC (g/kg)	LC (mg/kg)	SMBC ($\mu\text{g/gsoil}$)	TOC (mg/kg)	SOC Stock (mg/ha)
Pasture	2	0.0002	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	<0.0001
Grazing intensity (Pasture)	6	0.0230	<0.0001	<0.0001	<0.0001	<0.0001	0.0210	0.0586
Soil depth	3	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Pasture \times Soil depth	6	0.0137	0.9871	<0.0001	<0.0001	<0.0001	0.0132	0.0178
Grazing intensity \times Soil depth (Pasture)	18	0.9212	0.9939	0.0048	<0.0001	<0.0001	0.9128	0.9638

Table 4. Influence of pasture type, grazing intensity and soil depth on soil organic carbon (SOC), total organic carbon (TOC) and SOC stock.

Treatment			SOC (%)	TOC (g/kg)	SOC stock (mg/ha)	
a. Pasture	Natural pasture		0.44b	5.90b	12.07b	
	Improved pasture		0.53a	7.09a	14.90a	
	Sown pasture		0.46b	6.11b	12.93b	
	P value		0.0002	0.0002	<0.0001	
b. Grazing intensity (Pasture)	Natural pasture	I ₁	0.46bc	6.15c	12.46bc	
		I ₂	0.45bc	6.06bc	12.24bc	
		I ₃	0.41c	5.47c	11.54c	
	Improved pasture	I ₁	0.46bc	6.07bc	12.96bc	
		I ₂	0.59a	7.89a	16.20a	
		I ₃	0.54ba	7.29ba	15.53ba	
	Sown pasture	I ₁	0.47bc	6.28bac	13.69bac	
		I ₂	0.46bc	6.16bc	12.55bc	
		I ₃	0.44bc	5.89bc	12.54bc	
	P value		0.0230	0.0210	0.0586	
	c. Soil depth (cm)	0–20		0.76a	10.11a	20.49a
		20–40		0.48b	6.31b	13.09b
40–60		0.37c	4.93c	10.59c		
60–80		0.30d	4.11d	9.03c		
P value		<0.0001	<0.0001	<0.0001		
d. Pasture × Soil depth	Natural pasture	0–20	0.79a	10.60a	21.23a	
		20–40	0.43dc	5.74dc	11.71dc	
		40–60	0.32de	4.21de	8.84de	
		60–80	0.23e	3.02e	6.53e	
	Improved pasture	0–20	0.81a	10.79a	21.95a	
		20–40	0.54bc	7.15bc	14.89bc	
		40–60	0.43dc	5.77dc	12.50dc	
		60–80	0.35de	4.63de	10.26de	
	Sown pasture	0–20	0.67ba	8.92bc	18.28ba	
		20–40	0.46dc	6.03dc	12.68dc	
		40–60	0.36de	4.82de	10.43de	
		60–80	0.35de	4.67de	10.33de	
	P value		0.0137	0.0132	0.0178	

Values in each column followed by different lowercase letters are significantly different according to Tukey's test ($P < 0.05$). I₁, I₂ and I₃ grazing intensities were 1 ACU/ha, 2 ACU/ha and 3 ACU/ha, respectively.

Carbon fractions

Significant ($P < 0.05$) differences between pasture, grazing intensity and soil depth were recorded in HWSC. Higher HWSC (12.98 mg/kg) was found in sown pasture, which was 65.77 % and 68.35 % higher than improved and natural pastures, respectively. At 0–20 cm soil depth, HWSC was significantly ($P < 0.05$) higher (11.71 mg/kg) and decreased with successive soil depths (Figure 1).

I₁ grazing intensity in sown pasture had a significantly ($P < 0.05$) higher HWSC (16.41 mg/kg) compared with improved (9.15 mg/kg) and natural pastures (9.86 mg/kg), in which HWSC was not influenced by grazing intensities (Figure 2).

POC, LC and SMBC contents were significantly ($P < 0.05$) influenced by pasture type, grazing intensity and soil depth (Tables 5 and 6). At 0–20 cm soil depth, POC content was significantly ($P < 0.05$) higher (Table 5) in

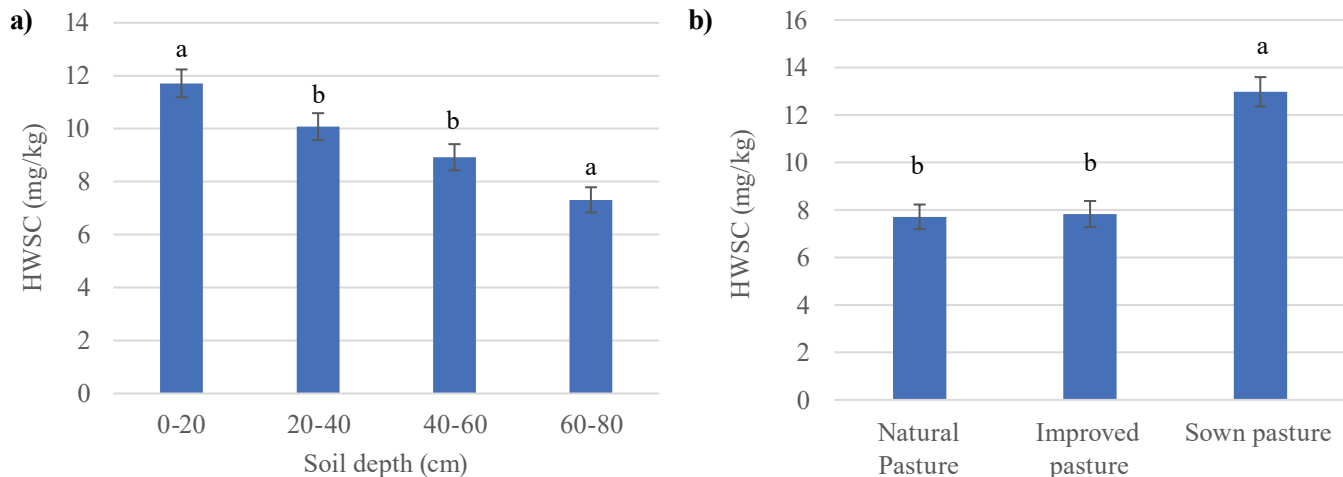


Figure 1. Hot-water-soluble carbon (HWSC mg/kg) influenced by soil depth (a) and different pastures at 0–80 cm soil depth (b). Bars with different lowercase letters indicate significant differences at $P < 0.05$ level based on Tukey's test.

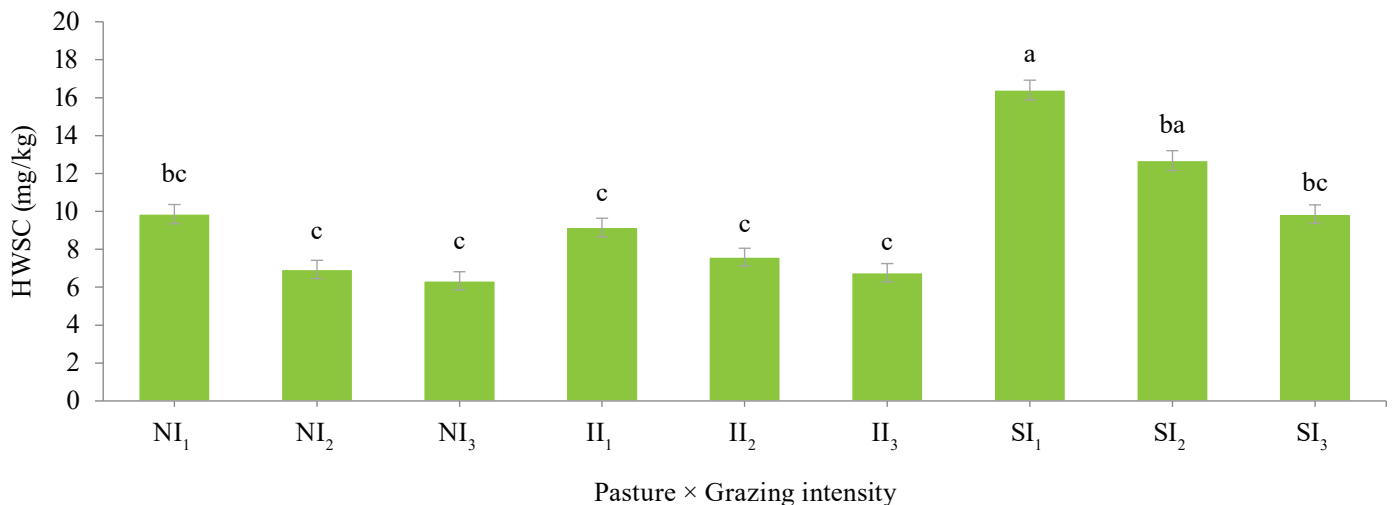


Figure 2. Hot-water-soluble carbon (HWSC mg/kg) influenced by different pastures and grazing intensities (NI₁, natural pasture I₁ grazing intensity; NI₂, natural pasture I₂ grazing intensity; NI₃, natural pasture I₃ grazing intensity; II₁, improved pasture I₁ grazing intensity; II₂, improved pasture I₂ grazing intensity; II₃, improved pasture I₃ grazing intensity; SI₁, sown pasture I₁ grazing intensity; SI₂, sown pasture I₂ grazing intensity; SI₃, sown pasture I₃ grazing intensity). Bars with different lowercase letters indicate significant differences at $P < 0.05$ level based on Tukey's test.

improved pasture (6.01 g/kg) as compared with sown (5.22 g/kg) and natural pastures (1.99 g/kg) whereas, LC were higher (24.40–30.79 %) in natural pasture as compared with improved and sown pastures. The SMBC was higher (37.63 %) in natural pasture compared with improved pasture but remained at par with sown pasture (Table 6). Among the grazing intensities, I₂ grazing intensity

resulted in significantly ($P < 0.05$) higher POC content at 0–20 cm soil depth in improved pasture while LC and SMBC were significantly higher in natural pasture with similar depth and grazing intensity. POC, LC and SMBC content in soil were significantly ($P < 0.05$) higher at 0–20 cm soil depth as compared with lower soil depths (20–80 cm) and decreased with increase in grazing intensity.

Table 5. Influence of grazing intensity (GI), pasture type and soil depth on particulate organic carbon (POC).

Treatment	GI	Soil depth (cm)	POC (g/kg)
a. Pasture × Soil depth			
Natural pasture		0–20	1.99ed
		20–40	2.22d
		40–60	2.05ed
		60–80	1.26e
Improved pasture		0–20	6.01a
		20–40	5.19b
		40–60	4.78cb
		60–80	2.63d
Sown pasture		0–20	5.22b
		20–40	4.66cb
		40–60	4.21c
		60–80	2.35d
P value			<0.0001
b. Soil depth × Grazing intensity (GI)			
Natural pasture	I ₁	0–20	3.09fghijh
		20–40	4.02fgecd
		40–60	3.81fgeidh
		60–80	1.68kj
	I ₂	0–20	1.72kj
		20–40	1.39k
		40–60	1.28k
		60–80	1.16k
	I ₃	0–20	1.15k
		20–40	1.24k
		40–60	1.06k
		60–80	0.95k
Improved pasture	I ₁	0–20	5.26bcd
		20–40	5.00bcd
		40–60	4.70becc
		60–80	2.51gkijh
	I ₂	0–20	7.31a
		20–40	5.79ba
		40–60	4.84bcd
		60–80	3.15fgeijh
	I ₃	0–20	5.46bc
		20–40	4.78bcd
		40–60	4.79bcd
		60–80	2.24kij
Sown pasture	I ₁	0–20	5.30bcd
		20–40	4.69fbeecc
		40–60	3.90fgecdh
		60–80	2.15kj
	I ₂	0–20	5.26bcd
		20–40	4.85bcd
		40–60	4.50fbeecc
		60–80	2.54gkijh
	I ₃	0–20	5.09bcd
		20–40	4.45fbeecc
		40–60	4.25fbeecc
		60–80	2.35kijh
P value			0.0048

Values in each column followed by different lowercase letters are significantly different according to Tukey’s test (P<0.05); I₁, I₂ and I₃ grazing intensities were 1 ACU/ ha, 2 ACU/ha and 3 ACU/ha, respectively.

Table 6. Influence of grazing intensity, pasture type and soil depth on labile carbon (LC) and soil microbial biomass carbon (SMBC).

Treatment	GI	Soil depth (cm)	LC (mg/kg)	SMBC (µg/g soil)
a. Pasture × Soil depth				
Natural pasture		0–20	314.42a	401.98a
		20–40	215.92c	321.63d
		40–60	116.52f	205.32h
		60–80	92.77h	179.53i
Improved pasture		0–20	252.54b	292.06f
		20–40	171.31d	312.57e
		40–60	115.56gf	256.23g
		60–80	102.63gh	248.99g
Sown pasture		0–20	240.40b	401.81a
		20–40	181.62d	377.30b
		40–60	173.94d	368.66cb
		60–80	144.44e	363.53c
P value			<0.0001	<0.0001
b. Soil depth × Grazing intensity (GI)				
Natural pasture	I ₁	0–20	316.56a	397.07cb
		20–40	152.38khji	314.70hg
		40–60	109.81op	197.85m
		60–80	93.37p	175.28n
	I ₂	0–20	326.44a	428.52a
		20–40	263.31c	325.39g
		40–60	122.25moln	224.80l
		60–80	95.00op	185.05nm
	I ₃	0–20	300.25ba	380.35cde
		20–40	232.06de	324.80g
		40–60	117.50mopn	197.33nm
		60–80	89.94p	178.28n
Improved pasture	I ₁	0–20	217.05e	288.63i
		20–40	171.69hjgi	368.19fde
		40–60	113.69opn	253.21k
		60–80	99.60op	245.76k
	I ₂	0–20	274.31bc	296.98hi
		20–40	180.25hg	288.88i
		40–60	122.75moln	263.50jk
		60–80	150.06op	254.60k
	I ₃	0–20	266.25c	290.56i
		20–40	162.00khjgi	280.63ji
		40–60	110.25op	251.99k
		60–80	103.19op	246.61k
Sown pasture	I ₁	0–20	209.13fe	397.02cb
		20–40	175.94hgi	374.70fde
		40–60	166.00khjgi	367.86fde
		60–80	138.81kmin	365.26fde
	I ₂	0–20	265.50c	403.15b
		20–40	188.44fg	382.40cd
		40–60	178.88hg	374.81fde
		60–80	150.31kjli	365.05fde
	I ₃	0–20	246.56dc	405.27b
		20–40	180.50hg	374.81fde
		40–60	176.94hgi	363.33fe
		60–80	144.19kmjl	360.28f
P value			<0.0001	<0.0001

Values in each column followed by different lowercase letters are significantly different according to Tukey’s test (P<0.05); I₁, I₂ and I₃ grazing intensities were 1 ACU/ ha, 2 ACU/ha and 3 ACU/ha, respectively.

Discussion

This study found greater accumulation of SOC, TOC and SOC stock in improved pastures compared with natural and sown pastures up to 80 cm soil depth. Topsoil layers (0–40 cm) had significantly higher SOC, TOC and SOC stock than the subsoil layers (40–80 cm) decreasing with increase in soil depths. The decreases in SOC, TOC and SOC stock were sharper in natural and sown pastures as compared with the improved pastures. It is believed that the greater TOC and SOC stock in improved pastures might be due to the addition of biomass and fine roots through perennial shrub pasture components like *Ailanthus excelsus*, *Leucaena leucocephala* and thornless cactus (*Opuntia* species). Banegas et al. (2019) also reported that *Leucaena leucocephala* introduction increased the SOC concentration in the subsoil by 45 % after 4 years in the Chaco region of Argentina. Similar findings were also reported by Carter et al. (1998) in *Leucaena* and *Stylosanthes* pastures in northern Australia. The sharp decline in SOC, TOC and SOC stock in natural and sown pastures might be attributed to changes in plant community (Fisher et al. 1998) and deposition of C inputs in the topsoil layers (Costa et al. 2009), which makes it more susceptible to loss into the environment. Saraiva et al. (2014) also reported higher C concentration and content at shallower soil depths is directly linked to litter deposition on the soil surface and greater root biomass in the topsoil layers. Similar results were also found for SOC, TOC and SOC stock, which were greater in topsoil layers as compared with subsoil layers under studied pastures. The sharp decline in SOC, TOC and SOC stock in the subsoil layers under natural and sown pastures may also be due to sharp declines in biomass and plant cover from heavy grazing resulting in low carbon sequestration in soil (Krishna and Mohan, 2017). Xie and Wittig (2004) and Abdalla et al. (2018) also found that overgrazing leads to significant SOC loss particularly in semi-arid regions, which impairs sustainability of grazing lands. This is because heavy grazing significantly reduces carbon uptake by grasses. In addition, pressure from trampling by animals leads to compaction of topsoil layers, increased bulk density and reduced infiltration and soil aeration. This limits the proper development of the root system of plants, which ultimately reduces SOC stock (Zhang et al. 2018). The higher SOC, TOC and SOC stocks in the subsoil layer (40–80 cm) in improved pasture could be attributed to the introduced shrubs' deep root systems, since a significant proportion of fine roots (>60 %) of shrubs

have been observed below 40 cm in soil compared with grasses (Radrizzani 2009). Pachas et al. (2018) also found that *Leucaena* had an abundance of roots in the deeper profile than grasses. Fine root carbon contributed significantly to the increase in subsoil layer SOC stock, particularly under high grazing pressure (Radrizzani and Nasca 2014). Results of this study clearly indicated that, in arid and semi-arid pasture lands, decline in herbaceous and shrub biomass (forage) is a key factor for pasture land deterioration. Improved pasture management practices play a vital role in grazing land sustainability, particularly under high grazing pressure in the semi-arid tropics. Many other authors also reported that shrub establishment and development offer an important safeguard against grazing land deterioration through increasing SOC, soil nutrients distribution and soil stability and by reducing soil erosion (Blaser et al. 2014; van Hall et al. 2017).

All the SOC fractions significantly decreased with successive soil depths and grazing intensities. The differences in HWSC in soils under different pasture types may be due to the difference in root exudation patterns in pasture species and the nature and amount of organic sources in soil (Campbell et al. 1999). A study conducted in Inner Mongolia, China by Cao et al. (2013) also reported that POC and LC at a depth of 0–15 cm decreased with increasing grazing intensity. The decreasing trend of POC, LC and SMBC with grazing intensity was due to over extraction of carbon for biomass and less carbon input (Li et al. 2015). Moderate grazing intensity resulting in significantly higher POC, LC and SMBC was also reported by Ma et al. (2005), where after 22 years grazing in *Leymus chinensis* steppe, LC and SMBC content at 0–10 cm soil depth decreased by 22.0 % and 27.9 %, respectively. Results of this study also indicate that POC, LC and SMBC contents in topsoil layers (0–40 cm) were significantly higher as compared with subsoil layers (40–80 cm). This was due to the fact that C contribution comes mainly from soil surface C accumulation by leaf litter, plant roots and animal excrements. The significant decline in various carbon fractions (HWSC, POC, LC and SMBC) under heavy grazing intensity (3 ACU/ha) in natural and sown pastures as compared with the improved pastures may be linked with lower input of forage biomass since the input of plant residue and vertical distribution of roots throughout the profile affect SOC stocks at depth (Jobbágy and Jackson 2000; Sampaio and Costa 2011). Carbon fractions responded more rapidly to land use and management activities and can provide a very sensitive indicator of changes in the dynamics of SOC (Su et al. 2009).

Conclusions

This study has provided valuable information on impact of different grazing intensities on SOC stock and its fractions under different pasture types in the semi-arid tropics of India. It has also highlighted the ability of different pastures to improve SOC stock for grazing land sustainability of improved pastures using improved grazing management practices. Heavy grazing intensity decreased TOC content and SOC stock of natural and sown pastures. It is proposed that inclusion of shrub species such as *Leucaena leucocephala*, *Ailanthus excelsus*, *Zizyphus nummularia* and thornless cactus (*Opuntia* species) and moderate grazing intensity should be adopted to prevent further degradation of pasture lands and improve carbon sequestration and soil sustainability in arid and semi-arid tropical pastures.

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Research Paper

Fermentation losses and aerobic stability of elephant grass silages containing *Parkia platycephala* pod meal and urea

*Pérdidas de fermentación y estabilidad aeróbica de ensilajes de pasto elefante que contienen harina de vaina de *Parkia platycephala* y urea*

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Abstract

Fermentation losses and aerobic stability of elephant grass silages with inclusion of 4 levels of *Parkia platycephala* pod meal (PP) (0, 10, 20 and 30 %) and 2 levels of urea (0 and 1.5 %) on as-fed basis were evaluated. The silages were analyzed for gas and effluent losses, dry matter recovery (DMR), pH and aerobic stability (pH and temperature) to determine effects of interactions between levels of urea and PP on gas losses, dry matter recovery, temperature and pH of the silages. The combination of urea and PP increased gas losses and reduced the DMR. PP reduced effluent losses and urea increased aerobic stability of the silages. pH values decreased with inclusion of PP and increased with use of urea. Silages containing 20 and 30 % PP showed greater stability over time, especially when associated with urea. PP with urea were effective in reducing effluent losses, maintaining DMR and allowing higher aerobic stability, with stability rupture from 40 h with the inclusion of 20 % of PP in elephant grass silages.

Keywords: Aerobic degradation, dry matter recovery, effluents, pH.

Resumen

Se evaluaron las pérdidas por fermentación y la estabilidad aeróbica de ensilajes de pasto elefante con inclusión de 4 niveles de harina de vaina (PP) de *Parkia platycephala* (0, 10, 20 y 30 %) y 2 niveles de urea (0 y 1.5 %) sobre la base del alimento en fresco. Los ensilajes se analizaron en cuanto a pérdidas de gas y efluentes, recuperación de materia seca, pH, nitrógeno amoniacal y estabilidad aeróbica (pH y temperatura) para determinar los efectos de las interacciones entre los niveles de urea y PP en las pérdidas de gas, recuperación de materia seca (DMR), temperatura y pH de los ensilajes. La combinación de urea y PP aumentó las pérdidas de gas y redujo la DMR. El PP redujo las pérdidas de efluentes y la urea aumentó la estabilidad aeróbica de los ensilajes. Los valores de pH disminuyeron con la inclusión de PP y aumentaron con el uso de urea. Los ensilajes que contenían 20 y 30 % de PP mostraron mayor estabilidad en el tiempo, especialmente cuando se asociaron con urea. La combinación de PP con urea fue efectiva para reducir las pérdidas de efluentes y mantener la recuperación de materia seca y permitir una mayor estabilidad aeróbica, con ruptura de la estabilidad a partir de las 40 h con la inclusión de 20 % de PP en los ensilajes de pasto elefante.

Palabras clave: Degradación aeróbica, efluentes, pH, recuperación de materia seca.

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Introduction

Elephant grass (*Cenchrus purpureus*) is one of the most important tropical forages cultivated in many regions of the world due to its high production potential, quality, acceptability and vigor (Fonseca and Martuscello 2010). Using surplus elephant grass for silage is an alternative to direct feeding to provide a feed with high nutritional value (Santos et al. 2008; Andrade et al. 2010).

Optimal nutritional value of silage from elephant grass harvested at 60–70 days is associated with high moisture in the grass, low levels of soluble carbohydrates and high buffering capacity, factors that inhibit the fermentation process and make it difficult to obtain good quality silage. This favors development of *Clostridium* bacteria and increases loss of nutrients from the large amount of effluent produced during undesirable secondary fermentation (Andrade et al. 2010; Pacheco et al. 2014). Wilting before the silage process has been shown to be beneficial for fermentation, in particular to increase lactic acid production (Santos et al. 2010). Additives have been tested in elephant grass silage to modify fermentation dynamics, inhibit growth of undesirable microorganisms during the fermentation process, reduce losses, improve aerobic stability, increase nutritional value of the silage and provide soluble carbohydrates for fermentation (Santos et al. 2008; Andrade et al. 2010; Rezende et al. 2011).

Moisture absorbing additives allow ensiling young forage plants, which are typically sources of carbohydrates. Cereals and feed meal can be used to raise dry matter content of silages, reduce effluent production and increase nutritional value of silages (Lopes et al. 2007). As an example, *Parkia platycephala* pods can be used because they contain 77 % dry matter, which can be used as an alternative to improve chemical and fermentation characteristics of silage. The genus *Parkia*, belongs to the subfamily Mimosoideae, and is found mainly in tropical rainforests. *Parkia platycephala* is a legume native to arid and semi-arid regions. Fallen pods are consumed by animals and contain high levels of soluble carbohydrates, about 69 % of DM (Barbosa et al. 2015), and about 8 % of crude protein with good biomass yield (Magalhães et al. 2014).

Use of urea in silages is based on its transformation into ammonia (NH₃), which reacts with water to form ammonium hydroxide, raising the pH and metabolism of microorganisms that cause secondary fermentation, such as yeasts, enterobacteria and *Clostridium* bacteria (Siqueira et al. 2007). Urea increases the aerobic stability of the silage (Rocha et al. 2006).

It was hypothesized that *Parkia platycephala* pod meal (PP) reduces fermentation losses, mainly by reducing effluents, and together with urea, increases aerobic stability of silages, extending the preservation period after opening of these silos. The aim in this study was to evaluate fermentation losses and aerobic stability of elephant grass silages with inclusion of different levels of PP and urea.

Materials and Methods

Experimental location and treatments

The experiment was carried out in the Federal University of Piauí (UFPI), located in Bom Jesus Piauí, Brazil (09°04'28" S, 44°21'31" W; 277 masl). The average annual rainfall is 875 mm and during forage harvesting the mean maximum and minimum temperatures were 32.7 and 21.5 °C, respectively (Inmet 2018).

The experimental design was completely randomized in a factorial arrangement (4 × 2) with 4 levels of inclusion of PP (0, 10, 20 and 30 % as-fed basis) and 2 levels of urea (0 and 1.5 % as-fed basis) with 5 replications. The treatments corresponded to 8 different types of silage:

- T1=100 % elephant grass + 0 % PP + 0 % urea (Control)
- T2=90 % elephant grass + 10 % PP + 0 % urea
- T3=80 % elephant grass + 20 % PP + 0 % urea
- T4=70 % elephant grass + 30 % PP + 0 % urea
- T5=98.5 % elephant grass + 0 % PP + 1.5 % urea
- T6=88.5 % elephant grass + 10 % PP + 1.5 % urea
- T7=78.5 % elephant grass + 20 % PP + 1.5 % urea
- T8=68.5 % elephant grass + 30 % PP + 1.5 % urea

Silage preparation

Elephant grass cultivar 'Napier' was manually harvested after 60 d regrowth and chopped in a forage chopper/grinder (model GTM-2001sb GARTHEN®) into 2 cm fragments. Mature pods of PP were harvested from local trees in Valencia, PI and air dried. Dry pods were ground in a disintegrator/chopper/grinder using a 10 mm sieve to obtain the meal. Samples of approximately 500 g of elephant grass and PP were collected, packed in labelled plastic bags and stored in a freezer before pre-drying in a forced ventilation oven at 60 °C for 72 h. Dry samples were ground in a Wiley mill to pass a 1 mm sieve for the determination of the chemical composition (Table 1) according to AOAC (2012) to determine dry matter (DM) (method 967.03), ash (method 942.05), crude protein (CP) (method 981.10) and ether

extract (EE) (method 920.29). To quantify the neutral detergent fiber (NDF), the methodology of Van Soest et al. (1991) was used with modifications proposed in the Ankon device manual (Ankon Technology Corporation, USA). Acid detergent fiber (ADF) was determined according to the method of Robertson and Van Soest (1981). Total carbohydrates (TCHO) were estimated using the equation proposed by Sniffen et al. (1992):

$$\text{TCHO} = 100 - (\% \text{ CP} + \% \text{ EE} + \% \text{ ash})$$

Non-fiber carbohydrates (NFC) were estimated using the equation recommended by Mertens (1997):

$$\text{NFC} = 100 - (\% \text{ ash} - \% \text{ CP} - \% \text{ EE} - \% \text{ NDF})$$

Plant materials for each treatment were manually homogenized and compressed to a density of 473 kg/m³ in experimental PVC silos of approximately 3 kg with 1 kg of sand in the bottom of each silo separated from the forage by a layer of cotton fabric. Silos were closed and sealed with plastic adhesive tape to exclude air and Bunsen valves were inserted in each silo to allow escape of gasses and loss of dry matter (DM) from the fermentation process to be quantified. Silos were stored at room temperature from 21 °C to 38 °C for 50 d.

Table 1. Chemical composition of elephant grass and PP at the time of ensiling.

Variable (% DM)	Elephant grass	PP
Dry matter (DM) as-fed	22.27	85.57
Ash	5.32	1.54
Organic matter (OM)	94.68	98.46
Ether extract (EE)	2.40	1.23
Crude protein (CP)	6.87	8.50
Neutral detergent fiber (NDF)	73.27	20.20
Acid detergent fiber (ADF)	42.76	10.55
Hemicellulose	30.51	9.65
Total carbohydrates (TCHO)	85.42	88.73
Non-fiber carbohydrates (NFC)	12.14	68.53

Fermentation losses

Fermentation losses of silages were quantified by weight difference of the mini silos before and after ensiling, and the respective dry matter contents estimated through equations according to Schmidt (2006).

Gaseous losses were quantified by subtracting the weight of the full silo before opening from the weight

directly after ensiling using the following equation:

$$G = \frac{[(\text{WFen} - \text{Wen}) \times \text{DMen}] - [(\text{WFao} - \text{Wen}) \times \text{DMao}] \times 100}{[(\text{WFen} - \text{Wen}) \times \text{DMen}]}$$

where:

G is gaseous losses in % of DM;

WFen is weight of the full silo at ensiling (kg);

Wen is total weight (silo + lid + sand + screen + cotton fabric) at ensiling (kg);

DMen is DM content of the forage at ensiling (%);

WFao is weight of the full silo at opening (kg); and

DMao is DM content in the forage at opening (%).

Production of effluents was calculated by using the sand as a collector at the bottom of silos, considering the total weight at opening and before ensiling in relation to the amount of fresh forage ensiled:

$$\text{EL (kg/t GM)} = \frac{(\text{EFW} \times 1000)}{\text{GMen}}$$

where:

EL is effluent losses;

GM is green mass;

EFW is effluent weight (weight of empty silo after opening-weight of empty silo before filling); and

GMen is weight of green mass of ensiled forage.

DM recovery was estimated through the equation:

$$\text{DMR (\%)} = \frac{(\text{FMao} \times \text{DMao}) \times 100}{(\text{FMac} \times \text{DMac})}$$

where:

DMR is dry matter recovery index;

FMao is forage mass at opening;

DMao is DM content at opening;

FMac is forage mass at closure; and

DMac is DM content of the forage at closure.

After 50 days of fermentation, silos were weighed and opened and approximately 10 cm from the upper and lower layers of the silages that could have been spoiled in the ensiling process were discarded. Silage samples were manually removed from the silos and stored in plastic bags for homogenization and transferred to an air-conditioned room at 25.23 ± 0.4 °C with an average air relative humidity of 63.23 ± 5.91 %. The samples were spread to allow greater penetration of air into the mass. Silage temperatures were taken every hour with a digital thermometer (INCOTERM®) inserted at 10 cm depth in the center of the silage mass, as proposed by Kung Jr. et al. (2003) and Bernardes et al. (2007).

Surface temperatures were measured by an infrared digital thermometer with laser targeting (BENETECH®).

Approximately 50 g of silage was taken every 8 hours (3 samples per day) from each treatment during 4 days of air exposure, to determine pH following the methodology described by Jobim et al. (2007). Aerobic stability was based on an increase in silage temperature of 2 °C in relation to the environment after opening of silos (Moran et al. 1996). Number of hours for the elevation of silage temperature to 2 °C in relation to room temperature, number of hours to reach maximum temperature and maximum temperature in silages exposed to air from 0 to 4 d were measured as proposed by O'Kiely et al. (1999).

Statistical analyses

All data were analyzed using the MIXED procedure of the SAS software as a 4 × 2 factorial design. Effects of level of inclusion of PP on fermentation losses and aerobic stability were evaluated using orthogonal contrasts to determine the linear and quadratic effects. The contrasts were significant when the P-value was ≤0.05. Effect of urea and days of air exposure were compared through the probability of the difference (PDIFF) using Fisher's LSD test. Statistical differences were declared significant at P-value<0.05 and trends accepted if P-value<0.1.

Residues were plotted against predicted values and used to verify the assumptions of the model such as homoscedasticity, independence and normality of errors. A

measurement was considered an outlier and removed when the studentized residue was outside the range of ± 2.5.

Results

Fermentation losses

Inclusion of PP promoted linear reduction of effluent losses and DMR (Table 2). Silage with 1.5 % of urea with 0, 20 % and 30 % PP showed the lowest DMR. PP levels and urea levels had significant effects (P<0.0001) on gaseous losses and DMR. A linear increase was observed in gaseous loss with increasing PP without and with urea. The interaction (P=0.32) between PP and urea levels did not influence effluent losses (Table 2). Silages containing urea showed higher effluent losses.

Aerobic stability

The temperature and pH of silages were affected (P<0.0001) by the interaction PP × U × Day (Table 3). A quadratic effect was observed with temperatures increasing proportionally to levels of PP. All silages, except the one with 20 % PP, showed higher fermentation temperatures when urea was added, with higher values being recorded on the third day.

pH values increased over the days regardless of level of inclusion of additives (Table 3). Silages with no PP nor urea showed the highest pH values depending on hours of air exposure, while silage with 30 % PP retained a pH value close to 4 (Figure 1).

Table 2. Fermentation losses of elephant grass silages containing urea and *Parkia platycephala* pod meal (PP).

Urea (% AF)	PP (% GM)				Mean	SEM	P-value		
	0	10	20	30			PP	U	PP×U
	Gas (% DM)								
0	3.24 ^{Db}	9.54 ^{Ca}	15.38 ^{Ba}	18.92 ^{Aa}	11.77	0.89	<0.0001	<0.0001	<0.0001
1.5	8.41 ^{Ca}	9.81 ^{Ca}	16.05 ^{Ba}	19.12 ^{Aa}	13.35				
Mean	5.82	9.67	15.72	19.02					
	Effluent (kg /t GM)								
0	53.86	32.60	32.01	7.08	31.38b	3.08	<0.0001	0.02	0.32
1.5	61.25	37.25	30.40	13.34	35.56a				
Mean	57.55	34.92	31.20	10.21					
	DMR (% DM)								
0	87.55 ^{Aa}	84.24 ^{Ba}	80.74 ^{Ca}	78.05 ^{Da}	82.64	0.60	<0.0001	<0.0001	<0.0001
1.5	82.42 ^{Bb}	84.82 ^{Aa}	78.80 ^{Cb}	77.05 ^{Da}	80.77				
Mean	84.98	84.53	79.77	77.54					

Means followed by different lowercase letters in the columns differ for the level of urea and by different uppercase letters in the rows differ for the level of PP by Fisher's LSD test (P< 0.05); AF=as-fed; GM=fresh matter; DM=dry matter.

Stability rupture occurred immediately after exposure to air in silages with PP and those containing 20 and 30 % PP had higher temperature variations over the hours of aerobic activity (Figure 2). Silages with 0 and 10 % PP without urea and 0 % PP + 1.5 % U had the maximum

temperatures immediately after opening and kept aerobic stability after 96 hours of air exposure. Treatments without urea and 0, 10 and 30 % PP showed rupture of stability immediately after air exposure.

Table 3. Temperature (°C) and pH of elephant grass silages containing urea (U) and *Parkia platycephala* pod meal (PP) during four days (D) of air exposure.

Urea (% AF)	Day	<i>Parkia platycephala</i> pod meal (% AF)				Mean	SEM	P-value					
		0	10	20	30			PP	U	D	PP×D	U×D	PP×U×D
Temperature (°C)													
0	1st	25.06 ^{Ac}	24.50 ^{Bc}	24.79 ^{Abc}	25.05 ^{Ac}	24.85							
	2nd	26.51 ^{Ba}	25.84 ^{Cb}	26.62 ^{Bab}	27.08 ^{Ab}	26.51							
	3rd	26.31 ^{Ca}	26.47 ^{Ca}	26.81 ^{Ba}	27.62 ^{Aa}	26.80							
	4th	25.93 ^{Cb}	26.00 ^{Cb}	26.32 ^{Bb}	27.16 ^{Ab}	26.35							
Mean		25.95 ^α	25.70 ^β	26.14 ^α	26.73 ^α	0.06	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	
1.5	1st	24.58 ^{Ac}	23.94 ^{Bc}	24.04 ^{Bd}	24.18 ^{Bd}	24.19							
	2nd	25.33 ^{Bb}	27.00 ^{Aa}	26.74 ^{Ab}	24.80 ^{Cc}	25.97							
	3rd	25.85 ^{Da}	26.86 ^{Ca}	28.00 ^{Ba}	28.56 ^{Aa}	27.32							
	4th	25.56 ^{Cab}	26.04 ^{Bb}	25.68 ^{BCc}	27.71 ^{Ab}	26.25							
Mean		25.33 ^β	25.96 ^α	26.12 ^α	26.32 ^β								
pH													
0	1st	3.74 ^{Ac}	3.76 ^{Ac}	3.84 ^{Ab}	3.93 ^{Aa}	3.82							
	2nd	4.08 ^{Ac}	3.83 ^{Ac}	3.88 ^{Ab}	4.02 ^{Aa}	3.95							
	3rd	5.35 ^{Ab}	4.20 ^{Bab}	4.06 ^{Bab}	4.13 ^{Ba}	4.43							
	4th	5.82 ^{Aa}	4.54 ^{Ba}	4.40 ^{Ba}	4.19 ^{Ba}	4.75							
Mean		4.76 ^β	4.08 ^β	4.05 ^α	4.07 ^α	0.08	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	
1.5	1st	6.43 ^{Ac}	3.80 ^{Bc}	3.76 ^{Bc}	3.77 ^{Bb}	4.44							
	2nd	6.65 ^{Ac}	4.04 ^{Bc}	3.83 ^{Bc}	3.75 ^{Bb}	4.57							
	3rd	7.07 ^{Ab}	4.70 ^{Bb}	4.64 ^{Bb}	3.94 ^{Cab}	5.10							
	4th	8.41 ^{Aa}	6.26 ^{Ba}	5.35 ^{Ca}	4.37 ^{Aa}	6.10							
Mean		7.14 ^α	4.70 ^α	4.40 ^α	3.96 ^α								

Means followed by different lowercase letters in the columns differ for day and uppercase in the rows differ for the level of *Parkia platycephala* and Greek letters in the columns differ for urea by the Fisher's LSD test (P< 0.050). AF=as-fed.

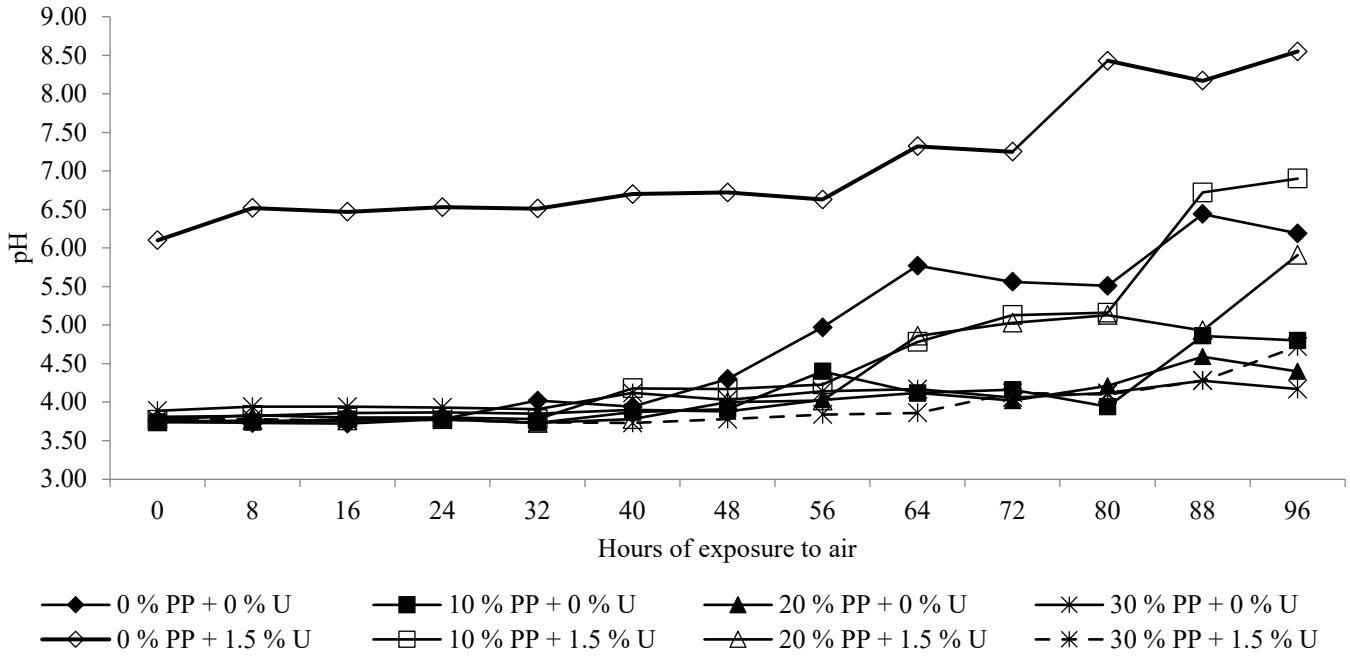


Figure 1. pH of elephant grass silages containing PP and urea after air exposure.

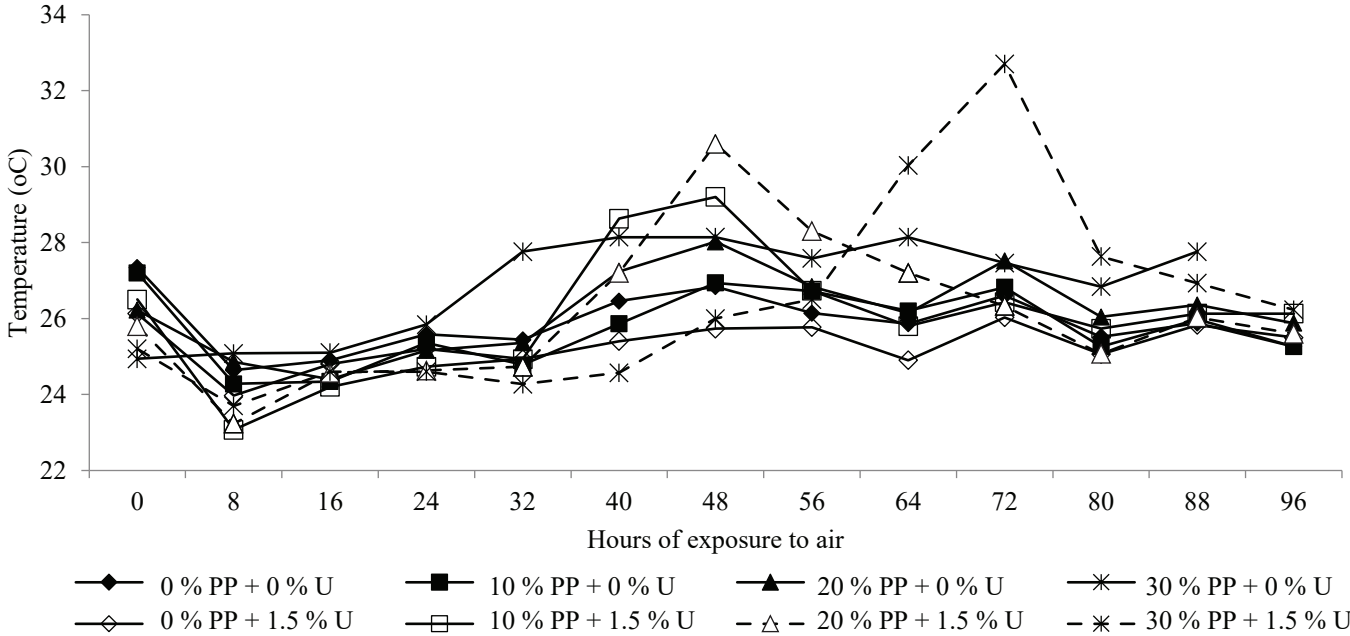


Figure 2. Temperature (°C) of elephant grass silages containing PP and urea with hours of air exposure.

Discussion

Results showed that gaseous losses increased with addition of PP in elephant grass silage regardless of use of urea. This effect may be related to increase in soluble carbohydrates from PP as described by Barbosa et al. (2015). When elephant grass was ensiled with urea and without PP, gaseous losses were higher than in silage

made with only elephant grass, possibly because of the higher percentage of water retained, promoting changes in the fermentation process (Rezende et al. 2011), together with further development of gas producing microorganisms in the presence of urea. In silages that contained urea, effluent losses were higher, probably because of enhanced biochemical reactions producing metabolic water with percolation

through the ensiled mass. According to Balieiro et al. (2009) some additives can alter the structural integrity of plant cells and their ability to retain water, leading to rupture and leakage of cellular contents. Effluent losses reduced with inclusion of PP, demonstrating its capacity to absorb moisture from the silage. Many additives with high DM content, such as coffee hulls (Bernardino et al. 2005), wheat meal (Zanine et al. 2006), cassava meal (Oliveira 2009) and *Parkia platycephala* pods (Barbosa et al. 2015), have been used to reduce moisture content of silage and thereby reduce losses by effluents. Reduction of losses by effluents also promotes reduction of nutrient losses by percolation with the effluent. Effluent loss values observed in this study were high when compared with results reported in the literature. Rezende et al. (2011) reported that plant respiration and growth of microorganisms produce water and contribute to formation of effluents. However, in this experiment, increased production of effluent may be due to the low dry matter content of elephant grass.

DMR reduced as PP was added to the silage. This reduction is associated with higher gaseous losses in treatments with PP. However, Barbosa et al. (2015) obtained an increase of DMR with inclusion of *Parkia platycephala* pods, which increased dry matter contents and promoted increased availability of soluble carbohydrates for fermentation inside silos with consequent reduction of CO₂ production. According to McDonald et al. (1991), fermentation by *Clostridium* bacteria involves decarboxylation and/or oxidation with high losses by gas (CO₂). The DMR values were considered sufficient to provide high DM recovery of the ensiled material. According to Pedroso et al. (2005), DM recovery is highly correlated with gaseous losses confirming the results obtained in this study, in which DMR was inversely proportional to gaseous losses.

Highest silage temperature was recorded for mixtures of elephant grass with PP, possibly due to increased fermentation of soluble carbohydrates present, showing higher heat production due to the elevated metabolism of microorganisms present. According to Lima et al. (2015), silages rich in soluble carbohydrates are more susceptible to aerobic deterioration. The increase in temperature of silages after opening of silos is due to the growth of aerobic microorganisms, which utilize organic acids and other soluble nutrients as an energy source, resulting in loss of nutrients (Rezende et al. 2011).

Inclusion of PP provided a suitable environment for

development of lactic acid bacteria and pH increases after opening silos is related to degradation of lactic acid by aerobic microorganisms for energy and loss of other organic acids by volatilization (Amaral et al. 2008). Increase of pH does not imply poorer quality silage (Rocha et al. 2006). Lower pH does not ensure that activity of undesirable microorganisms is prevented during fermentation unless pH reduction is achieved quickly (Oliveira 2009; Rezende et al. 2008). The final pH of the silage is related to length of the fermentation period and it becomes more difficult to reduce pH value as fermentation proceeds (Siqueira et al. 2007). Treatments with no inclusion of urea and inclusion of 10, 20 and 30 % PP and the treatment with 30 % PP and 1.5 % urea all presented mean pH values within the range recommended by McDonald (1981) for good preservation of ensiled material. However, only the treatment with 30 % PP without urea reached the maximum value after 88 h of air exposure. Silages containing urea had high pH after 96 h of air exposure.

Aerobic stability is important to retain nutrients. Rezende et al. (2011) evaluated the aerobic stability of sugarcane silages treated with lime and found the highest pH values in the treated silages due to the strong alkaline nature of the additive. Stability rupture immediately after exposure to air of silages with 0, 10 and 30 % of PP was probably due to the fast metabolism of yeasts during the first hours of aerobic activity and other spoilage microorganisms which caused the temperature to vary significantly during that period (Santos 2013). Treatments with 1.5 % urea showed higher aerobic stability, probably because of the effects of urea on development of microorganisms (Jobim et al. 2008). The highest DM contents and inclusion of urea may have hindered proliferation of microorganisms due to reduction of moisture in the silages. Bernardes et al. (2007) found that silages of tropical grasses exposed to air have a prevalence of aerobic bacteria due to fermentation stability at pH above 4.5, the high moisture content and the absence of substrates.

Conclusions

The combination of PP with urea is effective in decreasing losses by effluents as well as in keeping DMR at appropriate levels to maintain the characteristics of a good silage. Silages containing PP and urea were more stable when exposed to air, with stability rupture from 40 hours with the inclusion of 20 % of PP.

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(Note of the editors: All hyperlinks were verified 25 January 2023).

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Artículo Científico

Estimación de la disponibilidad de forraje y de parámetros asociados a la calidad nutricional del pasto *Urochloa humidicola* cv Llanero a partir de imágenes multispectrales

Estimation of forage availability and parameters associated with the nutritional quality of Urochloa humidicola cv Llanero based on multispectral images

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Resumen

El uso de imágenes multispectrales en la evaluación de pasturas se ha convertido en una herramienta práctica para la gestión de los sistemas pastoriles a nivel predial, ya que estas permiten construir índices de vegetación (IV) los cuales se relacionan con diferentes características productivas y fisiológicas de las plantas. El objetivo de este estudio fue estimar la oferta de forraje y la calidad nutricional de una pastura de *Urochloa humidicola* cv Llanero a partir de IV. La oferta de forraje (OF) y la altura de planta (ALP) fueron evaluadas en campo a los 28 días de rebrote, tomando muestras para análisis espectro radiométrico y para determinar los contenidos de proteína cruda (PC), fibra detergente neutro (FDN), fibra detergente ácido (FDA) y lignina (LIG). Los vuelos se realizaron a 70 metros de altura y se evaluaron siete IV (NDVI, GCI, SRPI, SR, GNDVI, SAVI y RDVI). El análisis de los datos se realizó por medio de componentes principales (CP) y modelos aditivos generalizados (GAM). Las variables que más contribuyeron a la formación del CP1 fueron las asociadas a la calidad nutricional del pasto y para el CP2 se agruparon las variables asociadas a la disponibilidad de forraje. El índice que mejor se relacionó con la OF fue el NDVI, con un efecto significativo por parte de la ALP ($p \leq 0.001$) y para la PC con el GNDVI. Los resultados para FND, FDA y LIG, presentaron un R^2 bajo.

Palabras claves: Ganadería de precisión, índice de vegetación, pasturas, teledetección, vehículo aéreo no tripulado.

Abstract

The use of multispectral images for pasture evaluation has become a practical tool for the management of pastoral systems at farm level, as those images allow the construction of vegetation indexes (IV) which are related to different productive and physiological characteristics of the plants. The objective of this study was to use IV for estimating the forage supply and nutritional quality of *Urochloa humidicola* cv Llanero pastures. Forage availability (OF) and plant height (ALP) were measured in the field after 28 days of regrowth, and samples were taken for spectrum radiometric analysis to determine the crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (LIG) contents. Flights were made at 70 meters and 7 vegetation indexes (NDVI, GCI, SRPI, SR, GNDVI, SAVI and RDVI) were evaluated. Data analysis was performed by using principal components (PC) and generalized additive models

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(GAM). The variables that contributed the most to PC1 were those associated with pasture nutritional quality and for PC2 were those associated with forage availability. NDVI was the index best related to OF with a significant effect by ALP ($p \leq 0.001$), and for CP with GNDVI. The NDF, ADF and LIG values showed low R^2 .

Keywords: Pastures, precision livestock, remote sensing, unmanned aerial vehicle, vegetation index.

Introducción

Los sistemas de pastoreo de gramíneas son la forma más económica de alimentación de rumiantes productores de carne o leche, apoyan la sostenibilidad de la producción bovina en general, contribuyen a disminuir costos, proporcionan mayor bienestar animal y no crean problemas de competencia con granos útiles para la alimentación humana (O'Mara 2012). La gestión adecuada de las áreas pastoriles es una tarea que puede ser compleja para ganaderos y técnicos, ya que en ella interactúan aspectos relacionados con la fisiología y crecimiento de las especies forrajeras, las condiciones intrínsecas de los suelos, aspectos de meteorología y de etología animal, y cada uno de ellos son agentes dinámicos que interactúan y se expresan en el resultado final (Fournel et al. 2017).

Con el advenimiento de la ganadería de precisión (GdP), entendida como una extensión de las estrategias productivas derivadas de la agricultura de precisión, se ha prestado más atención al uso de un conjunto de tecnologías tales como, los sistemas globales de navegación por satélite, la teledetección tanto satelital como mediante Aeronaves Tripuladas a Distancia (RPA, por sus siglas en inglés), los sistemas de información geográfica, el aprendizaje automático (*machine learning*) y otras técnicas de inteligencia artificial (Kharuf-Gutierrez et al. 2018). La teledetección es una de las herramientas fundamentales para la GdP, ya que tiene el potencial de promover un manejo más dinámico de las pasturas a partir de la respuesta espectral de las plantas, para generar índices de vegetación (IV) relacionados con la producción agrícola (Shanahan et al. 2001).

Los IV son medidas cuantitativas basadas en los niveles de reflectancia obtenidos por un sensor y corresponden a combinaciones algebraicas de varias bandas espectrales (Chuvieco 2002; Ramírez 2013), las cuales resaltan las propiedades específicas de la vegetación como son: la biomasa, la radiación absorbida y el contenido de clorofila (Mitsikostas 2017).

Los IV han sido utilizados para evaluar sistemas ganaderos, estudiando el comportamiento temporal y espacial de la disponibilidad forrajera. En Colombia, Posada-Asprilla et al. (2019) utilizaron un sensor

multiespectral para estimar la oferta y composición química del pasto kikuyo [*Cenchrus clandestinus* (Hochst. Ex Chiov) Morrene] con coeficientes de determinación (R^2) superiores a 0.90. Mientras que Pereira et al. (2015) en Brasil evaluaron la respuesta espectral y las características productivas en pasturas tropicales con diferentes dosis de fertilización. De forma similar en Alemania (Capolupo et al. 2015) estudiaron la capacidad de un sensor hiperespectral a bordo de un RPA, en la detección de variaciones estructurales y bioquímicas en parcelas de pastos que habían recibido diferentes dosis de nitrógeno.

También los IV han sido utilizados en estudios etológicos en Brasil para conocer patrones en el pastoreo de bovinos (Chiacchio 2017). En sistemas pastoriles de Escocia, Maire et al. (2018) evaluaron la capacidad de una cámara con lente modificado (RGNir) para la determinación de parches de orina y poder mejorar las estimaciones de óxido nítrico (N_2O) a nivel de potrero.

En el Cerrado Brasileiro (Pessi et al. 2020) utilizaron el algoritmo *k-mean* de clasificación no supervisada en imágenes multiespectrales, para identificar el pasto *Urochloa* spp; siendo el algoritmo asertivo para cada una de las clases observadas en campo, mientras que (Neves et al. 2019) en la región del Rio Grande del Sur en Brasil, determinaron el potencial de estos sensores a bordo de un RPA, para identificar y generar mapas de infestación de la hierba “capimannoni” (*Eragrostis plana* Nees) en pasturas nativas.

Mientras tanto Sankey et al. (2019) en Arizona central (EEUU), monitorearon las tendencias en la condición de la salud y la degradación de los pastizales a partir de imágenes multiespectrales obtenidas por un RPA y concluyeron que estas imágenes, junto a estudios de campo, permiten realizar estimaciones cuantitativas de la composición, cobertura y distribución espacial de la vegetación.

Los sensores a bordo de las RPA también han sido incorporados en diferentes sistemas de análisis. Insua et al. (2019) en Michigan (EEUU), integraron datos de NDVI recolectados por un sensor multiespectral en pasturas, a un modelo de predicción de crecimiento (SALUS, por sus siglas en inglés) y a un modelo predictivo del valor nutritivo del forraje (MDP, por sus

siglas en inglés), donde encontraron un alto grado de asociación entre el NDVI y la biomasa aforada en campo. Por otro lado, en Luxemburgo, Brenner et al. (2018) utilizaron imágenes termográficas de alta resolución a bordo de un RPA y datos micrometeorológicos obtenidos por una torre de flujo “Eddy Covariance”, para generar mapas de evapotranspiración en pastizales a partir de patrones térmicos, como resultado del comportamiento heterogéneo del flujo del calor (calor sensible y calor latente).

Mientras que Míchez et al. (2019) en Gembloux (Bélgica), desarrollaron un modelo para evaluar la biomasa en pasturas de *Lolium perenne* (ryegrass) a partir de los índices derivados de un sensor multiespectral junto a modelos digitales de superficie (DSM, por sus siglas en inglés) y concluyeron que este modelo puede ser integrado a programas de pastoreo de precisión.

Sobre la base de los trabajos citados previamente, el objetivo de este estudio fue evaluar el uso de diferentes índices de vegetación provenientes de un RPA para la estimación de la disponibilidad de forraje y composición química asociada a la calidad nutricional de una pastura de *Urochloa humidicola* cv Llanero, manejada bajo pastoreo, lo cual es un avance metodológico necesario para la gestión de las pasturas en la Orinoquia colombiana

Materiales y Métodos

Localización

El estudio se desarrolló en áreas de pastoreo bovino en el Centro de Investigación La Libertad de la Corporación Colombiana de Investigación Agropecuaria - Agrosavia, georreferenciado en las coordenadas 4°03'49.55" N y 73°27'44.16" W, a 328 m.s.n.m.

El área de estudio se ubica en la subregión del Piedemonte Llanero que hace parte de la región Orinoquia, en el municipio de Villavicencio, del departamento del Meta-Colombia (Figura 1).

La topografía es plana y homogénea, con un suelo Oxisol franco arcillo-arenoso de buen drenaje, caracterizado por alta saturación de aluminio (Al) y baja disponibilidad de fósforo (P) (Rincón et al. 2019).

La temperatura promedio es de 26 °C, la humedad relativa de 80 % y la precipitación promedio anual de 2,953 mm; la época seca va entre los meses de enero y mediados de marzo, con lluvias esporádicas que alcanzan los 110 mm y la época de lluvias va desde finales de marzo hasta el mediados de diciembre, con 322 mm/mes en promedio (Rincón y Álvarez 2010; Rincón et al. 2018).

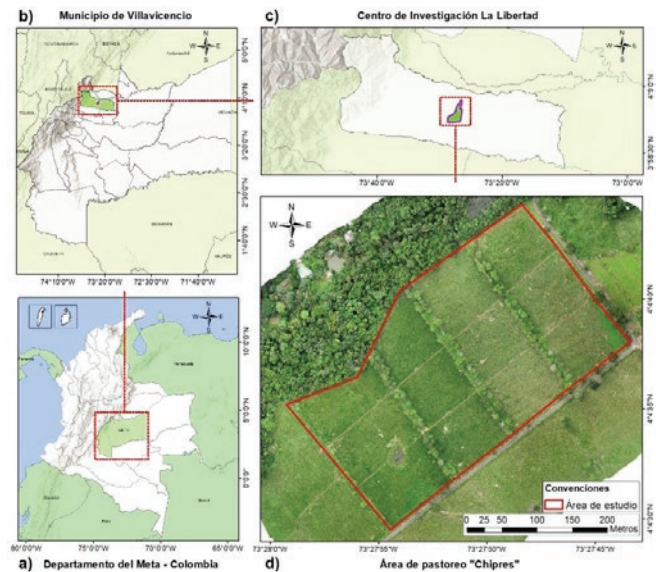


Figura 1. Ubicación del área de pastoreo en el C.I. La Libertad. a) Departamento del Meta en la República de Colombia; b) Municipio de Villavicencio en el Departamento del Meta; c) C.I. La Libertad; d) Área de pastoreo.

Imágenes ópticas del área de estudio

Se utilizó la cámara Mapir Survey 3W[®] de resolución espectral: verde (550nm), rojo (660nm) e infrarrojo cercano - NIR (850nm), la cual iba acoplada en un Vehículo Aéreo no Tripulado (VANT) Phantom 4Pro[®]; los vuelos se realizaron entre las 12:00 y 14:00 horas; previo a cada vuelo se tomaron imágenes al panel reflectante de calibración (versión 2) de Mapir[®] con el propósito de realizar las correcciones radiométricas, y reducir las distorsiones de la respuesta electromagnética del suelo y de los fenómenos atmosféricos. Los planes de vuelo fueron programados en el software Pix4D Capture[®]; se fijó la altura de vuelo a 70 metros, resolución espacial de 10.5 cm² por pixel, traslape ≥ 75 % y velocidad de la aeronave de 6 metros/segundo.

Los ortomosaicos fueron generados en el software Pix4D Mapper pro[®], y los niveles digitales de la imagen fueron convertidos a reflectancia en el aplicativo control de cámara Mapir[®] (versión 16/10/2019). El recorte de las imágenes en los puntos muestreados y la generación de los índices espectrales se realizó con el software ArcMap 10.8[®] (Cuadro 1).

Área de pastoreo

El área experimental incluyó potreros de *Urochloa humidicola* cv Llanero (pasto Llanero), (sin. *Brachiaria*

humidicola; antes considerada como *Brachiaria dictyoneura* (Cook y Schultze-Kraft 2015), manejados en un sistema de pastoreo rotacional por más de cinco años luego de haber sido establecida y con vocación productiva para la ceiba de ganado bovino. Se recolectaron un total de 200 muestras entre los meses de junio a agosto de 2019, que corresponde a la ventana meteorológica de la época de lluvias

Se determinó la altura de planta (ALP) (cm) y la oferta forrajera (OF) (kg MS/ha). Las evaluaciones se efectuaron a los 28 días de rebrote contados a partir del momento en que el ganado salió del potrero. Veintiocho días es el periodo modal de descanso para el pastoreo de esta gramínea en la región, tal como ha sido recomendado por Costa et al. (2019). La ALP fue evaluada mediante una regla desde la base la planta hasta la canopia y las muestras de forraje verde se cortaron a 20 cm del suelo de acuerdo con su hábito de crecimiento (Rincón 2011), se pesaron y posteriormente fueron secadas en una estufa de aire forzado por 72 horas a 60 °C; la materia seca (MS) se obtuvo por diferencia entre el peso verde y seco.

Cuadro 1. Índices de vegetación y relación de bandas espectrales propuestos.

Índice espectral de vegetación	Fuente
$NDVI = \frac{NIR - R}{NIR + R}$	Rouse et al. (1974)
$GCI = \left(\frac{NIR}{G}\right) - 1$	Gitelson et al. (2003)
$SRPI = \frac{R - G}{NIR}$	Peñuelas et al. (1995)
$SR = \frac{NIR}{R}$	Birth y McVey (1968)
$GNDVI = \frac{NIR - G}{NIR + G}$	Gitelson y Merzlyak (1998)
$SAVI = \frac{1.5 * (NIR - R)}{(NIR + R + 0.5)}$	Huete (1988)
$RDVI = \frac{(NIR - R)}{\sqrt{(NIR + R)}}$	Roujean y Breon (1995)

NDVI=Índice de vegetación de diferencia normalizada; GCI=Índice verde de clorofila; SRPI=Índice de reflectancia de planta senescente; SR=Proporción simple; GNDVI=Índice de vegetación de diferencia normalizada verde; SAVIA=Índice de vegetación de suelo ajustado; RDVI=Índice de vegetación de diferencia renormalizada; G=banda espectral del verde, R=banda espectral del rojo y NIR=banda espectral del infrarrojo cercano.

Los contenidos de proteína cruda (PC), fibra detergente neutro (FDN), fibra detergente acida (FDA) y lignina (LIG), se estimaron a partir de espectro radiometría del infrarrojo cercano (NIRS, por sus siglas en inglés) con el equipo de barrido VIS/NIR modelo 6500 FOSS® en el Laboratorio de Nutrición Animal del Centro de Investigación (C.I.) Tibaitatá, de Agrosavia, según la metodología propuesta por Ariza-Nieto et al. (2018).

Modelo de disponibilidad de forraje y composición química asociada a la calidad nutricional

Para identificar las relaciones existentes entre los IV y las variables evaluadas (PC, FDN, FDA, LIG), se realizó un análisis de componentes principales usando el software R (R Core Team 2016) mediante la librería FactorMinerR (Lê et al. 2008). Una vez realizado esto, se probaron tres modelos aditivos generalizados - GAM, por medio de la librería mgcv (Wood 2019), donde se evaluaron diferentes interacciones con funciones suavizadas no parametrizadas (*splines*) de regresión cúbica (Posada y Cerón 2019) entre el IV y ALP. En la Figura 2, se presenta el esquema de análisis y los modelos utilizados.

Para seleccionar el modelo que mejor se ajustaba a la estimación de la disponibilidad de forraje se utilizó el criterio de información Bayesiano (BIC), el criterio de información de Akaike (AIC), por medio de la librería MASS (Ripley et al. 2020) en el software R (R Core Team 2016).

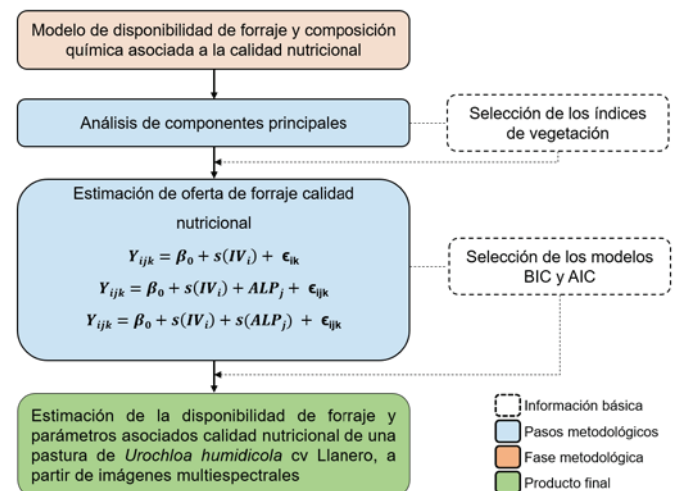


Figura 2. Modelos GAM utilizados en el estudio. Dónde: Y representa las variables evaluadas, β_0 es el intercepto, s es la función de suavizado para la variable, IV representa el índice espectral, ALP es la altura de la planta y ϵ es el efecto residual.

Resultados

Evaluación de la disponibilidad de forraje

Durante el periodo de evaluación, la precipitación en los meses de julio y agosto fue superior (9.2 % y 17.7 %, respectivamente) al promedio acumulado de los últimos 30 años, (Figura 2); esta condición favoreció el ataque poco común del mión de los pastos (*Aeneolamia* spp) (Calderón et al. 1982; CIAT 1991) (Figura 3).

El pasto Llanero presentó una ALP promedio de 44 ± 3 cm, resultado superior al reportado por Rincón (2011) para la época de lluvias en el departamento de Meta. Con relación a la OF se obtuvo un rendimiento promedio de 1,054 kg MS/ha, valor que se encuentra dentro del rango de 950 y 1,700 kg MS/ha reportado por el Instituto Colombiano Agropecuario [ICA] (1987) para la temporada de máxima precipitación.

Composición química asociada a la calidad nutricional del forraje

En el Cuadro 2, se presentan los resultados de la calidad nutricional del pasto Llanero obtenidos por medio de la técnica NIRS, en donde se evidencia que, a pesar de la alta precipitación y un ataque de mión de los pastos, la composición química del pasto no se vio afectada, lo cual demuestra su buena capacidad de recuperación y adaptación a condiciones desfavorables.

Índices de vegetación asociados a la disponibilidad de biomasa del forraje

En el Cuadro 3, se presenta los valores promedios, mínimos y máximos de los IV evaluados en esta investigación. Los resultados son relativamente bajos si se comparan con los que se pueden obtener con otras imágenes multiespectrales, lo cual es atribuido a la forma como el sensor Sony Exmor R IMX117 12MP (Bayer Red-Green-Blue) específico de la cámara Mapir Survey 3W[®] capta las imágenes (diseñado para obtener imágenes RGB). Según el fabricante: “las bandas del filtro se superponen con las del patrón Bayer en el sensor y eso afecta su transmisión. Esto puede producir un contraste diferente al esperado y valores inferiores de los que se pueden obtener con un índice proveniente de otros sensores” (MAPIR – Inc, comunicación personal - Re: [#6974402], 3 de febrero de 2020). Esta situación se evidenció a pesar de haber seguido el proceso de calibración de las imágenes recomendado por el fabricante.

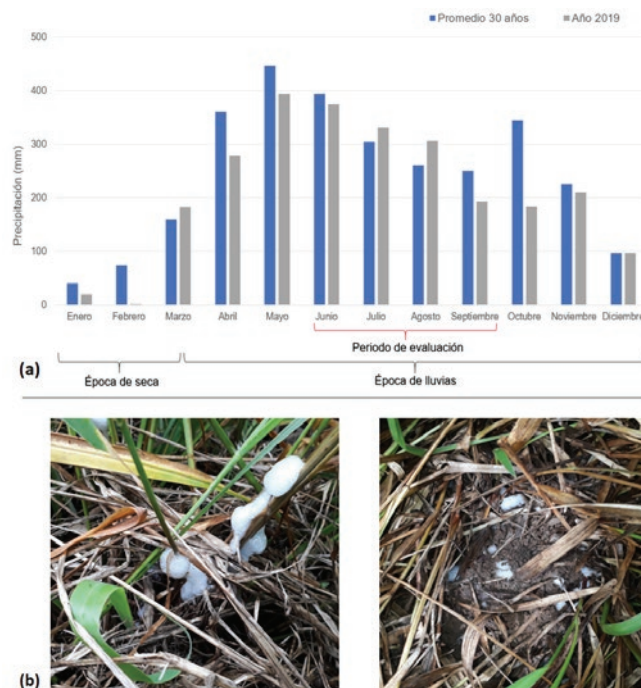


Figura 3. (a) Precipitación del 2019 comparada con el promedio de 30 años en el C.I. La Libertad, (b) presencia de *Aeneolamia* spp. en el pasto *Urochloa humidicola* cv Llanero.

Cuadro 2. Composición química del *Urochloa humidicola* cv Llanero en la época lluviosa.

Estadísticos	PC* (g/kg)	FDN* (g/kg)	FDA* (g/kg)	LIG* (g/kg)
Promedio	105	676	358	82
Mínimo	79	651	322	70
Máximo	134	712	391	92
Des	14	17	13	5

Des=desviación estándar. Dónde: PC=proteína cruda; FDN=fibra en detergente neutro; FDA=fibra en detergente acida y LIG=lignina. *Para transformar estos valores a % multiplicar por el factor 0.1

Cuadro 3. Índices de vegetación calculados para *Urochloa humidicola* cv Llanero.

Índice	Media ± DE	Mínimo	Máximo
NDVI	0.3542 ± 0.014	0.3099	0.3822
GCI	1.0040 ± 0.038	0.9014	1.0874
SRPI	0.0554 ± 0.011	0.0331	0.0899
SR	2.3432 ± 0.069	2.1663	2.4527
GNDVI	0.3334 ± 0.008	0.3107	0.3448
SAVI	0.3727 ± 0.055	0.2845	0.4469
RDVI	0.3621 ± 0.018	0.3165	0.3902

Estimación de la disponibilidad y composición química del forraje a partir imágenes multiespectrales

Se generó la matriz de correlación con el objetivo de identificar el IV que tiene mayor relación con la oferta de forraje y la calidad nutricional del pasto. En la Figura 4, se observa que la OF se correlacionó de forma positiva con la ALP (0.65) y con los índices NDVI y SR (0.74 y 0.68, respectivamente); la PC se correlacionó con el SR y GNDVI ($r=0.52$ y 0.69), mientras que para el FDN y LIG se observó una correlación negativa para el SR ($r=-0.52$ y -0.67), GNDVI ($r=-0.69$ y -0.55) y GCI ($r=-0.32$ y -0.44). Para el FDA, igualmente se presentaron correlaciones negativas de -0.53 , -0.55 , -0.78 y -0.40 para los índices SR, RDVI, GNDVI y GCI, respectivamente. Todos los coeficientes presentaron significancia de $p<0.001$.

Se realizó el test de Bartlett, que arrojó una $p < 0.001$, lo que indica que la matriz de correlaciones es distinta a la matriz de identidad, por lo que se procedió a realizar el análisis de componentes principales (ACP) a partir de la matriz de varianzas (Figura 5).

En el ACP, los dos primeros componentes explicaron el 64.18 % de la varianza total observada, donde el CP1 explica el 49 % de la varianza y en él se agruparon variables relacionadas con la composición química del pasto Llanero. Las variables más importantes fueron la PC, FND, FDA, LIG, y los IV GCI, SR, GNDVI y RDVI, donde la variable que más contribuyó a la formación de este componente fue el GNDVI (12.38). El CP2 explicó el 15.18 % de la varianza relacionada con la disponibilidad del forraje, las variables agrupadas son OF, ALP y los IV NDVI y GCI; donde el índice que más



Figura 4. Matriz de correlación de las variables de crecimiento y los índices de vegetación.

contribuyó a la conformación de este eje fue el NDVI (15.58). En el Cuadro 4, se presentan los *autovectores* que permiten identificar la contribución de cada una de las variables para la conformación de los componentes principales (CP1 y CP2).

A partir de los resultados obtenidos del ACP se construyeron los GAM para relacionar las características de OF y de composición química del pasto Llanero con los IV. Los IV utilizados corresponden a los que tuvieron mayor contribución en la formación del CP1 (GCI, SR, GNDVI y RDVI) y CP2 (NDVI y GCI), para un total 22 GAM probados (CP1=16 y CP2=6). En el Cuadro 5, se presenta la estructura de los modelos.

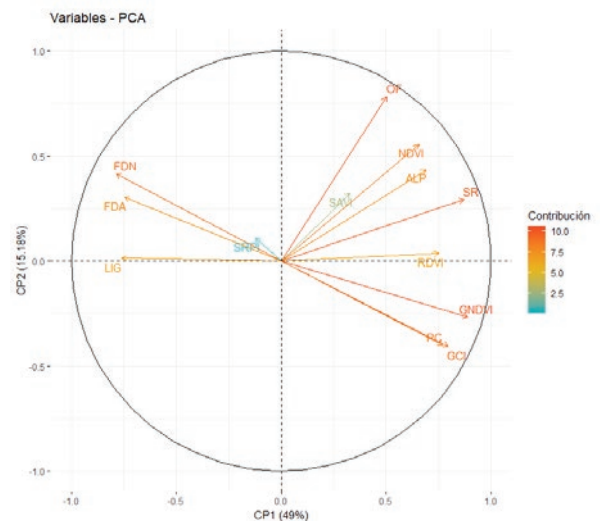


Figura 5. Análisis de componentes principales para las variables evaluadas

Cuadro 4. Autovectores reportados para cada una de las variables evaluadas.

Variables	CP1	CP2
NDVI	6.7671789	15.588463
GCI	9.9610747	8.3934135
SRPI	0.2340676	0.6038061
SR	11.902275	4.3834425
GNDVI	12.389612	3.6173191
SAVI	1.6580291	5.2876
RDVI	8.8789385	0.0697551
OF	3.9561736	31.005273
ALP	7.4469754	9.5775559
PC	9.3085735	8.1223782
FDN	9.6872631	8.6764744
FDA	8.7488051	4.6648082
LIG	9.0610334	0.0097109

Cuadro 5. Estructura de los GAM utilizados en el estudio.

ACP	Modelo	Ecuación
CP1	$Z_{ij} = \beta_0 + s(IV_i) + \epsilon_{ij}$	Ecuación 1
	$U_{ij} = \beta_0 + s(IV_i) + \epsilon_{ij}$	Ecuación 2
	$V_{ij} = \beta_0 + s(IV_i) + \epsilon_{ij}$	Ecuación 3
	$W_{ij} = \beta_0 + s(IV_i) + \epsilon_{ij}$	Ecuación 4
CP2	$Y_{ij} = \beta_0 + s(IV_i) + \epsilon_{ij}$	Ecuación 5
	$Y_{ijk} = \beta_0 + s(IV_i) + ALP_j + \epsilon_{ijk}$	Ecuación 6
	$Y_{ijk} = \beta_0 + s(IV_i) + s(ALP_j) + \epsilon_{ijk}$	Ecuación 7

El modelo que mejor explicó la OF fue el que consideró el IV y la ALP en función suavizada [$Y_{ijk} = \beta_0 + s(IV_i) + s(ALP_j) + \epsilon_{ijk}$] (Cuadro 5 - Ecuación 7), sin presentar una diferencia marcada; pero al probar este modelo con el NDVI se obtuvieron valores de R² superiores (0.78), desviación explicada (0.79) y con valores inferiores de BIC y AIC, en comparación con el GCI (Cuadro 6); para ambos casos, el IV y la ALP obtuvieron un valor de p≤0.001. En la Figura 6a, se

presenta los valores ajustados entre el NDVI y la OF, y se observa una respuesta curvilínea; en la Figura 6b, se presentan los valores ajustados entre el NDVI, ALP y la OF, y se observa que la OF está relacionada con diferentes rangos del índice de vegetación y a diferentes alturas, como se presenta en el Cuadro 7.

Con relación a la PC, el modelo que utilizó el GNDVI en función suavizada presentó un efecto significativo con la variable dependiente (p≤0.001) (Figura 7) con un R² de 0.764, desviación explicada de 0.77, menor BIC (2,558) y menor AIC (2,517), en comparación a los otros índices (Cuadro 6). El modelo presenta de la siguiente forma:

$$Z_{ij} = \beta_0 + s(IV_i) + \epsilon_{ij}$$

Donde, Z es la PC (g/kg), β_0 es el intercepto, IV es el índice GNDVI, s es la función de suavizado para la variable y ϵ es el efecto residual.

El uso del RDVI para estimar el FDN, presentó un R² con la variable dependiente de 0.583 y desviación explicada de 0.593 (Figura 8a); mientras que para estimar el FDA se obtuvo un R² de 0.378 y una desviación explicada de 0.392 con el IV SR (Figura 8b); así mismo, el SR contribuyó en la estimación de la LIG con valores de R² y desviación explica de 0.392 y 0.446 (Figura 8c) (Cuadro 6).

Cuadro 6. Criterios de selección para los modelos evaluados

Parámetro	Índice	BIC	AIC	R ²	Desviación explicada
OF	NDVI	5,238	5,177	0.788	0.795
	GCI	5,290	5,227	0.758	0.767
PC	RDVI	2,838	2,799	0.541	0.561
	SR	2,723	2,682	0.557	0.575
	GNDVI	2,490	2,449	0.764	0.77
	GCI	2,558	2,517	0.712	0.72
FDN	RDVI	2,981	2,938	0.583	0.593
	SR	3,051	3,013	0.491	0.501
	GNDVI	3,187	3,164	0.234	0.242
	GCI	3,142	3,100	0.361	0.375
FDA	RDVI	2,672	2,633	0.362	0.375
	SR	2,663	2,623	0.378	0.392
	GNDVI	2,415	2,773	0.278	0.286
	GCI	2,402	2,764	0.284	0.291
LIG	RDVI	2,111	2,068	0.366	0.381
	SR	1,993	1,951	0.435	0.446
	GNDVI	2,070	2,029	0.328	0.341
	GCI	2,037	1,994	0.379	0.391

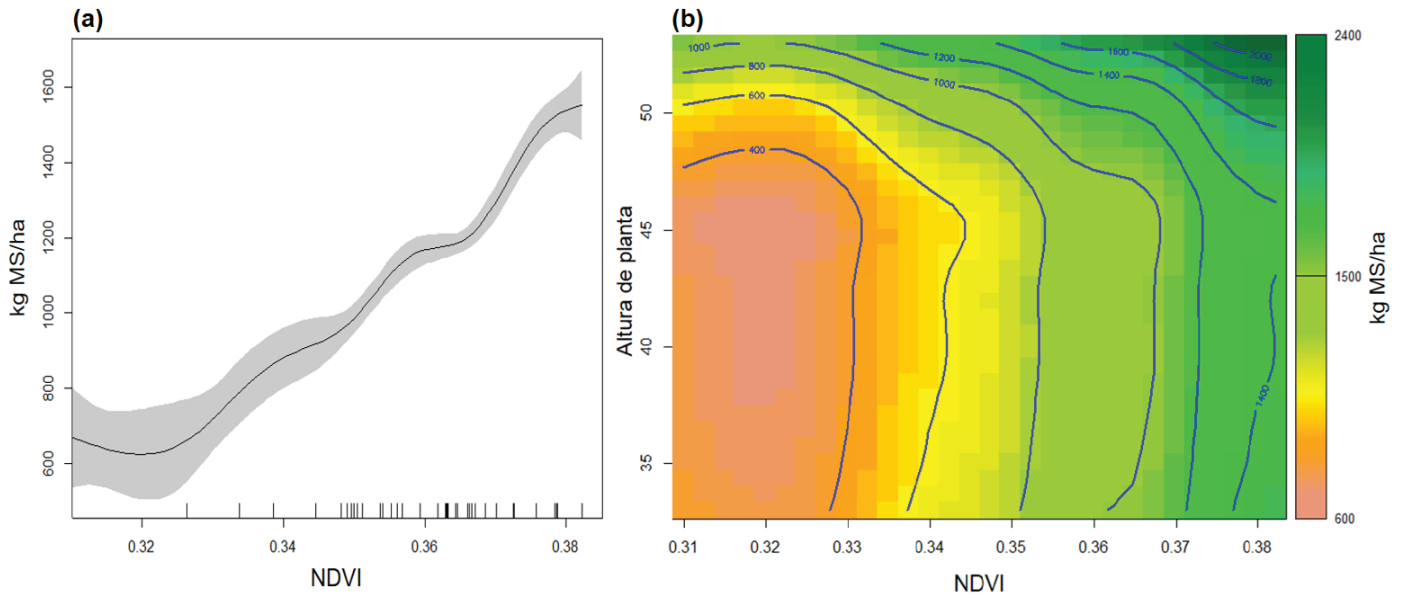


Figura 6. (a) Ajuste entre los valores del IV NDVI y la oferta de forraje del pasto Llanero, (b) Ajuste entre los valores del NDVI, la altura de planta y la oferta de forraje del pasto Llanero.

Cuadro 7. Relación de la oferta de forraje con respecto al NDVI y ALP.

NDVI	ALP (cm)	Oferta de forraje (kg MS/ha)
0.31–0.34	≤ 50	400–600
0.341–0.36	≥ 30	600–1,000
0.361–0.38	≥ 30	1,000–1,600
≥ 0.381	≥ 50	1,600–2,000

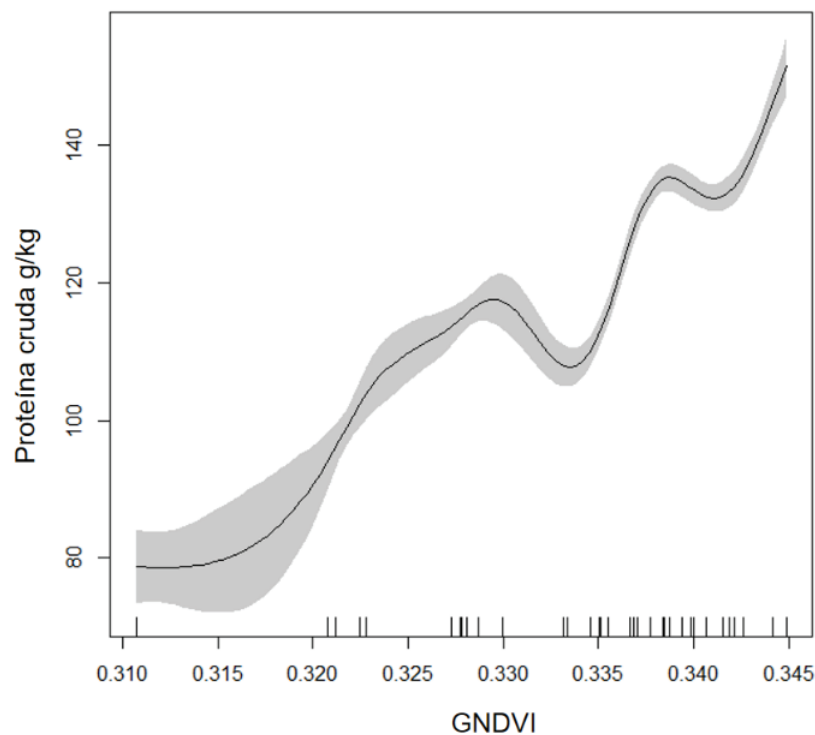


Figura 7. Ajuste entre los valores del IV GNDVI y la proteína cruda del pasto Llanero.

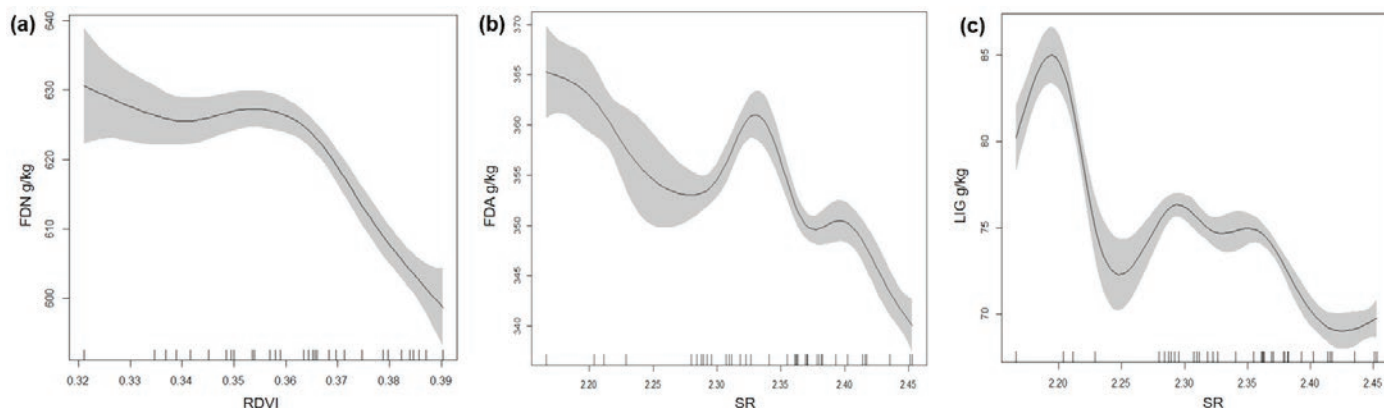


Figura 8. (a) Ajuste entre los valores del RDVI y la fibra en detergente neutra (FDN) del pasto Llanero, (b) Ajuste entre los valores del SR y la fibra en detergente acida (FDA) del pasto Llanero y (c) Ajuste entre los valores del SR y la lignina del pasto Llanero.

Discusión

Evaluación de la disponibilidad de forraje

En este estudio, la disponibilidad promedio de forraje fue de 1,054 kg MS/ha, valores muy cercanos a los reportados por Rincón et al. (2018), que obtuvieron una producción de 958 kg MS/ha para el pasto Llanero (testigo sin fertilizar), en el Piedemonte del departamento del Meta, mientras que Pérez et al. (2019), en la Altillanura colombiana obtuvieron una OF de 1,030 kg MS/ha.

Por su parte Giraldo et al. (1989) reportaron una producción de 750 kg MS/ha en el municipio de Amalfi, Antioquia, y Sánchez et al. (1989), Carulla et al. (1991), Passoni et al. (1992) y Vela y Flores (1996) presentaron valores más altos a los obtenidos en este estudio, con disponibilidades de biomasa de 1,500; 1,538; 1,200 y 2,000 kg MS/ha, respectivamente. Según Insua et al. (2019) las pasturas que no sobrepasan los 3,000 kg MS/ha antes de ser pastoreadas, pueden ser monitoreadas a partir de imágenes multiespectrales. Por consiguiente, los resultados obtenidos en la presente investigación están dentro del rango óptimo descritos por dichos autores.

Composición química asociada a la calidad nutricional del forraje

Los contenidos promedio de PC, FDN, FDA y lignina fueron 10.5 %, 67.6 %, 32.2 % y 8.2 %, respectivamente. Los valores de PC obtenidos en este estudio fueron similares a los reportados por Rincón et al. (2018), Pérez y Cuesta, (1994), Pardo y Pérez (2010) y Pérez et al. (2019) quienes obtuvieron valores de PC de 8.6 a 12 % en gramíneas que crecen en los Llanos Orientales. En relación con los contenidos de FDN y FDA, los valores son similares a los reportados por Giraldo et al. (2007), Rincón

et al. (2018), Canchila et al. (2009), Sánchez et al. (2000) y Nguku (2015) con valores para FDN que varían entre 63.4 a 75.4 % y FDA entre 35.1 y 40 %, respectivamente.

Índices de vegetación asociados a la disponibilidad de biomasa del forraje

Sinde et al. (2020) obtuvieron promedios de NDVI de 0.363, 0.371 y 0.362, en pasturas de kikuyo (*Cenchrus clandestinus*), ryegrass anual (*Lolium multiflorum*) y ryegrass perenne (*Lolium perenne*) respectivamente, que son similares a los obtenidos en este estudio usando el lente NIR GP39728 Green, que es fabricado por la casa comercial *Peauroductions* anexa a Mapir.

Las cámaras Mapir Survey[®] también han sido incorporadas con resultados satisfactorios en diferentes niveles de análisis; así por ejemplo, Maimaitjiang et al. (2020) obtuvieron un $R^2=0.72$ cuando estimaron el rendimiento de la producción de grano de soya (*Glicine max*) en un modelo de análisis de aprendizaje profundo, a partir de la respuesta espectral de la planta y características térmicas y de textura. Por otro lado, Kerkech et al. (2020) obtuvieron un 92 % en la detección de enfermedades en viñedos de la región del Valle de Loira en Francia a partir de imágenes RGB y multiespectrales

Estimación de la disponibilidad y composición química del forraje a partir imágenes multiespectrales

Las relaciones obtenidas en el ACP fueron inversas a las reportadas por Posada-Asprilla et al. (2019), quienes encontraron para el CP1 una varianza explicada de 46.2 %, con variables asociadas a la oferta de forraje del pasto kikuyo [*Cenchrus clandestinus* (Hochst. ex Chiov.) Morrone] y el CP2 explicó el 24.1 % de la varianza, donde se agruparon las variables relacionadas

con la composición química; sin embargo, este estudio coincidió que el NDVI, fue el que más contribuyó a la formación del componente asociado a la disponibilidad de forraje. El SRPI y SAVI fueron los que menos contribuyeron en la conformación del CPI (Tabla 4).

Para la estimación de la OF se probaron los índices de vegetación NDVI y GCI, junto con la interacción de la ALP, se obtuvo una correlación positiva con estos índices de 0.74 y 0.63, respectivamente (Figura 3).

La correlación del NDVI con la OF y la ALP concuerda con lo reportado por la literatura en relación con las características de crecimiento de las pasturas, donde el NDVI es un indicador de biomasa, en especial cuando la vegetación evaluada presenta buena cobertura (Zerbato et al. 2016). Esta característica es propia de una pastura bien manejada de *Urochloa humidicola* cv Llanero, que es un genotipo de crecimiento postrado, y que bajo esas condiciones presenta una alta producción de estolones y buena cobertura del suelo (Rincón 2011; Rincón et al. 2018).

La correlación de la ALP con la OF fue de 0.65, valor similar al reportado por Blanco et al. (2014), quienes indican que la altura de la especie (*U. decumbens* y *U. humidicola*) es la variable que mayor probabilidad tiene (0.59) de estar directamente relacionada con los valores de NDVI generados de la imagen de satélite. De forma similar Scarabotti et al. (2011) argumentaron que la ALP a pesar de ser un parámetro de fácil obtención, no ofrece mayor ventaja en la estimación de la biomasa en macollas de *Spartina argentinensis*; sin embargo, resaltan que la acción del pastoreo mejora el ajuste entre ALP y la OF. En contraposición a esto, Braga et al. (2009) especificaron que los modelos para estimar la OF de *Urochloa brizantha* cv Marandú, a partir de ALP obtenida por medidor de placa ascendente, deben ser específicos para cada mes o temporada de evaluación, frente a los modelos que cubren la temporada total, y autores como Santillán et al. (1979), Arruda et al. (2011) y Bernardi y Pérez (2014) encontraron resultados satisfactorios en la estimación de la biomasa con esta misma metodología.

Con respecto a la estimación de la disponibilidad de forraje a partir de índices espectrales, el coeficiente de determinación (R^2) obtenido en esta investigación, es similar a lo reportado por Díaz et al. (2021) quienes obtuvieron un R^2 de 0.712 en una pastura de *Urochloa humidicola* cv Llanero en la Altillanura colombiana entre el NDVI y la oferta de biomasa. En cambio, Gargiulo et al. (2020) encontraron una relación exponencial entre el NDVI y la oferta de

biomasa, con un R^2 (0.77) en pasturas de ryegrass anual (*Lolium multiflorum* Lam) en New South Wales, Australia. De igual manera Posada-Asprilla et al. (2019), estimaron la oferta de biomasa verde a partir de modelos GAM en pasto kikuyo [*Cenchrus clandestinus* (Hochst. ex Chiov.)], y encontraron que el NDVI explicaba mejor la biomasa, con un R^2 de 0.993.

En relación con la PC, el GNDVI utiliza la diferencia normalizada entre las bandas espectrales verde y NIR, lo que genera una medición en un rango menor (Cuadro 3) en comparación al NDVI; sin embargo, Gitelson y Merzlyak (1998) encontraron que este índice presenta alta sensibilidad a la clorofila en diferentes coberturas vegetales, obteniendo un valor de R^2 de 0.96. El contenido de clorofila en las hojas tiene alta relación con la concentración de nitrógeno (N) lo cual refleja el estado nutricional con respecto a este nutriente (Alonso et al. 2008; Rincón y Ligarreto 2010; Kharuf-Gutierrez et al. 2018), el cual está relacionado con el contenido proteico de las hojas (Rincón et al. 2019).

El modelo para estimar la FDN a partir del RDVI reportado en este estudio coincidió con lo encontrado por Pullanagari et al. (2012) en una pradera compuesta por ryegrass perenne (*Lolium perenne* L.) y trébol blanco (*Trifolium repens* L.), quienes obtuvieron un R^2 de 0.40 para un modelo exponencial. Estos autores también estimaron la relación entre FDA y LIG con IV y obtuvieron R^2 de 0.58 y 0.40, respectivamente, cuando usaron modelos lineales. Ellos argumentaron que estos valores bajos pueden estar relacionados con el hecho que longitudes de onda amplias enmascaran información espectral esencial y por consiguiente no permiten explicar las características detalladas de los pastos. En apoyo a lo anterior, Starks et al. (2006) trabajando con pasto Bermuda (*Cynodon dactylon*) recomendaron el uso de bandas más estrechas para evaluar sustancias químicas foliares, y recalcaron la importancia de la región del borde rojo en la predicción de la bioquímica foliar. Sin embargo, Posada-Asprilla et al. (2019) a partir de modelos GAM, trabajando con pasto kikuyo [*Cenchrus clandestinus* (Hochst. ex Chiov.)], no encontraron mayores diferencias con el uso del borde rojo para la estimación del FDN y FDA, logrando valores igualmente bajos de R^2 (0.43 y 0.24, respectivamente).

Conclusiones

Con los resultados obtenidos en pasturas de *Urochloa humidicola* cv Llanero fue posible determinar que el NDVI es el índice de vegetación que mejor se

correlaciona con la disponibilidad de forraje y el GNDVI con la proteína cruda.

Los modelos GAM propuestos para las estimaciones de disponibilidad de biomasa y contenido de proteína a partir de los índices NDVI y GNDVI generados con imágenes multiespectrales de un VANT, son un buen estimador de la disponibilidad forrajera y el contenido de proteína cruda en pasturas de *Urochloa humidicola* cv Llanero; en cambio, con el tipo de sensor evaluado las estimaciones de los contenidos de fibra detergente neutra, fibra detergente ácida y lignina no funcionan como buenos estimadores.

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Nota Técnica

Análisis económico de la producción de semilla gámica de *Tithonia diversifolia* (Hemsl.) Gray en Cuba

Economic analysis of the production of gametic seed of Tithonia diversifolia (Hemsl.) Gray in Cuba

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Resumen

La *Tithonia diversifolia* es una de las plantas proteicas más aceptada por los ganaderos en Cuba para alimentar a los animales. Encontrar una alternativa económica para fomentar sistemas de producción con esta planta es vital para disminuir los costos asociados a la plantación e indirectamente en la alimentación, por lo que el objetivo de este trabajo fue determinar qué método de propagación (por semilla vegetativa o gámica) de tithonia resulta económicamente más factible para la producción de semilla gámica en las condiciones de Cuba. Se determinaron las fichas de costo de la producción de semilla gámica de tithonia a partir de plantaciones por vía asexual. Luego, a partir de este valor se determinó el costo de producción de la semilla gámica sembrada por vía sexual. El costo de producción de la semilla gámica fue de 15.07 USD/kg cuando se sembró con material vegetativo, y de 11.04 USD/kg cuando se usó semilla gámica. Se demuestra que el costo de establecimiento de una hectárea de tithonia para la producción de semillas se reduce hasta 3 veces cuando se parte del uso de semilla gámica, comparado al uso de semilla vegetativa (US\$ 142.13 vs 472.05, respectivamente).

Palabras clave: Costos de producción, material vegetativo, métodos de propagación, semilla sexual.

Abstract

Tithonia diversifolia is one of the protein-rich plants most accepted for animal feeding by Cuban farmers. Identification of an economic alternative to promote production systems using this species is critical to reduce forage production costs, and indirectly feeding cost. The objective of this study was to identify the most economically feasible method for tithonia seed production using either vegetative planting materials or gametic seeds for plant propagation under Cuban conditions. Detailed gametic seeds production costs of tithonia from plots planted using either vegetative planting materials or gametic seeds were estimated. The cost of production per kilo of gametic seeds was US\$ 15.07 and 11.04, when multiplication plots were established using either vegetative planting materials or sexual seeds, respectively. The investment for establishing one hectare of a tithonia multiplication plot was reduced to one third when gametic seeds were used, as compared to the use of vegetative planting materials (US\$ 142.13 vs. 472.05, respectively).

Keywords: Costs of production, planting material, propagation methods, sexual seeds.

Introducción

En los últimos años se ha impulsado el empleo de plantas proteicas para la mejora de la alimentación animal en Cuba ([Alonso-Vázquez et al. 2021](#)) y otros países en desarrollo ([Ramírez et al. 2012](#); [Lopera et al. 2015](#);

[Hoyos-Rojas et al. 2021](#)). *Tithonia diversifolia* es una de estas plantas proteicas más aceptadas por los ganaderos cubanos ([Paniagua-Hernández et al. 2020](#); [Padilla y Rodríguez 2021](#); [Padilla et al. 2021](#)).

La tithonia posee un alto potencial de adaptación a múltiples condiciones ambientales, presenta una

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elevada capacidad de acumulación de nitrógeno, buen valor nutritivo con altos contenidos de proteína y minerales, alta digestibilidad de la materia seca y presencia de aceites tanto en hojas como en flores (Ruíz et al. 2016; Ruíz et al. 2017), lo que ha contribuido a su distribución a lo largo de las empresas ganaderas cubanas y en otros países tropicales (Mejía-Díaz et al. 2017; Guatusmal-Gelpud et al. 2020).

A nivel internacional son varias las investigaciones que muestran con indicadores económicos el impacto positivo del uso de esta especie en los sistemas silvopastoriles y en la alimentación animal a partir de material vegetativo. Así lo han demostrado en América Latina, Navarro (2018) y Fuente-Martínez et al. (2019) en la alimentación de aves y Arias-Gamboa (2018) y Angulo-Arizala et al. (2021) en bovinos. También en África, Katongole et al. (2016) en bovinos y Abiloro et al. (2020) en conejos han mostrado sus bondades económicas.

En Cuba, Iraola et al. (2022) evaluaron diferentes indicadores económicos en el comportamiento productivo de machos vacunos en silvopastoreo con *T. diversifolia*, demostrando la posibilidad de utilización de estos sistemas como alternativa para la producción de carne. De igual forma Iriban-Díaz y Alonso-Vázquez (2022) indican el impacto económico positivo en la producción de leche; por ello, la posibilidad de disminuir los costos de establecimiento contribuye a una mayor rentabilidad y difusión de estos sistemas con *T. diversifolia* y, por lo tanto, una mayor disponibilidad de carne y leche para la demanda de la población mundial creciente.

En los últimos años, tanto a nivel internacional como nacional, se ha producido un incremento de las investigaciones sobre la propagación por vía gámica de tithonia. Si bien se ha avanzado en aspectos de carácter agronómico como la siembra y establecimiento puede aumentar los rendimientos (Mahecha et al. 2015; Gallego-Castro, 2016; Mahecha et al. 2017; Padilla et al. 2018b; Mattar et al. 2019; Santos-Gally et al. 2019a,b), no ha sido así con los aspectos económicos. Resulta difícil encontrar en la literatura científica reportes sobre este aspecto, incluso en sitios de compra y venta de semillas, pues por lo general esta especie solo se comercializa para fines de jardinería y con precios bastante elevados. Esto demuestra que aún es insuficiente la disponibilidad y generalización de esta vía de propagación de tithonia, pese a que constituye un nicho de mercado y por lo tanto una oportunidad para la producción a gran escala y posterior comercialización de semilla gámica de esta especie.

En Cuba, la tithonia se considera una forma barata de suministrar proteína al ganado, por lo que la búsqueda

de alternativas económicas para la reproducción y propagación de esta especie por vía gámica constituye un reto y una prioridad para la comunidad científica (Rodríguez et al. 2021). Por tanto, el objetivo de este estudio fue determinar qué método de propagación (por semilla vegetativa o semilla sexual) resulta económicamente más factible para la producción de semilla gámica de *Tithonia diversifolia* en las condiciones de Cuba.

Materiales y Métodos

La investigación se realizó en el Centro Experimental de Pastos y Forrajes 'Miguel Sistachs Naya' del Instituto de Ciencia Animal (ICA), ubicado en el municipio de San José de las Lajas, actual provincia de Mayabeque, Cuba, situada a lo 23°55 Norte y a los 82°00 Oeste, a 92 msnm. El tipo de suelo es el Ferralítico Rojo Eútrico, de rápida desecación, arcilloso y profundo sobre calizas (Hernández et al. 2015).

Para el desarrollo del trabajo se tomaron los costos directos involucrados en el establecimiento de tithonia según investigaciones desarrolladas en el ICA (Ruíz et al. 2016). Se tuvo en cuenta los elementos involucrados en la preparación del suelo, la siembra y labores culturales de esta planta. Se confeccionó la ficha de costo en dólares (USD) a partir de la inversión en mano de obra, combustibles, maquinaria (depreciación) y otros gastos (compra de semillas y fertilizantes). El gasto por concepto de semillas proviene del precio informado por la Empresa Productora y Comercializadora de Semillas de Cuba y es de 0.04 USD la estaca (Ministerio de la Agricultura 2021). Se determinó que a partir de las necesidades de material vegetativo para la plantación de una hectárea (4.5 toneladas) se requirieron 10,000 estacas, para un costo de 416.67 USD. El costo de salario de un obrero se obtuvo de la Resolución No.29-2020 (Ministerio del Trabajo y la Seguridad Social 2020) y el costo del litro de combustible de la Resolución No.350-2020 (Ministerio de Finanzas y Precios 2020) vigentes en Cuba. Todos los datos convertidos a USD usando la tasa de cambio actual en el 2022 de 24 pesos cubanos (CUP) por 1 USD.

Para determinar la ficha de costo de la producción de semilla gámica se tuvieron en consideración los costos directos involucrados en la cosecha y poscosecha, a partir de las recomendaciones tecnológicas de Padilla et al. (2022). Posteriormente, una vez estimados los costos de establecimiento por vía asexual y teniendo en cuenta los costos de la cosecha y poscosecha se procedió a calcular la ficha de costo de un kilogramo de semilla gámica en condiciones experimentales y con el procesamiento de la semilla de forma manual, empleando métodos artesanales.

A partir de este valor y la información de siembras por vía sexual realizadas en la Dirección Científica Tecnológica y Productiva “El Guayabal” de la Universidad Agraria de La Habana, también en San José de las Lajas provincia Mayabeque, se procedió a determinar la ficha de costo del kg de semilla gámica obtenida por vía sexual según las recomendaciones tecnológicas de Padilla et al. (2022).

Resultados

El costo total de la plantación y establecimiento de una hectárea de tithonia usando material vegetativo ascendió a 472.05 USD (Cuadro 1). Teniendo en cuenta que una plantación de tithonia tiene una vida útil para la producción de semilla de tres años, se dividió el costo total de siembra y establecimiento entre las tres posibles cosechas de semilla gámica, por lo que este valor fue de 157.35 USD/ha.

Los costos de las labores culturales (28.91 USD/ha) y de cosecha y poscosecha de la semilla (265.79 USD/ha) (Cuadro 2 y 3) fueron inferiores al costo de establecimiento

total de la hectárea de tithonia.

El costo total de producción de semilla para una hectárea fue de 452.05 USD a partir de plantaciones con material vegetativo (Cuadro 3). Considerando que según Padilla et al. (2020) el rendimiento estimado promedio de la producción de semillas es de 30 kg/ha, el costo del kg de semilla gámica sembradas por vía vegetativa es de 15.07 USD/kg. Este rendimiento promedio oscila entre 24.0–53.26 kg/ha de semilla pura germinable (SPG) en dependencia del material (Padilla y Rodríguez 2021), por lo que además se muestran otros valores del costo del kg de semilla de acuerdo con diferentes niveles de rendimiento (Cuadro 4). Como es de esperar, a medida que se incrementen los rendimientos, menor será el costo de producción del kg de semilla gámica.

El cálculo del costo de siembra y establecimiento por semilla gámica se puede apreciar en el Cuadro 5. Este valor asciende a 142.13 USD/ha para los 3 años de vida útil del campo de semillas y para una cosecha el valor es de 47.38 USD/ha. Ambos valores resultan considerablemente menores que cuando se siembra con material vegetativo.

Cuadro 1. Ficha de costo en USD de la plantación y establecimiento de una hectárea de tithonia con material vegetativo.

Labores	Jornadas ¹	Salario	Combustible	Maquinaria	Otros insumos	Total (USD)	
I. Preparar Tierra	Pase de arado	5.33	3.08	12.24	0.46	15.78	
	Pase de grada medio	4	2.32	2.77	0.06	5.15	
	Cruce de arado	4.4	2.57	8.90	0.35	11.82	
	Pase de grada fino	3.2	1.85	1.98	0.06	3.90	
	Surcado	2	1.16	1.75	0.16	3.07	
	Sub-total	18.93	1.98	27.64	1.10	0.00	39.72
II. Labores de establecimiento	Corte de semilla	0.8	0.46	0.07	0.11	0.65	
	Acarreo de semilla	2	1.16	0.58	0.28	2.02	
	Plantación manual	8	4.63			4.63	
	Semilla tithonia ²	0				416.67	416.67
	Limpieza	10	5.79				5.79
	Tape Semilla	2	1.16	1.17	0.26		2.58
	Sub-total	22.8	13.20	1.82	0.65	416.67	432.33
Total (I+II)		24.17	29.46	1.75	416.67	472.05	
III. Valor para una cosecha						157.35	

¹Es la multiplicación de las horas trabajadas por la cantidad de obreros. Esto multiplicado por el salario por hora, es el monto de salario desembolsado en la labor; ² 4.5 t/ha (agámica)

Cuadro 2. Ficha de costo en USD de las labores culturales en una hectárea para la producción de semilla de tithonia con material vegetativo.

Labores	Jornadas	Salario	Combustible	Maquinaria	Otros insumos	Total (USD)	
IV Labores Culturales	Chapea	1.6	0.93	1.20	0.11	2.24	
	Aporque	4	2.32			2.32	
	Fertilización	2	1.16	0.90	0.14	22.17	24.36
	Sub-total	7.6	4.40	2.10	0.25	22.17	28.91

Cuadro 3. Ficha de costo en USD de las labores de cosecha y poscosecha en una hectárea para la producción de semilla de tithonia con material vegetativo.

Labores	Jornadas	Salario	Combustible	Maquinaria	Total (USD)	
V Cosecha	Recoger semilla	200	115.75		115.75	
	Acarreo	15	8.68	1.46	0.56	10.70
	Sub-total	215	124.43	1.46	0.56	126.45
VI Poscosecha	Secado natural	30	17.36			17.36
	Tratamiento	120	69.45			69.45
	Beneficio de la semilla	80	46.30		3.91	50.21
	Envasar y almacenar	4	2.32			2.32
	Sub-total	234	135.42	0	3.91	139.33
Total Cosecha y Poscosecha (Suma V+VI)					265.79	
Total Producción semilla (Suma III+IV+V+VI)					452.05	

Cuadro 4. Costo de producción del kg de semilla gámica de tithonia a partir de siembras con material vegetativo según niveles de rendimiento de SPG.

Rendimiento de SPG	Costo de producción del kg de semilla gámica (USD)
24	18.83
30	15.07
40	11.30
53.26	8.48

Cuadro 5. Ficha de costo en USD de la siembra y establecimiento de una hectárea de tithonia por semilla gámica.

Labores	Jornadas	Salario	Combustible	Maquinaria	Otros insumos	Total (USD)	
I. Preparación de suelo	Pase de arado	5.33	3.08	12.24	0.46	15.78	
	Pase de grada medio	4	2.32	2.77	0.06	5.15	
	Cruce de arado	4.4	2.57	8.90	0.35	11.82	
	Pase de grada fino	3.2	1.85	1.98	0.06	3.90	
	Surcado	2	1.16	1.75	0.16	3.07	
	Sub-total	18.93	10.98	27.64	1.10	0.00	39.72
II. Labores de siembra	Semilla botánica				15.07	15.07	
	Acarreo de semilla	1	0.58	0.58	0.28	1.44	
	Siembra manual	12.48	7.23			7.23	
	Acarreo de cobertura vegetal	1	0.58	0.58	0.28	70.00	71.44
	Aplicar cobertura vegetal	12.48	7.23			7.23	
	Sub-total	26.96	15.63	1.17	0.56	85.07	102.42
	Total (I+II)		26.61	28.81	1.65	85.07	142.13
III. Valor para una cosecha						47.38	

Las labores culturales (28.91 USD/ha), cosecha (126.45 USD/ha) y poscosecha (139.33 USD/ha) coinciden en ambos métodos de siembra (Cuadros 2 y 3 respectivamente). En este caso (siembra por vía sexual) el valor del costo de producción de una hectárea para semilla gámica de tithonia es de 342.08 USD/ha y el costo del kg de esta semilla es de 11.04 USD/kg con

un rendimiento promedio de 30 kg/ha de SPG. Se muestra además, otros valores de costos de producción teniendo en cuenta diferentes niveles de rendimiento (Cuadro 6). Los costos disminuyen con el incremento de los rendimientos en la producción de semillas.

El costo de producción de un kg de semilla gámica (Cuadro 7) varía según el método de siembra empleado,

siendo más económico en siembras por vía sexual, ya que los costos de las estacas y el volumen requerido son superiores.

Cuadro 6. Costo de producción del kg de semilla gámica de tithonia a partir de siembras por vía gámica según niveles de rendimiento de SPG.

Rendimiento de SPG	Costo de producción del kg de semilla gámica (USD)
24	14.25
30	11.04
40	8.55
53.26	6.42

Cuadro 7. Comparación económica de ambos métodos de propagación para la producción de semilla botánica de tithonia.

Costos (USD) Para una hectárea	Método de propagación	
	SA ²	SG ³
Semilla	416.67	15.07
Siembra y establecimiento	472.05	142.13
Producción total de semillas	452.05	342.08
Producción de 1 kg de semilla gámica ¹	15.07	11.04

¹Rendimiento estimado promedio de 30 kg/ha

²SA=Semilla agámica (dosis 4.5 t/ha)

³SG=Semilla gámica (dosis 1.0 kg/ha)

Discusión

Estudios realizados por Cino et al. (2012) en Cuba reportaron un costo de establecimiento de 790.62 USD/ha para siembras con semilla vegetativa, lo cual es mayor al reportado en este trabajo, pero aún se considera aceptable teniendo en cuenta que las condiciones económicas cuando se desarrolló dicho estudio (tasa de cambio vigente, sistema contable, entre otras) no eran las mismas que las actuales. En un trabajo más reciente, también en Cuba, Iriban-Díaz y Alonso-Vázquez (2022) reportaron un costo de establecimiento por esta misma vía de propagación de 336.20 USD/ha. En este último trabajo la densidad de siembra del sistema fue menor al que se considera aquí, lo cual explica su valor más bajo. Buitrago et al. (2017) reportaron en Colombia un costo de establecimiento de la hectárea de tithonia por vía vegetativa de entre 680 y 1,100 USD. Cabe recalcar que las condiciones de ambas investigaciones no son iguales, pero se considera un monto aceptable. Como se puede observar en la Cuadro 1, la adquisición del material

vegetativo para siembra representó la mayor inversión en este tipo de plantación.

Se asumió que la vida útil del campo de semillas es de tres años, a partir de las experiencias de investigación reportadas por Padilla et al. (2022) sobre la producción de semilla con cuatro variedades de tithonia en Cuba, en las que los semilleros mantuvieron un rendimiento aceptable por tres años; pero será necesario seguir realizando otros estudios de ese tipo con una duración mayor, para ver si mantienen la productividad por un mayor tiempo. Otros autores (Arias-Gamboa et al. 2018) han considerado hasta 10 años como la vida útil de una plantación de tithonia para la producción de biomasa forrajera; sin embargo, en este estudio se considera un período inferior (3 años), ya que para la producción de semilla es necesario disponer de una mayor población de plantas y una mayor cantidad de tallos fértiles para la floración. La experiencia práctica y los resultados de las investigaciones en otras especies como *Megathyrus maximus* (Jacq.) B. K. Simon & S. W. L. Jacobs, indican que a partir del tercer año la producción de semilla disminuye y hay que aplicar labores de recuperación de las áreas para mantener la vida útil en la producción de semilla (Padilla et al. 2018a).

Es necesario aclarar que la cosecha y poscosecha de la semilla fueron completamente manuales, excepto un último proceso de beneficio que requirió de equipo, lo que encarece el costo de producción de semilla al necesitar de un mayor uso de mano de obra en el proceso. Si se pudiera disponer del equipamiento necesario para todo el proceso es posible que se pueda abaratar aún más los costos. La recogida de las semillas (115.75 USD) fue la que tuvo un mayor peso dentro del costo de la cosecha y poscosecha.

Como se puede apreciar en la Cuadro 2, la aplicación de la fertilización constituye el 84.26 % del costo de las labores culturales. Si bien esta práctica no es común utilizarla en la producción de forraje para el ganado por el incremento en los costos; en cambio, se considera como un requisito indispensable en los sistemas de producción de semillas de cualquier especie. Existe evidencia de la respuesta de la tithonia a la fertilización nitrogenada (Astúa-Ureña et al. 2021) y a la aplicación de humus (Lugo-Soto et al. 2013) para la producción de biomasa en parcelas sembradas utilizando la propagación agámica, pero no hay información sobre su efecto en la producción de semilla, por lo que se fertilizaron para asegurar un buen crecimiento de las plantas. El tema de la fertilización de semilleros de tithonia queda por investigar.

Los costos de establecimiento y mantenimiento de las áreas de semillas, así como los beneficios económicos obtenidos por la producción de semilla de tithonia, varían

en función de la disponibilidad de maquinaria y equipos de calidad, así como de la contratación de servicios para las diferentes prácticas y labores culturales requeridas.

La dosis de semilla gámica a utilizar varía en función del propósito productivo de la siembra, lo cual está relacionado a su vez con la densidad de plantas/ha que se desea obtener. En Cuba, para el establecimiento de áreas de forraje y/o producción de semillas, Padilla et al. (2022) recomiendan una dosis de siembra de 0.75 a 1.0 kg/ha con semilla gámica que posee más del 50 % de germinación y 95 % de pureza; mientras que para el fomento de sistemas silvopastoriles con tithonia asociadas con gramíneas en pastoreo, la dosis de siembra recomendada es de 0.25 kg/ha. En cambio, en Colombia se proponen dosis de siembra superiores (5, 7 y hasta 10 kg/ha, con semillas no seleccionadas), aunque no se aclara el nivel de pureza de la semilla (Gallego et al. 2017 y Arguello-Rangel et al. 2020).

A lo anterior se suma que la dosis de siembra de la semilla a utilizar va a estar relacionada con la calidad de los lotes de semillas, donde las variables de SPG y los porcentajes de germinación definen la dosis a utilizar. Estos indicadores influyen directamente en los costos.

Desde el punto de vista agronómico también resulta más ventajosa la siembra por semilla gámica, ya que Padilla y Rodríguez (2021) en Cuba reportan una mayor rapidez en la germinación de las siembras por semilla gámica comparado a las plantaciones con material vegetativo, bajo condiciones iguales. Además, no se ha observado diferencias en cuanto al rendimiento de forraje y producción de semillas por una vía u otra.

Según Londoño et al. (2019) el porcentaje de pérdidas de plantas es significativamente menor con semilla sexual (5–10 %) que con material vegetal (30–40 %), y los costos de producción son menores reduciendo a la mitad los costos asociados al uso de la mano de obra.

Conclusiones

Se concluye que el costo de establecimiento de una hectárea de tithonia para la producción de semillas se reduce hasta 3 veces cuando se parte del uso de semilla gámica. Se demuestra los beneficios desde el punto de vista económico de las siembras por semilla gámica, lo cual reafirma los otros beneficios de esta vía de propagación como, por ejemplo, la necesidad de un menor volumen para la siembra y el mayor tiempo de conservación de la semilla gámica. La importancia de este trabajo radica en que se demuestra que es rentable producir semilla gámica fundamentalmente usando semilla sexual, lo

cual abre una ventana para la comercialización de esta especie e incrementar su acceso en el mercado que hoy es prácticamente nulo para los ganaderos.

Se recomienda continuar los estudios de la producción de semilla gámica de tithonia, donde las labores de poscosecha se realicen fundamentalmente con tecnologías de avanzada, como por ejemplo mediante el secado artificial, el empleo de zarandas para la selección y el envasado automático, entre otras. Esto permitirá alcanzar una mayor escala de producción y mayor disponibilidad de semilla sexual para la comercialización.

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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

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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. Søgaard 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 (Chapman et al. 2014). Humphreys (1996) 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 (Karki 2013).

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 (Ball et al. 2001), especially from vegetative to reproductive stage (Karki 2013). As forage crops mature, dry matter yield increases, but digestibility of

neutral detergent fiber (NDF), starch, sugar and crude protein declines (Kilcer et al. 2003). 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) (Google Earth Engine, 2022). 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 $[(\% \text{ Germination} \times \% \text{ Purity}) \div 10,000]$ 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 $CP \% \times DM \text{ yield}$. 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.

$$Y_{ijkl} = \mu + A_i + B_j + Y_k + A_i \times B_j + e_{ijkl},$$

where:

μ =population mean;

A_i =ith effect of legume species;

B_j =jth effect of growth stage;

Y_k =kth effect of year;

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

e_{ijkl} =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

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.

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

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

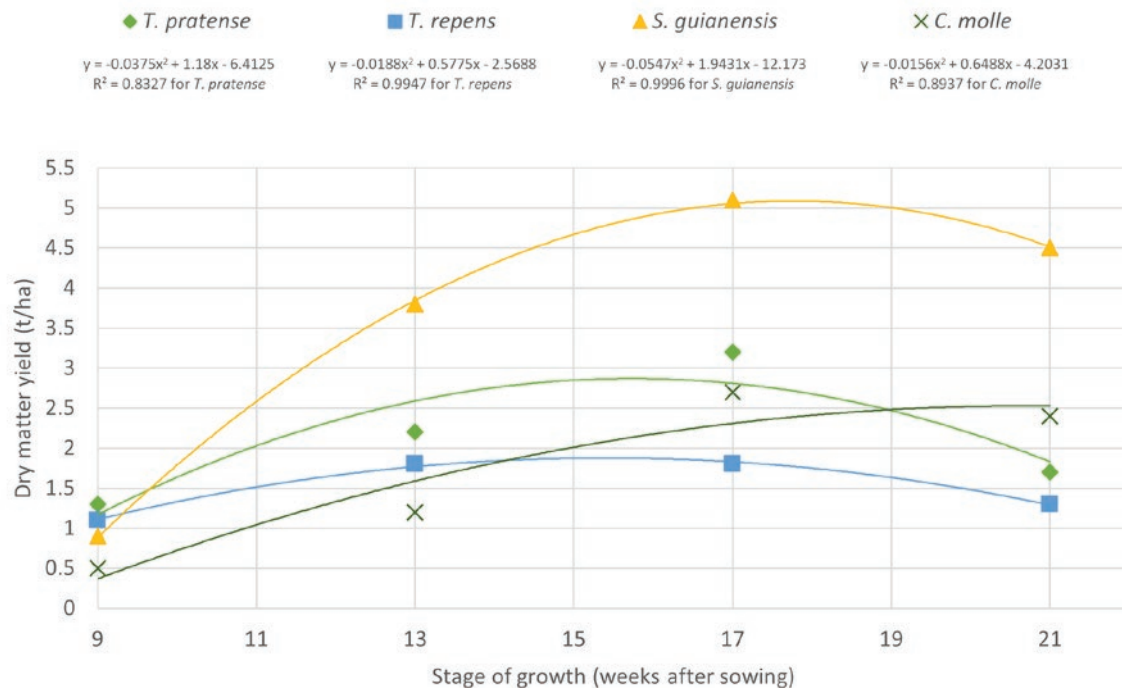
**Figure 1.** Correlations between dry matter yield and growth stage for 2 temperate and 2 tropical forage legumes. Data for *T. pratense*, *S. guianensis* and *C. molle* are averages for 2 years (2015 and 2016), while *T. repens* data are for 1 year (2016).

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

Parameter	Leaf no./plant		Leaf:stem ratio	
	2015	2016	2015	2016
Species				
<i>T. pratense</i>	27.8 ^c	53.4 ^b	0.6 ^b	0.5 ^b
<i>T. repens</i>	na	156.9 ^a	na	0.4 ^c
<i>S. guianensis</i>	85.6 ^a	46.8 ^b	0.6 ^b	0.3 ^c
<i>C. molle</i>	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	15.9 ^d	23.7 ^c	1.1 ^a	1.0 ^a
13	33.7 ^c	57.5 ^b	0.7 ^b	0.6 ^b
17	71.5 ^a	115.6 ^a	0.5 ^c	0.3 ^c
21	65.2 ^b	117.2 ^a	0.3 ^d	0.2 ^c
s.e.m.	2.2	11.89	0.02	0.06
P value	<0.01	<0.01	<0.01	<0.01

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	CP	CPY	Ash	ADF	NDF
<i>T. pratense</i>	9	208.8 ^d	275.0 ^{efgh}	118.0 ^{bc}	234.7 ^{gh}	341.2 ⁱ
	13	198.2 ^e	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 ^e
	21	180.3 ^{hi}	453.7 ^{bcde}	96.0 ^{fg}	282.4 ^e	435.2 ^e
<i>T. repens</i>	9	256.2 ^a	292.7 ^{efgh}	132.5 ^a	198.8 ⁱ	278.3 ^k
	13	246.5 ^b	452.9 ^{bcde}	129.9 ^a	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 ^c	280.2 ^{efgh}	111.2 ^{de}	261.4 ^f	377.7 ^{fg}
<i>S. guianensis</i>	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 ^c	434.5 ^c
	17	162.2 ^{jk}	816.3 ^a	76.9 ^{ij}	361.2 ^b	470.5 ^b
	21	143.6 ^l	640.0 ^{ab}	71.2 ^{ij}	391.3 ^a	494.1 ^a
<i>C. molle</i>	9	195.1 ^{ef}	106.3 ^h	108.3 ^{bc}	275.4 ^e	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 ^c	430.9 ^e
	21	169.2 ^{jk}	384.7 ^{defgh}	84.6 ^h	357.3 ^b	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.

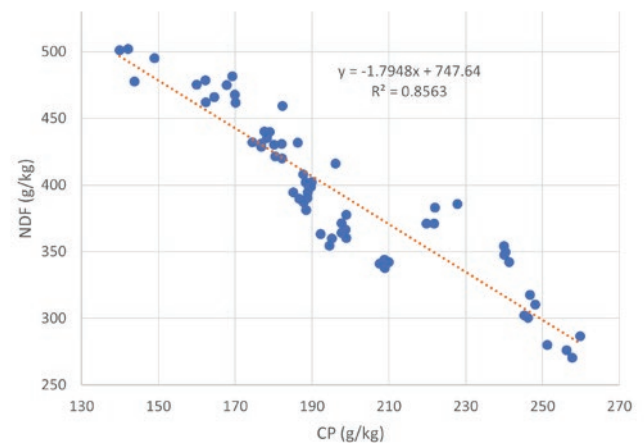


Figure 2. Correlation between neutral detergent fiber and crude protein concentrations for 2 temperate (*T. pratense* and *T. repens*) and 2 tropical (*S. guianensis* and *C. molle*) legumes.

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

Legume	WAS	Calcium	Phosphorus	Magnesium	Potassium	Sodium
<i>T. pratense</i>	9	18.3 ^a	3.6 ^{abc}	5.1 ^a	20.1 ^c	1.6 ^b
	13	18.1 ^a	3.2 ^{abc}	4.5 ^b	19.1 ^e	1.0 ^c
	17	17.8 ^{ab}	2.4 ^{cd}	3.9 ^c	18.6 ^f	0.8 ^{de}
	21	17.7 ^{ab}	2.4 ^{cd}	3.5 ^d	18.0 ^{gh}	0.2 ^f
<i>T. repens</i>	9	17.2 ^{bc}	4.7 ^a	3.8 ^c	25.2 ^a	2.6 ^a
	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 ^c	0.8 ^{de}
<i>S. guianensis</i>	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 ^e	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
<i>C. molle</i>	9	14.8 ^e	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 ^l	0.4 ^{ef}
	21	13.6 ^f	1.2 ^d	1.0 ^l	13.2 ^m	0.2 ^f
s.e.m.		0.4	0.8	0.1	0.2	0.3

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 (Ratnayake 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 yields 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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Obituary Peter Campbell Kerridge



Peter Kerridge, an internationally distinguished plant nutrition scientist and pasture research leader, died in Queensland in September 2022 at the age of 87 years.

Peter was born in Stanthorpe, Queensland in 1935 and grew up with his 3 sisters on their parents' orchard, where fruit and table grapes were grown. He was educated at Scots College, Warwick, where he boarded from an early age, and at the University of Queensland, Brisbane (B.Agr.Sc. 1957).

After graduating Peter worked for 3 more years with the University of Queensland on fodder conservation in western Queensland and on the ecology of a native legume, *Psoralea eriantha* (now called *Cullen patens*). During those years he formed a lasting friendship with Dr. Percy Skerman, a legendary agricultural scientist of that era. Percy's influence probably strengthened Peter's lifelong commitment to achieving practical outcomes for farmers from his research.

For the next 3 years (1961-64) Peter worked as a volunteer in Indonesia, located at the agricultural university in Bogor and studied the adaptation of forage legumes to acid soils. There he first experienced the challenges of conducting agricultural research in and for developing countries, and that was probably where he developed his research interest in plant nutrition and response to fertilizer applications.

His interest in plant nutrition took him next to Oregon State University, Corvallis, USA where, from 1964, he undertook Ph.D. studies on aluminum toxicity in wheat and identified genetic diversity in tolerance of high aluminum levels.

In 1968 Peter joined the CSIRO Division of Tropical Crops and Pastures to study the fertilizer requirements of dairy pastures, initially in north Queensland. His research on the plant nutrient status of soils on the Atherton Plateau demonstrated acute and widespread deficiencies of molybdenum, with deficiencies of potassium and phosphorus also common. With colleagues in the Queensland Department of Primary Industries he extended these studies to the Queensland subtropics.

In 1973 CSIRO commenced a program of scientific support to the then newly-established Malaysian Agricultural Research and Development Institute (MARDI). Part of this program involved the development of a pasture research capability within MARDI. Plant nutrition was an obvious and urgent priority, and Peter was seconded to Serdang, Malaysia for 5 years to identify plant nutrient deficiencies in the main pasture soils and to develop fertilizer recommendations for the livestock-production experiments that were to follow. His research indicated that: both phosphorus and nitrogen deficiencies were widespread on the main pasture soil types; potassium and molybdenum were often limiting; and deficiencies of copper and magnesium also occurred on some soils.

Returning to CSIRO in Brisbane in mid-1978, Peter commenced a new research program on the maintenance fertilizer requirements of grazed tropical grass-legume pastures. With colleagues, he planned and personally led the so-called "Nutrient Cycling Trial" (NCT) at CSIRO's Narayen Research Station near Mundubbera, Queensland. This large-scale experiment commenced in January 1981, when pastures containing buffel grass (*Cenchrus ciliaris*) and either shrubby stylo (*Stylosanthes scabra*) or Siratro (*Macroptilium atropurpureum*) were sown. Various maintenance fertilizer treatments were applied and the paddocks were grazed by cattle. The research soon showed that it was necessary to provide



Peter in his garden around 2015

maintenance fertilizers (in this case, both phosphorus and sulphur) in order to retain legumes – and hence a productive grass – in pastures on these infertile soils. Research showed that cattle liveweight gain not only responded to maintenance fertilizers, but also somewhat unexpectedly was closely linked to phosphorus intake by cattle. Dietary phosphorus supplements were also beneficial, especially during drought conditions that prevailed in the later years of the experiment, which ended in 1989.

In about 1990 Peter visited CIAT (Centro Internacional de Agricultura Tropical), Colombia as a consultant. In September 1992 he returned to CIAT as head of their Tropical Forages Program, where his energy and vision led to several new initiatives. Among these was the so-called “Forages for Smallholders Project” (FSP), which he led. This participatory research program involved both CIAT and CSIRO, along with research workers in Indonesia, Laos, Malaysia, the Philippines, Thailand, Vietnam and southern China. It was funded by AusAID during 1995-99, and subsequently by the Asian Development Bank (2000-2003). Its focus was on the development of technologies (particularly forage plants) for use by smallholder upland farmers. While Peter was based at CIAT’s headquarters (Cali, Colombia) for most of this time, in 1999 he shifted to the Philippines (briefly) and then to Vientiane, Laos, where he lived for more than 2 years. Thousands of smallholders benefitted from this research.

Peter retired in 2002 and returned to Australia, where he purchased a small farm near Nambour in SE Queensland. This became a showplace for Australian

native plants, providing foliage to local florists. Peter also took great pleasure in restoring the 1926 Armstrong Siddeley utility that had been used as a farm vehicle when he was a boy.

Peter left a legacy of about 100 research publications, which are evidence of his major contribution to the development of tropical pasture research in Australia, South America and SE Asia. His approach was strongly collaborative; most of his contribution occurred as a member of teams of professional research workers and there was always a practical focus to his research. It ranged in scale from the laboratory to the field and from the development of measurement techniques to the increased productivity of grazing cattle. His contribution to tropical pasture research was recognized in 1995 when he was made a fellow of the Tropical Grassland Society of Australia. He was a tenacious individual who did not allow the challenges (and sometimes the monotony) of research to stop him from finishing the tasks he accepted. He believed strongly in the adage “the work is not finished until the paper work is done”.

His efforts in the research field were not confined to conducting and publishing his own research and in 1989, 1990 and part of 1991 Peter was editor of *Tropical Grasslands*, the journal of the Tropical Grassland Society of Australia, which has now been replaced by *Tropical Grasslands-Forrajes Tropicales*. It is appropriate that this contribution should be recognised specifically in this brief biography.

Bob Clements
December 2022

Obituary Richard (Dick) Arthur Date



It is with great sorrow that we report the death of our friend and colleague Dr Dick Date, an eminent CSIRO (Commonwealth Scientific and Industrial Research Organisation) scientist and member of the Australian and international rhizobial research communities, who passed away in Brisbane, Queensland on 5 December 2022.

Dick was born in Wollongong, New South Wales on 14 November 1934 and developed a lifelong dedication to the plant sciences and agriculture through his early years on the family farm, attending Hurlstone Agricultural High School and completing undergraduate and M.Sc. Ag. (1959) degrees at The University of Sydney. He then travelled overseas to obtain his Ph.D. (1962) from College Park at The University of Maryland. Dick's postgraduate research focussed on growth, survival, serological classification and competitiveness of rhizobia, particularly in relation to the soybean-rhizobia symbiosis.

Dick went on to obtain a wealth of practical experience in rhizobial inoculant technology and its delivery through his work with FAO (Food and Agriculture Organization) to develop an inoculant industry and investigate problems with legume establishment in Uruguay. On return to Australia (1965), Dick was appointed Officer-in-Charge of The University of Sydney-Department of Agriculture

Laboratory Service, the lead organization responsible for quality control of rhizobial inoculants produced in Australia at that time.

In 1969, Dick accepted a position at CSIRO Division of Tropical Pastures in Brisbane, where his focus shifted to selection of rhizobial inoculant strains for crop and pasture legumes grown in tropical and subtropical (northern) Australia.

Continuing the pioneering work of Dr Don Norris and others, Dick set about to increase the number and diversity of rhizobia held in the CSIRO Brisbane (CB) *Rhizobium/Bradyrhizobium* strain collection to meet the needs of rapidly expanding tropical pasture research programs across northern Australia. He targeted his collection activities on legumes that nodulated ineffectively with rhizobia present in Australian soils or failed to nodulate at all, particularly species of *Stylosanthes*, *Desmodium*, *Leucaena*, *Centrosema*, *Desmanthus*, *Glycine* and *Macroptilium*. This was an enormous undertaking, as the selection of tropical legume inoculant strains lagged behind the underpinning knowledge associated with temperate and Mediterranean legumes of southern Australia and involved a far greater number of legume genera and species, many of which were new to agriculture at that time.

Because of these challenges, Dick sought ways to reduce the number of rhizobial strains and legume accessions under study and increase the likelihood of selecting inoculants that were highly effective in their nitrogen-fixing capacity with the target host legume(s). By applying what was then newly developed Pattern Analysis to results of his glasshouse studies, he identified key strains that typified nitrogen-fixing effectiveness response groups and, consequently, broadly effective strains with commercial potential. Dick also embarked on targeted field collection trips to regions of the world with similar soil and climatic conditions to northern Australia. He did this to increase the chance of finding inoculant strains with desirable attributes, such as tolerance to neutral to alkaline fertile clay soils common to Queensland grain-cropping regions. These approaches proved highly successful in selecting inoculants for species of *Stylosanthes* and *Desmanthus* that exhibited specific rhizobial strain requirements.

A second focus of Dick's research was to improve understanding of rhizobial diversity and ecology in soil and especially persistence of inoculants in hot and dry conditions prevalent in northern Australia. Dick and colleagues refined serological strain typing and identified antibiotic resistance markers that enabled the



With Alison McInnes during retirement

fate of rhizobial inoculants to be followed in non-sterile environments including on seed, in plant rhizospheres and in soil. Dick applied that research to develop new options for delivery of inoculants, such as introducing rhizobia into soil at depth in winter prior to sowing the legume in hot soils the following summer. Dick also overcame difficulties in obtaining inoculant strains for some *Stylosanthes* species collected overseas by isolating them directly from nodules in the field and then growing them in acidic laboratory culture under low-oxygen conditions.

In addition to his own work, Dick was actively engaged in the national rhizobial research agenda, participating in successive government-industry steering committees established to provide oversight of commercial inoculant strain release and quality control. He also contributed extensively to the Australian Rhizobium Newsletter and was a co-editor of this publication from 1978 to 1981. As President of the Australian Society for Nitrogen Fixation from 1991 to 1993, he played a lead role in organizing the 10th Australian Nitrogen Fixation Conference, the proceedings of which were published in *Soil Biology and Biochemistry* (Volume 27 p. 381-738). He was always extraordinarily generous with his time, providing invaluable advice to post-graduate students and emerging researchers through both his own work and as part of the wider Australian and international research communities.

Dick retired in 2000 after more than 30 years of service to CSIRO but continued to publish his research findings until 2016. He also continued to advise on both conservation of the CB *Rhizobium/Bradyrhizobium*

strain collection and techniques for inoculant delivery in northern Australia up until his death. Dick's lasting legacies include an extensive body of peer-reviewed literature, the CB collection (now held at Legume Rhizobium Sciences, Murdoch University, Western Australia) and the ongoing nitrogen benefit to the northern Australian grain and cattle industries from the 20 commercial tropical inoculant strains. We remember Dick fondly as a warm, approachable and generous man, and an ethical, rigorous and hardworking scientist, who provided wonderful mentoring and companionship to his research colleagues throughout all phases of his professional life.

Retirement allowed Dick and his wife of 60 years, Jan, to pursue many other interests, including camping adventures with the Queensland Naturalists Club and volunteer guiding at Mt Coot-tha Botanic Gardens and Sherwood Arboretum in Brisbane. He was also an accomplished club golfer and continued playing until late in 2022. The long-term friendships Dick formed through these activities were a great comfort and support to him in the final years of his life following the death of Jan in 2018.

Dick is survived by his children Cathy, Elizabeth and Andrew, grandchildren Ursula, Emma, Erin and Julian and their partners. We convey our deepest sympathies to all members of Dick's family for their recent loss.

Alison McInnes and Bruce Pengelly
January 2023

Obituary Wang Yanrong



We are sad to report the death of Dr Wang Yanrong, an internationally respected forage seed scientist, who died in Beijing on 12 January 2023. She was 66 years old, just short of planned retirement.

Dr Wang was born in Da'an County, Jilin Province, NE China in 1956. She was educated at local primary, middle and high schools, after which she became a teacher. In 1977, she was accepted to attend Gansu Agricultural University in Lanzhou where she graduated as a pasture scientist (BAGSc, 1982). There she came under the influence of renowned Professor Ren Jizhou, who established the Gansu Grassland Ecological Research Institute (GGERI) in 1981 and recruited Yanrong while she was an undergraduate honours student. She was among the first researchers appointed.

In 1982/83 Yanrong spent 3–4 months learning English language at Beijing No. 2 Foreign Language University. During the 1980s, on leave from GGERI she studied at the Seed Technology Centre, Massey University, New Zealand where she was fortunate to work with two eminent seed scientists, Professors John Hampton and Murray Hill. She obtained a Certificate in Seed Technology from her brief first visit (1984/85). She returned for a longer visit (1987–1990) when she accompanied her husband Nan ZhiBiao, who is one of the leading grassland scientists in China, when he was doing his graduate studies there. She graduated with

a Masters degree (1st Class Honours) in Agricultural Science. Later in her career, Yanrong was awarded a PhD in seed physiology from Nanjing Agricultural University in 2003 for her research on the measurement and control of membrane damage during seed deterioration.

In 1986, at the age of 30 and still working within GGERI, Yanrong was appointed as Director of the Herbage Seed Testing Centre of the Ministry of Agriculture in Lanzhou, responsible for forage seed quality assessment throughout western China. She held that position for more than 30 years, leading a staff of 15 researchers and training dozens of seed analysts. While her personal research extended to grassland ecology, forage germplasm assessment, domestication of native forage plants (especially from arid regions of China) and assessment of turfgrasses, her main focus was on seed and seedling physiology, seed quality, seed production and seed storage. Late in her career, her postgraduate students included molecular techniques in their joint research.

Most of her research focussed on temperate forage species, especially alfalfa (lucerne) seed technology. This research supported the development of an alfalfa seed industry in the Hexi Corridor of Gansu, an arid environment dotted with oases originating in rivers and streams from the Qilian Mountains. In 2001 she became a Chinese co-leader of an ACIAR-funded project (2001–2006) on the development of alfalfa adapted to adverse environments in China and Australia. Research in that project showed that a simple change to the irrigation regime used for alfalfa seed production in the Hexi Corridor could increase seed yields by 40 %. Five new alfalfa cultivars emerged from this project, including one produced by GGERI.

Towards the end of the ACIAR project Yanrong worked more on tropical forage species and spent two years (2004–2006) on a post-doctoral appointment at the International Livestock Research Institute (ILRI) in Addis Ababa. There she joined the Forage Diversity Project, leading a team of 7 technicians working on developing improved methods for seed germination of tropical legumes and carrying out research on the effects of temperature and dormancy breaking treatments to improve germination of *Vigna* and *Sesbania*. This research was summarised in several publications. She also jointly supervised a PhD student from LZU working on morphological and nutritional diversity of *Lathyrus* at ILRI to identify accessions with potential for use in dryland areas of Gansu. Later still, towards the end of her career she supervised some research on treatment of



Yanrong and Zhibiao outside GGERI, Lanzhou

Leucaena leucocephala seeds collected from southern China.

In her 40-year research career Yanrong published more than 220 refereed scientific papers. At first, most of her publications were in Chinese language journals, although she did also publish in English. In 2002 when GGERI was merged with Lanzhou University (LZU) to establish the College of Pastoral Agriculture Science and Technology, Yanrong became a Professor and her access to students greatly increased her research and publication opportunities. In addition to about 60 postgraduate students, she had research collaborators in many countries. She travelled widely, undertaking long periods of research in Ethiopia (ILRI), England (University of London), and Denmark (National Seed Testing Station), in addition to her years in New Zealand.

Yanrong was active in several international scientific organisations. She attended numerous international conferences, raising awareness of research being conducted in China and extending her personal network. For many years China was not a member country of the International Seed Testing Association (ISTA), so Yanrong was not able to hold office. However, she was a personal member of ISTA and she was an active member of their Vigour and Germination Committees. She attended ISTA Congresses in Argentina, Denmark, France, Turkey and New Zealand. Yanrong was also a long-time member of the Executive Committee of the International Herbage Seeds Group (IHSG) and served as coordinator for China and Asia. Her influence and personal reputation encouraged IHSG to hold its 8th International Herbage Seeds Conference at LZU in 2015. It attracted 160 delegates from 14 countries. Yanrong chaired the local organising committee, co-

edited the proceedings, presented the opening plenary paper on China's herbage seed industry and co-authored 14 papers. It was perhaps the highlight of her career. John Hampton, who attended the conference, said recently that the 5-day post-conference trip along the Hexi Corridor was remarkable for the extent to which Yanrong's research and contribution to the seed industry was everywhere acknowledged. Yanrong attended two International Grassland Congresses. At the 21st IGC in China in 2008 she presented some of the research on tropical *Vigna* species and *Lathyrus* diversity from her research at ILRI. In 2013 she was an invited speaker at the 22nd IGC in Sydney, Australia, where she reviewed the management of seed dormancy in forage grasses and legumes. She also co-chaired a session on turfgrasses.

Yanrong received several awards within China for her research achievements. In 1992 she became Vice-President of the Chinese Society for Forage Seed Science and Technology. Although the herbage seed industry in China is still at an early stage of development, its progress owes much to her lifetime of scientific support. Her legacy includes nine new cultivated plant varieties, a comprehensive seed testing protocol for forage species and a generation of trained researchers and technicians. Commenting on her career, Professor Ren described her as a leading scientist, always optimistic. He said her death was a great loss for herbage seed science and technology in China.

Yanrong is survived by her husband, Nan ZhiBiao, daughter Fang and husband Song Fangwei and granddaughter Shun er.

Bob Clements
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