ISSN: 2346-3775

Tropical Grasslands -Forrajes Tropicales

Online Journal

Vol. 9 No. 2

May 2021

Published by:

Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia

International Center for Tropical Agriculture
Since 1967 Science to cultivate change

In association with:

Chinese Academy of Tropical Agricultural Sciences (CATAS), Haikou, Hainan, P.R. China

CATAS 1954

www.tropicalgrasslands.info

Tropical Grasslands-Forrajes Tropicales Vol 9, No 2 (May 2021)

Announcement

This is the last issue edited by Rainer Schultze-Kraft and Lyle Winks and we would like to give all our gratitude, admiration and respect to them for their outstanding work as editors of the journal. Their committed work to publish excellence in all aspects of tropical forages research is reflected in the quality of the papers published, both in shape and substance, and the steady increase of the rankings and metrics of the journal:

- During their role as editors, *Tropical Grasslands-Forrajes Tropicales* has published 332 papers, 179 in special issues (119 contributions to the International Grassland Congress 2013 and 60 contributions to the International Leucaena Conference 2018), and 153 in regular issues.
- The Journal is indexed in the core collection of Science Citation Index Expanded and two additional indexes of Clarivate Web of Science, which provides the best-known impact factor indicator (formerly: ISI Journal Impact Factor). The Impact Factor of the Journal for 2019 was 0.703 (for 2018 was 0.441 and for 2017 was 0.389, showing a constant increase)**.** The Impact Factor 2020 will be available in mid-2021.
- TGFT is also indexed in Scopus, and the CiteScore for 2020 is currently 1.60, making a considerable progress since 2017 (0.44), 2018 (0.69) and 2019 (1.30). CiteScore is the way how Scopus measures the impact of its indexed journals.
- The SCImago Journal & Country Rank is a portal that includes the journals and country scientific indicators developed from the information contained in the Scopus® database (Elsevier B.V.). These indicators can be used to assess and analyze scientific domains. For 2019, the Journal had a score of 0.37 (in 2018 was 0.28), and ranked in the second highest value amongst the set of journals ranked by SJR (Q2).

The journal is in a transition period to the new editors, Dr. Jean Hanson, English and Managing Editor and Dr. Danilo Pezo, Spanish Editor. Both have worked with the International Livestock Research Institute (ILRI) for many years and have long and broad experience in tropical forages. Jean is an Emeritus fellow with ILRI in Ethiopia and Danilo is currently working with CATIE in Costa Rica. Jean and Danilo bring complimentary expertise to their roles as editors. Jean is a forage scientist working with forage germplasm and seed production, while Danilo is a livestock scientist working with animal nutrition, pastures and silvopastoral options in tropical livestock systems. The new editorial team will continue to be well supported by Jose Luis Urrea Communications Specialist at The Alliance of Bioversity International and CIAT, Colombia.

During the transition period, there have been some delays in the publication process and the journal has been unable to accept submissions in Spanish. The new editors are working hard to deal with the pending submissions, but we do apologise for the delays and aim to be back on schedule by the next issue in September 2021.

Tropical Grasslands-Forrajes Tropicales Vol 9, No 2 (May 2021)

Anuncio

Esta es la última edición a cargo de Rainer Schultze-Kraft y Lyle Winks como editores y queremos expresarles todo nuestro agradecimiento, admiración y respeto por su destacada labor como editores de la revista. Su trabajo comprometido con la excelencia en todos los aspectos de la investigación de forrajes tropicales se refleja en la calidad de los artículos publicados, tanto de forma como de fondo, y el incremento constante de las clasificaciones y métricas de la revista:

- *Durante su rol como editores,* Tropical Grasslands-Forrajes Tropicales *ha publicado 332 artículos, 179 en ediciones especiales (119 contribuciones al Congreso Internacional de Pasturas 2013 y 60 contribuciones a la Conferencia Internacional Leucaena 2018) y 153 en ediciones regulares.*
- *La revista está indexada en la colección principal de Science Citation Index Expanded y dos índices adicionales de Clarivate Web of Science, que proporciona el indicador de factor de impacto más conocido (anteriormente: ISI Journal Impact Factor). El Factor de Impacto de la Revista para 2019 fue 0.703 (para 2018 fue 0.441 y para 2017 fue 0.389, mostrando un aumento constante). El Factor de impacto 2020 estará disponible a mediados de 2021.*
- *TGFT también está indexado en Scopus, y el CiteScore para 2020 es actualmente de 1.60, con un progreso considerable desde 2017 (0.44), 2018 (0.69) y 2019 (1.30). CiteScore es la forma en que Scopus mide el impacto de sus revistas indexadas.*
- *El SCImago Journal & Country Rank es un portal que incluye indicadores científicos por revistas y por países, desarrollado a partir de la información contenida en la base de datos Scopus® (Elsevier B.V.). Estos indicadores se pueden utilizar para evaluar y analizar dominios científicos. Para 2019, la Revista tuvo una puntuación de 0.37 (en 2018 fue 0.28) y se ubicó en el segundo valor más alto entre el conjunto de revistas clasificadas por SJR (Q2).*

La revista se encuentra en un período de transición a los nuevos editores. La Dra. Jean Hanson, directora y editora en inglés y el Dr. Danilo Pezo, editor en español. Ambos trabajaron con el International Livestock Research Institute (ILRI) durante años y tienen una amplia experiencia en forrajes tropicales. Jean es miembro emérito del ILRI en Etiopía y Danilo trabaja actualmente con CATIE en Costa Rica. Jean y Danilo aportan su experiencia complementaria a sus funciones como editores. El trabajo de investigación de Jean se ha centrado en germoplasma de forrajes y producción de semillas, mientras que el de Danilo se ha enfocado en nutrición animal, pasturas y opciones silvopastoriles en sistemas ganaderos tropicales. El nuevo equipo editorial seguirá contando con el apoyo de José Luis Urrea, Especialista en Comunicaciones de la Alianza de Bioversity International y CIAT, Colombia.

Durante el período de transición, se han presentado algunos retrasos en el proceso de publicación y la revista no está aceptando contribuciones en español. Los nuevos editores están trabajando arduamente para lidiar con las presentaciones pendientes, pero nos disculpamos por las demoras y nuestro objetivo es volver a lo programado para el próximo número en septiembre de 2021.

Principal Contacts

Michael Peters
The Alliance of Bioversity International and CIAT
Kenya
Phone: +254 709 134 130
Email: CIAT-TGFT-Journal@cgiar.org

Technical Support
José Luis Urrea Benítez
The Alliance of Bioversity International and CIAT
Colombia
Phone: +57 2 4450100 Ext. 3354
Email: CIAT-TGFT-Journal@cgiar.org

Table of Contents

Research Papers

Short Communications

doi: 10.17138/TGFT(9)144-158

Research Paper

Root production in a subtropical pasture is mediated by cultivar identity and defoliation severity

La producción de raíces en una pastura subtropical es mediada por el genotipo y la severidad de defoliación

CHRIS H. WILSON[1], JOAO M. VENDRAMINI[2], LYNN E. SOLLENBERGER[1] AND S. LUKE FLORY[1]

[1]*Agronomy Department, University of Florida, Gainesville, FL, USA.* agronomy.ifas.ufl.edu
[2]*Range Cattle Research and Education Center, University of Florida, Ona, FL, USA.* rcrec-ona.ifas.ufl.edu

Abstract

Grasslands occupy significant land area and account for a large proportion of the global soil carbon stock, yet the direct effects of grazing and genotypic composition on relationships between shoot and root production are poorly resolved. This lack of understanding hinders the development of models for predicting root production in managed grasslands, a critical variable for determining soil carbon stocks. We quantified the effects of season-long defoliation treatments on both shoot and root production across 4 cultivars of a widely planted pasture grass species (*Paspalum notatum* Flüggé) in a common garden setting in South Florida, USA. We found that infrequently applied (4 weekly) severe defoliation (to 5 cm) substantially enhanced shoot production for all cultivars, while severe defoliation reduced root production across cultivars, regardless of frequency. Overall, there was no significant relationship between shoot and root production. Our results showed that above-ground and below-ground productivity are only weakly coupled, suggesting caution against use of simple above-ground proxies to predict variations in root production in grasslands. More broadly, our results demonstrated that improved modeling and management of grasslands for below-ground ecosystem services, including soil carbon sequestration/stocks, must account for intraspecific genetic variation and responses to defoliation management.

Keywords: Below-ground production, genotypic variability, grazing management, *Paspalum notatum*.

Resumen

Los pastizales ocupan una superficie considerable de tierra y representan una gran proporción de las reservas mundiales de carbono del suelo, pero los efectos directos del pastoreo y la composición genotípica sobre las relaciones entre la producción de brotes y raíces no están bien resueltos. Esta falta de comprensión dificulta el desarrollo de modelos para predecir la producción de raíces en pastizales gestionados, una variable crítica para determinar las reservas de carbono del suelo. Cuantificamos los efectos de los tratamientos de defoliación durante toda la temporada en la producción de brotes y raíces en 4 cultivares de una especie de gramínea ampliamente plantada (*Paspalum notatum* Flüggé) en un jardín común en el sur de Florida, EE.UU. Encontramos que la defoliación severa (hasta 5 cm) aplicada con poca frecuencia (4 semanas) mejoró sustancialmente la producción de brotes para todos los cultivares, mientras que la defoliación severa redujo la producción de raíces entre los cultivares, independientemente de la frecuencia. En general, no hubo una relación significativa entre la producción de brotes y raíces. Nuestros resultados mostraron que la productividad por encima y por debajo del suelo están débilmente acopladas, lo que sugiere precaución contra el uso de simples sustitutos por encima del suelo para predecir variaciones en la producción de raíces en los pastizales. En términos más generales, nuestros resultados demostraron que el modelado y la gestión mejorados de los pastizales para los servicios ecosistémicos

Correspondence: Chris H. Wilson, 3119 McCarty Hall B, Agronomy Department, University of Florida, Gainesville, FL 32611, USA. Email: chwilson@ufl.edu

subterráneos, incluido el secuestro/almacenamiento de carbono del suelo, deben tener en cuenta la variación genética intraespecífica y las respuestas a la gestión de la defoliación.

Palabras clave: Manejo del pastoreo, *Paspalum notatum*, producción subterránea, variabilidad genotípica.

Introduction

Grassland ecosystems occupy more than a fifth of earth's land area and account for a large proportion of the global soil organic carbon (SOC) stocks (Scurlock and Hall 1998; Lal 2010). However, there is considerable uncertainty in predictions of net ecosystem exchange, and hence carbon sequestration services from grasslands (Gilmanov et al. 2007; Nicholas et al. 2009). One significant source of uncertainty is that, while large herbivore grazing is known to mediate patterns of plant species composition, diversity and above-ground primary productivity (McNaughton 1985; Knapp et al. 1999; Fuhlendorf and Engle 2001), the effects of grazing on below-ground processes and soil carbon are less clear (McNaughton et al. 1998; Hamilton and Frank 2001; McSherry and Ritchie 2013; Balogianni et al. 2014). In particular, there are limited field studies where the impact of grazing on root production in grassland systems has been directly measured (e.g. via root ingrowth cores or minirhizotron technology; see Ziter and MacDougall 2012; Balogianni et al. 2014; Cooley et al. 2019). Since below-ground production may be the largest component of total net primary productivity (NPP) for many grasslands (Gill et al. 2002; Hui and Jackson 2006), determining how grazing affects root production will help to predict when grassland ecosystems will behave as carbon sinks, and whether grazing is likely to promote or inhibit carbon sequestration services.

Root carbon inputs may constitute a disproportionate amount of the total SOC stock compared with shoot carbon (Rasse et al. 2005; Poirier et al. 2018; Sokol et al. 2019), and are especially critical in grassland ecosystems where above-ground tissue is susceptible to frequent removal by fire and grazing (Johnson and Matchett 2001). Current understanding of how grazing affects root production is ambiguous. For example, one temperate mesocosm study showed that intense defoliation inhibited root production and accelerated the loss of SOC (Klumpp et al. 2009), whereas some field studies have documented greater below-ground allocation and root production under grazing in the Tibetan plateau (Hafner et al. 2012) and in subtropical pasture (Wilson et al. 2018). Augustine et al. (2011) found that defoliation reduced below-ground carbon allocation in one grazing-adapted North American grass species (*Pascopyrum smithii*, western wheatgrass) but not in another (*Bouteloua gracilis*, blue grama), highlighting interspecific variations in response to a given defoliation regime. In general, laboratory and mesocosm studies have found that frequent grazing/defoliation leads to declines in standing root biomass over the long term (Bardgett et al. 1998), whereas a global synthesis of data comparing grazed and ungrazed grasslands found a mix of positive and negative effects on standing root biomass (Milchunas and Lauenroth 1993). Overall, this discordance suggests that variations in plant composition, underlying environmental factors, grazing severity or some combination of these factors significantly mediates the effects of grazing on root production.

Grazing effects on below-ground production may vary based on not only plant species but also the genotypic composition of a grazed stand, given the increasing evidence of the importance of intraspecific variation in driving ecosystem structure and function (Madritch and Hunter 2002; Whitham et al. 2006). In general, some literature suggests that reduced allocation of photosynthates to roots (and increased allocation to shoots) following grazing may represent an evolutionarily adaptive trait for grazing tolerance (Briske and Richards 1995). For instance, Carman (1985) noted that short-leaved genotypes of *Schizachyrium scoparium*, selected from a long-term grazed site, exhibited lower rates of root elongation post-grazing than longer-leaved genotypes from a site where grazing was excluded for a long period. Planted pasture grasses have been shown also to exhibit genotypic variability in shoot and root production in response to grazing (e.g. Dawson et al. 2000). For example, Interrante et al. (2009) observed significantly less plant cover in recently-selected, upright-growing *Paspalum notatum* (bahiagrass) cultivars in response to severe, frequent defoliation, but cover was not reduced with the same defoliation treatments on widely-naturalized cultivars, suggesting significant intraspecific variability in grazing tolerance and below-ground biomass allocation.

Although root production is a critical component in predicting the carbon cycle in grassland ecosystems, it is difficult to monitor or predict over large spatial scales. Thus, regional-scale grassland models have been developed that predict total NPP and/or greenhouse gas exchange on the basis of above-ground canopy characteristics estimated from remote sensing (Houborg and Soegaard 2004; Zhao et al. 2014; Gu et al. 2013). Similarly, some previous work has sought to predict below-ground net primary productivity (BNPP) on the basis of readily obtained above-ground measurements in both grasslands (Gill et al. 2002) and forests (Chen et al. 2004). Recently, concerted efforts have been made to link fine root traits with other plant traits, across species and environments, by compiling and analyzing global-scale large datasets (Iversen et al. 2017). The goal is to have reliable above-ground proxies for predicting critical below-ground root processes (Malhotra et al. 2018). However, given the evidence for potentially significant genotypic and defoliation effects on below-ground carbon allocation, it is unclear whether above-ground proxies can ever reliably approximate root production. Given the central importance of root system carbon inputs to maintaining SOC, especially in grasslands, we need more data from experimental systems where genotypic composition and defoliation management have been manipulated, and the relationships between above- and below-ground biomass allocation have been quantified.

In this study, we tested the independent and combined effects of defoliation severity and frequency, and cultivar on root production of a widely utilized pasture grass species of the southeastern United States, *Paspalum notatum* Flüggé (bahiagrass). Bahiagrass is a perennial C4 pasture grass that was introduced to Florida in the 1920s from South America and constitutes the primary forage for the Florida cow-calf industry (Silveira et al. 2011). 'Argentine' and 'Pensacola' are widely-distributed, naturalized cultivars in the US Gulf Coast region with a prostrate growth habit, whereas 'Tifton-9' and 'UF-Riata' are recently-released cultivars selected for improved agronomic characteristics, including more upright growth habit and less sensitivity to photoperiod (Interrante et al. 2009; Vendramini et al. 2013). Bahiagrass cultivars can be broadly delineated on the basis of growth habit, as historically older, widely-naturalized cultivars tend to be prostrate, whereas modern cultivars tend to be upright, reflecting selection for improved forage growth characteristics (Vendramini et al. 2013). Previous work and considerable producer experience suggest that bahiagrass has a remarkable resilience to severe grazing, wherein forage growth and quality are maximized with severe defoliation (close to ground level) so long as regrowth intervals are adequate (Beaty et al. 1968; Stanley et al. 1977). However, impacts of defoliation severity on root production across cultivars, and their associated growth habits, have not been studied directly, reflecting a general lack of information on below-ground growth responses in subtropical pasture during the warm season (Cooley et al. 2019). To redress this gap in knowledge, we conducted an experiment in a common garden setting under realistic conditions of limited soil fertility in an endeavor to: 1) isolate the effects of defoliation severity and frequency plus cultivar on below-ground production; and 2) evaluate the relationships between above-ground and below-ground growth.

Consistent with the literature on compensatory growth responses by natural and planted pastures (Stanley et al. 1977; McNaughton 1983; Zhao et al. 2008), and also with the literature on genotypic variability (e.g. Dawson et al. 2000), we hypothesized that:

1) Severe defoliation, applied infrequently, would stimulate increases in above-ground primary productivity (via compensatory response mechanisms), but would have neutral effects on root productivity across all cultivars;
2) Severe defoliation, applied frequently, would significantly suppress root production across all cultivars as a consequence of plant requirements to prioritize photosynthate allocation to regrowing shoots. Shoot production would either be unaffected or decrease somewhat, as the high level of stress over-rides compensatory growth mechanisms;
3) Widely-naturalized, prostrate cultivars would show proportionally greater reductions in root production under severe defoliation than the more upright cultivars, reflecting a beneficial adaptation for increased allocation to shoots following severe defoliation events; and
4) Despite alterations to below-ground biomass, allocation on the basis of cultivar and defoliation treatment, shoot production and root production would positively correlate at the plot level, reflecting variations in underlying soil factors determining total production.

Materials and Methods

To evaluate the independent and potential interactive effects of defoliation severity and plant cultivar on root production, we established thirty-two 3 × 7 m experimental plots at the University of Florida Range Cattle Research and Education Center, Ona, FL (27°26' N, 82°55' W) in 2009. The soils were uniform and classified as Pomona fine sand (sandy, siliceous, hyperthermic Ultic Alaquod). First, we seeded plots with 1 of 4 bahiagrass cultivars (Argentine, Pensacola, Tifton-9 and UF-Riata). Plots were fully established by the onset of the 2010 summer growing season with complete, uniform plant cover. More details, including soil fertility characteristics can be found in Vendramini et al. (2013). Weather data for the experimental site are presented in Table 1; all fell within normal ranges.

Table 1. Meteorological data from the study site, Ona Range Cattle Research and Education Center [Source: Florida Automated Weather Network (FAWN), fawn.ifas.ufl.edu/].

Period	Temp Avg (°C)	Rel. Humid. Avg. (%)	Precip. Total (mm)	ET Avg (cm, daily)
Jun 2013	25.9	86	246	0.38
Jul 2013	25.8	88	264	0.38
Aug 2013	26.8	86	188	0.416
Sep 2013	25.8	87	185	0.33

We initiated defoliation treatments on 13 June 2013 and concluded field sampling 16 weeks later on 5 October 2013. Although we did not measure soil moisture, all soils were visibly waterlogged from July until the end of the experiment, as is typical in Florida Spodosol soils (Silveira et al. 2011). We therefore assumed that plant growth was not limited by low water availability during the sampling period; it may even have been slightly limited by anaerobiosis. However, these production conditions are totally normal during the growing season in south Florida, and at the very least water availability was essentially constant across plots. Each plot (n = 32) was randomly assigned to either a frequent (2 weekly) or an infrequent (4 weekly) defoliation treatment to simulate grazing stress and was halved to receive 2 defoliation severities (severe at 5 cm residual height, and mild at 15 cm residual height) resulting in n = 64 experimental units (Figure 1). Residual heights were chosen based on personal observation (C.H. Wilson, L.E. Sollenberger and J.M. Vendramini) to represent the extremes of pasture defoliation under grazing by beef cattle in Florida. Thus, our design was effectively split-plot with 2 main-plot treatments (cultivar and defoliation frequency), while our subplot factor was defoliation severity. Overall, each cultivar × defoliation severity × defoliation frequency treatment was replicated 4 times.

We harvested forage from a 0.92 m^2 quadrat within each subplot during each defoliation treatment using a rotary mower (Sensation Mow-Blo Model 11F4-0) at the target cutting heights. To quantify above-ground production, harvested material was oven-dried at 60 °C to constant mass and weighed on an analytical scale. At the final harvest, all subplots were harvested at 5 cm. Total above-ground production was determined by summing values for each subplot across all dates, including the final harvest.

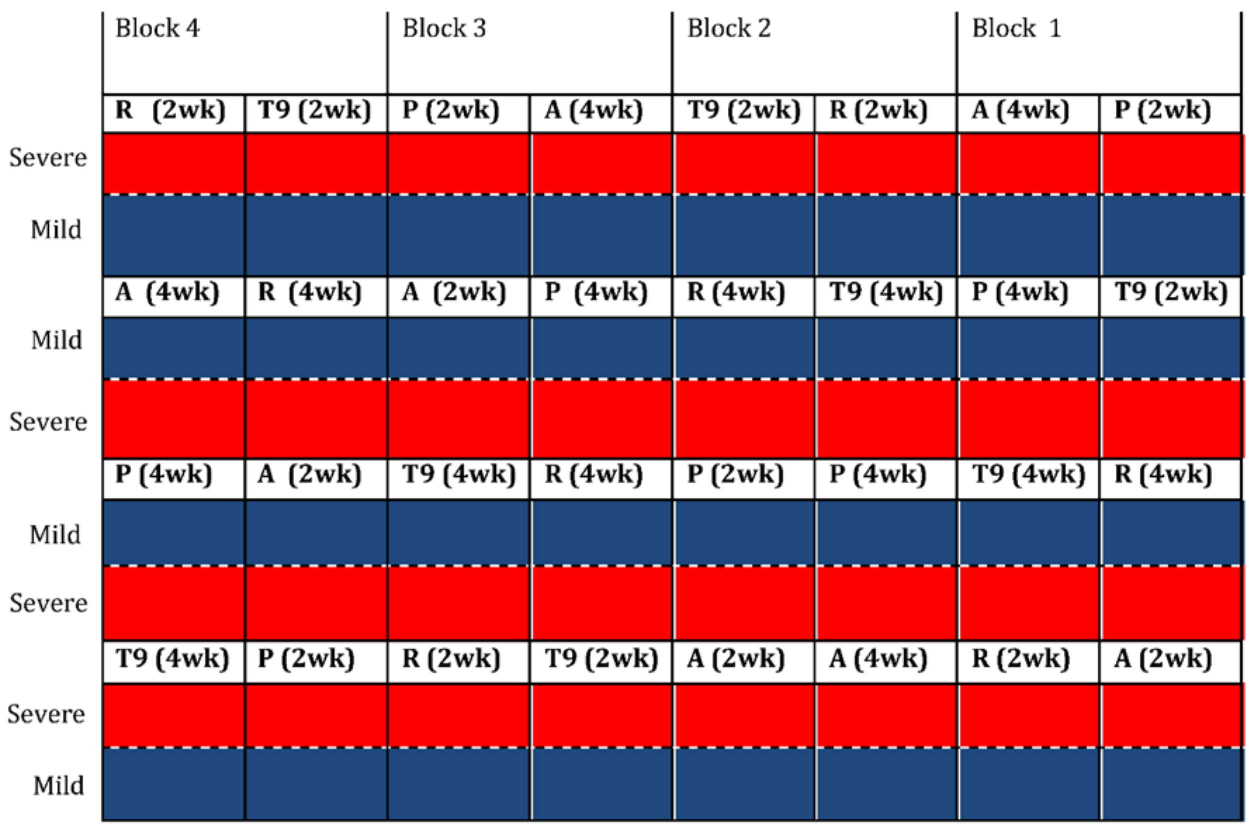


Figure 1: Diagram showing the layout of plots. North is top of the page. Legend: Defoliation severity - Red = Severe Defoliation (5 cm), Blue = Lenient Defoliation (15 cm). Defoliation frequency - 2wk = Defoliated every 2 weeks, 4wk = Defoliated every 4 weeks. Bahiagrass cultivar identity - A = Argentine, P = Pensacola, T9 = Tifton 9, R = UF-Riata.

To quantify root primary production in response to the defoliation treatments, we installed 2 mm mesh root ingrowth cores (Makkonen and Helmisaari 1999) on 7 June 2013, prior to imposing the defoliation treatments. Cores were 7.5 cm diameter × 25 cm deep and constructed of fiberglass mesh. They were installed by first excavating soil with a soil auger to target 25 cm depth, placing the mesh cores into the holes produced so that the upper edge of the core was just below the soil surface, and then re-filling the cores with sieved, root-free soil from the same plot. We retrieved the cores at the end of the growing season on 5 October 2013, 16 weeks after installation. The final volume of soil contained in each core was quantified prior to washing the roots free of soil on a 250 μm sieve. Roots were then oven-dried at 60 °C to constant mass and weighed. To correct for variation in core volume, root biomass was multiplied by a correction factor determined as the inverse of the ratio of each core volume to a reference core (a cylinder of 7.5 cm diameter and 25 cm depth). Finally, we visually verified that almost all root biomass was contained within the depth we evaluated (i.e. 25 cm depth) by digging several test pits around our study area. We noted from personal observation that wet pastures tended to result in shallower root distribution, consistent with early literature (Doss et al. 1960). Therefore, we multiplied root biomass by a constant ${}^{10{,}000}/_{\pi \times 3.75^2}$ to convert our measurements to g/m^2, putting them on an easily interpretable scale.

Statistical analysis

Response variables for analyses were shoot and root production, and a measure of allocation of photosynthate to root biomass defined as:

$$\frac{\text{Root production}}{\text{Root production} + \text{Shoot production}}$$

To analyze 'among-cultivar' variability in response to our treatments, we parameterized a varying-intercept/varying-slope Bayesian hierarchical model that we applied to both response variables. In this model, we estimated intercept and slope (i.e. treatment effects) coefficients for each cultivar, where each batch of coefficients was modeled as a draw from a normal distribution with an estimated variance component (Gelman and Hill 2007). We included binary predictor variables using a -0.5/0.5 'effect coding' for our experimentally imposed treatments: lenient (15 cm) and infrequent (4 weekly) defoliations were assigned -0.5 values, while frequent (2 weekly) and severe (5 cm) defoliations were assigned 0.5 values. Under this coding, the model intercept represents the grand mean, and the coefficients for defoliation severity and frequency represent the main effects of severe and/or frequent defoliation across both levels of the other treatment (see e.g. Schabenberger et al. 2000). We also included a term for the interaction of severe and frequent defoliation treatments and a random effect of plot to allow for correlation in observations from the same plot. Our varying-intercept/varying-slope model therefore included 4 separate estimates of grand means (1 for each cultivar), each of which represented an estimate of performance for that cultivar across all defoliation treatment conditions, and 4 treatment effect estimates (1 for each cultivar) for frequent defoliation, severe defoliation and their interaction. Since these coefficients were drawn from distributions with estimated variance components, the separate estimates were partially pooled towards their common mean, which also was estimated from the data, a property that built in an automatic correction for multiple comparisons among cultivars and obviated the need for arbitrary post-hoc adjustments such as the Bonferonni correction (Gelman et al. 2012). Finally, because growth data are naturally constrained to be positive only and because we observed a pattern of variance increasing with the mean, we used a Gamma distribution to model our data, which naturally accounts for this nearly universal pattern in biomass data. We used the standard log-link in our parameterization of the Gamma regression model, and thus our model coefficients represent multiplicative effects, and are reported on the log-link scale (Gelman and Hill 2007). Values greater than zero indicate positive effects on the response variable, whereas values less than zero indicate negative effects. As applies in all cases where the log-link is used, exponentiation of these regression coefficients returns the multiplicative effect, which can be naturally interpreted as a % effect.

We display treatment effects graphically by first plotting estimated fixed effect coefficients (i.e. frequency, severity and frequency × severity) centered on the median, and include both 50% (thick) and 95% (thin line) uncertainty (credible) intervals. These coefficients represent the overall average effects of treatment or the interaction effect across all cultivars. In addition, we graphically present the varying intercepts portion of our model, which represents the overall average deviation of each cultivar from the grand mean across all cultivars, and is thus naturally centered at zero. Here again, we include both 50% (thick) and 95% (thin line) credible intervals. The proportion of the credible interval above or below zero can be interpreted as the Bayesian probability of that cultivar differing in response from the average across all cultivars. In the case of root allocation, we further analyzed all the pair-wise contrasts among cultivars (n = 6 contrasts), by taking the difference for each coefficient at each iteration of the Markov Chain Monte Carlo sampler, the computational algorithm by which Bayesian models are fitted (Gelman et al. 2013). These pairwise contrasts thus represented the differences between each pair of cultivars in their overall root allocation, averaged across all treatment conditions.

We estimated these models in a Bayesian framework via Hamiltonian Monte Carlo in the packaged 'rstanarm' (v 2.18.2) called from R (v 3.5.3) via R-studio (v 1.1.463). Prior to analysis, shoot and root production responses were standardized by dividing by their mean, resulting in this case with response variables with scale ~O (1) to facilitate faster sampling, and to help specify weakly-regularizing Normal (0,1) priors for all treatment effects. For all models, we sampled the target (posterior) distribution with 4 chains of 2,000 iterations each. Model convergence was assessed via use of the R-hat <1.01 criterion (Gelman and Hill 2007) as well as by visual inspection for chain blending and stability, and monitoring of the powerful diagnostics built into 'rstanarm' (i.e. divergent transitions and the Energy Bayesian Fraction of Missing Information, or E-BFMI, Carpenter et al. 2017).

In addition to the log-linear Gamma regression models, we performed a standard split-plot analysis of variance on the root production response variable, being careful to assign the cultivar, frequency and cultivar × frequency terms to the plot-level error term, while residual cutting height, and its various interaction terms were assigned to the residual error term. The results of this ANOVA complement our analysis of the treatment effects from the log-linear regression models, but in common with all strictly ANOVA analyses do not provide quantitative insight beyond the P-value significance. For readers who are interested, we have provided these results in supplemental form (Appendix).

To understand the relative importance of defoliation treatment and cultivar compared with shoot production for predicting root production, we first fitted a simple univariate regression model using only above-ground biomass from each subplot (n = 64) as a continuous covariate. We then refitted our varying-intercepts/varying-slopes model, while including shoot production as a continuous covariate alongside treatment and cultivar effects. We compared a Bayesian R^2 metric between the models (Gelman et al. 2018). Since the visual and R^2 comparisons were so clear, there was no need to evaluate additional metrics of model predictive performance.

Results

Shoot production model

Average shoot DM production across all cultivars and treatment combinations in our study was 290 g/m^2, with the highest values observed in the infrequent, severe defoliation treatment, which averaged 384 g/m^2 (Figure 2). The fixed main effect estimate (on log-link scale, and reported as posterior median ± posterior standard error) for severe defoliation was positive (0.28 ± 0.07; Figure 3a), while the estimate for frequent defoliation was negative (-0.18 ± 0.08; Figure 3a); however, the interaction was negative as well (-0.25 ± 0.15; Figure 3a), consistent with a readily observable pattern (Figure 2) that the combination of severe + infrequent (4 weekly) defoliation leads to over-yielding. Overall, we did not estimate substantial variability in shoot production among cultivars across all treatments, although the upright cultivars (UF-Riata and Tifton-9) had slightly higher production than the prostrate cultivars Argentine and Pensacola (Figure 4a).

Root production model

We observed an average root DM production of 224 g/m^2, where mild defoliation treatments were the highest with 262 g/m^2 averaged across 2 and 4 weekly defoliation frequencies, compared with severe defoliation with an average of 186 g/m^2 (Figure 2). The fixed main effect estimate for severe defoliation was negative (-0.33 ± 0.12; Figure 3b), with >97.5% of posterior probability below 0, while the main effects of frequent defoliation and the interaction of frequent × severe defoliation were highly uncertain, with 95% credible intervals spanning a similar range above and below zero. Average root production across all treatment groups varied by cultivar more substantially than shoot production (Figure 4b), with the prostrate cultivars Argentine and Pensacola having greater root production than the upright cultivars UF-Riata and Tifton-9 (Figure 4b; Figure 5). The greatest contrast was between Argentine and UF-Riata, which had a median posterior difference of -0.36 on the log-link scale (Figure 5), which represents a 30% lower root production.

Root allocation

The fixed main effect estimate for severe defoliation on root biomass allocation proportion was -0.34 ± 0.09 (Figure 3c), a very similar median estimate to that for root production, although with a smaller uncertainty (s.e. = 0.09 vs. 0.12). This result represents a median estimate of 29% reduced allocation proportion to roots overall among cultivars and across both frequencies of defoliation with severe defoliation. Variation among cultivars was similar to that observed for root production (Figure 4c vs. Figure 4b), so we did not repeat the pairwise analysis since it would convey redundant information.

Root production predictions

The univariate regression between shoot and root production revealed a very weak ($R^2 = 0.09$) relationship (Figure 6a). The full model that included treatment indicators and cultivar identity (as in the analyses above) yielded a median R^2 of 0.45 (Figure 6b). After removing the varying intercepts/slopes by cultivar, this R^2 value declined to 0.21, indicating that accounting for cultivar identity doubles model fit. Close examination of Figure 6b reveals that the full model accounted for observed variations in root production quite well in the range of 100–300 g DM/m^2 but severely under-predicted root production when >300 g DM/m^2.

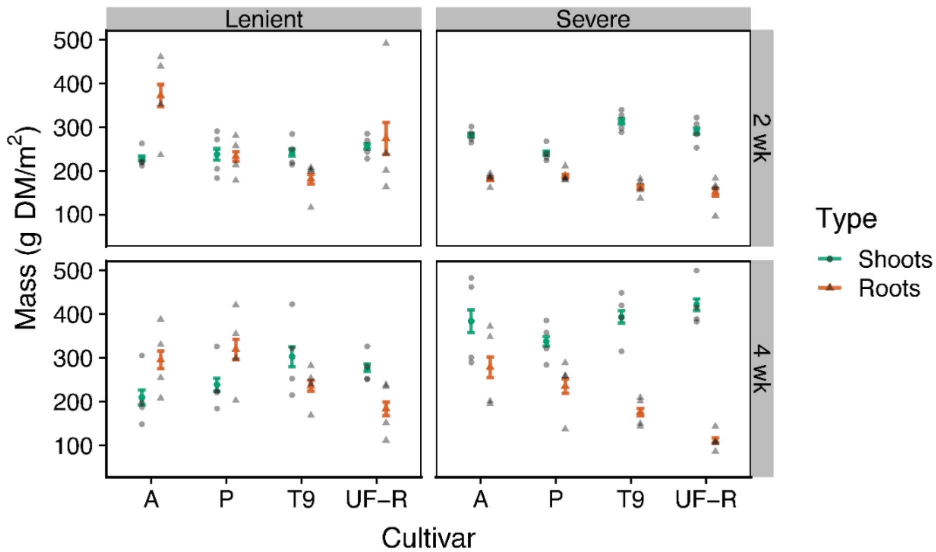


Figure 2. Raw data (g DM/m^2) plotted as circles (shoots) and triangles (roots). Error bars show mean biomass (g DM/m^2) ± 1 s.e. for shoots (purple error bars) and roots (brown error bars). The panels are faceted by treatment combinations: severity of defoliation on top (lenient 15 cm or severe 5 cm on top); and frequency of defoliation labeled on the right hand side (2 or 4 weekly). The x-axis groups responses by cultivar: A = Argentine; P = Pensacola; T9 = Tifton-9; and UF-R = UF-Riata.

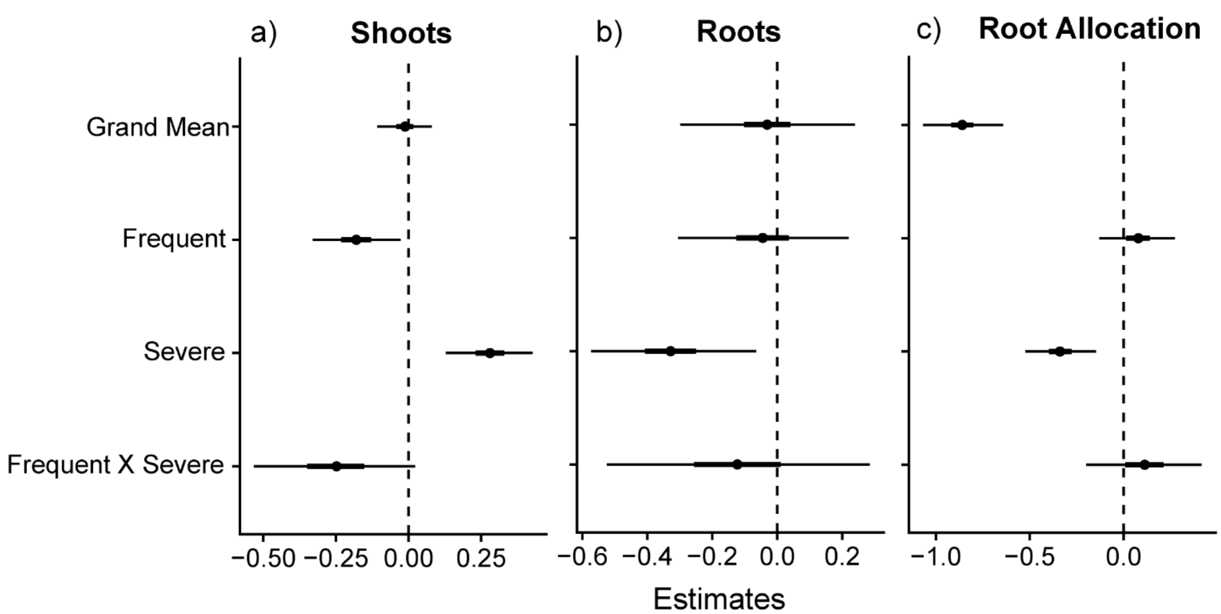


Figure 3. Fixed effects from varying-intercepts/varying-slopes Gamma regression model. Coefficients are plotted on the log-link scale and include a median (point), plus 50% (thick line) and 95% (thin line) credible intervals for: **a**) shoot production; **b**) root production; and **c**) root allocation. Where the entire 95% credible interval falls above or below zero, we can interpret that as a 97.5+% Bayesian probability of that coefficient having a positive or negative effect on the response, respectively.

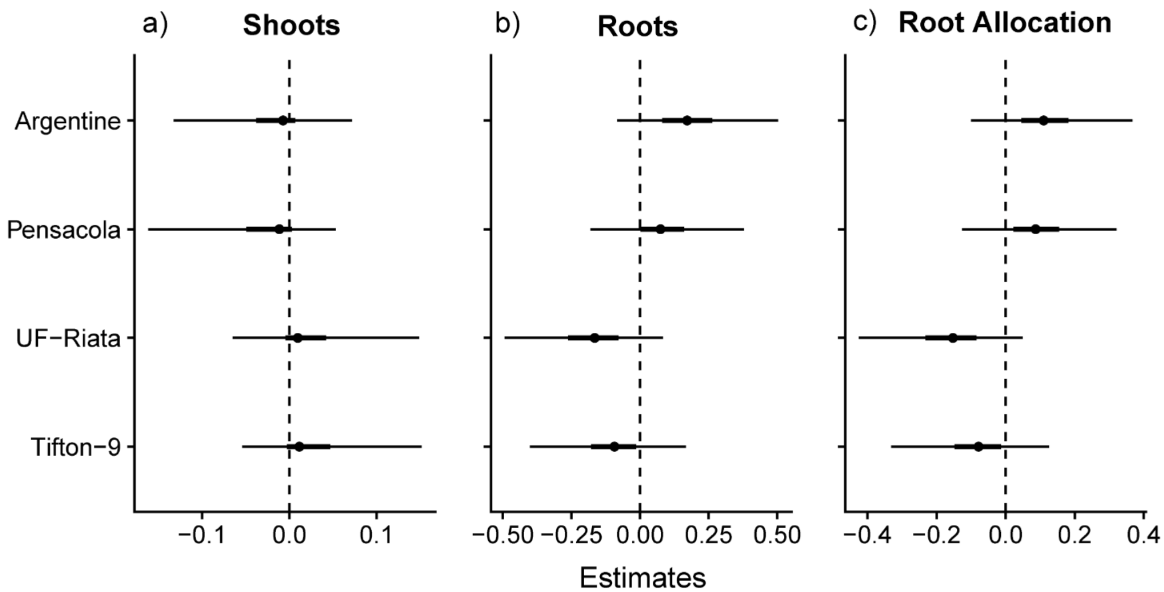


Figure 4. Varying-intercepts from the Gamma regression model for root production. Coefficients represent deviations of each cultivar from the overall mean (fixed effect coefficient), and are thus naturally centered at 0, where negative values represent lower than average performance and positive values higher than average performance. Plots include a median (point) plus 50% (thick line) and 95% (thin line) credible intervals. Where the entire 95% credible interval falls above or below zero, we can interpret that as a 97.5+% Bayesian probability of the cultivar having a higher or lower overall root production than the mean among all cultivars.

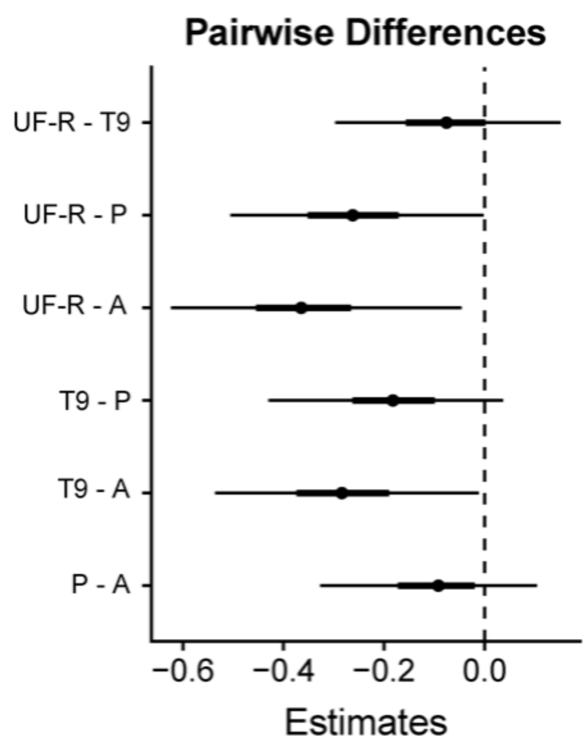


Figure 5. Pairwise contrasts among cultivars for the varying intercepts of the root allocation model. Key: A = Argentine; P = Pensacola; T9 = Tifton-9; and UF-R = UF-Riata. Plots include a median (point) plus 50% (thick line) and 95% (thin line) credible intervals. Where the entire 95% credible interval falls above or below zero, we can interpret that as a 97.5+% Bayesian probability of the first cultivar having a higher or lower root allocation, respectively, than the second cultivar.

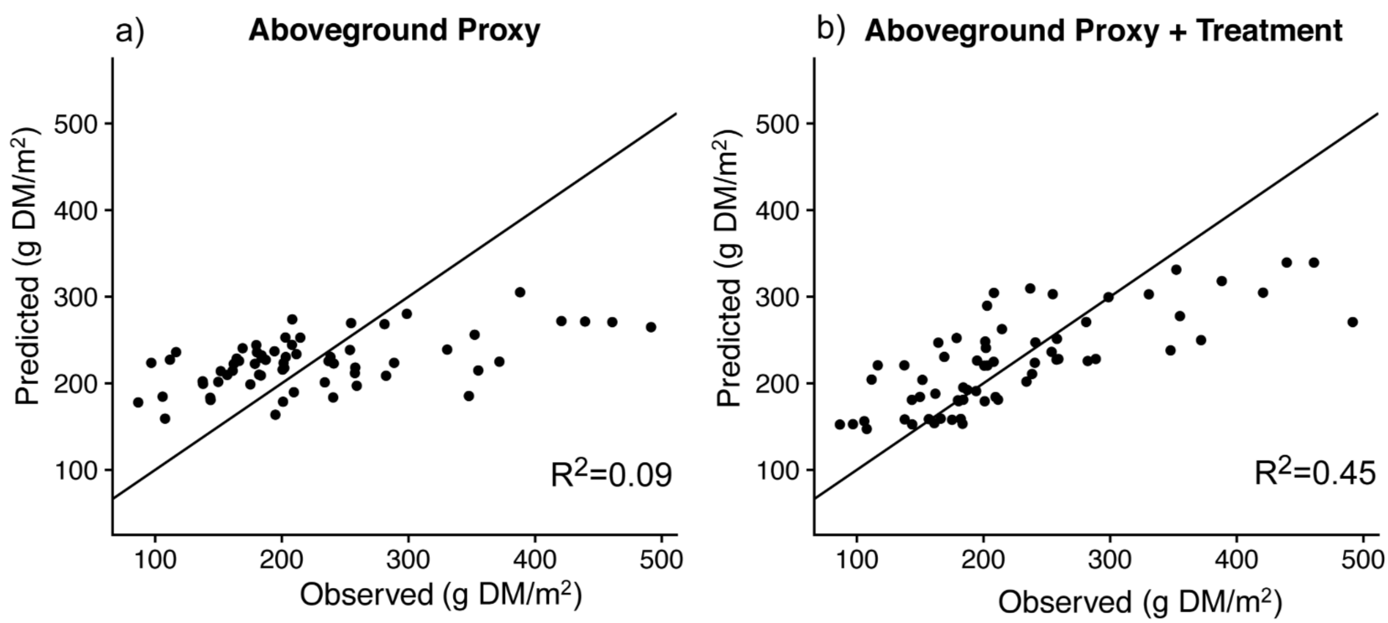


Figure 6. Shoot DM production does not predict root DM production. **a**) Predicted vs. observed scatterplot for root production as predicted by shoot production as an above-ground proxy; and **b**) Predicted vs. observed scatterplot for root production as predicted by defoliation treatment, cultivar identity and shoot production. For reference, the 1:1 line of 'perfect fit' is plotted along with an in-sample median Bayesian R^2 for both predictive models.

Discussion

Severe defoliation resulted in substantially greater shoot production when applied infrequently, but reduced root production among the bahiagrass cultivars. Averaged across all defoliation treatments, root production was also more strongly variable among cultivars than was shoot production. Thus, our results suggest that severe defoliation can trigger a trade-off between above-ground and below-ground allocation of photosynthate in managed subtropical pastures, and that the extent of this trade-off depends in part on cultivar identity. Contrary to Georgiadis et al. (1989) and Briske and Richards (1995), who suggested that over-compensation was likely to occur only under water-limitation, or given concomitant fertilizer application, we found significantly greater shoot production in response to severe defoliation under limited fertility and abundant soil water. Compared with mild defoliation, all cultivars exhibited this compensatory above-ground growth response following severe defoliation, but only when defoliation was applied infrequently [similar to Gates et al. (1999)]. However, the severe but infrequent defoliation treatment that led to above-ground compensatory growth also suppressed root production. Thus, under low-input conditions, manipulating defoliation severity and frequency to enhance forage production could evoke a trade-off between shoot and root production. Given the substantial literature demonstrating the importance of root carbon for maintenance of soil carbon pools (Rasse et al. 2005; Wilson et al. 2018; Sokol et al. 2019), these altered patterns of allocation of photosynthate may have significant consequences for carbon cycling, and hence soil carbon sequestration services, in managed subtropical pastures. Moreover, use of simple above-ground proxies, such as leaf area/biomass, is unlikely to yield accurate predictions of root production over large spatial scales.

Impacts of severe defoliation on shoot and root responses (Hypotheses 1 and 2)

In terms of our Hypothesis 1, we found support for the existence of a compensatory growth response mechanism in bahiagrass, but rather than an indifferent effect on root production, we observed a somewhat reduced production (~28% reduction on average) of roots. Our results differ from the short-term responses measured by Ziter and MacDougall (2012) and Hamilton et al. (2008), where a single defoliation event stimulated root production and root exudation, respectively. Additionally, the results reported here appear to conflict with measurements of standing root biomass, root exudation rates and their connections to microbial biomass and soil carbon, across a system of long-term grazing exclosures on a similar pasture site, as reported in Wilson et al. (2018). These discrepancies suggest that root responses to short-term grazing/

defoliation events can differ strongly from season-long responses to grazing regimens, where both severity and frequency of defoliation are expected to mediate plant regrowth strategies (Briske and Richards 1995). Moreover, longer-term impacts of grazing exclusion (over many years) in bahiagrass-dominated subtropical pasture appear to involve pronounced phenotypic shifts in root:shoot ratios, whereby absence of grazing favors lower root:shoot ratios, even when holding species composition constant (Wilson et al. 2018). On the other hand, Thornton and Millard (1996) found that greater severity of defoliation resulted in lower root mass (but greater N uptake per unit of root mass), which is consistent with our findings.

Dawson et al. (2000) reported that weekly defoliation over a growing season reduced root biomass compared with no defoliation, but infrequent defoliation (every 8 weeks) had no effect. Our ambivalent findings on the role of frequency of defoliation were thus somewhat surprising. Although we observed marked suppression of variability of production under our severe + frequent treatment (see e.g. Figure 2), root production was not markedly lower than in our severe + infrequent treatment. However, our second hypothesis was upheld, in that the decrease in root production we observed was less variable across cultivars under severe + frequent defoliation. Despite this result, it appears that in our system, severity of grazing is a more important determinant of root production of grass than frequency of grazing.

Cultivar variability in response to defoliation treatment (Hypothesis 3)

We observed substantial overall variability in root production among the grass cultivars. However, it does not appear possible to predict cultivar-level below-ground responses to specific grazing regimens based on observations of above-ground compensatory growth responses. As we hypothesized (Hypothesis 3), the cultivars selected for enhanced upright growth habit [Tifton 9, UF-Riata; Interrante et al. (2009)], especially Tifton-9, exhibited less overall root production than the widely-naturalized prostrate types (Argentine, Pensacola), especially Argentine. On the other hand, all cultivars responded negatively to severe defoliation per se, and we observed similar total root production among all cultivars in the severe + frequent defoliation treatment, a scenario reasonably representative of overstocked pastures. These results contradict the hypothesis that more grazing-tolerant genotypes, in our case Argentine and Pensacola, will have lower root production as a consequence of greater post-grazing allocation of resources to shoot regrowth (Briske and Richards 1995; Dawson et al. 2000). Instead, it appears that cultivars simply vary in root growth potential, but that severe defoliation, especially when applied frequently, overwhelms this variability.

Spatial correlation of shoot and root production (Hypothesis 4)

Contrary to our Hypothesis 4, our study revealed that shoot and root production are decoupled at fine spatial scales, at least in our experimental plots, with shoot production explaining only 8% of the in-sample variation in root production. By contrast, defoliation treatment and especially cultivar identity appeared to be very important for predicting root production in this system, together accounting for roughly half the observed variance in root production. Gill et al. (2002) reported some success in predicting below-ground NPP using an algorithm based only on above-ground biomass and climate, but their model consistently under-predicted root production in more productive sites. Interestingly, we observed a similar severe under-prediction of root production in our more productive plots. Thus, we caution against using above-ground proxies to predict below-ground production, even within uniform and homogeneous ecosystems, such as the planted pasture system where we worked. Our results suggest that knowledge of grazing management and cultivar identity, in addition to species-level variations in composition (Steinbeiss et al. 2008; Tilman et al. 2012), are critical for generating accurate predictions of BNPP. Moreover, half of the variance in below-ground production was unexplained, even in our best model, suggesting significant spatial heterogeneity in root system productivity that should be investigated further. Given recent calls highlighting the importance of plant roots and their production in achieving future progress in biogeochemical modeling and the quest to find reliable, scalable above-ground proxies to infer root processes indirectly (Iversen et al. 2017; Malhotra et al. 2018), our results are a sobering reminder of the challenges inherent to linking above- and below-ground production. Accordingly, we suggest that a high priority for future research is to study below-ground root-rhizosphere processes using spatially explicit sampling protocols designed to maximize insight into heterogeneity at various spatial and temporal scales.

On a large scale, McNaughton et al. (1998) found that grazing severity was uncorrelated with standing root biomass or productivity in the Serengeti. However, in speciose natural grasslands plant diversity may confer a stabilizing influence on root production (Fornara et al.

2009; Tilman et al. 2012). By contrast, monoculture pasture systems may respond more like mesocosm systems, where high defoliation severity is associated with reduced root biomass (Bardgett et al. 1998). Moreover, since a large proportion of planted pastures (particularly in the subtropics) are dominated by single species, variation in root production among cultivars may represent an especially important component of diversity. Grazing management may need to be matched to cultivar-level characteristics to optimize both forage and root production, and establishment of planted pastures with multiple cultivars or genotypes may be a viable, yet under-appreciated, strategy for enhancing functional diversity. For instance, combining upright and prostrate cultivars may introduce beneficial genotypic diversity that could maximize utilization of both above- and below-ground resources via niche complementarity (Avolio et al. 2011; Chang and Smith 2014). Additionally, cultivar-level variability suggests the potential for ecologists, agronomists and physiologists to collaborate with plant breeders to improve the sustainability of grassland agroecosystems by development of improved forage cultivars selected for superior below-ground traits.

Overall, our results suggest that intermittent severe defoliation can elicit much greater shoot growth but have neutral or negative effects on root production. It is possible that a more moderate defoliation severity than we tested would have led to similar stimulation of above-ground compensation without the negative consequences for root production, a possibility our study was not designed to test. Neither did our study consider impacts of defoliation on rhizome biomass, but we stress that our intent was to focus on root production, since it appears to be of greater relevance for soil carbon sequestration than other compartments of plant biomass (Rasse et al. 2005). Likewise, it is also possible that the lower levels of fine root production we measured under severe defoliation may have been compensated for by greater root exudation, or rhizodeposition more generally. However, this possibility seems unlikely given that rates of root exudation generally correlate closely with fine root surface area (Jones et al. 2009; Wilson et al. 2018).

The main limitation of our work is that realistic animal grazing management can differ from experimentally imposed defoliation in 2 major ways: 1) grazing impacts will fall along a spectrum of timing and severity with more intermediate values than can be tested in a randomized factorial experiment; and 2) grazers will return a certain fraction of consumed carbon and nutrients in the form of manure and urine, creating heterogeneous patches of varying nutrient availability. Moreover, we also caution that year-to-year variability in growing conditions can induce variability in experimental effects. Ideally, we recommend that medium-term (3+ years) field studies of controlled grazing (or defoliation) be conducted to properly estimate the random effects of such year-to-year environmental fluctuations. In addition to recommending greater future consideration of intraspecific variations in below-ground responses to grazing, our work supports the need to perform season-long measures of below-ground production to obtain reliable estimates of below-ground production that can be used to parameterize soil carbon models.

Conclusions

Across the 4 cultivars we tested, severe defoliation, regardless of frequency, suppressed root production, while infrequently applied severe defoliation increased shoot production. Thus, it appears that manipulating timing and severity of grazing to optimize forage production might evoke a negative trade-off with root production. Unfortunately, our data suggest that reliance on above-ground proxies to predict below-ground processes is not justified, at least for subtropical pastures.

We suggest that longer-term field manipulations are necessary to evaluate a suite of defoliation management scenarios across plant composition treatments to improve our ability to design management strategies for grassland agroecosystems to meet above-ground (forage) production goals while optimizing below-ground production, so as to improve soil carbon sequestration, nutrient retention and water cycling.

Acknowledgments

For significant assistance with field work and data collection we thank Carly Althoff, Jessica Wilson, Trevor Caughlin, Anand Roopsind, James Estrada and Bryan Tarbox.

References

(Note of the editors: All hyperlinks were verified 10 March 2021).

Augustine DJ; Dijkstra FA; Iii EWH; Morgan JA. 2011. Rhizosphere interactions, carbon allocation, and nitrogen acquisition of two perennial North American grasses in response to defoliation and elevated atmospheric CO_2. Oecologia 165(3):755–770. doi: 10.1007/s00442-010-1845-4

Avolio ML; Chang CC; Smith MD. 2011. Assessing fine-scale genotypic structure of a dominant species in native grasslands. The American Midland Naturalist 165(2):211–224. doi: 10.1674/0003-0031-165.2.211

Balogianni VG; Wilson SD; Vaness BM; MacDougall AS; Pinno BD. 2014. Different root and shoot responses to mowing and fertility in native and invaded grassland.

Rangeland Ecology & Management 67(1):39–45. doi: 10.2111/REM-D-13-00080.1

Bardgett RD; Wardle DA; Yeates GW. 1998. Linking above-ground and below-ground interactions: how plant responses to foliar herbivory influence soil organisms. Soil Biology and Biochemistry 30(14):1867–1878. doi: 10.1016/S0038-0717(98)00069-8

Beaty ER; Stanley RL; Powell J. 1968. Effect of height of cut on yield of Pensacola bahiagrass. Agronomy Journal 60(4):356–358. doi: 10.2134/agronj1968.00021962006000040007x

Briske D; Richards J. 1995. Plant responses to defoliation: a physiological, morphological and demographic evaluation. In: Bedunah DJ; Sosebee RE, eds. Wildland plants: physiological ecology and developmental morphology.Society for Range Management, Littleton, CO. p. 635–710. bit.ly/3bxMArb

Carman JG. 1985. Morphological characterization and defoliation responses of selected *Schizachyrium scoparium* genotypes. The American Midland Naturalist 114(1):37–43. doi: 10.2307/2425238

Carpenter B; Gelman A; Hoffman MD; Lee D; Goodrich B; Betancourt M; Brubaker M; Guo J; Li P; Riddell A. 2017. Stan: A probabilistic programming language. Journal of Statistical Software 76(1). doi: 10.18637/jss.v076.i01

Chang CC; Smith MD. 2014. Resource availability modulates above- and below-ground competitive interactions between genotypes of a dominant C_4 grass. Functional Ecology 28(4):1041–1051. doi: 10.1111/1365-2435.12227

Chen W; Zhang Q; Cihlar J; Bauhus J; Price DT. 2004. Estimating fine-root biomass and production of boreal and cool temperate forests using aboveground measurements: A new approach. Plant Soil 265(1):31–46. doi: 10.1007/s11104-005-8503-3

Cooley KD; Sollenberger LE; Blount AR; da Silva LS; Kohmann MM; Aryal P; Dubeux JCB; Silveira ML. 2019. A modified ingrowth core to measure root-rhizome accumulation of perennial forage species. Agronomy Journal 111(6):3393–3397. doi: 10.2134/agronj2019.01.0051

Dawson LA; Grayston SJ; Paterson E. 2000. Effects of grazing on the roots and rhizosphere of grasses. In: Lemaire G; Hodgson J; de Moraes A; Nabinger C; Carvalho PC de F, eds. Grassland Ecophysiology and Grazing Ecology. CABI, Wallingford. p. 61–84. bit.ly/3vazB6f

Doss BD; Ashley DA; Bennett OL. 1960. Effect of soil moisture regime on root distribution of warm season forage species. Agronomy Journal 52(10):569–572. doi: 10.2134/agronj1960.00021962005200100005x

Fornara DA; Tilman D; Hobbie SE. 2009. Linkages between plant functional composition, fine root processes and potential soil N mineralization rates. Journal of Ecology 97(1):48–56. doi: 10.1111/j.1365-2745.2008.01453.x

Fuhlendorf SD; Engle DM. 2001. Restoring heterogeneity on rangelands: ecosystem management based on evolutionary grazing patterns. BioScience 51(8):625–632. doi: 10.1641/0006-3568(2001)051[0625:RHOREM]2.0.CO;2

Gates RN; Hill GM; Burton GW. 1999. Response of selected and unselected bahiagrass populations to defoliation. Agronomy Journal 91:787–795. doi: 10.2134/agronj1999.915787x

Gelman A; Carlin JB; Stern HS; Dunson DB; Vehtari A; Rubin DB. 2013. Bayesian data analysis. CRC Press, Boca Raton, FL.

Gelman A; Goodrich B; Gabry J; Vehtari A. 2018. R-squared for Bayesian regression models. The American Statistician 73:307–309. doi: 10.1080/00031305.2018.1549100

Gelman A; Hill J. 2007. Data analysis using regression and multilevel/hierarchical models. Cambridge University Press, Cambridge, England. doi: 10.1017/CBO9780511790942

Gelman A; Hill J; Yajima M. 2012. Why we (usually) don't have to worry about multiple comparisons. Journal of Research on Educational Effectiveness 5:189–211. doi: 10.1080/19345747.2011.618213

Georgiadis NJ; Ruess RW; McNaughton SJ; Western D. 1989. Ecological conditions that determine when grazing stimulates grass production. Oecologia 81(3):316–322. doi: 10.1007/BF00377077

Gill RA; Kelly RH; Parton WJ; Day KA; Jackson RB; Morgan JA; Scurlock JMO; Tieszen LL; Castle JV; Ojima DS; Zhang XS. 2002. Using simple environmental variables to estimate below-ground productivity in grasslands. Global Ecology and Biogeography 11:79–86. bit.ly/3cdQTXH

Gilmanov TG; Soussana JF; Aires L; Allard V; Ammann C; Balzarolo M; Barcza Z; Bernhofer C; Campbell CL; Cernusca A; Cescatti A; Clifton-Brown J; Dirks BOM; Dore S; Eugster W; Fuhrer J; Gimeno C; Gruenwald W; Haszpra L; Hensen A; Ibrom A; Jacobs AFG; Jones MB; Lanigan G; Laurila T; Lohila A; Manca G; Marcolla B; Nagy Z; Pilegaard K; Pinter K; Pio C; Raschi A; Rogiers N; Sanz MJ; Stefani P; Sutton M; Tuba Z; Valentini R; Williams ML; Wohlfahrt G. 2007. Partitioning European grassland net ecosystem CO_2 exchange into gross primary productivity and ecosystem respiration using light response function analysis. Agriculture, Ecosystems & Environment 121:93–120. doi: 10.1016/j.agee.2006.12.008

Gu Y; Wylie BK; Bliss NB. 2013. Mapping grassland productivity with 250-m eMODIS NDVI and SSURGO database over the Greater Platte River Basin, USA. Ecological Indicators 24:31–36. doi: 10.1016/j.ecolind.2012.05.024

Hafner S; Unteregelsbacher S; Seeber E; Lena B; Xu X; Li X; Guggenberger G; Miehe G; Kuzyakov Y. 2012. Effect of grazing on carbon stocks and assimilate partitioning in a Tibetan montane pasture revealed by $^{13}CO_2$ pulse labeling. Global Change Biology 18(2):528–538. doi: 10.1111/j.1365-2486.2011.02557.x

Hamilton EW; Frank DA. 2001. Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. Ecology 82(9):2397–2402. doi: doi.org/10.2307/2679923

Hamilton III EW; Frank DA; Hinchey PM; Murray TR. 2008. Defoliation induces root exudation and triggers positive rhizospheric feedbacks in a temperate grassland. Soil Biology and Biochemistry 40(11):2865–2873. doi: 10.1016/j.soilbio.2008.08.007

Houborg RM; Soegaard H. 2004. Regional simulation of ecosystem CO_2 and water vapor exchange for agricultural land using NOAA AVHRR and Terra MODIS satellite data. Application to Zealand, Denmark. Remote Sensing of Environment 93:150–167. doi: 10.1016/j.rse.2004.07.001

Hui D; Jackson RB. 2006. Geographical and interannual variability in biomass partitioning in grassland ecosystems: a synthesis of field data. New Phytologist 169(1):85–93. doi: 10.1111/j.1469-8137.2005.01569.x

Interrante SM; Sollenberger LE; Blount AR; Coleman SW; White UR; Liu K. 2009. Defoliation management of bahiagrass germplasm affects cover and persistence-related responses. Agronomy Journal 101(6):1381–1387. doi: 10.2134/agronj2009.0126

Iversen CM; McCormack ML; Powell AS; Blackwood CB; Freschet GT; Kattge J; Roumet C; Stover DB; Soudzilovskaia NA; Valverde-Barrantes OJ; van Bodegom PM; Violle C. 2017. A global fine-root ecology database to address below-ground challenges in plant ecology. New Phytologist 215(1):15–26. doi: 10.1111/nph.14486

Johnson LC; Matchett JR. 2001. Fire and grazing regulate belowground processes in tallgrass prairie. Ecology 82(12):3377–3389. doi: 10.2307/2680159

Jones DL; Nguyen C; Finlay RD. 2009. Carbon flow in the rhizosphere: carbon trading at the soil–root interface. Plant Soil 321:5–33. doi: 10.1007/s11104-009-9925-0

Klumpp K; Fontaine S; Attard E; Le Roux X; Gleixner G; Soussana J-F. 2009. Grazing triggers soil carbon loss by altering plant roots and their control on soil microbial community. Journal of Ecology 97(5):876–885. doi: 10.1111/j.1365-2745.2009.01549.x

Knapp AK; Blair JM; Briggs JM; Collins SL; Hartnett DC; Johnson LC; Towne EG. 1999. The keystone role of bison in North American tallgrass prairie. BioScience 49:39–50. doi: 10.1525/bisi.1999.49.1.39

Lal R. 2010. Managing soils and ecosystems for mitigating anthropogenic carbon emissions and advancing global food security. BioScience 60(9):708–721. doi: 10.1525/bio.2010.60.9.8

Madritch MD; Hunter MD. 2002. Phenotypic diversity influences ecosystem functioning in an Oak sandhills community. Ecology 83(8):2084–2090. doi: 10.1890/0012-9658(2002)083[2084:PDIEFI]2.0.CO;2

Makkonen K; Helmisaari H-S. 1999. Assessing fine-root biomass and production in a Scots pine stand – comparison of soil core and root ingrowth core methods. Plant and Soil 210:43–50. doi: 10.1023/A:1004629212604

Malhotra A; Sihi D; Iversen C. 2018. The fate of root carbon in soil: data and model gaps. Eos. Accessed (22 January 2020). doi:10.1029/2018EO112593

McNaughton SJ. 1983. Compensatory plant growth as a response to herbivory. Oikos 40(3):329–336. doi: 10.2307/3544305

McNaughton SJ. 1985. Ecology of a grazing ecosystem: the Serengeti. Ecological Monographs 55(3):259–294. doi: 10.2307/1942578

McNaughton SJ; Banyikwa FF; McNaughton MM. 1998. Root biomass and productivity in a grazing ecosystem: the Serengeti. Ecology 79(2):587–592. doi: 10.1890/0012-9658(1998)079[0587:RBAPIA]2.0.CO;2

McSherry ME; Ritchie ME. 2013. Effects of grazing on grassland soil carbon: a global review. Global Change Biology 19:1347–1357. doi: 10.1111/gcb.12144

Milchunas DG; Lauenroth WK. 1993. Quantitative effects of grazing on vegetation and soils over a global range of environments. Ecological Monographs 63(4):327–366. doi: 10.2307/2937150

Nicholas K.A; Kucharik CJ; Foley JA. 2009. Prairie restoration and carbon sequestration: difficulties quantifying C sources and sinks using a biometric approach. Ecological Applications 19(8):2185–2201. doi: 10.1890/08-0069.1

Poirier V; Roumet C; Munson AD. 2018. The root of the matter: Linking root traits and soil organic matter stabilization processes. Soil Biology and Biochemistry 120:246–259. doi: 10.1016/j.soilbio.2018.02.016

Rasse DP; Rumpel C; Dignac M-F. 2005. Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. Plant Soil 269:341–356. doi: 10.1007/s11104-004-0907-y

Schabenberger O; Gregoire TG; Kong F. 2000. Collections of simple effects and their relationship to main effects and interactions in factorials. The American Statistician 54(3):210–214. doi: 10.2307/2685592

Scurlock JMO; Hall DO. 1998. The global carbon sink: a grassland perspective. Global Change Biology 4:229–233. doi: 10.1046/j.1365-2486.1998.00151.x

Silveira ML; Obour AK; Arthington J; Sollenberger LE. 2011. The cow-calf industry and water quality in South Florida, USA: a review. Nutrient Cycling in Agroecosystems 89:439–452. doi: 10.1007/s10705-010-9407-z

Sokol NW; Kuebbing SE; Karlsen-Ayala E; Bradford MA. 2019. Evidence for the primacy of living root inputs, not root or shoot litter, in forming soil organic carbon. New Phytologist 221(1):233–246. doi: 10.1111/nph.15361

Stanley RL; Beaty ER; Powell JD. 1977. Forage yield and percent cell wall constituents of Pensacola bahiagrass as related to N fertilization and clipping height. Agronomy Journal 69(3):501–504.doi: 10.2134/agronj1977.00021962006900030041x

Steinbeiss S; Beßler H; Engels C; Temperton VM; Buchmann N; Roscher C; Kreutziger Y; Baade J; Habekost M; Gleixner G. 2008. Plant diversity positively affects short-term soil carbon storage in experimental grasslands. Global Change Biology 14(12):2937–2949. doi: 10.1111/j.1365-2486.2008.01697.x

Thornton B; Millard P. 1996. Effects of severity of defoliation on root functioning in grasses. Journal of Range Management 49:443–447. doi: 10.2307/4002927

Tilman D; Reich PB; Isbell F. 2012. Biodiversity impacts ecosystem productivity as much as resources, disturbance, or herbivory. PNAS 109(26):10394–10397. doi: 10.1073/pnas.1208240109

Vendramini JMB; Sollenberger LE; Blount AR; Aguiar AD; Galzerano L; Valente ALS; Alves E; Custodio L. 2013. Bahiagrass cultivar response to grazing frequency with limited nitrogen fertilization. Agronomy Journal 105(4):938–944. doi: 10.2134/agronj2012.0404

Whitham TG; Bailey JK; Schweitzer JA; Shuster SM; Bangert RK; LeRoy CJ; Lonsdorf EV; Allan GJ; DiFazio SP; Potts BM; Fischer DG; Gehring CA; Lindroth RL; Marks JC; Hart SC; Wimp GM; Wooley SC. 2006. A framework for community and ecosystem genetics: from genes to ecosystems. Nature Reviews Genetics 7:510–523. doi: 10.1038/nrg1877

Wilson CH; Strickland MS; Hutchings JA; Bianchi TS; Flory SL. 2018. Grazing enhances belowground carbon allocation, microbial biomass, and soil carbon in a subtropical grassland. Global Change Biology 24(7):2997–3009. doi: 10.1111/gcb.14070

Zhao W; Chen S-P; Lin G-H. 2008. Compensatory growth responses to clipping defoliation in *Leymus chinensis* (Poaceae) under nutrient addition and water deficiency conditions. Plant Ecology 196:85–99. doi: 10.1007/s11258-007-9336-3

Zhao, Fen, Bin Xu, Xiuchun Yang, Yunxiang Jin, Jinya Li, Lang Xia, Shi Chen, and Hailong Ma. 2014. Remote sensing estimates of grassland aboveground biomass based on MODIS net primary productivity (NPP): A case study in the Xilingol grassland of Northern China. Remote sensing 6(2014):5368-5386. doi: 10.3390/rs6065368

Ziter C; MacDougall AS. 2012. Nutrients and defoliation increase soil carbon inputs in grassland. Ecology 94:106–116. doi: 10.1890/11-2070.1

Appendix

Supplemental ANOVA analysis

Results of split-plot ANOVA analysis on root biomass from our study. Note that cutting frequency (Freq) and cultivar (Cults) were assigned at the plot level, and thus the F-ratio denominator should be based on the estimate of plot-level variance, rather than residual variance.

Split_plot <- aov(rootmass ~ Freq*Resid*Cults + Error(Plots), data = dat_lmer)
Summary (Split plot)

Error: Plots	Df	Sum	Sq Mean	Sq F value	Pr (>F)
Freq	1	1970	1970	0.483	0.493540
Cults	3	110334	36778	9.024	0.000351***
Freq:Cults	3	38017	12672	3.109	0.045248*
Residuals	24	97814	4076		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Error: Within

	Df	Sum	Sq Mean	Sq F value	Pr (>F)
Resid	1	93015	93015	17.703	0.000312***
Freq: Resid	1	5116	5116	0.974	0.333613
Resid: Cults	3	10763	3588	0.683	0.571228
Freq: Resid Cults	3	30931	10310	1.962	0.146580
Residuals	24	126099	5254		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Received for publication 24 February 2020; accepted 19 October 2020; published 31 May 2021)

doi: 10.17138/TGFT(9)159-170

Research Paper

Anatomical and nutritional characteristics of *Megathyrsus maximus* genotypes under a silvopastoral system

Características anatómicas y nutricionales de genotipos de Megathyrsus maximus *en un sistema silvopastoril*

MARIANA PEREIRA[1,4], ROBERTO GIOLO DE ALMEIDA[2], MANUEL CLAUDIO MOTTA MACEDO[2], VALÉRIA ANA CORVALÃ DOS SANTOS[3], ÉRICK LEMES GAMARRA[1], JOAQUÍN CASTRO-MONTOYA[4], BEATRIZ LEMPP[5] AND MARIA DA GRAÇA MORAIS[1]

[1]*Faculdade de Medicina Veterinária e Zootecnia, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil.* famez.ufms.br
[2]*Embrapa Gado de Corte, Campo Grande, MS, Brazil.* embrapa.br/gado-de-corte
[3]*Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, SP, Brazil.* esalq.usp.br
[4]*Institute of Agricultural Sciences in the Tropics, University of Hohenheim, Stuttgart, Germany.* tropen.uni-hohenheim.de
[5]*Faculdade de Ciências Agrárias, Universidade Federal da Grande Dourados, Dourados, MS, Brazil.* ufgd.edu.br

Abstract

Our objective was to measure chemical composition and anatomy of 5 *Megathyrsus maximus* (syn. *Panicum maximum*) genotypes, when grown in combination with eucalypts in a silvopastoral system. Cultivars Massai, Mombaça, BRS Tamani, Tanzânia and intraspecific hybrid accession PM44 were evaluated in full sun and a silvopastoral system at 5 different distances from eucalyptus tree rows. The experimental design was a randomized block in split plot with 2 replications. Plots corresponded with genotypes and subplots with sampling points within the system. Total forage and leaf biomass as well as nutritive value und tissue proportions were evaluated. Our results showed a decrease in biomass as radiation incidence decreased. Forage biomass was greatest in BRS Tamani and Mombaça and lowest in PM44. There was a significant interaction between sampling points and genotype for nutritive value variables, such as crude protein, in vitro digestibility of organic matter, cellulose, hemicellulose and lignin-S, while tissue proportions were not affected by the interaction between sampling points and genotypes. Genotype had more pronounced effects on chemical composition and anatomical characteristics than did sampling points. The leaves of Mombaça were the longest and had greatest total cross-sectional area, and this genotype showed greater proportions of sclerenchyma and vascular tissues than other cultivars and the lowest proportion of mesophyll. The greatest proportion of parenchyma bundle sheaths was also found in Mombaça leaves. Genotypes PM44 and Tanzânia had the lowest proportions of sclerenchyma, and PM44 and BRS Tamani had the lowest proportions of vascular tissues. On the other hand, PM44 and Tanzânia had the greatest proportions of mesophyll. BRS Tamani was comparable with the most used cultivars, Mombaça and Tanzânia, and had forage quality slightly superior to that of Mombaça. Tropical grasses growing under shade can potentially produce less forage but with better nutritive value, in terms of chemical composition and tissue proportions, than grasses grown under full sun. However, as the degree of shading in silvopastoral systems does not occur uniformly across the whole area, the improved nutritive value would not be uniform and may not be very prominent overall.

Keywords: Agroforestry, anatomical tissues, chemical composition, *Panicum maximum*, shading, tropical grasses.

Present address of senior author: University of Hohenheim, Institute of Agricultural Sciences in the Tropics, Garbenstr 13, 70599 Stuttgart, Germany.
Correspondence: Mariana Pereira, Federal University of Mato Grosso do Sul, UFMS, Faculty of Veterinary Medicine and Animal Science, Av. Senador Filinto Müler 2443, 79070-900, Campo Grande, Brazil.
E-mail: mariana.pereira@uni-hohenheim.de

Resumen

Nuestro objetivo fue medir la composición química y la anatomía de 5 genotipos de *Megathyrsus maximus* (syn. *Panicum maximum*), cuando se cultivan en combinación con eucaliptos en un sistema silvopastoril. Los cultivares Massai, Mombaça, BRS Tamani, Tanzânia y la accesión híbrida intraespecífica PM44 se evaluaron a pleno sol y en un sistema silvopastoril a 5 distancias diferentes de las hileras de eucaliptos. El diseño experimental fue un bloque al azar en parcela dividida con 2 repeticiones. Las parcelas se correspondieron con genotipos y subparcelas con puntos de muestreo dentro del sistema. Se evaluó la biomasa total de forrajes y hojas, así como el valor nutritivo y las proporciones de tejido. Nuestros resultados mostraron una disminución en la biomasa a medida que disminuyó la incidencia de radiación. La biomasa del forraje fue mayor en BRS Tamani y Mombaça y menor en PM44. Hubo una interacción significativa entre los puntos de muestreo y el genotipo para las variables de valor nutritivo, como proteína cruda, digestibilidad in vitro de materia orgánica, celulosa, hemicelulosa y lignina-S, mientras que las proporciones de los tejidos no se vieron afectadas por la interacción entre los puntos de muestreo y los genotipos. El genotipo tuvo efectos más pronunciados sobre la composición química y las características anatómicas que los puntos de muestreo. Las hojas de Mombaça fueron las más largas y tuvieron mayor área de corte transversal total, y este genotipo mostró mayores proporciones de esclerénquima y tejidos vasculares que otros cultivares y la menor proporción de mesófilo. La mayor proporción de vainas de haces de parénquima también se encontró en las hojas de Mombaça. Los genotipos PM44 y Tanzânia tenían las proporciones más bajas de esclerénquima, y PM44 y BRS Tamani tenían las proporciones más bajas de tejidos vasculares. Por otro lado, PM44 y Tanzânia tuvieron las mayores proporciones de mesófilo. BRS Tamani fue comparable con los cultivares más utilizados, Mombaça y Tanzânia, y tuvo una calidad de forraje ligeramente superior a la de Mombaça. Los pastos tropicales que crecen bajo sombra pueden producir potencialmente menos forraje pero con mejor valor nutritivo, en términos de composición química y proporciones de tejido, que los pastos cultivados a pleno sol. Sin embargo, como el grado de sombreado en los sistemas silvopastoriles no ocurre de manera uniforme en toda el área, el valor nutritivo mejorado no sería uniforme y podría no ser muy prominente en general.

Palabras clave: Agroforestería, composición química, *Panicum maximum*, pastos tropicales, sombreado, tejidos anatómicos.

Introduction

In the tropics, Guinea grass [*Megathyrsus maximus* (syn. *Panicum maximum*)] is one of the major grasses used as forage for cattle feeding, characterized by both high forage biomass and high nutritive quality, which favors weight gain of bovines in grazing systems (Euclides et al. 2015). Although 24 cultivars are registered in the official site of the Brazilian Ministry of Agriculture, Livestock and Food Supply (http://sistemas.agricultura.gov.br/snpc/cultivarweb/cultivares_registradas.php), the most used *M. maximus* cultivars in Brazil are Tanzânia and Mombaça, comprising about 90% of the seed of this species being sold (Fernandes et al. 2014). This lack of diversity carries the risk that traditional cultivars might succumb to a disease or pest, such as *Bipolaris maydis* fungus, which has caused significant losses in cv. Tanzânia (Braz et al. 2015). Therefore, there is an ongoing need for research to develop and evaluate new cultivars, in a range of different environmental conditions, in order to promote pasture diversification in livestock production systems.

Aiming at achieving resource-efficient and sustainable animal production in the tropics, integrated crop-livestock and integrated livestock-forestry and crop-livestock-forestry systems have been studied by Brazilian Agricultural Research Corporation (Embrapa). The standard silvopastoral systems combine livestock and forestry in the same location, providing benefits in terms of soil quality (Sousa Neto et al. 2014), forage quality (Gamarra et al. 2017), greenhouse gas balance (Alves et al. 2015) and animal welfare (Karvatte Junior et al. 2016).

The presence of trees in the systems modifies the environment for forage grass development and, consequently, its growth patterns. When exposed to shade, some forage grasses can modify their leaf anatomy, in order to increase light absorption efficiency and continue growing under tree canopies (Gobbi et al. 2011). Further factors like gaseous exchanges and, consequently, the forage primary production process and nutritive value are also affected (Batistoti et al. 2012).

An increase in the specific leaf area of plants growing under shade has been reported widely in the literature (Gomes et al. 2019; Pezzopane et al. 2019). This greater specific leaf area under shade than in full sun may affect the leaf blade proportion of tissues, such as lower mesophyll proportion, decrease in sclerenchyma and vascular tissues plus thinner cell walls and bulliform cells

(Lambers et al. 1998). Quantifying the proportion of various tissues in leaf blades should indicate the potential digestibility of forage, although some leaves are highly digestible, while others are poorly digestible or even indigestible (Batistoti et al. 2012). In addition, leaf anatomy consists of morphologically and functionally distinct types of photosynthetic cells (Berry and Patel 2008). For instance, C_4 leaf anatomy was identified with lower proportions of mesophyll and greater less-digestible epidermis, bundle sheath, sclerenchyma and vascular tissues than C_3 leaf anatomy, which negatively affects pasture digestibility of C_4 species (Wilson and Hattersley 1989).

Several studies have been conducted with tropical grasses under different shading levels, focusing on responses in biomass yield, nutritive value, physiology and morphology of forage (Gobbi et al. 2011; Baldissera et al. 2016; Geremia et al. 2018). For these parameters, *M. maximus* cultivars have shown medium tolerance of shaded environments (Santiago-Hernández et al. 2016), which indicates that they have potential for use in silvopastoral systems (Paciullo et al. 2016). However, those studies are more focused on cv. Tanzânia and Mombaça, although cv. BRS Tamani, launched in 2014, has shown high performance under shading (Pereira et al. 2015).

The increased interest in silvopastoral systems has boosted research into forage grasses suitable for use in shaded environments. We aimed at investigating the leaf anatomy of different *M. maximus* genotypes under shaded conditions in a silvopastoral system, and the relationship between anatomical and nutritional characteristics of leaf blades of these genotypes and different degrees of shade.

Materials and Methods

Experimental design

The field experiment was conducted at Embrapa Beef Cattle, located in the municipality of Campo Grande - MS (20º24'54.9 S, 54º42'25.8 W; 530 masl). The climate corresponds to the humid tropical type, sub-type Aw (Köppen classification), with hot and rainy summers. Soil is classified as clayey Oxisol. The evaluations were carried out in full sun and in a silvopastoral system, both established in 2008, and briefly described as follows:

- Full sun, representing the Control (CON), with soybean (*Glycine max*) planted every 4 years in a no-tillage system in rotation with tropical grass pasture for 3 consecutive years.
- A silvopastoral system with *Eucalyptus urograndis* H13 clone trees (*Eucalyptus grandis* × *E. urophylla*) planted in rows in an east-west direction. Distance between rows was 22 m and between trees within rows 2 m, totalling 227 eucalyptus trees/ha. During the experimental period, the average tree height was 22 m measured by a Haglöf compass clinometer (Haglöf Sweden AB, Långsele, Sweden). As for the CON treatment, soybean was sown every 4 years by sod-seeding, in rotation with tropical grass pasture for 3 consecutive years.

A detailed description of the establishment and design for both the silvopastoral system and CON is given by Oliveira et al. (2014) and Gamarra et al. (2017). The management of the systems was similar throughout. The soybean was harvested in April 2013, and in October of the same year, 5 *Megathyrsus maximus* genotypes, i.e. cvv. Massai, Mombaça, BRS Tamani, Tanzânia and hybrid accession PM44 were sown in plots of 20 × 1.5 m, at a row spacing of 0.25 m, as an understory for eucalyptus trees. There was an application of 109 kg urea/ha and 300 kg N:P:K/ha (0:20:20) at seeding. Grass seeding rates were adjusted to apply 200 pure viable seeds/m^2. Grasses were first harvested at 70 days after sowing and 30 cm above ground level, and then 35 days after the first cut, in order to evaluate regrowth potential. The average height of the cultivars at harvest was 60 cm. Evaluation of the anatomical and chemical characteristics of the genotypes was conducted during the rainy season at the beginning of February 2014.

The experimental design was a randomized block in split plot with 2 replications/ paddock. Plots corresponded with *M. maximus* genotypes and the subplots with sampling points within each plot. Five sampling points were defined: 1 m from the northern tree rows (P1N); 1 m from the southern tree rows (P1S); 6 m from the northern tree rows (P6N); 6 m from the southern tree rows (P6S); and midway between the tree rows, i.e. 11 m from each (P11). For the CON system 2 sampling points were randomly collected in the system. The photosynthetically active radiation (PAR) was recorded at grass canopy height at each sampling point by a portable ceptometer (model AccuPAR-LP 80, Decagon Devices Inc., Pullman, WA, USA), in the morning and afternoon on sunny days (Figure 1), and at random points under full-sun conditions. The leaf greenness index was measured with a Soil Plant Analysis Development (SPAD) optical chlorophyll meter (SPAD-502 Plus, Minolta, Japan) on 3 leaves of randomly selected plants at each sampling point.

Field plots were mechanically defoliated 70 days after establishment and 35 days later at a cutting height of 30 cm above ground to determine forage biomass (total dry matter, TDM) within a metallic frame of 1 × 1 m. All forage samples collected were individually weighed after harvesting. A subsample from each sampling point was taken, placed in a paper bag and dried in a forced-air oven at 65 ºC until constant mass for determination of DM. Another subsample from each sampling point was selected and separated into its morphological components – leaf blade, stem with sheath and senescent material, which were weighed and subsequently dried in a forced-air oven at 65 ºC for DM determination. Leaf biomass (LDM) was then estimated for the purpose of this study.

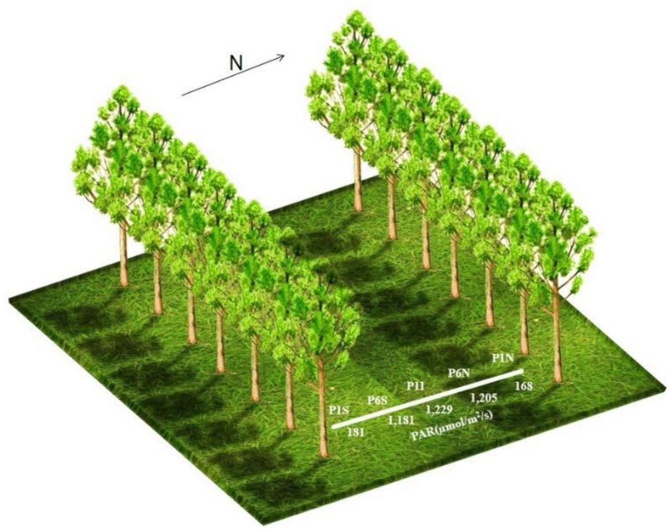


Figure 1. Schematic representation of forage sampling points (P1N: 1 m from the northern tree rows; P1S: 1 m from the southern tree rows; P6N: 6 m from the northern tree rows; P6S: 6 m from the southern tree rows; P11: midway between the tree rows, i.e. 11 m from each) sited in a silvopastoral system (grass + 227 trees/ha), with values for respective photosynthetically active radiation reaching pasture surface. (Adapted from Oliveira et al. 2019).

Anatomical parameters

The penultimate expanded leaf blade, with exposed ligules, cut from the basal region of the main vegetative shoot of 5 tillers randomly selected from each cultivar, was taken at each sampling point. Leaf blades were identified and width at the central point and length (from the apex of the blade to the base of the ligule insertion) were measured. Sample fragments of 1 cm were taken from the central area of 5 blades and stored in bottles filled with formalin aceto-alcohol solution. The 1 cm leaf blade fragments were stored in a tertiary butyl ethanol series (Daykin and Hussey 1985).

After dehydration, fragments were processed with paraplast. Fragments were sectioned with a thickness of 10 µm, using a manual rotary microtome, followed by triarch quadruple staining of tissues before permanent blade mounting, following the methodology proposed by Hagquist (1974). The image analyzing system (AxioVision version 3.1) was coupled to the binocular optical microscope to estimate proportions of each tissue in the leaf blades. Total cross-sectional area projected in the video was measured first (TA), followed by determination of the area occupied by abaxial (ABAep) and adaxial (ADAep) epidermal tissues from the parenchyma bundle sheath (PBS), the sclerenchyma (SCL) and the vascular (VT) system. The mesophyll (MES) area was calculated as the difference between total anatomical area and the area of other tissues.

Nutritional parameters

Leaf samples from the respective sampling points (P1N, P1S, P6N, P6S, P11 and CON) as for anatomical parameters were weighed and dried in a forced-air oven at 65 ºC until constant weight was reached. After grinding to pass a 1-mm sieve, samples were analyzed for crude protein (CP), in vitro organic matter digestibility (IVOMD) and cellulose, while hemicellulose was calculated by subtracting acid detergent fiber values from neutral detergent fiber values, and lignin-S values determined for the predictive equation by the method of solubilization of cellulose with sulfuric acid, using near infrared reflectance spectroscopy (FOSS NIR System 5000), according to Marten et al. (1985), at the Embrapa Beef Cattle facilities.

Statistical analysis

Shapiro-Wilk test was used to assess the normality of the residual data. No transformation was required. Pearson correlation was used to evaluate the relationship between anatomical and chemical characteristics of data, and was also applied for the PAR, SPAD index, forage biomass, and leaf biomass of the genotypes. These data were also subjected to an analysis of variance using the General Linear Model (GLM) procedure of SAS with genotype, sampling point and their interaction as main effects. Statistical significances were defined for P<0.05, and mean differences were analyzed by the Tukey test.

Results

The anatomy of the penultimate expanded leaf blade of the 5 *Urochloa maximus* genotypes evaluated is depicted in Table 1 and Figure 2. A distinguishing feature of C_4 pathways of photosynthesis, which occurs in tropical grasses, is the Kranz anatomy, i.e. 2 morphologically and functionally different types of photosynthetic cells, PBS and MES, with numerous well-developed chloroplasts. The bundle sheath of specialized cells, with normally thick walls, surrounds the VT and MES cells, surrounding the bundle sheath beneath the epidermis (Epi). The carbon

Table 1. Means of anatomical traits and length of leaf blades of *Megathyrsus maximus* genotypes, in a silvopastoral system.

Variable[1]	Massai	Mombaça	PM44	BRS Tamani	Tanzânia	s.e.m.	P-value
TA (μm²)	98,477ab	131,631a	120,054ab	89,044b	120,035ab	4.56	<0.01
			Relative tissue proportion (%)				
Epi	28.6	25.9	26.1	28.4	24.9	7.24	<0.01
ADAep	18.8a	16.9a	16.4a	17.9a	16.0a	5.37	<0.01
ABAep	9.8ab	9.0b	9.8ab	10.5a	8.9b	0.38	<0.01
PBS	36.3b	40.0a	36.7b	36.0b	38.0ab	2.25	0.01
VT	7.6ab	9.3a	6.8b	6.9b	7.3ab	1.62	0.01
SCL	4.8a	4.5a	3.4b	4.4a	3.5b	0.22	<0.01
MES	22.7ab	19.7b	27.0a	24.3ab	26.3a	7.20	<0.01
SPAD	40.7a	40.1a	43.9a	35.4a	43.6a	49.7	<0.01
Length (cm)	53.4ab	66.3a	51.0b	53.7ab	56.0ab	49.5	0.01

[1]Variables: TA, total anatomical area of the leaves; Epi, epidermis; ADAep, adaxial epidermis; ABAep, abaxial epidermis; PBS, parenchyma bundle sheath; VT, vascular tissue; SCL, sclerenchyma; MES, mesophyll; SPAD, Soil Plant Analysis Development. Within rows, genotypes without a common letter are significantly different (P<0.05).

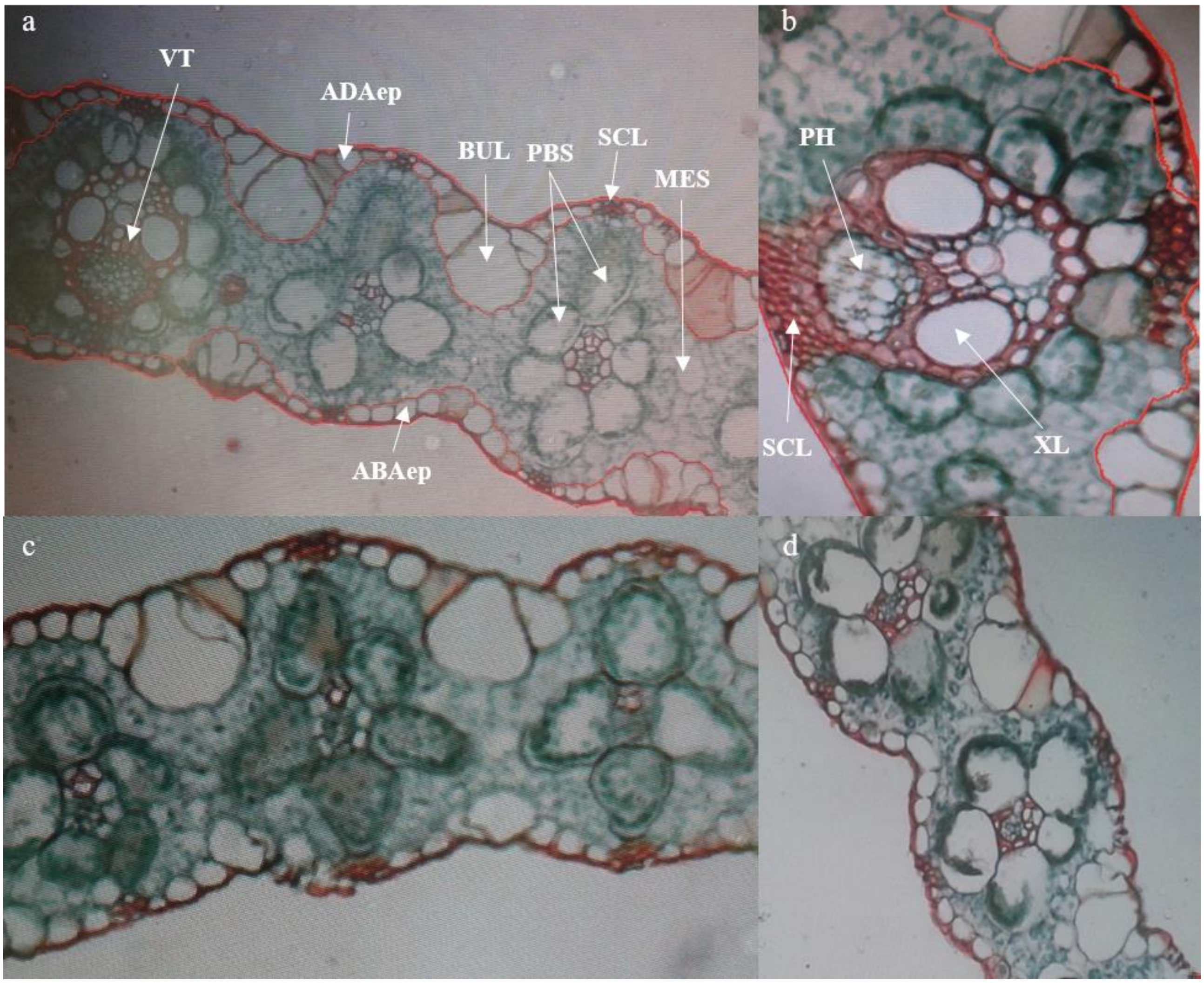


Figure 2. Cross-sectional area of leaves of *Megathyrsus maximus* genotypes in a silvopastoral system (grass + 227 trees/ha) and full sun conditions. a. ADAep, adaxial epidermis; ABAep, abaxial epidermis; PBS, parenchyma bundle sheath; VT, vascular tissue; SCL, sclerenchyma; MES, mesophyll; BUL, bulliform cells. b. An example of a leaf of cv. Massai with the Girder structure. PH, phloem; XL, xylem. c and d. Examples of leaves with PBS and MES with chloroplasts.

dioxide-assimilation C_4 pathway utilizes 2 different sets of biochemical reactions, and the compartmentalization and functional separation of those structural frameworks are provided by this wreath-like arrangement (Berry and Patel 2008). *M. maximus* (biochemical characterization of phosphoenolpyruvate carboxykinase, PEP-CK subtype of C_4) has an undifferentiated MES with few intercellular spaces and a single layer of concentric bundle sheath cells around VT. The chloroplasts in the bundle sheath cell are centrifugal and the leaves contain stomata in both surfaces (amphistomatic). The epidermis contains a single layer of juxtaposed cells with bulliform cells placed between the vascular bundles in the ADAep (Habermann et al. 2019). While their leaves are relatively resistant to degradation by rumen microorganisms, they afford great photosynthetic carbon conversion efficiency and hence above-ground biomass production (Wilson et al. 1983).

In our study the level of PAR that reached the forage canopy varied greatly according to the distance from the trees, with pasture close to the tree lines receiving only 10% as much radiation as open pasture received. While points P6N, P11 and P6S in the silvopastoral system received levels of PAR above 1,100 µmol/m^2/s, or the amount required for tropical forage grass development, [for instance, 800 µmol/m^2/s for the genus *Brachiaria* (Gifford 1974; Sousa et al. 2010)], points P1N and P1S received only 22% of this requirement (Figure 1). The PAR of full sun conditions was on average 1,697 µmol/m^2/s. As expected, forage growth decreased as level of PAR reaching the grass canopy also decreased, so leaf biomass was lower in the silvopastoral than the full-sun environment.

Total forage biomass (TDM) and leaf biomass (LDM) were greater (P<0.05) in CON (means of 6,249 kg DM/ha and 2,408 kg DM/ha, respectively) than at the silvopastoral sampling points, where TDM and LDM decreased as distance from tree lines decreased (Pereira et al. 2021). Means for TDM were 4,915, 4,211, 5,699, 4,656 and 5,613 kg DM/ha and for LDM were 1,511, 1,339, 1,694, 1,192 and 1,814 kg DM/ha, at points P1N, P1S, P6N, P6S and P11, respectively (data not shown). By comparing the genotypes, TDM of BRS Tamani (6,490 kg DM/ha) and Mombaça (6,033 kg DM/ha) was greater (P<0.05) than that of PM44 (3,596 kg DM/ha), while those of Massai (5,356 kg DM/ha) and Tanzania (4,644 kg DM/ha) were intermediate (P>0.05). Massai (2,385 kg DM/ha) showed the greatest LDM and Tanzania (1,142 kg DM/ha) the lowest. Mombaça (1,873 kg DM/ha) was greater (P<0.05) than PM44 (1,189 kg DM/ha), while BRS Tamani (1,708 kg DM/ha) was intermediate (P>0.05) (data not shown).

There were significant interactions (P<0.05) between sampling point and genotype for all chemical parameters measured, so data are presented for all combinations in Table 2. The CP concentration for BRS Tamani for P1N exceeded that for P6S (P<0.05). Similarly, IVOMD for PM44 at P1N exceeded that at P6S (P<0.05) and hemicellulose concentration of BRS Tamani at P1N exceeded those for P1S, P6S and CON (P<0.05).

Effects of genotype were much more pronounced, although genotype had no significant effect on CP concentration or IVOMD (P>0.05). At P6S, cellulose concentration of BRS Tamani exceeded those for PM44 and Tanzania and that of Mombaça exceeded that of PM44 (P<0.05). At P1N hemicellulose concentration of BRS Tamani exceeded that of PM44 and at P6N concentration of Tanzania exceeded that of PM44 (P<0.05). Lignin concentration of PM44 at P1N and P6S exceeded that of Tanzania and lignin concentration of BRS Tamani at P1S and P6N exceeded that of Tanzania (P<0.05).

Regarding anatomical characteristics, effects of genotype were much more pronounced than effects of sampling points. Mombaça showed the highest total cross-sectional area (TA), which was significantly greater (P<0.01) than that of BRS Tamani, and leaves of Mombaca were longer than those of PM44 (Table 1). In general, Mombaca showed higher proportions of poorly digestible or even indigestible tissues, e.g. SCL and VT, than other cultivars and lower proportion of mesophyll. Genotypes PM44 and Tanzânia had the lowest proportions of sclerenchyma, while PM44 and BRS Tamani had the lowest proportions of vascular tissues. On the other hand, PM44 and Tanzânia had higher proportions of MES, a rapidly and completely digested tissue, than Mombaça (P<0.01). In relation to partially degradable tissues, BRS Tamani showed higher ABAep than Mombaça and Tanzânia, while Mombaça had higher PBS than Massai, PM44 and BRS Tamani. Total cross-sectional area of leaf blades in the full-sun system (CON) was greater than for the sampling points near trees (P1N, P1S and P6S) (Table 3). Effects of sampling points on the remaining anatomical parameters were not significant (P>0.05).

Overall, weak correlations were found between anatomical and nutritional parameters (P≤0.49; Table 4). CP was positively related to IVOMD, hemicellulose and MES, but negatively related to cellulose, Epi, ADAep and SCL. These final 3 tissues were positively related to cellulose, whereas lignin-S was positively correlated with Epi and ADAep. As expected, IVOMD showed positive correlation with hemicellulose, and negative correlation with cellulose and lignin-S. SPAD index was positively related to CP, IVOMD, TA and MES, and negatively related to cellulose and Epi (Table 4).

PAR was positively related to LDM and TA, and negatively related to CP, IVOMD, hemicellulose and ABAep. In addition, LDM showed positive correlation with SCL, and negative with MES.

Table 2. Chemical composition of leaf blades of *Megathyrsus maximus* genotypes at each sampling point in a silvopastoral system.

Parameter	Sampling point[1]	Genotype					Average	s.e.m.	P-value		
		Massai	Mombaça	PM44	BRS Tamani	Tanzânia			SP	Genotype	SP × Genotype
CP (g/kg DM)	P1N	142Aa	146Aa	135Aa	143Aa	162Aa	146	0.95	<0.01	<0.01	0.01
	P1S	129Aa	141Aa	147Aa	124ABa	147Aa	138				
	P6N	115Aa	137Aa	127Aa	131ABa	151Aa	132				
	P6S	118Abc	110Ac	159Aa	100Bc	151Aab	128				
	P11	115Aa	120Aa	128Aa	131ABa	133Aa	125				
	CON	127Aa	123Aa	128Aa	122ABa	133Aa	127				
	Average	124	130	137	125	146					
IVOMD (g/kg DM)	P1N	634Aa	583Aa	695Aa	628Aa	662Aa	640	3.20	0.01	<0.01	0.04
	P1S	555Aa	595Aa	594ABa	570Aa	645Aa	592				
	P6N	612Aa	607Aa	617ABa	564Aa	619Aa	604				
	P6S	636Aa	524Aa	524Ba	550Aa	636Aa	574				
	P11	561Aa	567Aa	567ABa	636Aa	602Aa	587				
	CON	581Aa	542Aa	542ABa	539Aa	557Aa	552				
	Average	597	570	590	581	620					
Cellulose (g/kg DM)	P1N	304Aa	304Aa	296Aa	300Aa	284Aa	298	0.64	0.19	<0.01	0.02
	P1S	316Aa	307Aa	309Aa	332Aa	294Aa	312				
	P6N	325Aa	306Aa	310Aa	315Aa	289Aa	309				
	P6S	317Aabc	326Aab	282Ac	335Aa	290Abc	310				
	P11	329Aa	318Aa	295Aa	310Aa	317Aa	314				
	CON	309Aa	294Aa	291Aa	305Aa	289Aa	298				
	Average	317	309	297	316	294					
Hemicellulose (g/kg DM)	P1N	343Aab	375Aab	334Ab	404Aa	392Aab	370	1.46	<0.01	<0.01	0.02
	P1S	361Aa	370Aa	355Aa	326Ba	364Aa	355				
	P6N	330Aab	347Aab	308Ab	349ABab	382Aa	343				
	P6S	339Aa	332Aa	300Aa	340Ba	353Aa	333				
	P11	320Aa	343Aa	309Aa	359Aba	367Aa	340				
	CON	343Aa	347Aa	317Aa	332Ba	345Aa	337				
	Average	339	352	321	352	367					
Lignin-S (g/kg DM)	P1N	33Aab	33Aab	36Aa	31Aab	25Ab	32	0.24	<0.01	0.01	0.01
	P1S	37Aab	31Aab	35Aab	40Aa	29Ab	34				
	P6N	34Aab	32Aab	32Aab	38Aa	27Ab	33				
	P6S	27Aab	34Aab	36Aa	31Aab	25Ab	31				
	P11	35Aa	30Aa	29Aa	30Aa	27Aa	30				
	CON	33Aa	31Aa	27Aa	34Aa	30Aa	31				
	Average	33	32	33	34	27					

[1]Sampling points: P1N, 1 m from the northern tree rows; P1S, 1 m from the southern tree rows; P6N, 6 m from the northern tree rows; P6S, 6 m from the southern tree rows; P11, midway between the tree rows; CP, crude protein; IVOMD, in vitro organic matter digestibility. Within parameters and columns means without a common upper-case letter and within rows means without a common lower-case letter are significantly different by Tukey test (P<0.05).

Table 3. Means of anatomical traits and length of leaf blades of *Megathyrsus maximus* genotypes at various sampling points[1], in a silvopastoral system.

Variable[2]	P1N	P1S	P6N	P6S	P11	CON	s.e.m.	P-value
TA (μm²)	97,259b	105,178b	114,790ab	108,796b	117,163ab	130,304a	1.17	0.01
			Relative tissue proportion (%)					
Epi	27.5	27.4	26.3	27.1	26.3	26.1	1.75	0.25
ADAep	17.2	17.2	17.0	17.8	17.0	17.0	1.46	0.63
ABAep	10.3a	10.2a	9.4a	9.3a	9.3a	9.1a	0.45	0.03
PBS	37.6	37.3	37.7	36.8	35.8	38.8	4.80	0.19
VT	7.7	7.1	7.0	7.8	7.4	8.5	2.81	0.46
SCL	4.2	4.0	4.5	3.9	4.1	4.1	0.33	0.56
MES	22.9	24.2	24.6	24.3	25.3	22.6	10.5	0.56
SPAD	40.2	40.6	44.4	38.4	41.9	38.9	15.1	0.19
Length (cm)	58.8	58.8	61.1	51.8	49.9	53.2	43.2	0.07

[1]Sampling points: P1N, 1 m from the northern tree rows; P1S, 1 m from the southern tree rows; P6N, 6 m from the northern tree rows; P6S, 6 m from the southern tree rows; P11, midway between the tree rows, i.e. 11 m from each. [2]Variables: TA, total anatomical area of the leaves; Epi, epidermis; ADAep, adaxial epidermis; ABAep, abaxial epidermis; PBS, parenchyma bundle sheath; VT, vascular tissue; SCL, sclerenchyma; MES, mesophyll; SPAD, Soil Plant Analysis Development. Within rows, means without a common letter are significantly different (P<0.05).

Table 4. Correlation coefficients among anatomical and chemical characteristics of leaf blades of *Megathursus maximus* genotypes in a silvopastoral system.

	PAR	TDM	SPAD	IVOMD	Cel	Hemi	LigS	Epi	ADAep	ABAep	PBS	VT	SCL	MES
LDM	0.33	0.69	-	-	-	-	-	-	-	-	-	-	0.37	-0.27
CP	-0.40	-	0.30	0.49	-0.76	0.43	-	-0.26	-0.34	-	-	-	-0.35	0.29
IVOMD	-0.44	-	0.30		-0.37	0.44	-0.34	-	-	-	-	-	-	-
Cel	-	-	-0.28			-	0.37			-	-	-		-
Hemi	-0.42	-	-		-		0.40	-	-	-	-	-	-	-
TA	0.38	-	0.34	-	-0.28	-	-	-0.73	-0.49	-0.78	0.44	0.26	-0.26	-
Epi	-	-	-0.27	-	0.37	-	0.28				-0.47	-0.30	-	-
ADAep	-	-	-	-	0.40	-	0.26	0.90			-0.38	-	-	-
ABAep	-0.44	-	-	-	-	-	-	0.71	0.33	-	-0.40	-0.35		
VT	-	-	-	-	-	-	-		-		0.76	-		
SCL	-	0.31	-	-	0.36	-	-	-	-	-	-	0.37	-	
MES	-	-	0.26	-	-	-	-	-	-	-	-0.70	-0.82	-0.60	-
Length	-	-	-	-	-	-	-	-	-	-	0.38	-	-	-

PAR, photosynthetically active radiation; TDM, total dry matter; SPAD, Soil Plant Analysis Development; IVOMD, in vitro organic matter digestibility; Cel, cellulose; Hemi, hemicellulose; LigS, lignin determined by solubilization of cellulose with sulfuric acid; Epi, epidermis; ADAep, adaxial epidermis; ABAep, abaxial epidermis; PBS, parenchyma bundle sheath; VT, vascular tissue; SCL, sclerenchyma; MES, mesophyll; LDM, leaf dry matter; CP, crude protein; TA, total anatomical area of the leaves. Values are shown when P<0.05 and are highlighted when correlations are negative.

Discussion

The complex adjustments in plant physiology and the structure of leaf tissues provide the mechanism for plants to survive in a limited or impaired environment. Despite neglecting leaf anatomy in the studies evaluating plant responses to different conditions (Xu et al. 2012), plant physiology, productivity and animal performance are closely related to leaf anatomy (Habermann et al. 2019). Mesophyll characteristics influence carbon assimilation rates and leaf function, while leaf tissue thickness plays a vital role in thermal regulation, light interception and CO_2 and water vapor diffusion (Terashima et al. 2011; Habermann et al. 2019) and different proportions of leaf tissues are important in the digestibility and nutritional value of forage for animals (Batistoti et al. 2012).

There was a positive correlation between forage biomass and leaf biomass plus indigestible tissues, such as SCL and VT and a negative correlation between leaf biomass and MES. In agreement with our findings, Britto de Assis Prado et al. (2016) reported that large biomass for expanded leaves tended to result in thinner MES. Even though those authors assessed the effects of temperature and CO_2 concentrations on foliage development, our findings of leaf biomass being negatively correlated with MES suggest further studies are warranted to measure differences in ambient air temperature between full-sun and silvopastoral systems, which are likely to favor full sun (Karvatte Junior et al. 2016). Although some genotypes had a greater quantity of highly digestible tissues, their forage biomass, leaf:stem ratio and tiller density, as the case of PM44 in contrast to Mombaça, should be considered for use in systems with a forest component, to support grazing.

The negative correlation between level of radiation and CP was expected due to the increase of nitrogen concentration of forage in shaded environments, already widely reported in the literature (Lin et al. 2001; Soares et al. 2009; Gobbi et al. 2010; Paciullo et al. 2016), but still undefined for IVOMD (Lima et al. 2018). In addition, the negative correlation between radiation and hemicellulose is indicative of increase of primary wall in forage developing with light restriction due to changes in the partitioning of photosynthate. Light restriction decreases the availability of photosynthate, resulting in the partitioning of photosynthate to priority areas to improve the efficiency of the photosynthesis, instead of developing secondary walls. In addition, leaves grown in full sun tend to be thicker than shaded leaves (Deinum et al. 1996).

The positive correlations between SPAD index, CP and MES result from high amounts of photosynthesis-related enzymes in MES tissues, because most of the nitrogen in MES is present in the chloroplasts and involved in photosynthesis, which can be measured by SPAD (Yamamoto et al. 2002; Batistoti et al. 2012). Moreover, the positive relationships between cellulose and lignin-S and Epi, ADAep, SCL and VT were also expected, because these tissues are cell types with thickened secondary walls, and are highly lignified, contributing to poor quality of forage (Wilson 1994). Positive correlation between PBS and VT is the reason for thickened secondary walls and the lignification of parenchyma bundle sheath tissues (Batistoti et al. 2012).

Although shaded plants displayed changes in anatomical development relative to plants grown in full sun, such as lower mesophyll proportion, decrease of sclerenchyma and vascular tissues, thinner cell walls and bulliform cells (Lambers et al. 1998), in silvopastoral systems the light environment in the understory of trees is dynamic, varying according to the incline of the earth's axis in relation to the sun, as well as the growth of the trees. Consequently, the amount of light reaching plants under trees could have varied with time (Geremia et al. 2018). However, most of the chemical and anatomical differences between locations and cultivars were likely due to genetic variability. Some interaction was found between genotype and sampling points, meaning an interaction between the genotype and the PAR reaching the canopy or degree of competition for nutrients and water. Presumably, effects of soil moisture competition between trees and forage grasses would be minimal since the measurements occurred in the rainy season. Nevertheless, we propose that research should be undertaken in the areas of soil moisture and nutrient uptake in silvopastoral systems, especially since eucalypts have a very large root system.

Depending on the plant's response, shading will not necessarily result in improvements in the forage nutritive value. While we failed to record any significant effects of degree of shading on CP% (except for cv. BRS Tamani), according to Paciullo et al. (2016), there was an increase in both CP and acid detergent insoluble protein in leaves of cv. Massai under intense shading. The nitrogen fraction accounting for the unavailable protein contained in the cell walls is undegraded in the rumen and is non-digestible in ruminant intestines, Sniffen et al. (1992) suggested that shading may compromise forage nutritive value. Our study failed to clarify this hypothesis.

The high proportion of tissues with thickened cell walls and increased lignification for Mombaça is related to the greater length of leaves. Lengthy leaf blades require strong structural support to maintain their erect configuration, and this support is provided by xylem and SCL (Paciullo et al. 2002). Mombaça presents visually erect leaf configuration and cespitose growth habit. Gomes et al. (2011) found greater structural tissues, such as SCL and VT, in genotypes with lengthy leaf blades and lower MES, in agreement with our finding as MES of Mombaça leaf blade was the lowest reported in this study.

Regarding cv. Massai, the high SCL is associated with the Girder structure present in its leaves. This structure has an arrangement of SCL cells among Epi and PBS (Brâncio et al. 2002), reducing the nutritive value of forage produced (Lempp 2007). BRS Tamani showed results similar to those of Massai, being slightly superior to Mombaça, but slightly inferior to Tanzânia and PM44. According to Martuscello et al. (2015), BRS Tamani was clustered together with Massai based on graphic dispersion of canonical variables, indicating also a similarity among them for low plant height, but high leaf:stem ratio and leaf proportion. However, our study did not evaluate the presence of Girder structure in the genotypes, as those authors evaluated genotypes of *M. maximus* for productive characteristics.

Conclusions

This study has shown that the tropical forages evaluated had similar nutritive value in both full sun and shade in contrast to other published findings of better nutritive value in terms of chemical composition and tissue proportions in grasses grown in shade. Since the degree of sunlight reaching the grass canopy under trees can vary dramatically depending on time of day and distance from the tree lines, differences in nutritive value may not be very prominent. This issue seems to warrant more studies.

Acknowledgments

We thank Embrapa Beef Cattle, Fundect, Unipasto (Associação para o Fomento à Pesquisa de Melhoramento de Forrageiras) and the Research Training Group 'Water-People-Agriculture' at the University of Hohenheim funded by Anton & Petra Ehrmann Foundation for financial support, and Roberta Alves Gomes for contributions with laboratory analyses.

References

(Note of the editors: All hyperlinks were verified 15 April 2021.)

Alves FV; Almeida RG; Laura VA; Porfirio-da-Silva V; Macedo MCM; Medeiros SR; Ferreira AD; Gomes RC; Bungenstab DJ; Reis M. 2015. Carbon neutral Brazilian beef: a sustainable concept for beef production. Proceedings of the World Congress on Integrated crop-livestock-forestry system, Brasilia, Brazil, 12-17 July 2015.

Baldissera TC; Pontesc LS; Giostri AF; Barro RS; Lustosa SBC; Moraes A; Carvalho PCF. 2016. Sward structure and relationship between canopy height and light interception for tropical C_4 grasses growing under trees. Crop & Pasture Science 67:1199–1207. doi: 10.1071/CP16067

Batistoti C; Lempp B; Jank L; Morais MG; Cubas AC; Gomes RA; Ferreira MVB. 2012. Correlations among anatomical, morphological, chemical and agronomic characteristics of leaf blades in *Panicum maximum* genotypes. Animal Feed Science and Technology 171:173–180. doi: 10.1016/j.anifeedsci.2011.11.008

Berry JO; Patel M. 2008. Kranz anatomy and the C_4 pathway. Encyclopedia of life sciences. John Wiley & Sons Ltd, Chichester. p. 1–6. doi:10.1002/9780470015902.a0001295.pub2

Brâncio PA; Nascimento Jr D; Euclides VPB; Regazzi AJ; Almeida RG; Fonseca DM; Barbosa RA. 2002. Avaliação de três cultivares de *Panicum maximum* Jacq. sob pastejo: Composição Química e Digestibilidade da Forragem. Revista Brasileira de Zootecnia 31:1605–1613. doi:10.1590/S1516-35982002000700002

Braz TGS; Fonseca DM; Jank L; Cruz CD; Martuscello JA. 2015. Genotypic stabilization of agronomic traits in *Panicum maximum* (Jacq.) hybrids. Revista Brasileira de Zootecnia 44:377–383. doi: 10.1590/S1806-92902015001100001

Britto de Assis Prado CH; Haik Guedes de Camargo-Bortolin L; Castro É; Martinez CA. 2016. Leaf Dynamics of *Panicum maximum* under Future Climatic Changes. PLoS ONE 11(2): e0149620. doi: 10.1371/journal.pone.0149620

Daykin ME; Hussey RS. 1985. Staining and histopathological techniques in nematology. In: Barker KR; Carter CC; Sasser JN, An advanced treatise on Meloidogyne. North Carolina State University, Raleigh. p. 39–48.

Deinum B; Sulastri RD; Zeinab MHJ; Maassen A. 1996. Effects of light intensity on growth, anatomy and forage quality of two tropical grasses (*Brachiaria brizantha* and *Panicum maximum* var. trichoglume). Netherlands Journal of Agricultural Science 44:111–124. doi: 10.18174/njas.v44i2.551

Euclides VPB; Lopes FC; Nascimento Junior D; Silva SC; Difante GS; Barbora RA. 2015. Steer performance on *Panicum maximum* (cv. Mombaça) pasture under two grazing intensities. Animal Production Science 56, 1849-1856. doi: 10.1071/AN14721

Fernandes FD; Ramos AKB; Jank L; Carvalho MA; Martha Jr GB; Braga GJ. 2014. Forage yield and nutritive value of *Panicum maximum* genotypes in the Brazilian savannah. Scientia Agricola 71:23–29. doi: 10.1590/S0103-90162014000100003

Gamarra EL; Morais MG; Almeida RG; Paludetto NA; Pereira M; Oliveira CC. 2017. Beef cattle production in established integrated systems. Semina: Ciências Agrárias 38:3241–3252. doi: 10.5433/1679-0359.2017v38n5p3241

Geremia EV; Crestani S; Mascheroni JD; Carnevalli RA; Mourão GB; Silva SC. 2018. Sward structure and herbage intake of *Brachiaria brizantha* cv. Piatã in a crop-livestock-forestry integration area. Livestock Science 212:83–92. doi: 10.1016/j.livsci.2018.03.020

Gifford RMA. 1974. Comparison of potential photosynthesis, productivity and yield of plant species with different photosynthetic metabolism. Australian Journal of Plant Physiology 1:107–117. doi: 10.1071/PP9740107

Gobbi KF; Garcia R; Garcez Neto AF; Pereira OG; Rocha GC. 2010. Valor nutritivo do capim-braquiária e do amendoim-forrageiro submetidos ao sombreamento. Archivos de Zootecnia 59:379–390.

Gobbi KF; Garcia R; Ventrella MC; Garcez Neto AF; Rocha GC. 2011. Área foliar específica e anatomia foliar quantitativa do capim-braquiária e do amendoim-forrageiro submetidos a sombreamento. Revista Brasileira de Zootecnia 40:1436–1444. doi: 10.1590/S1516-35982011000700006

Gomes FJ; Pedreira CGS; Bosi C; Cavalli J; Holschuch SG; Mourão GB; Pereira DH; Pedreira BC. 2019. Shading Effects on Marandu Palisadegrass in a Silvopastoral System: Plant Morphological and Physiological Responses. Agronomy Journal 111:2332–2340. doi: 10.2134/agronj2019.01.0052

Gomes RA; Lempp B; Jank L; Carpejani GC; Morais MG. 2011. Características anatômicas e morfofisiológicas de lâminas foliares de genótipos de *Panicum maximum*. Pesquisa Agropecuária Brasileira 46:205–211. doi: 10.1590/S0100-204X2011000200013

Habermann E; San Martin JAB; Contin DR; Bossan VP; Barboza A; Braga MR; Groppo M; Martinez CA. 2019. Increasing atmospheric CO_2 and canopy temperature induces anatomical and physiological changes in leaves of the C_4 forage species *Panicum maximum*. PLoS ONE 14(2):e0212506. doi:10.1371/journal.pone.0212506

Hagquist CW. 1974. Preparation and care of microscopy slides. American Biology Teacher 36:414–417. doi: 10.2307/4444888

Karvatte Junior N; Klosowski ES; Almeida RG; Mesquista EE; Oliveira CC; Alves FV. 2016. Shading effect on microclimate and thermal comfort indexes in integrated crop-livestock-forest systems in the Brazilian Midwest. International Journal of Biometeorology 60:1933–1941. doi: 10.1007/s00484-016-1180-5

Lambers H; Chapim III FS; Pons TL. 1998. Plant physiological ecology. Springer, New York.

Lempp B. 2007. Avanços metodológicos da microscopia na avaliação de alimentos. Revista Brasileira de Zootecnia 36:315–329. doi: 10.1590/S1516-35982007001000029

Lima MA; Paciullo DSC; Morenz MJF; Gomide CAM; Rodrigues RAR; Chizzoti FHM. 2018. Productivity and nutritive value of *Brachiaria decumbens* and performance of dairy heifers in a long-term silvopastoral system. Grass and Forage Science 74:160–170. doi: 10.1111/gfs.12395

Lin CH; McGraw ML; George MF; Garret HE. 2001. Nutritive quality and morphological development under partial shade of some forage species with agroforestry potential. Agroforestry Systems 53:269–281. doi: 10.1023/A:1013323409839

Marten GC; Shenk JS; Barton Ii FE. 1985. Near-infrared reflectance spectroscopy (NIRS): analysis of forage quality. USDA Agriculture handbook No 643. USDA, Washington.

Martuscello JA; Braz TGS; Jank L; Cunha DNFV; Carvalho ALS. 2015. Identification of ideotypes by canonical analysis in *Panicum maximum*. Ciência e agrotecnologia 39:147–153. doi: 10.1590/S1413-70542015000200006

Oliveira CC de; Villela SDJ; Almeida RG de; Alves FV; Behling-Neto A; Martins PGMA. 2014. Performance of Nellore heifers, forage mass, and structural and nutritional characteristics of *Brachiaria brizantha* grass in integrated production systems. Tropical Animal Health and Production 46:167–172. doi: 10.1007/s11250-013-0469-1

Oliveira CC de; Alves FV; Martins PGMA; Nivaldo Jr K; Alves GF; Almeida RG; Mastelaro AP; Costa e Silva EVC. 2019. Vaginal temperature as indicative of thermoregulatory response in Nellore heifers under different microclimatic conditions. PlosOne 14(10): e0223190. doi: 10.1371/journal.pone.0223190

Paciullo DSC; Gomide JA; Silva EAM; Queiroz DS; Gomide CAM. 2002. Degradação *in vitro* de tecidos da lâmina foliar e do colmo de gramíneas forrageiras tropicais, em função do estádio de desenvolvimento. Revista Brasileira de Zootecnia 31:900–907. doi: 10.1590/S1516-35982002000400013

Paciullo DSC; Gomide CAM; Castro CRT; Mauricio RM; Fernandes PB; Morenz MJF. 2016. Morphogenesis, biomass and nutritive value of *Panicum maximum* under different shade levels and fertilizer nitrogen rates. Grass and Forage Science 72:590–600. doi: 10.1111/gfs.12264

Pereira M; Almeida RG; Bungenstab DJ; Morais MG; Lempp B; Macedo MCM. 2015. *Panicum maximum* genotypes yield under integrated crop-livestock-forest system in the Brazilian Cerrado. In: Proceedings of World Congress on Integrated crop-livestock-forestry system. Embrapa, Brasilia, DF.

Pereira M; Morais MDG; Fernandes PB; Santos AVC, Glatzle S, Almeida RG. 2021. Beef cattle production on Piatã grass pastures in silvopastoral systems. Tropical Grasslands-Forrajes Tropicales 9:1–12. doi: 10.17138/tgft(9)1-12

Pezzopane JRM; Bernardi ACC; Bosi C; Oliveira PPA; Marconato MH; Pedroso AF; Esteves SN. 2019. Forage productivity and nutritive value during pasture renovation in integrated systems. Agroforest Systems 93:39–49. doi: 10.1007/s10457-017-0149-7

Santiago-Hernández F; López-Ortiz S; Ávila-Reséndiz C; Jarillo-Rodríguez J; Pérez-Hernández P; Guerrero-Rodríguez JD. 2016. Physiological and production responses of four grasses from the genera *Urochloa* and *Megathyrsus* to shade from *Melia azedarach* L. Agroforestry Systems 90:339–349. doi: 10.1007/s10457-015-9858-y

Sniffen CJ; O'Connor JD; Van Soest PJ; Fox DG; Russel JB. 1992. A net carbohydrate and protein system for evaluating cattle diets. II. Carbohydrate and protein availability. Journal of Animal Science 70:3562–3577. doi: 10.2527/1992.70113562x

Soares AB; Sartor LR; Adami PF; Varella AC; Fonseca L; Mezzalira JC. 2009. Influência da luminosidade no comportamento de onze espécies forrageiras perenes de verão. Revista Brasileira de Zootecnia 38:443–451. doi: 10.1590/S1516-35982009000300007

Sousa LF; Maurício RM; Moreira GR; Gonçalves LC; Borges I; Pereira LGR. 2010. Nutritional evaluation of "Braquiarão" grass in association with "Aroeira" trees in a silvopastoral system. Agroforestry Systems 79:189–199. doi: 10.1007/s10457-010-9297-8

Sousa Neto EL; Andrioli I; Almeida RG; Macedo MCM; Lal R. 2014. Physical quality of an oxisol under an integrated crop-livestock-forest system in the Brazilian Cerrado. Revista Brasileira de Ciência do Solo 38:608–618. doi: 10.1590/S0100-06832014000200025

Terashima I; Hanba YT; Tholen D; Niinemets U. 2011. Leaf Functional Anatomy in Relation to Photosynthesis. Plant Physiology 155:108–116. doi: 10.1104/pp.110.165472

Wilson JR. 1994. Cell wall characteristics in relation to forage digestion by ruminants. Journal of Agricultural Science 122:173–182. doi: 10.1017/S0021859600087347

Wilson JR; Brown RH; Windham WR. 1983. Influence of leaf anatomy on the dry matter digestibility of C_3 and C_4, and C_3/C_4 intermediate types of *Panicum* species. Crop Sciences 23:141–146. doi:10.2135/cropsci1983.0011183X002300010041x

Wilson JR; Hattersley PW. 1989. Anatomical characters and digestibility of leaves of *Panicum* and other grass genera with C_3 and different types of C_4 photosynthetic pathway. Australian Journal of Agricultural Research 40:125–136. doi: 10.1071/AR9890125

Yamamoto A; Nakamura T; Adu-Gyamfi JJ; Saigusa M. 2002. Relationship between chlorophyll content in leaves of sorghum and pigeonpea determined by extraction method and by chlorophyll meter (SPAD-502). Journal of Plant Nutrition 25:2295–2301. doi: 10.1081/PLN-120014076

Xu C; Salih A; Ghannoum O; Tissue D. 2012. Leaf structural characteristics are less important than leaf chemical properties in determining the response of leaf mass per area and photosynthesis of *Eucalyptus saligna* to industrial-age changes in CO_2 and temperature. Journal of Experimental Botany 63:695–709. doi: 10.1093/jxb/ers231

(Received for publication 28 January 2020; accepted 3 January 2021; published 31 May 2021)

doi: 10.17138/TGFT(9)171-181

Research Paper

Prospective genetic gain to improve salinity tolerance in a population of *Panicum coloratum* var. *coloratum* with two different selection methods

Ganancia genética potencial para mejorar la tolerancia a salinidad en una población de Panicum coloratum *var.* coloratum *con dos diferentes métodos de selección*

GABRIELA PITTARO[1], MAURO LIFSCHITZ[2,3], MIGUEL SÁNCHEZ[5], DOLORES BUSTOS[1], JOSÉ OTONDO[4] AND MARÍA ANDREA TOMÁS[2]

[1]*Instituto Nacional de Tecnología Agropecuaria (INTA), Instituto de Fisiología y Recursos Genéticos Vegetales, Unidad de Estudios Agropecuarios (INTA-CONICET), Córdoba, Argentina.* inta.gob.ar/ifrgv
[2]*Instituto de Investigación de la Cadena Láctea (INTA-CONICET), Estación Experimental Agropecuaria Rafaela, Rafaela, Santa Fe, Argentina.* inta.gob.ar/rafaela
[3]*Consejo Nacional de Investigaciones Científicas y Técnicas.* conicet.gov.ar
[4]*Instituto Nacional de Tecnología Agropecuaria, (INTA), EEA Cuenca del Salado, Buenos Aires.* inta.gob.ar/cuencadelsalado
[5]*Unidad de Estudios Agropecuarios, INTA-CONICET, Córdoba, Argentina.* conicet.gov.ar

Abstract

Panicum coloratum var. *coloratum* is a subtropical grass for potentially increasing forage production in lowly productive environments where cattle-raising activities have been relocated. Heritability was estimated for characters related to salinity tolerance under saline and non-saline conditions to explore the possibility of improving tolerance by selection. From a base germplasm collected in a very harsh environment, heritability and gain after selection were calculated using 2 recombination units: individual and phenotypic family mean (PFM). Heritability estimates were very low for all characters both in saline and non-saline conditions, suggesting a complex genetic control of salinity tolerance, with a high proportion of non-additive genetic effects. Estimates were higher using individual selection than with PFM and expected genetic gains were higher for individual selection. When compared in both saline and non-saline conditions, predicted means were greater than for plants of cv. Klein, the most common cultivar in use. It appears that the analyzed germplasm would be a valuable source of genes to be included in breeding programs to increase salinity tolerance in *Panicum coloratum*.

Keywords: Familial selection, heritability, individual selection, phenotypic and genetic variability.

Resumen

Panicum coloratum var. *coloratum* es una gramínea forrajera subtropical adecuada para incrementar la producción de forraje en ambientes de baja productividad donde la ganadería ha sido relocalizada. En un estudio realizado en Buenos Aires y Córdoba, Argentina, se estimó la heredabilidad para caracteres relacionados a la tolerancia a salinidad en condiciones salinas y no salinas para explorar la posibilidad de mejorar la tolerancia por selección. A partir de un germoplasma base recolectado de un ambiente con condiciones restrictivas de crecimiento, la heredabilidad y la ganancia

Correspondence: M.A. Tomás, Instituto de Investigación de la Cadena Láctea (INTA-CONICET), Estación Experimental Agropecuaria Rafaela, Ruta 34, Km 227, Rafaela, Santa Fe, Argentina. Email: tomas.maria@inta.gob.ar

genética luego de un ciclo de selección fueron calculadas usando dos unidades de recombinación: individual y media fenotípica familiar (PFM en inglés). Las estimaciones de heredabilidad fueron bajas para todos los caracteres tanto en condiciones salinas como no salinas sugiriendo un complejo control genético de la tolerancia a salinidad con alta proporción de efectos genéticos no aditivos. Las estimaciones fueron superiores usando la selección individual en comparación con la media fenotípica familiar. La ganancia genética esperada fue mayor para la selección individual. Tanto en condiciones salinas como en no salinas, la media predicha fue superior al cv. Klein, el cultivar más común de la variedad. El germoplasma analizado sería un buen recurso para ser incluido en programas de mejoramiento para incrementar la tolerancia a salinidad en *Panicum coloratum*.

Palabras clave: Heredabilidad, selección familiar, selección individual, variabilidad fenotípica y genotípica.

Introduction

Nearly one billion hectares of the earth's land surface (13.2×10^9 ha) are made up of saline and/or sodic soils, and between 25 and 30% of irrigated lands are salt-affected, making them essentially commercially unproductive or lowly productive (Shahid et al. 2018). In recent decades, due to the expansion of cropping, cattle-raising activities have moved to less-productive areas, many of which are affected to some degree by salinity, sodicity or a combination of both (Kraemer et al. 2013). These areas are covered by native species adapted to harsh conditions, which generally produce poor quality forage. Sowing of a warm-season grass like *Panicum coloratum*, tolerant of halomorphic conditions, could represent a potential option for increasing the productivity of such areas (Rossi 2005).

Panicum coloratum is a cross-pollinated, perennial C_4 grass, native to South Africa (Tischler and Ocumpaugh 2004) and P. *coloratum* var. *coloratum* has been grown in Africa, Australia and Southern USA to improve pasture lands and for erosion control and re-vegetation (Conrad 1976; Lodge et al. 2009). It exhibits above-average forage quality compared with other warm-season grasses (Rouquette et al. 1974, Ricci 2007) and has high resistance to drought, frost (Petruzzi et al. 2003) and salinity stress (Taleisnik et al. 2002; Pittaro et al. 2015), along with tolerance of waterlogging (Imaz et al. 2013; 2015) and alkaline soils as well (Otondo 2011; Luna et al. 2016).

As in other allogamous forage grasses, *P. coloratum* cultivars are highly heterozygous and represent panmictic populations, where internal genotypic variability offers the possibility of selecting individuals with improved performance in specific traits (Vogel and Burson 2004). Breeding efforts allowed the development of experimental lines displaying the following attributes: improved seed retention (Young 1991); reduced post-harvest dormancy (Tischler and Young 1987); differences in the length of the subcoleoptilar node (Tischler and Voigt 1995); and increased seed weight (Hussey and Holt 1986; Giordano et al. 2013). In *P. coloratum* var. *makarikariense* genetic variation in tolerance of abiotic stresses such as drought (Giordano et al. 2019) and salinity has been reported recently (Cardamone et al. 2018). However, breeding efforts to increase salinity tolerance in *P. coloratum* var. *coloratum* have still not been made.

Panicum coloratum var. *coloratum* is widely known as Klein grass and Verde is the most popular cultivar used extensively in Texas and Central Argentina (Ruiz et al. 2004; Tischler and Ocumpaugh 2004). This material has been introduced to Argentina as cultivar Klein and has been sown in different soil and climatic conditions during the 20th century, resulting in long-lasting pastures given its wide adaptation to various environments (Burgos et al. 2018). Severe selection pressures like extreme droughts or frosts have produced races or local populations that are common in central Argentina. These local populations may become the starting point to initiate a breeding program aimed at generating germplasm tolerant of different stressful conditions, if they have retained enough genetic variation.

The breeding method most commonly used to improve quantitatively inherited characters in populations of outcrossing plants is recurrent phenotypic selection (Vogel and Burson 2004). However, whether selection is exerted on individual plants or in families depends on the structure of the population and the source of genetic variation (Nguyen and Sleper 1983). Then, estimates of variance components and their distribution in the traits of interest of the germplasm source provide priceless information to decide on selection strategy in any breeding program. In addition, estimating additive genetic effects and heritability may allow prediction of genetic gain as a result of applying selection at a given intensity (Casler et al. 2002).

In a previous study, plants from an old pasture of *P. coloratum* cv. Klein, which had survived in a saline site during periods of severe droughts and frosts were collected, cloned, multiplied and evaluated in a greenhouse setting (Pittaro et al. 2015). The collected plants were found to be more tolerant (33%) of salinity than plants produced from commercial seeds of the same cultivar (Pittaro et al. 2015), supporting the hypothesis that strong selection pressure had been exerted by the stressful environment. Although the number of plants collected at this stressful site was relatively low, analysis with random genetic markers (ISSRs) revealed significant genetic variability (Pittaro et al. 2015). Tolerance of salinity combined with genetic variation suggests that this material may be a valuable source of germplasm to start a breeding program to further increase salinity tolerance. Nonetheless, it is necessary to confirm that genetic variation for specific traits related to salinity tolerance exists, as there is a possibility that the intense selection pressure exerted by drought and frost might have substantially reduced it.

In any breeding program, quantitative genetic information regarding the base population or the source of germplasm is required to effectively design the breeding strategy (Vogel and Burson 2004). Then, it is necessary to acquire information on the extent, nature and distribution of genetic variation and heritability of the traits of interest in order to make predictions of the possible genetic progress to be achieved (Dudley and Moll 1969). Estimates of variance components may be achieved by analyzing the performance of half-sib (HS) families as has been reported in other forage grasses, e.g. wheatgrass, ochardgrass (Casler and Brummer 2008) and tall fescue (Majidi et al. 2009). However, whether selection is performed on an individual plant basis or based on means for families depends on the structure of genetic variation and its distribution in the available germplasm (Fehr 1987). In addition, flowering synchronicity should be evaluated before pursuing any breeding strategy to ensure that estimates are made on the actual mating population (Majidi et al. 2009).

Prior to launching a breeding program to increase salt tolerance in *P. coloratum* var. *coloratum*, we were interested in characterizing the germplasm that survived extreme environments in terms of flowering time, biomass accumulation and plant structure under field conditions. The objectives were: 1. to characterize genotypes according to flowering synchronicity, biomass production and plant structure to narrow down the population that would be actually breeding; 2. to estimate narrow-sense heritability and genetic gain per cycle of traits associated with salinity tolerance in saline and non-saline conditions in a prospective breeding population; 3. to compare attainable genetic progress towards increasing productivity in saline conditions from individual and familial selection; and 4. to compare the calculated probable means with the performance of a widely used cultivar.

Materials and Methods

Plant material and clone production

The plants used for the trials were initially collected from an established pasture on a typical Natracualf soil (35°25′0″ S, 57°43′0″ W), which survived saline conditions and severe droughts (Pittaro et al. 2015). Then, these plants, identified here as genotypes, were grown in a greenhouse and propagated to obtain clonal groups as described by Pittaro et al. (2015).

Evaluation of the P. coloratum *genotypes under field conditions*

Twelve replicates of each of 15 genotypes were planted in December 2013 in the experimental field at 'Chacra Experimental Integrada Chascomús, INTA-MAA', Buenos Aires Province. The historical annual precipitation at the site is 1,038 mm, while soil is characterized by an electrical conductivity (EC) of 4.7 dS/m and a pH of 9.5. The genotypes were planted at random at 1 m spacing in a plot of 200 m^2. In the first year (accumulated precipitation 661 mm), height and diameter of each individual plant were registered. Height was measured from the base of the plant to the ligula of the flag leaf and diameter as the length of the longitudinal projection of the plant canopy on the soil surface at the widest part. Fresh biomass accumulation of the plants was recorded, and subsamples were taken to the laboratory, weighed and dried to calculate moisture content of each sample. In the second year (mean annual precipitation 788 mm), the first growth was cut in early October and discarded, then flowering synchrony was evaluated at the end of November, using an arbitrary scale based on the number of open spikelets per panicle at the time of evaluation, when all genotypes presented panicles. All plants were evaluated and were categorized with the following taxonomy: 'late' - fewer than 50% open spikelets per panicle; 'intermediate' - 50% open spikelets per panicle; and 'early' - more than 50% open spikelets per panicle plus older anthers.

Evaluation of salinity tolerance in the offspring of synchronic clones of P. coloratum

Ten genotypes previously classified as early- and intermediate-flowering were clonally propagated in a greenhouse and transferred to an isolated polycross nursery in the experimental 'Chacra Experimental Sinsacate' situated in Córdoba (30°56' S, 64°05' W), 1,000 km from the collection site. The historical annual precipitation at the site is 780 mm, with mean temperature 15.8 ºC; soil is an Udic Argiustoll, developed on silty loess in a rolling landscape, poorly drained with neutral pH. Twelve replicates of each genotype were planted in a crossing block at a spacing of 1 m to facilitate pollen exchange throughout. In January 2015, after anthesis had completed, several inflorescences on each plant were enclosed in seed traps to facilitate harvest of seeds from each genotype to produce half-sib families (HS fam). The collected seeds were manually threshed and blown to remove glumes and other residuals in a laboratory. Seeds from all replicates of each genotype were bulked to constitute one HS fam. Fifty seeds of each HS fam were germinated in a petri box containing vermiculite.

Genetic variation for salinity tolerance among HS in greenhouse.

The aim of this assay was to evaluate the performance of seedlings from 10 HS fam in saline and non-saline conditions, and compare them with commercial cv. Klein. Two-leaved seedlings were transplanted into forest tube pots (55 cm diameter and 20 cm height), containing a mixture of perlite and washed river sand (2:1), and placed in plastic trays. The experimental design was a split-plot completely randomized with 3 blocks and 12 seedlings per HS fam in total. Salinity treatments consisted of non-saline (EC 0.0 dS/m) and saline conditions (with the addition of 0.2 M NaCl to get EC 20.0 dS/m). Pots were irrigated twice a week with Hoagland solution (Hoagland and Arnon 1950) and gradually salinized with increasing NaCl concentrations (0.05, 0.1, 0.15 and 0.2 M) during a 2-week period. Once 0.2 M NaCl was reached, the trays were placed in a system with automatic irrigation. Concentration of the solutions in each tray was controlled once a week by measuring EC of the drained water, using a digital conductivity meter (Altronix CTX-II, Buenos Aires, Argentina). Leaf number (LN) and tiller number (TN) per plant were counted twice a week. Leaf emergence rate (LR) and tillering rate (TR) were calculated as a function of time, i.e. x/day, where x is either number of leaves or tillers. Plants were harvested 45 days after transplanting to collect above-ground biomass, which was then dried in an oven until constant weight to obtain shoot dry weight (SDW). Numbers of leaves (L) and tillers (T) per plant were also counted at harvest. For all variables, the values in saline and non-saline conditions were calculated (saline/non-saline) on a per cent basis and were named as %SDW, %TR, %TN, %LR and %LN.

Statistics

A general mixed linear model was used to estimate variance components in HS fam of *P. coloratum* var. *coloratum* seedlings growing in both saline and non-saline conditions with Infostat software with R interface (Di Rienzo et al. 2008).

The model used was:

$$Xij = \mu + Bi + Fj + (BF)ij + Eij,$$

where:

X*ij* is the *ij*th observation of the *i*th block and the *j*th family;

μ is the general mean;

B*i* is the fixed *i*th block effect;

F*j* is the random *j*th family effect;

BF*ij* is the random *j*th family by *i*th block interaction effect; and

E*ij* is the error term.

Narrow-sense heritability on an individual plant basis was estimated as follows:

$$h^2 = \sigma^2_A / \sigma^2_P,$$

where:

σ^2_A is the additive variance calculated as $4\sigma^2_F$ (σ^2_F: family variance component);

σ^2_P is the phenotypic variance corresponding to the sum of $\sigma^2_F + \sigma^2_E + \sigma^2_W$, where σ^2_E is ambient variation (σ^2_E) plus the family by block interaction of both (σ^2_w) (Nguyen and Sleper 1983).

Genetic gain per cycle (AG) of individual plant selection (mass selection in one year at one location) was predicted as follows:

$$AG = c\,k\,h^2\sigma_P,$$

where:

c = parental control factor (Sprague and Eberhart 1977);

k = standardized selection differential;

h^2 = narrow-sense heritability on an individual basis; and

σ_P = phenotypic standard deviation among individual plants.

Parental control factor c = 1 if selected plants are planted together in the field and both maternal and paternal selections are in place.

Heritability estimates were also calculated on a phenotypic family mean (PFM) basis as follows:

$$h^2_{PFM} = \sigma^2_F / (\sigma^2_F + \sigma^2_E/r + \sigma^2_W/r{*}n)$$

where:

σ^2_F is the family variance;

σ^2_E is the ambient variation plus the within block variance σ^2_W;

r and n are the number of blocks and plants per block, respectively.

Genetic gain per cycle of familial selection with 30% intensity was calculated in the same way as $AG = c\ k\ h^2_{PFM}\ \sigma_{PFM}$. Genetic progress was calculated under the assumption that superior parents were selected on the basis of the mean performance of their HS progenies, then replicated parents inter-mated in an isolated polycross (c = 2).

Standard error of heritability was calculated with Dickerson approximation (Dickerson 1969).

Individual and family selection methods

Two selection methods were used to calculate prospective means after the selected individuals were allowed to inter-mate: for individual selection, individuals were chosen according to their performance both in saline and non-saline conditions; for familial selection, parents were chosen according to the performance of their progeny. To select individuals or families a principal component analysis (PCA) based on the Euclidean distance matrix (Cardamone et al. 2018) was calculated from 5 variables: SDW, LN, TN, LR and TR and individuals were ranked considering all variables.

The predicted values derived after selection both on individual and phenotypic family mean (PFM) bases were compared with the mean values in the base population to calculate the progress made. In addition, predicted values were compared with the mean values registered by seedlings of cv. Klein growing in saline and non-saline conditions.

Results

Variability in biomass accumulation, plant structure and flowering synchronicity in clones of P. coloratum *grown in field plots*

Biomass accumulation showed significant differences among genotypes growing in the field (P<0.05) as indicated in Figure 1. Genotype 2 had the highest biomass accumulation, while Genotypes 11, 6 and 10 showed the lowest values. When evaluating plant structure by the ratio of plant diameter:height (D:H), all genotypes ranged from 1 to 1.5 (Figure 1). Genotypes 1, 16, 6 and 10 showed a compact structure (ratio close to 1), while Genotypes 12 and 9 had the highest D:H, indicating a prostrate habit.

It was possible to classify genotypes on the basis of flowering time into 3 groups (early, intermediate and late) (Figure 1), with 5 genotypes identified as late-flowering, while the other 10 were grouped as early- and intermediate-flowering. The early- plus intermediate-flowering group retained much of the phenotypic variation as it congregated genotypes differing in biomass production and plant structure.

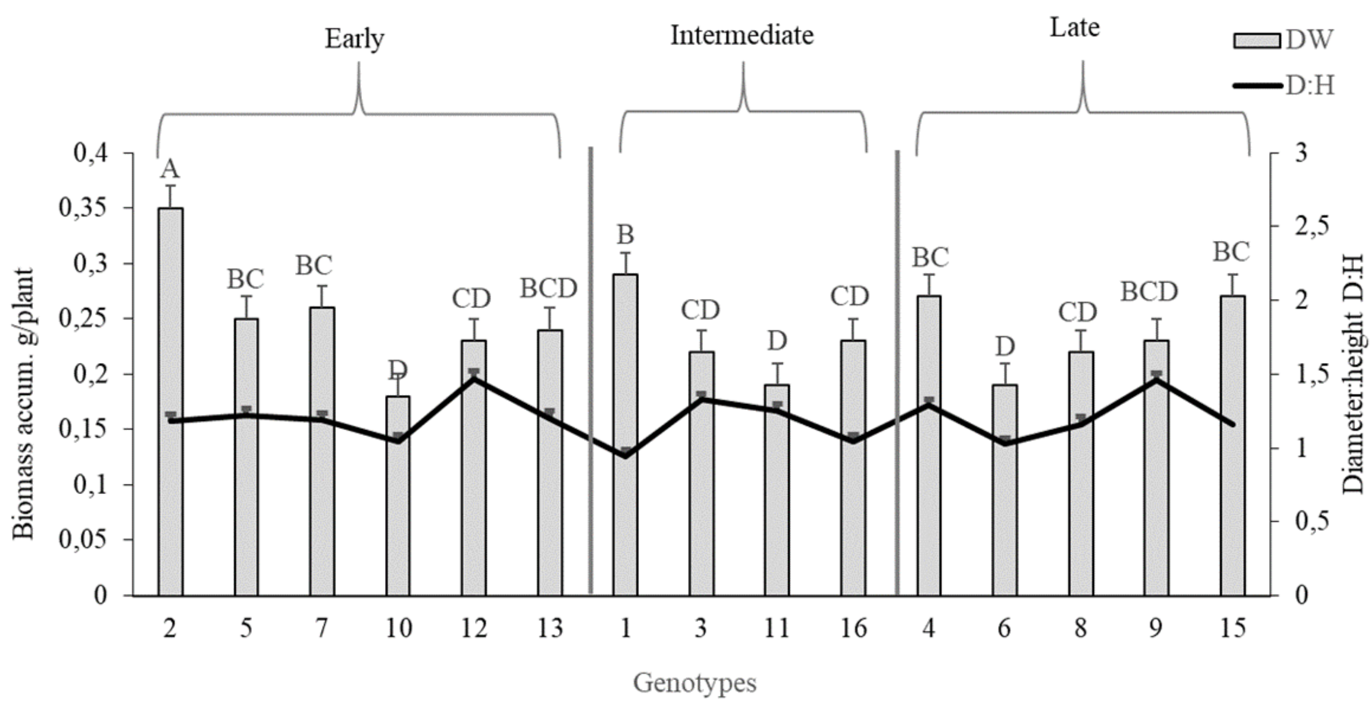


Figure 1. Biomass accumulation (DW; g/plant) and diameter:height (D:H) ratio in genotypes of *Panicum coloratum* growing in the field. Vertical lines separate groups according to flowering synchronicity into late-, intermediate- and early-flowering. Each column shows the mean ± s.d. Different letters on columns indicate significant differences at P<0.05 by Fisher's LSD test.

Performance of half-sib families in the greenhouse

Half-sib families from a crossing block of replicated genotypes belonging to the late- and intermediate-flowering groups were evaluated in the greenhouse in saline and non-saline conditions. No interaction between salinity condition and HS-family was evident for any of the evaluated traits. The parameters SDW and TN were negatively affected by salinity ($P = 0.0001$ and $P = 0.01$, respectively), while LN and LR were similar in both saline and non-saline conditions ($P = 0.029$ and $P = 0.140$, respectively). Only TR was higher in saline than non-saline conditions ($P = 0.0001$). Differences among families were evident in SDW ($P = 0.0194$), while no differences were observed for TN, TR, LR and LN. The most productive family regarding shoot biomass both in saline and non-saline conditions was HS 3 (data not shown). In addition, this family showed the highest salinity tolerance (saline/non-saline) for %SDW, %TN, %LR and %TR ($P = 0.0145$; $P = 0.0161$; $P = 0.0019$; $P = 0.0021$, respectively).

Estimates of genetic parameters

Variance components were estimated from HS family values in both saline and non-saline conditions. Estimates of heritabilities were calculated for all evaluated variables on both individual plant and phenotypic family mean (PFM) bases. Given that the base population was already proven tolerant of salinity, estimations of heritability were calculated in saline and non-saline conditions (Table 1). Estimates of heritabilities were low in non-saline and saline conditions for SDW, TN, TR and LN ($h^2<0.25$), on both individual plant and PMF bases. For LR, heritability estimate was higher than the variables mentioned before ($h^2 = 0.521$) but only in non-saline conditions and on an individual plant basis, while it was close to null in saline conditions. Heritability estimates calculated on an individual plant basis were always higher than on a PFM basis in both saline and non-saline conditions (Table 1).

Regarding expected genetic gains, progress was greater when calculated on an individual plant basis (Figure 2), by selecting individual plants in both saline and non-saline conditions for all variables measured. Progress in SDW, TN and LR is expected to be greater when selected in non-saline conditions, while higher expectations would occur in variables such as LN and TR when selected under saline conditions. However, genetic gains were negligible for all variables in saline conditions for both individual and familial selection procedures (Figure 2).

Table 1. Narrow-sense heritability (h^2) for individual and familial selection methods in saline and non-saline conditions, for shoot dry weight (SDW), leaf number (LN), tiller number (TN), leaf emergence rate (LR) and tillering rate (TR) of *Panicum coloratum*.

		h^2	
		Individual selection	Familial selection (PFM)
Non-saline	SDW	0.134 ± 0.016	0.025 ± 0.65
	LN	0 ± 0	0 ± 0
	TN	0.19 ± 0	0.037 ± 0.52
	LR	0.521 ± 0.32	0.095 ± 0.47
	TR	0.22 ± 0.226	0.040 ± 0.52
Saline	SDW	0.059 ± 0.23	0.010 ± 0.58
	LN	0.090 ± 0.29	0.015 ± 0.47
	TN	0 ± 0	0 ± 0
	LR	0 ± 0	0 ± 0
	TR	0.110 ± 0.27	0.019 ± 0.64

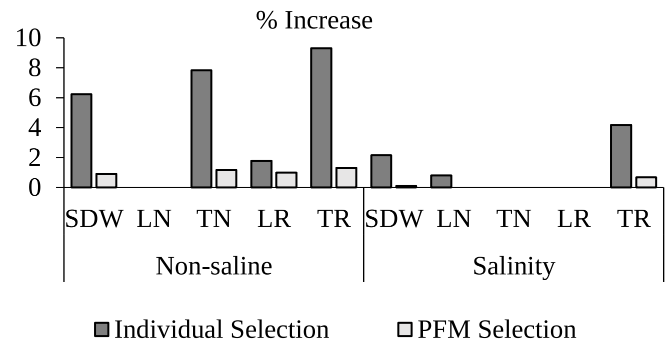


Figure 2. Percentage increase in predicted population of *Panicum coloratum* using individual and PFM selection methods, in non-saline and saline conditions for: shoot dry weight (SDW), leaf number (LN), tiller number (TN), leaf emergence rate (LR) and tillering rate (TR).

To visualize the prospective increase in performance through selection, we compared the predicted means from individual selection with the values obtained when growing seedlings of cv. Klein under the same conditions as described before, in saline and non-saline conditions. Means were plotted as radar graphs, where values for cv. Klein seedlings represent 100%. In both saline and non-saline conditions (Figure 3A; Figure 3B), the means predicted after selection were higher than the means of cultivar Klein in the same condition. Means after selection would be higher for TN (43.6%), TR (53.1%) and SDW (23.0%) than for cv. Klein seedlings under saline conditions. A more remarkable advance would be observed when selection was performed on plants growing in non-saline conditions. That is, the predicted population showed a better performance than cv. Klein (86.4% for TN, 82.2% for TR and 65.3% for SDW; Figure 3B).

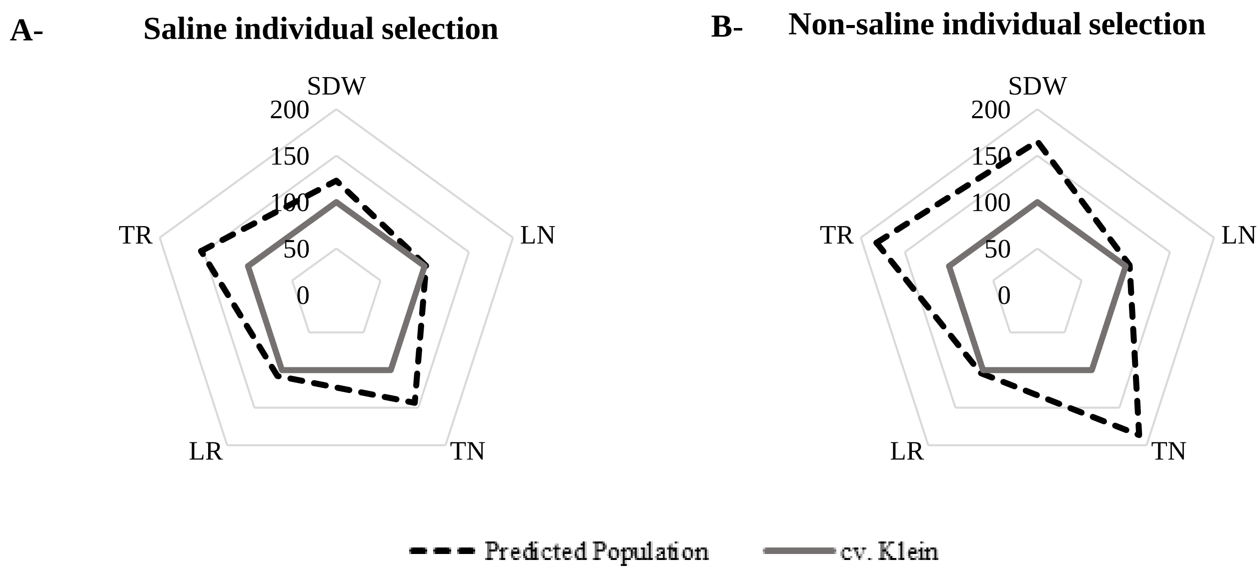


Figure 3. Radar plot showing a comparison of *Panicum coloratum* cv. Klein indicated as 100% and the predicted means after 30% selection intensity in % shoot dry weight (SDW), % leaf appearance rate (LR), % tillering rate (TR), % tiller number (TN) and % leaf number (LN) in (A) saline and (B) non-saline conditions with the individual selection method.

Discussion

In many forage species, progress in breeding has been accomplished by means of phenotypic recurrent selection (Vogel and Pedersen 1993; Marcón et al. 2020). This approach has been used to increase forage biomass (Dhaliwal 2009) and other traits such as seedling vigor (Tischler et al. 1989) and seed weight (Giordano et al. 2013). Relocation of cattle-raising activities into less-productive environments, frequently subjected to restrictions in growth by salinity, heavy soils, frequent droughts or flooding, has moved breeding objectives to plant survival and forage production under restrictive conditions (Griffa et al. 2010). In this study, we explored the possibility of initiating a breeding program involving the subtropical forage grass *Panicum coloratum* var. *coloratum* to increase forage production when growing in saline conditions. We considered using a set of plants collected in a very restricted environment as a source germplasm, assuming the selected individuals were already adapted to these conditions. The collected plants were analyzed and proved to be variable with neutral molecular markers (Pittaro et al. 2015). To analyze the possible outcome of the breeding program, we studied the base germplasm to choose mating genotypes according to phenology, estimated the variability related to additive effects and estimated the progress to be expected after selection. Finally, we evaluated the chance that the prospective germplasm we would obtain through selection might surpass the performance of the most common and widely known cultivar of *P. coloratum* var. *coloratum* (cv. Klein).

Variability among genotypes under field conditions.

Genotypes of *P. coloratum* transplanted to the field in a saline soil were highly variable for traits such as biomass production and plant structure (Figure 1). Phenotypic variation confirmed that *P. coloratum* is an allogamous species, quite variable in morphology and molecular markers (Burgos et al. 2018). As with other subtropical forage grasses, e.g. *Chloris gayana* (Ortega and Taleisnik 2003) and *Cenchrus ciliaris* (Tommasino et al. 2012), *P. coloratum* is adapted to thrive under stressful conditions. Even though individuals studied here were collected in a very stressful environment and had been exposed to quite strong selection pressure that might have been expected to reduce genetic variation, differences in phenology, plant structure and biomass production were apparent even in the reduced number of genotypes.

The synchrony in reproductive structures is of fundamental importance for the reproductive success of the plant and a point to consider when selecting genotypes to cross in a breeding program (Oleques et al. 2017). In the genotypes analyzed here, we identified 3 groups for flower synchrony (early, medium and late) when growing in the field (Figure 1). Then, individuals grouped as early- and intermediate-flowering were crossed and their progeny tested for salinity tolerance under controlled conditions.

Phenotyping and genetic variability to salinity tolerance in half-sib P. coloratum.

To pursue our breeding objective, i.e. to increase salinity tolerance, we then evaluated the extent of additive genetic variance among individuals and estimated the possible gain to be achieved after selection. To do that, we analyzed half-sib families from the individuals resulting from crosses.

Agronomic aspect of salinity tolerance. Both growth and biomass yield were adversely affected by salinity, a common feature in most plant species (Ashraf 2004), although not all traits were equally affected in *P. coloratum*. In our study, seedlings showed a higher tillering rate in saline conditions than in a non-saline situation. Tiller outgrowth may be related to a high appearance rate of low rank tillers associated with stressful conditions determined by several morphogenetic components (Gastal and Lemaire 2015). Regarding genetic variation related to these traits, heritability estimates were in general very low. Contrary to other studies that showed estimates of heritability and additive genetic component estimates increased as stress conditions increased (Kacser and Burns 1981; Blum 1988), in this study, estimates were higher in non-saline than in saline conditions. However, Mohammadi et al. (2014) showed that narrow-sense heritability estimates were higher in saline than in non-saline conditions for traits related to salinity tolerance in rice. In addition, *P. coloratum* var. *makarikariense*, a botanical variety of the same species, displayed high values for heritability of morphological traits in saline (Cardamone 2020) and drought conditions (Giordano 2018). On the contrary, our results suggested that additive variation for salinity tolerance had already been exploited in this source of germplasm as the population had been subjected to strong selection pressure in a very harsh environment; as a result, heritability estimates under saline conditions were very low. It is well accepted that estimates of heritability of any given character refer only to the germplasm evaluated under the particular experimental conditions (Falconer and Mackay 1996). Low heritability may be caused by low additive effects and/or high dominant gene action (Falconer and Mackay 1996). Salinity tolerance was suggested to have a complex pattern of inheritance related to a high non-additive component, associated with low heritabilities in highly saline concentrations in sorghum (Azhar and McNeilly 1988) and pearl millet (Kebebew and McNeilly 1996) adjudged to non-additive components. It was suggested that changes in genetic variation related to increases or decreases in salt concentrations might be driven by the action of different genes that contribute to the same trait in different environments (Rao and McNeilly 1999).

Selection methods and growth conditions. Phenotypic recurrent selection is the most efficient and commonly used form of plant breeding in perennial forage grasses (Vogel and Burson 2004). Estimates of heritable genetic variation and the degree of genetic progress within breeding populations are helpful to breeders in determining the probable effectiveness of pursuing selection strategies over time. In characters exhibiting high heritability, selection is more likely to be effective (Degefa et al. 2014). However, the outcome may differ depending on the recombination unit on which selection is performed. Depending on whether selection is based on values expressed by individual members of the population (Mariotti and Collavino 2014) or on the family mean, recombination would involve different sets of alleles. In PFM selection, the parents of the best families are polycrossed in isolation. Nguyen and Sleper (1983) recommend family selection over individual selection, when estimates of heritability are low because of large environmental effects. We compared the outcomes from these 2 selection methods estimating heritability on both individual plant and PFM bases and calculating genetic gain after 30% selection intensity. These methods have been used in breeding *P. coloratum* and other forage grasses (Giordano et al. 2013; Simeão et al. 2013; Cardamone et al. 2018). Both methods showed low narrow-sense heritability values for almost all parameters evaluated, but, in general, individual-based values were higher than family-based with minor differences. Accordingly, genetic gain per cycle was low for all parameters using both selection methods, although the standardized selection differential was higher in individual selection (data not shown).

Phenotypic family mean selection is recommended over individual selection for characters with low heritability (Basigalup 2007). When environmental deviations of individuals within the family tend to cancel each other out in determining the mean value of the family, the mean phenotypic value of the family approaches the genotypic mean (Falconer and Mackay1996). In this study, the experimental system allowed control of growth conditions and reduction in environmental variance (Broman and Sen 2009; Quero et al. 2013). Therefore, the low heritability values found here are due to limited genetic variability explained by the narrow genetic base in the population and not to environmental variation. Extreme conditions of drought, frosts and salinity at the site where the plants were collected might have exerted a strong selection pressure and reduced genetic variation among surviving individuals. As a result, heritability estimates were low for all characters, and minor differences were found depending on the method for calculating them (PFM or individual basis) (Table 1). In this situation, the recommendation for pursuing selection in this germplasm would be to perform individual selection, since it is more practical, easier and more straightforward than the PFM-based one (Falconer and Mackay 1996; Rose et al. 2008).

One interesting feature of this germplasm was that, while genetic gain was low for all characters and considering 2 selection methods, predicted means after selection were higher than the means observed in plants of cv. Klein for all parameters evaluated, in both non-saline and saline growth conditions (Figure 3). This constitutes further evidence that, in the collected germplasm which had been exposed to extreme conditions, only tolerant genotypes with limited variation survived, making them promising germplasm not only to be introduced in a breeding program to improve salinity tolerance, but also to study mechanisms and genes involved in salinity tolerance in forage grasses.

Conclusion

Although germplasm of *Panicum coloratum* var. *coloratum* studied here showed variability in morphology and phenology as well as molecular markers, variation in salinity tolerance was limited and estimates of narrow-sense heritability were low, even when calculated on a PFM basis. Estimates of genetic advance were also low, indicating that no further progress could be made in salinity tolerance by recurrent selection in this material. However, the predicted population of *P. coloratum* showed higher dry weight, tiller number, tillering rate and leaf production rate than cv. Klein under saline conditions. We consider the collected germplasm represents a source of salinity-tolerance genes in establishing a breeding program for this species. This material might also be valuable in studies to understand mechanisms of salinity tolerance in this and other species.

Acknowledgments

The authors thank Rosalba Pemán from Oscar Pemán y Asociados Semillas for financial support and for providing the experimental site.

References

(Note of the editors: All hyperlinks were verified 15 April 2021.)

Ashraf M. 2004. Some important physiological selection criteria for salt tolerance in plants. Flora 199 (5):361-376. doi: 10.1078/0367-2530-00165

Azhar FM; McNeilly T. 1988. The genetic basis of variation for salt tolerance in *Sorghum bicolor* (L.) Moench, seedlings. Plant Breeding 101(2):114-121. doi: 10.1111/j.1439-0523.1988.tb00275.x

Basigalup DH. 2007. Mejoramiento genético y desarrollo de variedades. In: Basigalup DH, ed. El cultivo de alfalfa en la Argentina. Ediciones INTA, Buenos Aires, Argentina. p. 83–103. ISBN: 978-987-521-242-8. bit.ly/38ui2EN

Blum A, ed. 1988. Plant breeding for stress environment. CRC Press. ISBN: 08-493-63888. bit.ly/3tmqqyv

Broman KW; Sen S. 2009. Experimental design and power. In: Gail M; Krickeberg K; Samet J; Tsiatis A; Wong W, eds. A guide to QTL mapping with R/qtl. Statistics for biology and health. Springer, New York, NY. doi:10.1007/978-0-387-92125-9_6

Burgos E; Thompson C; Giordano M; Thomas MA. 2018. Pre-breeding studies in *Panicum coloratum* var. *coloratum*: Characterization using agro-morphological traits and molecular markers. Tropical Grasslands-Forrajes Tropicales 6:82-92. doi: 10.17138/tgft(6)82-92

Cardamone L. 2020. Caracterización agronómica, variabilidad genética y ganancia por selección en caracteres relacionados con tolerancia a salinidad en una colección de *Panicum coloratum* var. *makarikariense*. Ph.D. Thesis. Universidad Nacional de Córdoba.

Cardamone L; Cuatrin A; Grunberg K; Tomas MA. 2018. Variability for salt tolerance in a collection of *Panicum coloratum* var. *makarikariense* during early growth stages. Tropical Grasslands-Forrajes Tropicales 6:134-147. doi: 10.17138/tgft(6)134-147

Casler M; Brummer EC. 2008. Theoretical expected genetic gains for among and within family selection methods in perennial forage crops. Crop Science 48(3):890-902. doi: 10.2135/cropsci2007.09.0499

Casler MD; Fales SL; McElroy AR; Hall MH; Hoffman LD; Undersander DJ; Leath KT. 2002. Half-sib family selection for forage yield in orchardgrass. Plant Breeding 121(1):43-48. doi: 10.1046/j.1439-0523.2002.00665.x

Conrad BE. 1976. Forage and animal production programs for South Texas. In: Holt EC; Lewis RD, eds. Grasses and Legumes in Texas - development, production and utilization. Vol 6. Texas Agricultural Experiment Station Research Monograph. p. 420-431. bit.ly/2Oc46sd

Degefa I; Petros Y; Andargie M. 2014. Genetic variability, heritability and genetic advance in Mungbean (*Vigna radiata* L. Wilczek) Accessions. Plant Science Today 1(2):94-98. doi: 10.14719/pst.2014.1.2.54

Dhaliwal A. 2009. Recurrent phenotypic selection for increased winter productivity in annual ryegrass (*Lolium multiflorum* Lam.). M.Sc. Thesis. Auburn University. bit.ly/3cn0ziq

Di Rienzo JA; Casanoves F; Balzarini MG; Gonzalez L; Tablada M; Robledo CW. 2008. InfoStat, Grupo infostat, FCA, Universidad Nacional de Córdoba, Argentina. Versiones 2008. infostat.com.ar

Dickerson GE. 1969. Techniques for research in quantitative animal genetics. In: Chapman AB, ed. Techniques and procedures in animal science research. American Society of Animal Science, Albany, NY.

Dudley J; Moll R. 1969. Interpretation and use of estimates of heritability and genetics variances to plant breeding. Crop Science 9(3):257–262. doi: 10.2135/cropsci1969.0011183X000900030001x

Falconer DS; Mackay TFC. 1996. Introduction to Quantitative Genetics. 4th Edn. Addison Wesley Longman, Harlow.

Fehr WR. 1987. Principles of cultivar development. Vol 1. Theory and Technique. Macmillan Publishing Company, New York, Iowa State University. ISBN: 0-02-949920-8. bit.ly/3sZuUum

Gastal F; Lemaire G. 2015. Defoliation, shoot plasticity, sward structure and herbage utilization in pasture: Review of the underlying ecophysiological processes. Agriculture 5(4):1146–1171. doi :10.3390/agriculture5041146

Giordano MC; Berone GD; Tomás MA. 2013. Selection by seed weight improves traits related to seedling establishment in *Panicum coloratum* L. var. *makarikariense*. Plant Breeding 132(6):620-624. doi : 10.1111/pbr.12106

Giordano, M. 2018. Plasticidad fenotípica en *Panicum coloratum*: variabilidad genética, heredabilidad y expresión diferencial de la plasticidad. Ph.D. Thesis. Universidad de Buenos Aires, Buenos Aires, Argentina.

Giordano MC; Grimoldi AA; Tomás MA. 2019. Phenotypic plasticity to drought in seedlings of the warm season grass *Panicum coloratum* is related to collection site. Annals of Applied Biology 175(2):164-171. doi: 10.1111/aab.12525

Griffa S; Ribotta A; López Colomba E; Tommasino E; Carloni E; Luna C; Grunberg K. 2010. Evaluation of seedling biomass and its components as selection criteria for improving salt tolerance in Buffel grass genotypes. Grass and Forage Science 65(3):358-361. doi: 10.1111/j.1365-2494.2010.00754.x

Hoagland DR; Arnon DI. 1950. The water-culture method for growing plants without soil. California Agricultural Experiment Station Circular 347. University of California, Berkeley, CA, USA. goo.gl/Mee8fK

Hussey MA; Holt EC. 1986. Selection for increased seed weight in Kleingrass. Crop Science 26(6):1162–1163. doi: 10.2135/cropsci1986.0011183X002600060017x

Imaz J; Giménez D; Grimoldi A; Striker G. 2015. Ability to recover overrides the negative effects of flooding on growth of tropical grasses *Chloris gayana* and *Panicum coloratum*. Crop and Pasture Science 66(1):100–106. doi: 10.1071/CP14172

Imaz JA; Giménez DO; Grimoldi AA; Striker GG. 2013. The effects of submergence on anatomical, morphological and biomass allocation responses of tropical grasses *Chloris gayana* and *Panicum coloratum* at seedling stage. Crop and Pasture Science 63(12):1145-1155. doi: 10.1071/CP12335

Kacser H; Burns JA. 1981. The molecular basis of dominance. Genetics 97(3-4):639–666. bit.ly/3ryHxMw

Kebebew F; McNeilly T. 1996. The genetic basis of variation in salt tolerance in Pearl Millet, *Pennisetum americanum* (L.) Leeke. Journal of Genetics & Breeding 50:129–136. (Italy). ISSN: 0394-9257. bit.ly/3t8gZSJ

Kraemer FB; Chagas CI; Marré G; Palacín EA; Santanatoglia O. 2013. Desplazamiento de la ganadería por la agricultura en una cuenca de la pampa ondulada: efectos sobre el escurrimiento superficial y erosión hídrica. Ciencia del Suelo (Argentina) 31(1):83-92. bit.ly/3bDoThf

Lodge GM; Boschma SP; Harden S. 2009. Replacement series studies of competition between tropical perennial and annual grasses and perennial grass mixtures in northern New South Wales. Crop and Pasture Science 68:526–531. doi: 10.1071/CP08374

Luna DF; Aguirre A; Pittaro G; Bustos D; Ciacci B; Taleisnik E. 2016. Nutrient deficiency and hypoxia as constraints to *Panicum coloratum* growth in alkaline soils. Grass and Forage Science 72(4):640-653. doi: 10.1111/gfs.12263

Majidi MM; Mirlohi A; Amini F. 2009. Genetic variation, heritability and correlations of agro-morphological traits in tall fescue (*Festuca arundinacea* Schreb.). Euphytica 167(3):323-331. doi: 10.1007/s10681-009-9887-6

Marcón F; Martínez EJ; Zilli AL; Rodríguez GR; Brugnoli EA; Acuña CA. 2020. Recurrent phenotypic selection and recurrent selection based on combining ability in tetraploid bahiagrass. Crop Science 60:1386-1397. doi: 10.1002/csc2.20137

Mariotti JA; Collavino NG. 2014. Los caracteres cuantitativos en la mejora genética de los cultivos. 1ra Edn. Orientación gráfica editora, Buenos Aires, Argentina. ISBN: 978-987-1922-09-3. bit.ly/3cyk08r

Mohammadi R; Mendioro MS; Diaz G Q; Gregorio GB; Singh RK. 2014. Genetic analysis of salt tolerance at seedling and reproductive stages in rice (*Oryza sativa*). Plant Breeding 133(5):548-559. doi: 10.1111/pbr.12210

Nguyen HT; Sleper DA. 1983. Theory and application of half-sib matings in forage grass breeding. Theoretical and Applied Genetics 64(3):187-196. doi: 10.1007/BF00303763

Oleques SS; Overbeck GE; De Avia RS Jr. 2017. Flowering phenology and plant-pollinator interactions in a grassland community of Southern Brazil. Flora 229:141-146. doi: 10.1016/j.flora.2017.02.024

Ortega L; Taleisnik E. 2003. Elongation growth in leaf blades of *Chloris gayana* under saline conditions. Journal of Plant Physiology 160(5):517–522. doi: 10.1078/0176-1617-00827

Otondo J. 2011. Efectos de la introducción de especies megatérmicas sobre características agronómicas y edáficas de un ambiente halomórfico de la Pampa Inundable. M.Sc. Thesis. Universidad de Buenos Aires. bit.ly/2OGH6RW

Petruzzi HJ; Stritzler NP; Adema EO; Ferri CM; Pagella JH. 2003. Mijoperenne- *Panicum coloratum*. Informe Técnico N51 EEA INTA Anguil, La Pampa, Argentina, Sitio argentino de producción animal. bit.ly/3bArNDq

Pittaro G; Cáceres L; Bruno C; Tomás A; Bustos D; Monteoliva M; Ortega L; Taleisnik E. 2015. Salt tolerance variability among stress-selected *Panicum coloratum* cv. Klein plants. Grass and Forage Science 71:683–698. doi: 10.1111/gfs.12206

Quero GE; Borsani O; Gutiérrez L; Melchiorre M; Monza J; Lascano R. 2013. Sistemas de fenotipado para la evaluación de las respuestas al estrés salino en Lotus. Agrociencia Uruguay 17(1):11-21 scielo.edu.uy/scielo.php?script=sci_arttext&pid=S2301-15482013000100002

Rao SA; McNeilly T. 1999. Genetic basis of variation for salt tolerance in maize (*Zea mays* L). Euphytica 108:145–150. doi: 10.1023/A:1003612411293
Ricci H. 2007. Producción primaria de cuatro gramíneas tropicales perennes cultivadas en la llanura deprimida de Tucumán. ISSN-13 N° 978-987-05-3688-8
Rose LW; Das MK; Taliaferro CM. 2008. Estimation of genetic variability and heritability for biofuel feedstock yield in several populations of switchgrass. Annals of Applied Biology 152:11-17. ISSN 0003-4746. doi: 10.1111/j.1744-7348.2007.00186.x
Rossi C. 2005. Mejoramiento de la condición forrajera en bajos alcalino-sódicos con especies subtropicales en la cuenca del salado. Informe de resultado del proyecto. Sitio Argentino de Producción Animal. bit.ly/3eqr5Kt
Rouquette FM; Holt EC; Ellis WC. 1974. Nutrient characteristics of kleingrass at various stages of maturity. II. In vivo and in vitro evaluations of selected varieties. Agronomy Journal 66(4):510-513. doi: 10.2134/agronj1974.00021962006600040010x
Ruiz MA; Babinec FJ; Adema EO; Rucci T. 2004. Producción de forraje y contenido de proteína de gramíneas estivales introducidas en Chacharramendi (La Pampa). Revista Argentina de Producción Animal 24(1-2):41-51. bit.ly/3rC4e2y
Shahid SA; Zaman M; Heng L. 2018. Soil Salinity: Historical Perspectives and a World Overview of the Problem. In: Guideline for Salinity Assessment, Mitigation and Adaptation Using Nuclear and Related Techniques. Springer, Cham. pp 43–53 doi: 10.1007/978-3-319-96190-3_2
Simeão Resende RM; Casler MD; Vilela de Resende MD. 2013. Selection methods in forage breeding: a quantitative appraisal. Crop Science 53(5):1925–1936. doi: 10.2135/cropsci2013.03.0143
Sprague GF; Eberhart SA. 1977. Corn breeding. In: Sprague GF, ed. Corn and Corn Improvement. 2nd Edn. American Society of Agronomy, Madison, Wisconsin, USA. p. 305-362.
Taleisnik E; Pérez H; Córdoba A; Moreno H; García Seffino L; Arias C; Grunberg K; Bravo S; Zenoff A. 2002. Salinity effects on the early development stages of *Panicum coloratum*: Cultivar differences. Grass and Forage Science 53(3):270–278. doi: 10.1046/j.1365-2494.1998.00139.x
Tischler CR; Ocumpaugh WR. 2004. Kleingrass, Blue Panic, and Vine Mesquite. In: Moser LE; Burson BL; Sollenberger LE, eds. Warm-season (C4) grasses. American Society of Agronomy, Madison, Wisconsin, USA. p. 623-649. doi: 10.2134/agronmonogr45.c18
Tischler CR; Voigt PW. 1995. Modifying seedling photomorphogenesis in Kleingrass by recurrent selection. Crop Science 35(6):1613-1617. doi: 10.2135/cropsci1995.0011183X003500060016x
Tischler CR; Voigt PW; Holt EC.1989. Adventitious root initiation in Kleingrass in relation to seedling size and age. Crop Science 29(1):180-183. doi: 10.2135/cropsci1989.0011183X002900010039x
Tischler CR; Young BA. 1987. Development and characteristics of a kleingrass population with reduced post-harvest seed dormancy. Crop Science 27(6):1238-1241. doi: 10.2135/cropsci1987.0011183X002700060030x
Tommasino E; Griffa S; Grunberg K; Ribotta A; López Colomba E; Carloni E; Quiroga M; Luna CM. 2012. Malondialdehyde content as a potential biochemical indicator of tolerant *Cenchrus ciliaris* L. genotypes under heat stress treatment. Grass and Forage Science 67:456-459. doi: 10.1111/j.1365-2494.2012.00851.x
Vogel KP; Burson BL. 2004. Breeding and genetics. In: Moser LE; Burson BL; Sollenberger LE, eds. Warm-season (C_4) grasses. American Society of Agronomy, Madison, Wisconsin, USA. p. 51-94. doi: 10.2134/agronmonogr45.c1
Vogel KP; Pedersen JF. 1993. Breeding systems for cross-pollinated perennial grasses. Agronomy & Horticulture. Faculty Publications, University of Nebraska, Lincoln. 984. Plant Breeding Reviews 11. bit.ly/3rwIMvI
Young BA. 1991. Heritability of resistance to seed shattering in kleingrass. Crop Science 31(5):1156-1158. doi: 10.2135/cropsci1991.0011183X003100050015x

(Received for publication 9 October 2020; accepted 3 March 2021; published 31 May 2021)

doi: 10.17138/TGFT(9)182-191

Research Paper

Effects of timing of nitrogen fertilizer application on responses by tropical grasses

Efectos del tiempo adecuado de fertilización con nitrógeno en la respuesta de gramíneas tropicales

CARLOS E.A. CABRAL[1], ALINE M. MOTTA[1], ALYCE R.M. SANTOS[2], FAGNER J. GOMES[3], BRUNO C. PEDREIRA[4] AND CARLA H.A. CABRAL[1]

[1]*Instituto de Ciências Agrárias e Tecnológicas, Universidade Federal de Mato Grosso, Rondonópolis, MT, Brazil.* ufmt.br
[2]*Laboratório de Nutrição Animal, Centro de Energia Nuclear na Agricultura (CENA), Universidade de São Paulo, Piracicaba, SP, Brazil.* cena.usp.br/nutricao-animal
[3]*Departamento de Zootecnia, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, SP, Brazil.* zootecnia.esalq.usp.br
[4]*Embrapa Agrossilvipastoril, Sinop, MT, Brazil.* embrapa.br/agrossilvipastoril

Abstract

Timing of nitrogen (N) fertilizer application can influence grass regrowth, so it is important to identify how tropical grasses respond to delays in applying fertilizer after defoliation. Our objective was to identify the effects of timing of N fertilizer application after harvest on the productive, morphogenic and structural characteristics of 3 tropical grasses: 'Xaraés' (*Urochloa brizantha* [Hochst. ex A. Rich.] Stapf cv. Xaraés), 'Marandu' (*Urochloa brizantha* [Hochst. ex A. Rich.] Stapf cv. Marandu) and 'Tanzânia' (*Megathyrsus maximus* [Jacq.] cv. Tanzânia). The experiments were performed in a greenhouse, in a completely randomized design, with 5 delays in applying N after harvesting (0, 3, 6, 9 and 12 days). Delaying fertilizer application did not affect the forage mass of Xaraés and Marandu palisade grass (7.4 and 7.8 g/pot, respectively). There was a linear decrease in number of leaves per tiller and leaf appearance rate, but tiller population density and phyllochron increased linearly as fertilizer application was delayed. Grass forage mass (12.2–10.6 g/pot), number of leaves per tiller (3.1–2.6 leaves/tiller) and forage accumulation rate (0.47 to 0.41 g DM/d) of Tanzânia guinea decreased linearly as N application was delayed, but tiller population density was unaffected (25 tillers/pot). Based on our results, N fertilizer should be applied to Tanzânia guinea grass pastures as soon as possible after harvest and certainly before 3 days, while there is not the same urgency with Xaraés and Marandu where fertilization could be delayed up to 12 days without significant detriment. These suggestions need to be tested in a field study before being recommended widely.

Keywords: Maintenance fertilizer, *Megathyrsus maximus*, nitrogen fertilizer, root mass, tiller density, tropical pastures, *Urochloa brizantha*.

Resumen

El momento de la fertilización nitrogenada puede influir en la regeneración de la pastura, lo que requiere identificar cómo responden las gramíneas tropicales a la fertilización después de una defoliación. Por lo tanto, nuestro objetivo fue identificar el impacto del tiempo adecuado de fertilización nitrogenada en las características productivas, morfogénicas y estructurales después de cosechar tres pasturas tropicales: 'Xaraés' (*Urochloa brizantha* [Hochst. ex A. Rich.] Stapf cv. Xaraés), 'Marandu' (*Urochloa brizantha* [Hochst. ex A. Rich.] Stapf cv. Marandu) y 'Tanzânia' (*Megathyrsus maximus* [Jacq.] cv. Tanzânia).

Correspondence: Carlos E.A. Cabral, Agricultural and Technological Sciences Institute, Federal University of Mato Grosso, Rondonópolis, MT, 78735-901, Brazil. Email: carlos.eduardocabral@hotmail.com

Los experimentos se realizaron en invernadero, en un diseño completamente al azar, con cinco tiempos de fertilización nitrogenada (0, 3, 6, 9 y 12 días). El tiempo de fertilización nitrogenada no afectó la masa forrajera de los pastos Xaraés y Marandu (7.4 y 7.8 g/maceta, respectivamente). Hubo una disminución lineal en el número de hojas por macolla y la tasa de aparición de la hoja, sin embargo, la densidad de población de macolla y el filocrón aumentaron linealmente de acuerdo con el momento de la fertilización nitrogenada. La masa de forraje de Tanzânia guinea (12.2 a 10.6 g/maceta), el número de hojas por macolla (3.1 a 2.6 hojas/macolla) y la tasa de acumulación de forraje (0.47 a 0.41 g/d) disminuyen linealmente de acuerdo con el tiempo de fertilización nitrogenada , sin embargo, la densidad poblacional de la macolla fue similar (25 rebrotes/maceta). Según nuestros resultados, el fertilizante N debe aplicarse a los pastizales de pasto Tanzânia guinea tan pronto como sea posible después de la cosecha y ciertamente antes de los 3 días, mientras que la fertilización de los cultivares de *B. brizantha* se puede realizar hasta 12 días después de la cosecha. Estas sugerencias deben probarse en un estudio de campo antes de ser recomendadas ampliamente.

Palabras clave: Densidad de rebrotes, fertilización de mantenimiento, fertilización nitrogenada, masa radicular, *Megathyrsus maximus*, *Urochloa brizantha*.

Introduction

Grasslands are among the most abundant ecosystems in the world (Hewins et al. 2018), where livestock production occurs in areas with native or exotic grasses. As a result, pastures are the main forage source in animal production systems in most tropical countries (Jank et al. 2014), with wide diversity in grazing systems, species and grazing management.

Periodic soil nutrient replacement is important, especially in intensified systems with high stocking rate, (Bourscheidt et al. 2019) which is commonly a combination of intermittent stocking management and a grass with high potential production. Thus, fertilizer application becomes an essential practice, especially when pastures have been established in infertile soils, such as the Oxisols (Chaves et al. 2017).

In grasslands, nitrogen (N) is the most limiting nutrient for plant growth, because it is one of the most extracted nutrients by grasses (Costa et al. 2016) and is a constituent of many cell components associated with the photosynthetic process (Taiz et al. 2017). The N requirement of grass is greater than that of other nutrients, so applying N fertilizer should be given priority to maintain pasture productivity and persistence (Rosado et al. 2017).

Timing of application of N fertilizer can be an important factor affecting grass regrowth (Marques et al. 2016), especially in highly productive systems. Immediately after harvesting, there is a period during which the grass is less responsive to N fertilizer due to the reduction in leaf area and root mass, where tillers need to rely on organic reserves (Faria et al. 2019). Each cultivar has a genetic characteristic driving the recovery potential after harvest and the timing of fertilizer application can contribute to enhancing regrowth (Gomide et al. 2019) and productivity, especially in systems where plants have high demand for N.

The most efficient usage of N has been reported in pastures under intermittent stocking, where adequate N results in rapid regrowth. Faster regrowth after N fertilizer application is attributed to the increased leaf appearance rate (LAR) and, therefore, reduction in phyllochron (Soares Filho et al. 2015; Paciullo et al. 2017). Although there are many studies in the literature on the optimal rates of N to apply to pastures, there is limited information regarding optimal timing of N application.

Approximately 85% of Brazil's pasture lands are cultivated with Urochloa (formerly: *Brachiaria*) species (Valle et al. 2014). The second most important genus is *Megathyrsus*, which is recommended for systems with medium to high fertility soils (Muir and Jank 2004). 'Xaraés' palisade grass (*Urochloa brizantha* syn *Brachiaria brizantha* cv. Xaraés), 'Marandu' (*Urochloa brizantha* syn *Brachiaria brizantha* cv. Marandu) and 'Tanzânia' guinea grass (*Megathyrsus maximus* syn *Panicum maximum* cv. Tanzânia) are widely used in forage-livestock systems in Central Brazil (Jank et al. 2011; 2014).

With the overall goal of increasing efficiency of N fertilizer usage in forage-livestock systems, we hypothesized that timing of applying N fertilizer can affect regrowth and, consequently, productivity of pastures. Therefore, our objective was to identify the effects of different timing of N fertilizer application after harvesting on the productive, morphogenic and structural characteristics of 3 tropical grasses (Xaraés, Marandu and Tanzânia).

Materials and Methods

Three experiments were carried out in a greenhouse at Federal University of Mato Grosso, Cuiabá, Mato Grosso State, in a completely randomized design with 5 treatments and 12 replicates. The experiments were conducted from April to December 2016, where each experiment studied the effects in one grass: Experiment I –Xaraés palisade grass; Experiment II – Marandu palisade grass; and Experiment III – Tanzânia guinea grass. In all experiments, treatments were 5 times post-harvest for applying N fertilizer (0, 3, 6, 9 and 12 days), where zero represented application immediately after harvest, i.e. on the same day. The same procedure was followed for each experiment.

The soil was an Oxisol (Santos et al. 2018) and was collected from the 0–20 cm layer and chemical and granulometric analyses were performed according to Teixeira et al. (2017): pH (H_2O): 6.5; P: 7.9 mg/dm³; K: 25.3 mg/dm³; Ca: 1.7 cmolc/dm³; Mg: 0.6 cmolc/dm³; H+Al: 1.2 cmolc/dm³; CEC: 3.5 cmolc/dm³; base saturation: 66%; organic matter: 9.7 mg/dm³; sand: 790 g/kg; silt: 41 g/kg; and clay: 169 g/kg.

The soil was sieved in a 4 mm mesh and used to fill plastic pots (5 dm^3 experimental units). Phosphorus was applied at sowing at the rate of 87 mg P/dm^3 in the form of simple superphosphate. Twenty seeds of each grass were planted in each pot, and 10 days after sowing, the plants were thinned to leave 5 plants per pot, using plant uniformity as the criterion. Fifteen days after thinning, 30 mg K_2O/dm³ (25 mg K/dm^3) in form of potassium chloride and 50 mg N/dm³ in form of urea was applied to all treatments.

Thirty-five days after thinning, plants in all pots were clipped at 15-cm stubble height for Marandu and Xaraés and 30 cm for Tanzânia. This was considered day 0 for each experiment, when 100 mg N/dm^3 (as urea) was applied to the Control and, following the chronogram, the same N fertilizer regime (dose and source) was applied at 3, 6, 9 and 12 days after clipping for the various treatments.

Four regrowth cycles (27-d) were evaluated for Xaraés palisade grass (April 22, May 18, June 14 and July 11), and 3 regrowth cycles for Marandu palisade grass (June 13, July 10 and August 6) and Tanzânia guinea grass (September 18, October 15 and November 11). For the third regrowth cycle of all experiments, 25 mg K/dm^3 was applied to reduce the incidence of foliage necrosis at leaf edges attributed to K deficiency.

The gravimetric method was used for irrigation, maintaining soil moisture near field capacity, as described by Cabral et al (2018).

Measurements

Every 27 days (last day of the regrowth cycle), Xaraés and Marandu were clipped at 15 cm and Tanzânia at 30 cm. Prior to clipping, in each pot, canopy height was measured with a graduated rule, and number of tillers and number of leaves (exposed ligule) above the clipping height were counted. The number of leaves per tiller (NLT) was obtained by dividing number of leaves (NL) by number of tillers in each pot. Leaf appearance rate (LAR) was estimated by the ratio between NLT and the interval between harvests. Phyllochron (PHY) was the inverse of LAR.

Forage mass (FM) was separated into morphological components, i.e. leaf (LM), stem (sheath + pseudostem; SM) and dead material (Dead). No dead material was observed in Marandu and Tanzânia in any regrowth cycle. Morphological components were dried at 55 °C in a forced-air dryer for 72 hours and weighed. Forage accumulation rate (FAR) was calculated by dividing FM by the length of the rest period. The mean weight of a leaf (individual leaf mass; ILM) was estimated by dividing leaf dry mass by NL. Individual tiller mass (ITM) was obtained by dividing FM by the number of tillers.

In the last regrowth period for each experiment (the third for Marandu and Tanzânia and the fourth for Xaraés), after harvesting the forage mass, the residue and root masses were collected. The soil was washed away on 4-mm sieves and the roots were dried following the same drying procedure and root mass (RM) was estimated.

Statistical analyses

Data were analyzed using general linear mixed model method, using the PROC MIXED command (SAS® Institute Inc., Cary, NC). Timing of N fertilizer application was considered a fixed effect. Regrowth cycle and replication (blocks) were considered random effects. Degrees of freedom were corrected using the Satterthwaite method and the variance and covariance structures were chosen based on Akaike Information Criterion. An orthogonal polynomial contrast ($P \leq 0.05$) was used to evaluate the effects (linear or quadratic) of timing of N fertilizer application.

The model used was as follows:

$y_{ijk} = \mu + T_i + e_{ij} + C_k + \varepsilon_{ijk}$;

where:

y_{ijk} = expected response;

μ = average/constant, associated with the experiment;

T_i = treatment effect (fertilizer timing) i;

e_{ij} = treatment error i, in replicate j, normally and independently distributed;

C_k = random effect associated with regrowth cycle k, normally distributed; and

ε_{ijk} = experimental error associated with treatment i, in replicate j, in cycle k, normally distributed.

When significant for the quadratic effect, the non-linear regression procedure (PROC NLIN) of the SAS® software was used to identify whether a plateau ($P \leq 0.05$) was reached for timing of N fertilizer application.

Principal component analysis (PCA) was performed and clusters were constructed using R software, v 4.0.2 (R Development Core Team 2015) and 'Cluster', 'FactoMiner' and 'Factoextra' packages, in order to characterize the response variables for the grasses and the N fertilizer timing. The biplots resulting from PCA are interpreted by visualizing inversely correlated quadrants (vectors in opposite directions). The larger the size of the arrows (vectors), the greater the variation in the data, and the closer the vectors, the greater the relationship between them. With the same database used for PCA analysis, one cluster analysis was run for Xaraés (X), Marandu (M) and Tanzânia (T), at different timing of N application (0, 3, 6, 9 and 12 days) being shown in the factor map. A heatmap ('heatmap.2' command, 'gplots' package) was constructed, and to facilitate the reading of the heatmap, cluster analyses were performed using Euclidean distance.

Results

Marandu and Xaraés palisade grass

Timing of N fertilizer application did not affect (P>0.05) FM, LM, SM, plant height and NL for Xaraés and Marandu (Tables 1 and 2).

There was a linear effect of N application timing on Dead, LAR and PHY for Xaraés (Table 1) and Marandu (Table 2). With increase in time elapsed following harvesting before N was applied, PHY increased but LAR decreased.

Individual tiller mass and TPD were affected by the length of delay in applying N for both Xaraés and Marandu (Tables 1 and 2). While ITM in Xaraés showed a quadratic effect, with a lesser value for the 9 days delay in applying N after harvest, linear effects were observed with TPD for Xaraés and Marandu and NLT and ITM for Marandu.

Tanzânia guinea grass

Delaying N application after harvest did not affect (P>0.05) SM, ILM, RM and LAR for Tanzânia (Table 3). However, delaying N application had linear effects on FM, LM, NL, FAR and PHY with the relationship being positive for PHY but negative for FM, LM, NL and FAR (Table 3). Plant height and length of delay followed a quadratic relationship, with the lowest point reached between 6 and 9 days delay after harvest (Table 3).

ITM and NLT for Tanzânia guinea grass were negatively and linearly related to length of delay in applying N after harvest (Table 3) but TPD was not affected (mean 25.2 tillers/pot).

Common responses

The only variables affected similarly by timing of N fertilizer application after harvest in all 3 grasses were ITM and PHY. The latest fertilizer application (12 days after harvest) caused a reduction in NLT and an enhancement in PHY.

Table 1. Productive, morphogenic and structural variables of Xaraés palisade grass according to time after harvesting before nitrogen fertilizer was applied.

Parameter	Nitrogen fertilizer timing (d)[1]					P-value[2]			s.e.m.
	0	3	6	9	12	L	Q	P	
Productive characteristic									
FM (g DM/pot)	6.8	7.1	7.9	7.4	7.8	0.07	0.36	-	1.6
LM (g DM/pot)	4.0	4.5	5.0	4.5	4.9	0.10	0.28	-	0.4
SM (g DM/pot)	1.2	1.0	1.1	1.1	1.1	0.61	0.14	-	0.5
Dead (g DM/pot)	1.6	1.7	1.8	1.9	1.9	0.003	0.32	-	1.4
ITM (g DM)	0.32	0.40	0.36	0.29	0.30	0.02	0.01	0.78	0.1
ILM (g DM)	0.08	0.09	0.11	0.08	0.09	0.10	0.06	-	0.01
RM (g DM/pot)	11.8	4.9	9.9	7.9	12.5	0.49	0.05	0.46	2.3
Morphogenic and structural characteristic									
Height (cm)	47	47	48	49	48	0.42	0.57	-	3
NLT	1.6	1.8	1.6	1.6	1.6	0.20	0.25	-	0.2
LAR (leaves/tiller/d)	0.08	0.107	0.078	0.076	0.075	0.002	0.32	-	0.008
NL (leaves/pot)	48	46	50	54	54	0.13	0.62	-	3
TPD (tillers/pot)	30	26	31	33	33	<0.0001	0.15	-	2
FAR (g DM/d)	0.25	0.26	0.29	0.27	0.29	0.07	0.36	-	0.06
PHY (days/leaf)	12.3	10.7	13.3	13.6	14.0	0.001	0.27	-	1.3

[1]Timing of N fertilizer application after harvesting (0: immediately after harvest; 3, 6, 9 and 12 = number of days after harvest).
[2]Orthogonal polynomial contrast: L = linear; Q = quadratic; Non-linear regression: P = plateau.
FM: forage mass; LM: leaf mass; SM: stem mass; Dead: dead material mass; ITM: individual tiller mass; ILM: individual leaf mass; RM: root mass; NLT: number of leaves per tiller; LAR: leaf appearance rate; NL: number of leaves; TPD: tiller population density; FAR: forage accumulation rate; PHY: phyllochron.

Table 2. Productive, morphogenic and structural variables of Marandu palisade grass according to time after harvesting before nitrogen fertilizer was applied.

Parameter	Nitrogen fertilizer timing (d)[1]					P-value[2]			s.e.m.
	0	3	6	9	12	L	Q	P	
Productive characteristic									
FM (g DM/pot)	7.6	7.8	7.7	7.9	7.8	0.45	0.71	-	0.9
LM (g DM/pot)	5.8	6.0	6.0	5.9	6.2	0.36	0.95	-	0.5
SM (g DM/pot)	1.7	1.8	1.7	1.9	1.7	0.80	0.29	-	0.4
ITM (g DM)	0.32	0.33	0.28	0.30	0.26	<0.0001	0.33	-	0.04
ILM (g DM)	0.09	0.09	0.09	0.09	0.09	0.75	0.82	-	0.01
RM (g DM/pot)	12.7	12.5	12.8	11.6	17.8	0.15	0.14	-	2.0
Morphogenic and structural characteristic									
Height (cm)	48	48	47	49	49	0.31	0.25	-	4.15
NLT	2.5	2.6	2.2	2.3	2.1	<0.0001	0.74	-	0.1
LAR (leaves/tiller/d)	0.092	0.098	0.081	0.084	0.079	<0.0001	0.83	-	0.005
NL (leaves/pot)	59	62	62	60	65	0.23	0.73	-	3.73
TPD (tillers/pot)	24	24	28	27	31	<0.0001	0.69	-	2.26
FAR (g DM/d)	0.3	0.3	0.3	0.3	0.3	0.45	0.71	-	0.03
PHY (days/leaf)	11.2	10.6	12.8	12.2	12.6	<0.0001	0.57	-	0.8

[1]Timing of N fertilizer application after harvesting (0: immediately after harvest; 3, 6, 9 and 12: number of days after harvest).
[2]Orthogonal polynomial contrast: L = linear; Q = quadratic; Non-linear regression: P = plateau.
FM: forage mass; LM: leaf mass; SM: stem mass; ITM: individual tiller mass; ILM: individual leaf mass; RM: root mass; NLT: number of leaves per tiller; LAR: leaf appearance rate; NL: number of leaves; TPD: tiller population density; FAR: forage accumulation rate; PHY: phyllochron.

Table 3. Productive, morphogenic and structural variables of Tanzânia guinea grass according to time after harvesting before nitrogen fertilizer was applied.

Parameter	Nitrogen fertilizer timing (d)[1]					P-value[2]			s.e.m.
	0	3	6	9	12	L	Q	P	
Productive characteristic									
FM (g DM/pot)	12.2	11.8	11.4	10.4	10.6	<0.0001	0.59	-	1.1
LM (g DM/pot)	12.2	11.8	11.4	10.4	10.5	<0.0001	0.64	-	0.7
SM (g DM/pot)	0.04	0.02	0.06	0.02	0.08	0.42	0.38	-	0.15
ITM (g DM)	0.54	0.49	0.50	0.45	0.46	0.0009	0.37	-	0.54
ILM (g DM)	0.18	0.16	0.18	0.16	0.17	0.12	0.34	-	0.16
RM (g DM/pot)	26.7	35.7	33.2	23.4	26.3	0.44	0.34	-	5.2
Morphogenic and structural characteristic									
Height (cm)	70	69	68	66	72	0.01	0.01	0.93	2
NLT	3.1	3.1	2.8	2.8	2.6	0.002	0.97	-	0.2
LAR (leaves/ tiller/d)	0.11	0.11	0.10	0.10	0.09	0.10	0.74	-	0.01
NL (leaves/pot)	71	76	66	67	64	0.01	0.60	-	4
TPD (tillers/pot)	24	26	25	25	26	0.51	0.95	-	3
FAR (g DM/d)	0.47	0.45	0.44	0.40	0.41	0.0019	0.71	-	0.003
PHY (days/leaf)	9.1	9.1	10.0	10.1	10.7	0.001	0.81	-	0.7

[1]Timing of N fertilizer application after harvesting (0: immediately after harvest; 3, 6, 9 and 12 = number of days after harvest).
[2]Orthogonal polynomial contrast: L = linear; Q = quadratic; Non-linear regression: P = plateau.
FM: forage mass; LM: leaf mass; SM: stem mass; ITM: individual tiller mass; ILM: individual leaf mass; RM: root mass; NLT: number of leaves per tiller; LAR: leaf appearance rate; NL: number of leaves; TPD: tiller population density; FAR: forage accumulation rate; PHY: phyllochron.

The PCA explained 90.6% of the data variance in PC1 and PC2 (Figure 1). PC1 expressed 79.7% of the data's variability, suggesting that this axis was sufficient to explain all variability (Figure 1). In this PCA, LM, FM and FAR were more correlated (0.96, 0.92 and 0.92, respectively).

The factor map indicates a solution of 2 clusters (Figure 2). The combinations identified in Cluster 1 included Tanzânia guinea grass at different N application timings. The combinations that were grouped in Cluster 2 included Marandu and Xaraés palisade grass at different N application timings. Cluster 1 is characterized by greater values for height, LM, ILM, FM, FAR, ITM, LAR, NLT and LN (variables classified among the strongest, Figure 1) and lesser values for the variables SM and PHY (variables classified among the weakest, Figure 1). Cluster 2 is characterized by greater values for SM and PHY (variables classified among the strongest, Figure 1) and lesser values for height, LM, ILM, FM, FAR, ITM, LAR, NLT and LN (variables classified as the weakest, Figure 1).

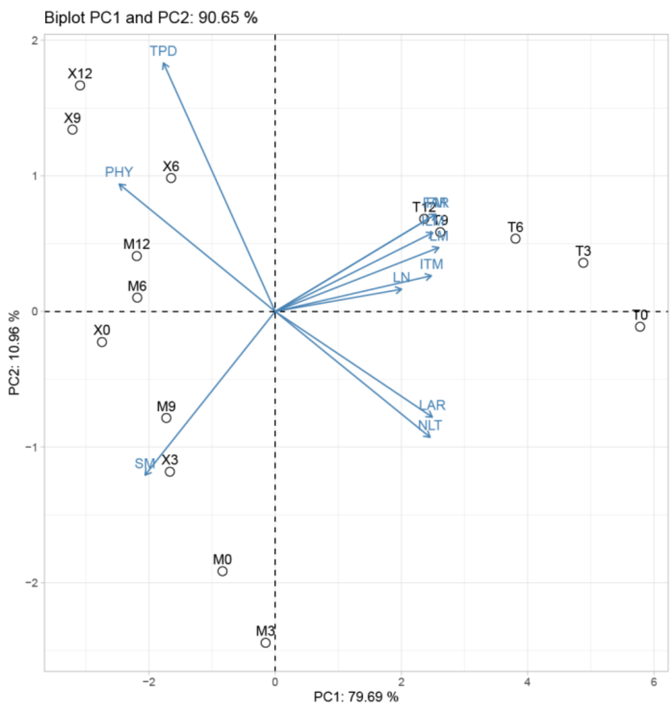


Figure 1. Biplot for the productive and morphogenic characteristics of Xaraés (X) and Marandu (M) palisade grass and Tanzânia (T) guinea grass with different delays in applying nitrogen fertilizer after harvest (0, 3, 6, 9 and 12 days). Height (cm); FM: forage mass (g DM/pot); LM: leaf mass (g DM/pot); SM: stem mass (g DM/pot); ITM: individual tiller mass (g DM); ILM: individual leaf mass (g DM); NLT: number of leaves per tiller; LAR: leaf appearance rate (leaves/tiller/d); NL: number of leaves (leaves/pot); TPD: tiller population density (tillers/pot); FAR: forage accumulation rate (g DM/d); PHY: phyllochron (days/leaf).

Two-way dendrograms generated from hierarchical cluster analysis are illustrated as a heatmap (Figure 3).

Green color denotes variable (rows) with relatively greater corresponding content (classified among the strongest) in treatments (column). In contrast, gray color denotes variable with lesser content of the corresponding element (classified among the weaker). The heatmap indicates that Xaraés with 9 and 12 d N application timing after harvest are characterized by the group with greater values for TPD and PHY (classified among the strongest). Xaraés and Marandu with 0, 3, 6, 9 and 12 d N application timing after harvest are characterized by lesser values for height, FM, FAR, LM, NLT and LAR (classified among the weaker). Tanzânia with 6, 9 and 12 d N application timing after harvest is characterized by the greatest values for height, ILM, LM, FM and FAR (classified among the strongest) and lesser values for SM. Tanzânia with 0 and 3 d N fertilizer timing after harvest are characterized by greater values for LAR, NLT, FAR, FM, LM, LN, ITM and height (classified among the strongest) and lesser values for PHY and SM (classified among the weaker).

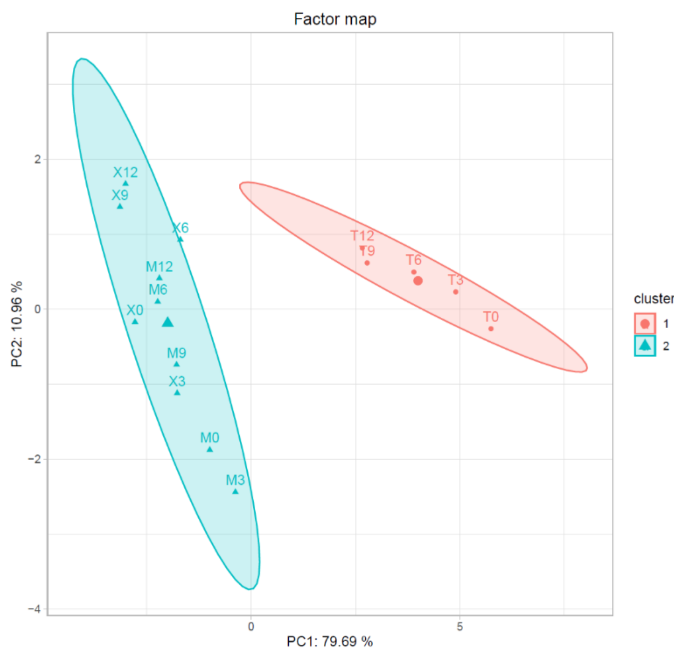


Figure 2. Factor map with combination of Xaraés (X) and Marandu (M) palisade grass and Tanzânia (T) guinea grass, at different nitrogen application times (0, 3, 6, 9 and 12 days after harvest).

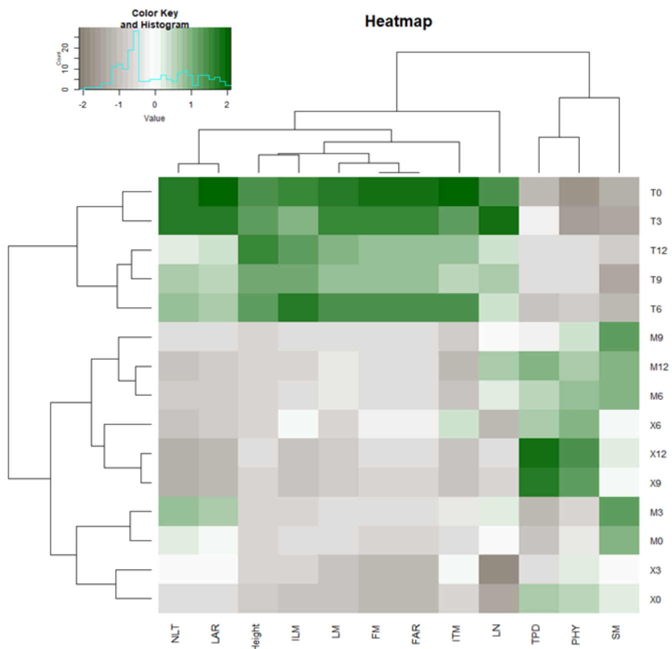


Figure 3. Heatmap analysis of plant responses in Xaraés (X) and Marandu (M) palisade grass and Tanzânia (T) guinea grass, at different nitrogen application times (0, 3, 6, 9 and 12 days after harvest).

Discussion

This study has provided interesting data on the differing responses of grasses from different genera, when N application after harvest is delayed. The results suggest that these different grasses need to be treated differently following harvesting, or at least will be affected differently by different post-harvest management, especially N application. As there was no effect of delayed application of N on forage mass for Xaraés and Marandu, it is likely shoot carbohydrate synthesis was not reduced (mainly leaf blades), which maintained root mass at similar levels among treatments (Tables 1 and 2). Roots are responsible for absorption of water and nutrients, as well as accumulating reserve carbohydrates during stress (Xiao and Jespersen 2019), including periods of low photosynthetic activity (after defoliation, water deficit and shading).

The increase in tillering without change in ITM, NLT and TPD highlights the phenotypic plasticity (Lopes et al. 2018) of Xaraés and Marandu, because even with modification in tillering, FM was maintained, reflecting a tiller size/density compensation (Sbrissia and Silva 2008).

Fertilizing Xaraés and Marandu with N shortly after harvesting provided fewer tillers with more leaves per tiller and therefore, heavier tillers. In contrast, when fertilizer application was delayed, there was an increase in TPD, with fewer leaves per tiller, resulting in lighter tillers. Higher TPD increases soil cover, which reduces the opportunity for weeds to appear and can increase the longevity of perennial pasture. Overall, weeds appear in areas with low tiller density and heavier tillers (Carvalho et al. 2016).

Marques et al. (2016) also observed a greater TPD in 'Massai' guinea grass (*Megathyrsus maximus* × *M. infestus* syn *Panicum maximum* × *P. infestum* cv. Massai) pastures when fertilized 7 days after harvesting. This phenomenon is known as tiller size/density compensation (Matthew et al. 1995), and has been reported in several forage species, such as: 'Mombaça' guinea grass (*Megathyrsus maximus* cv. Mombaça) (Alexandrino et al. 2011), oats (*Avena sativa*) and annual ryegrass (*Lolium perenne*) (Duchini et al. 2014), tifton 68 bermuda grass (*Cynodon nlemfuensis*) (Sbrissia et al. 2003) and Marandu palisade grass (Sbrissia and Silva 2008).

Besides tiller size/density compensation, delaying N fertilizer application influenced the growth pattern of Xaraés, as NLT declined as N application was delayed. This showed that although FAR and FM were not influenced by the timing of fertilizer application, as the delay increased, the emission of leaves was slower, but there was compensation with the larger TPD.

Tanzânia guinea grass forage mass, LM and plant height reduced as N application was delayed, along with number of leaves per pot and per tiller, but individual leaf mass did not alter. The reduction in leaf numbers was due to the increased PHY, which delayed leaf emission and resulted in fewer leaves per tiller and therefore reduced mass of individual tillers. Thus, Tanzânia was strongly influenced by N fertilizer application in the early days after harvest, which is different from the situation with Xaraés and Marandu. This suggested that fertilizer application for Tanzania must be carried out as soon as possible after harvesting. Grasses vary in their need for early application of N after harvest because of variation in organic reserve levels, which include N and carbohydrates accumulated in the base of the stem and roots (Pedreira et al. 2017).

Although these grasses were not strictly compared in the same experiment, this was a pot experiment in a glasshouse, which should minimize environmental differences. In addition, there is existing evidence that Tanzânia guinea grass has lower non-structural carbohydrate concentration (Soares Filho et al 2013) than Xaraés (Rodrigues et al. 2007) and Marandu (Alexandrino et al. 2008). Faria et al (2019) also suggested grasses with greater root starch concentration (non-structural total carbohydrate) are not disadvantaged by delaying N fertilizer application after harvesting as opposed to those with lower root starch concentration.

Although there was a 13% reduction in FM in Tanzânia with delay in N application, there was no effect on root mass (Table 3). Maintaining adequate root and residue masses improves the response potential of a grass under stress conditions (harvest, water stress, shading), because these organs are responsible for accumulation of reserve carbohydrates, which drives the regrowth under reduced photosynthetic activity (White 1973).

While delaying application of N fertilizer after harvest did not affect TPD of Tanzania, it did reduce individual tiller mass. In addition, there was a reduction in number of leaves per pot and per tiller as N fertilizer application was performed later, without altering individual leaf mass. Other studies reported a slight effect of the timing of fertilizer application on forage mass of Tanzânia guinea grass (Marques et al. 2016; Faria et al. 2019; Gomide et al. 2019).

While the longest delay (12 days after harvest) caused a reduction in number of leaves per tiller, it did increase the PHY. Increased PHY has been reported as being associated with N stress (Teixeira et al. 2014; Paciullo et al. 2017), which shows that delay in applying N after harvest may result in a short-term N deficiency. It is known that N reserves can influence the regrowth process (Schnyder and Viser 1999; Lehmeier et al. 2013); however, the physiological processes are still not well understood (Silva et al. 2015).

Forage accumulation rate, height, FM, ILM and ITM were grouped for Tanzânia, while PHY, TPD and SM were grouped for Xaraés and Marandu. For Xaraés and Marandu, individual tiller mass was inversely correlated with tiller population density, which was a reflection of an increase in PHY and reduction in LAR as application of N after harvest was delayed. The heatmap complemented the PCA and Cluster analysis results, in the differentiation between the forages and the timing of N application. There was an indication that tiller population density and PHY were greater for Xaraés and Marandu palisade grass if application of N after harvest was delayed by more than 6 days. In the case of Tanzânia guinea grass LAR, NLT, FAR, FM, ITM and LN were greatest if N was applied within 3 d after harvest.

Thus, grasses with low organic reserves or rapid growth potential demand rapid nutrient supply after harvest. Another hypothesis is that the reduction in forage production due to delayed fertilizer application will result in greater accumulation of nonstructural carbohydrates in the roots and stem base. This is a response reported when grasses are under flooding stress conditions (Ramos et al. 2011). In this case, the longer interval before N fertilizer application would promote nutritional stress due to the deficit of N reserves. Studies on N reserves in tropical grasses are scarce compared with studies in temperate forages (Avice et al. 1996; 1997).

This flexible response to fertilizer application several days after harvest in pastures like Xaraés and Marandu enables flexibility in management of grazing systems, mainly in intermittent stocking. A reduction in FM is avoided if a delay in fertilization is required, such as delay in fertilizer delivery, machinery breakdowns and/or maintenance and several other limitations that occur regularly on a farm.

Conclusion

In all grasses, there is an increase in PHY and reduction in NLT as N fertilizer application is delayed after harvest. While growth of Tanzânia guinea grass was reduced as fertilizer application was delayed, there was no effect on yield of Marandu and Xaraés palisade grass, since an increase in tillering compensated for any reduction in growth per tiller. Based on our results, N fertilizer application to Tanzânia guinea grass should be carried out as soon as possible after harvest, and certainly within 3 days, while fertilizing of *U. brizantha* cultivars may be carried out as late as 6–12 days after harvest without any negative impact. These findings should be tested in a field study before being recommended widely.

References

(Note of the editors: All hyperlinks were verified 8 March 2021).

Alexandrino E; Mosquim PR; Nascimento Júnior D; Vaz RGMV; Detmann E. 2008. Evolução da biomassa e do perfil da reserva orgânica durante a rebrotação da *Brachiaria brizantha* cv. Marandu submetida a doses de nitrogênio. Revista Brasileira de Saúde e Produção Animal 9(2):190–200.

Alexandrino E; Candido MJD; Gomide JA. 2011. Biomass flow and herbage net accumulation rate in Mombaca Grass under different heights. Revista Brasileira de Saúde e Produção Animal 12(1):59–71.

Avice JC; Ourry A; Lemaire G; Boucaud J. 1996. Nitrogen and carbon flows estimated by 15N and 13C pulse-chase labeling during regrowth of alfalfa. Plant Physiology 112:281–290. doi: 10.1104/pp.112.1.281

Avice JC; Ourry A; Lemaire G; Volenec JJ; Boucaud J. 1997 Root protein and vegetative storage protein are key organic nutrients for alfalfa shoot regrowth. Crop Science 37(4):1187–1193. doi: 10.2135/cropsci1997.0011183X003700040027x

Bourscheidt MLB; Pedreira BC; Pereira DH; Zanette MC; Devens J. 2019. Nitrogen input strategies in pastures: mineral fertilizer, bacterial inoculant and consortium with forage peanuts. Scientific Electronic Archives 12(3):137–147. doi: 10.36560/1232019784

Cabral CEA; Cabral LS; Bonfim-Silva EM; Carvalho KS; Abreu JG; Cabral CHA. 2018. Reactive natural phosphate and nitrogen fertilizers in Marandu grass fertilization. Comunicata Scientiae 9(4):729–736. doi: 10.14295/cs.v9i4.1170

Carvalho RM; Pimentel RM; Fonseca DM; Santos MER. 2016. Caracterização de perfilhos em relação à planta daninha no pasto de capim-braquiária. Boletim de Indústria Animal 73(2):103–110. doi: 10.17523/bia.v73n2p103

Chaves HML; Lozada CMC; Gaspar RO. 2017. Soil quality index of an Oxisol under different land uses in the Brazilian savannah. Geoderma Regional 10:183–190. doi: 10.1016/j.geodrs.2017.07.007

Costa CHM; Crusciol CAC; Soratto RP; Ferrari Neto J. 2016. Phytomass decomposition and nutrients release from pearl millet, guinea grass and palisade grass. Bioscience Journal 32(5):1191–1203. doi: 10.14393/BJ-v32n5a2016-32982

Duchini PG; Guzatti GC; Ribeiro Filho HMN; Sbrissia AF. 2014. Tiller size/density compensation in temperate climate grasses grown in monoculture or in intercropping systems under intermittent grazing. Grass and Forage Science 69(4):655–665. doi: 10.1111/gfs.12095

Faria DA; Avelino ACD; Cabral CEA; Abreu JG; Barros LV; Cabral CHA; Dantas VGV; Guarnieri SF; Behling Neto A; Assis LMB. 2019. Investigating the optimal day for nitrogen fertilization on Piatã palisadegrass and Quênia guineagrass after defoliation. Journal of Experimental Agriculture International 34(6):1–11. doi: 10.9734/jeai/2019/v34i630192

Gomide CAM; Paciullo DSC; Morenz MJF; Costa IA; Lanzoni CL. 2019. Productive and morphophysiological responses of *Panicum maximum* Jacq. cv. BRS Zuri to timing and doses of nitrogen application and defoliation intensity. Grassland Science 65(2):93–100. doi: 10.1111/grs.12226

Hewins DB; Lyseng MP; Schoderbek DF; Alexander M; Willms WD; Carlyle CN; Chang SX; Bork EW. 2018. Grazing and climate effects on soil organic carbon concentration and particle-size association in northern grasslands. Scientific Reports 8:1–9. doi: 10.1038/s41598-018-19785-1

Jank L; Do Valle CB; Resende RMS. 2011. Breeding tropical forages. Crop Breeding and Applied Biotechnology 11:27–34. doi: 10.1590/S1984-70332011000500005

Jank L; Barrios SC; Do Valle CB; Simeão RM; Alves GF. 2014. The value of improved pastures to Brazilian beef production. Crop and Pasture Science 65(11):1132–1137. doi: 10.1071/CP13319

Lehmeier CA; Wild M; Schnyder H. 2013. Nitrogen stress affects the turnover and size of nitrogen pools supplying leaf growth in a grass. Plant Physiology 162:2095–2105. doi: 10.1104/pp.113.219311

Lopes MJS; Dias-Filho MB; Castro THR; Silva GB. 2018. Light and plant growth-promoting rhizobacteria effects on *Brachiaria brizantha* growth and phenotypic plasticity to shade. Grass and Forage Science 73(2):493–499. doi: 10.1111/gfs.12336

Marques MF; Romualdo LM; Martinez JF; Lima CG; Lunardi LJ; Luz PHC; Herling VR. 2016. Momento de aplicação do nitrogênio e algumas variáveis estruturais e bromatológicas do capim-massai. Arquivo Brasileiro de Medicina Veterinaria e Zootecnia 68(3):776–784. doi: 10.1590/1678-4162-8500

Matthew C; Lemaire G; Sackville Hamilton NR; Hernandez-Garay A. 1995. A modified self-thinning equation to describe size/density relationships for defoliated swards. Annals of Botany 76(6):579–587. doi: 10.1006/anbo.1995.1135

Muir JP; Jank L. 2004. Guineagrass. In: Moser LE; Burson BL; Sollenberger LE, eds. Warm-Season (C_4) Grasses. Agronomy Monograph 45. ASA, CSSA, SSSA: Madison, WI. p. 589–621.

Paciullo DSC; Gomide CAM; Castro CRT; Maurício RM; Fernandes PB; Morenz MJF. 2017. Morphogenesis, biomass and nutritive value of *Panicum maximum* under different shade levels and fertilizer nitrogen rates. Grass and Forage Science 72(3):590–600. doi: 10.1111/gfs.12264

Pedreira CGS; Silva VJ; Pedreira BC; Sollenberger LE. 2017. Herbage accumulation and organic reserves of palisadegrass in response to grazing management based on canopy targets. Crop Science 57(4):2283-2293. doi: 10.2135/cropsci2016.11.0957

Ramos TJN; Souza CMA; Carvalho CJR; Vieira IMS. 2011. Respostas fisiológicas e metabólicas de gramíneas ao alagamento. Revista de Ciências Agrárias 54:80–86. doi: 10.4322/rca.2011.041

Rodrigues RC; Mourão GB; Valinote AC; Herling VR. 2007. Reservas orgânicas, relação parte aérea–raiz e C–N e eliminação do meristema apical no capim-xaraés sob doses de nitrogênio e potássio. Ciência Animal Brasileira 8(3):505–514.

Rosado TL; Gontijo I; Passos RR; Almeida MS. 2017. Nutrient extraction by mombaça grass submitted to sources and doses of nitrogen. Idesia 35(1):63–72. doi: 10.4067/S0718-34292017005000009

Santos HG; Jacomine PKT; Anjos LHC; Oliveira VA; Lumbreras JF; Coelho MR; Almeida JA; Araújo Filho JC; Oliveira JB; Cunha TJF. 2018. Sistema brasileiro de classificação de solos. 5th Edn. Embrapa, Brasília, DF.

Sbrissia AF; Silva SC; Matthew C; Carvalho CAB; Carnevalli RA; Pinto LFM; Fagundes JL; Pedreira CGS. 2003. Tiller size/density compensation in grazed Tifton 85 bermudagrass swards. Pesquisa Agropecuaria Brasileira 38(12):1459–1468. doi: 10.1590/S0100-204X2003001200013

Sbrissia AF; Silva SC. 2008. Tiller size/density compensation in Marandu palisadegrass swards. Revista Brasileira De Zootecnia 37(1):35–47. doi: 10.1590/S1516-35982008000100005

Schnyder H; Visser R. 1999. Fluxes of Reserve-Derived and Currently Assimilated Carbon and Nitrogen in Perennial Ryegrass Recovering from Defoliation. The Regrowing Tiller and Its Component Functionally Distinct Zones. Plant Physiology 119:1423–1436. doi: 10.1104/pp.119.4.1423

Silva SC; Sbrissia AF; Pereira LET. 2015. Ecophysiology of C_4 forage grasses—Understanding plant growth for optimising their use and management. Agriculture 5(3):598–625. doi: 10.3390/agriculture5030598

Soares Filho CV; Cecato U; Ribeiro OL; Roma CFC; Jobim CC; Beloni T; Perri SHV. 2013. Root system and root and stem base organic reserves of pasture Tanzania grass fertilizer with nitrogen under grazing. Semina: Ciências

Agrárias 34(2):2415–2426. doi: 10.5433/1679-0359.2013v34n5p2415
Soares Filho CV; Cecato U; Ribeiro OL; Roma CFC; Beloni T. 2015. Morphogenesis in pastures with Tanzania grass fertilized with nitrogen doses under a grazing system. Acta Scientiarum. Animal Sciences 37(3):235–241. doi: 10.4025/actascianimsci.v37i3.27101
Taiz L; Zeiger E; Møller IM; Murphy A. 2017. Plant Physiology and Development. 6th Edn. Artmed, Porto Alegre, RS. (in Portuguese)
Teixeira FA; Pires AJV; Silva FF; Fries DD; Rezende CP; Costa ACPR; Santos TC; Nascimento PVN. 2014. Nitrogen fertilization strategies, morphogenetic and structural features in *Brachiaria decumbens* deferred for 95 days. Semina: Ciências Agrárias 35(2):987–998. doi: 10.5433/1679-0359.2014v35n2p987
Teixeira PC; Donagemma GK; Fontana A; Teixeira WG. 2017. Manual de métodos de análise de solo. Embrapa, Brasília, DF.
Valle CB; Barrios SCL; Jank L; Santos MF. 2014. Melhoramento de plantas forrageiras para uma pecuária de baixa emissão de carbono. In: Pedreira BC; Pereira DH; Pina DS; Carnevalli RA; Lopes LB, eds. Intensificação da produção animal em pastagens. GEPI, Sinop, MT. p. 109–140.
White LM. 1973. Carbohydrate reserves of grasses: A review. Journal of Range Management 26:13-18. doi: 10.2307/3896873
Xiao B; Jespersen D. 2019. Morphological and physiological responses of seashore paspalum and bermudagrass to waterlogging stress. Journal of the American Society for Horticultural Science 144(5):305–313. doi: 10.21273/JASHS04737-19

(Received for publication 2 August 2020; accepted 19 February 2021; published 31 May 2021)

Tropical Grasslands-Forrajes Tropicales (2021) Vol. 9(2):192–205
doi: 10.17138/TGFT(9)192-205

Artículo científico

Producción animal en pasturas de tres leguminosas asociadas con *Urochloa decumbens* en los Llanos Orientales de Colombia

Animal production in three Urochloa decumbens*-legume pastures in the Eastern Plains of Colombia*

ÁLVARO RINCÓN CASTILLO[1] y MAYRA VILLALOBOS[2]

[1]*Corporación Colombiana de Investigación Agropecuaria (Agrosavia), C.I. La Libertad, Villavicencio, Colombia.* agrosavia.co
[2]*Corporación Colombiana de Investigación Agropecuaria (Agrosavia), C.I. Carimagua, Puerto Gaitán, Colombia.* agrosavia.co

Resumen

En un suelo franco-arcilloso de la Altillanura colombiana fue evaluada bajo pastoreo rotacional con novillos cebú Brahman la producción animal en pasturas con las leguminosas *Centrosema molle* accesión CIAT 15160, *C. macrocarpum* accesión CIAT 15799 y *Grona heterocarpa* subsp. *ovalifolia* (sin. *Desmodium ovalifolium*) cv. Maquenque, en asociación con *Urochloa decumbens* (sin. *Brachiaria decumbens*) cv. Decumbens. El experimento se estableció en sabana nativa del Centro de Investigaciones Carimagua de Agrosavia, en un diseño de bloques completos al azar con tres repeticiones. Durante tres años de pastoreo, *C. molle* sobresalió por su buena producción y capacidad de asociación con la gramínea, llegando a 40% en la composición botánica en el primer año y 24% en el tercer año. La presencia de esta leguminosa permitió obtener una mayor ganancia de peso vivo (PV), en promedio 760 g/animal/día en época lluviosa y 500 g/animal/día en época seca. Con una carga animal promedio de 800 kg/ha de PV, la productividad anual por hectárea de las pasturas con *C. molle* alcanzó 600 kg de PV frente a un promedio de 380 kg/ha/año obtenido en las otras asociaciones y en la pastura de gramínea sola. Se concluye que *C. molle* CIAT 15160 es una leguminosa promisoria para la región y se recomienda comprobar su productividad y persistencia a nivel de productor, también en asociaciones con otras gramíneas.

Palabras clave: *Brachiaria decumbens, Centrosema molle,* composición botánica, ganancia de peso, persistencia.

Abstract

In a loamy clay soil of the Colombian Altillanura, animal production of the legumes *Centrosema molle* accession CIAT 15160, *C. macrocarpum* accession CIAT 15799 and *Grona heterocarpa* subsp. *ovalifolia* (syn. *Desmodium ovalifolium*) cv. Maquenque, in association with *Urochloa decumbens* (syn. *Brachiaria decumbens*) cv. Decumbens, was evaluated under rotational grazing with zebu Brahman steers. The experiment was established in a native savanna area at the Carimagua Research Center of Agrosavia, in a randomized complete block design with three replications. During three years of grazing, *C. molle* stood out over the other legumes, due to its good forage production and ability to associate and persist with the grass, reaching 40% in the botanical composition in the first year and 24% in the third year. The highest animal liveweight (LW) gains were achieved with this legume with, on average, 760 g/animal/day in the rainy season and 500 g/animal/day in the dry season. With an average stocking rate equivalent to 800 kg LW/ha, mean annual hectare productivity of the pasture with *C. molle* reached 600 kg LW compared with an average of about 380 kg/ha/year obtained in the other associations and in the grass-only pasture. It is concluded that *C. molle* CIAT 15160 is a promising pasture legume for the region and confirming its productivity and persistence at farm level and in association with other grasses seems warranted.

Keywords: Botanical composition, *Brachiaria decumbens, Centrosema molle,* liveweight gain, persistence.

Correspondencia: Álvaro Rincón**,** Agrosavia, C.I. La Libertad, Km 22
vía Villavicencio – Puerto López, Meta, Colombia.
Correo electrónico: arincon@agrosavia.co

Introducción

Es reconocido el aporte que hacen las leguminosas a la agricultura y a la ganadería por la fijación de nitrógeno atmosférico mediante la simbiosis entre las plantas y las bacterias *Rhizobium*, resultando en un incremento de proteína vegetal y animal (Dean 2013; Andrade et al. 2015). La utilización de leguminosas forrajeras en la alimentación de los bovinos ha sido estudiada en el trópico desde hace muchos años y se han demostrado los beneficios que se obtienen en: la reducción del uso de fertilizantes nitrogenados; el consumo voluntario por los rumiantes en pastoreo debido a mayor disponibilidad y calidad del forraje (Barahona y Sánchez 2005); la mayor producción de carne o leche que se traduce en beneficio económico para el productor (Lazier y Clatworthy 1997; Rincón 2004; Sánchez 2013); y el potencial de impacto positivo en el sistema suelo-pasto (Lok Mejías et al. 2017) y en el medio ambiente, mediante su contribución a la intensificación sostenible de la producción ganadera, junto con la provisión de servicios ecosistémicos (Schultze-Kraft et al. 2018). Sin embargo; la adopción de tecnologías basadas en leguminosas forrajeras tropicales ha sido baja (Vázquez et al. 2019).

El aporte de las leguminosas es de especial importancia si se tiene en cuenta que el nitrógeno es el nutriente más deficitario no solo en los forrajes tropicales, sino también en los suelos Oxisoles como los de los Llanos Orientales de Colombia. Su deficiencia se manifiesta en una clorosis y baja concentración de proteína en las hojas, y, en general, limitada producción de forraje y lenta recuperación después de realizado un pastoreo.

En la Orinoquia colombiana (Llanos Orientales) se han liberado cuatro leguminosas forrajeras para pastoreo: *Stylosanthes capitata* cv. Capica, *Centrosema acutifolium* cv. Vichada, *Arachis pintoi* cv. Maní Forrajero y *Desmodium ovalifolium* (nuevo nombre: *Grona heterocarpa* subsp. *ovalifolia*) cv. Maquenque. Estas leguminosas, entregadas a los productores hace más de 20 años, han tenido limitantes en disponibilidad de semilla comercial y en su mayoría han tenido baja persistencia bajo pastoreo en asociación con gramíneas, afectando su uso por parte de los productores. En los últimos 10 años se han retomado esfuerzos en investigación orientada al desarrollo de nuevas opciones de germoplasma de leguminosas forrajeras adaptadas a las condiciones de los suelos de baja fertilidad de los Llanos Orientales de Colombia. Estas investigaciones se retomaron considerando: la baja disponibilidad de leguminosas comerciales; el actual interés por parte de los ganaderos en pasturas de asociaciones gramíneas-leguminosas; el alto costo de los fertilizantes nitrogenados; y la necesidad del desarrollo de sistemas de producción sostenibles y amigables con el ambiente.

En etapas anteriores de evaluación agronómica de leguminosas herbáceas para asociarlas con gramíneas en condiciones de la Altillanura plana, fueron seleccionadas *Centrosema molle* (sin. *Centrosema pubescens*) accesión CIAT 15160 y *Centrosema macrocarpum* accesión CIAT 15799. Estos materiales sobresalieron por su adaptación a las condiciones de suelos ácidos, buena producción y calidad de forraje, tolerancia a plagas y enfermedades y producción de semilla. Posteriormente presentaron buena respuesta en asociación con gramíneas bajo pastoreo con ganado bovino, en términos de rendimiento de forraje y persistencia (A. Rincón, datos sin publicar).

En este trabajo se presenta la continuación del proceso de investigación que tuvo como objetivo determinar el efecto de las leguminosas seleccionadas, en asociación con una gramínea comercial, en la producción de peso vivo bovino, durante tres años de pastoreo. La hipótesis del trabajo fue que, en un sitio representativo para la Altillanura colombiana, *Centrosema molle* CIAT 15160 y *C. macrocarpum* CIAT 15799, en asociación con *Urochloa decumbens* (sin. *Brachiaria decumbens*), producen mayores ganancias de peso vivo bovino que la gramínea sola o asociada con una leguminosa testigo, *Grona heterocarpa* subsp. *ovalifolia* (sin. *Desmodium ovalifolium*) cv. Maquenque.

Materiales y Métodos

Localización

El experimento se realizó entre 2017 y 2019 en el Centro de Investigaciones Carimagua de Agrosavia localizado en la Altillanura colombiana a 300 km de la ciudad de Villavicencio, Departamento del Meta (4º03.500' N, 73º28.152' O; 150 msnm).

Suelo y vegetación

El suelo del área experimental es de textura franco-arcillosa y clasificado como Oxisol de baja fertilidad natural (Cuadro 1).

Precipitación y temperatura

Las lluvias ocurridas durante los tres años del experimento y, para comparación, el promedio de los tres años anteriores, se presentan en la Figura 1. Mientras que en 2017 la precipitación total fue de 2,277 mm, algo inferior al promedio anterior (2,416 mm), en los años 2018 y 2019 se presentó un incremento importante especialmente durante junio, julio y agosto que ocasionó inundaciones en el área experimental.

La temperatura anual promedio en el C.I. Carimagua es de 26 °C. En la época seca, la temperatura máxima fue de 32–33 °C, mientras que en el periodo de lluvias estuvo entre 29 y 31 °C. La temperatura mínima ocurrida en la noche fue de 22.5 y 21.8 °C en las épocas seca y lluviosa, respectivamente.

Materiales forrajeros utilizados

- *Centrosema molle* (sin. *C. pubescens*), accesión CIAT 15160 (de aquí en adelante denominada '*C. molle*')
- *Centrosema macrocarpum*, accesión CIAT 15799 (de aquí en adelante denominada '*C. macrocarpum*')
- Leguminosa testigo: *Grona heterocarpa* subsp. *ovalifolia* (sin. *Desmodium ovalifolium*) cv. Maquenque (de aquí en adelante denominada 'cv. Maquenque')
- Gramínea acompañante con todas las leguminosas: *Urochloa decumbens* (sin. *Brachiaria decumbens)* cv. Decumbens (de aquí en adelante denominada 'Decumbens')

La nomenclatura aquí utilizada se rige por la taxonomía del USDA Genetic Resources Information Network (GRIN; npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysearch).

Las dos accesiones de *Centrosema* fueron seleccionadas con base en evaluaciones agronómicas en los tres años anteriores.

Tratamientos

Los tratamientos estuvieron constituidos por las siguientes pasturas:

1. Asociación *Centrosema molle* + *Urochloa decumbens*
2. Asociación *Centrosema macrocarpum* + *Urochloa decumbens*
3. Asociación cv. Maquenque + *Urochloa decumbens*
4. Decumbens solo (sin leguminosa)

Cuadro 1. Características químicas del suelo bajo sabana nativa (con predominio de la gramínea *Trachypogon vestitus*) en el sitio experimental. C.I. Carimagua, Altillanura colombiana.

pH	M.O.	P	S	Al	Ca	Mg	K	Sat. Al
	%	(mg/kg)		(cmol/kg)				(%)
5.0	2.9	<3.87	1.8	2.2	0.6	0.20	0.09	65

M.O. = materia orgánica; P = fósforo; S = azufre; Al = aluminio; Ca = calcio; Mg = magnesio; K = potasio; Sat. Al = saturación de aluminio.

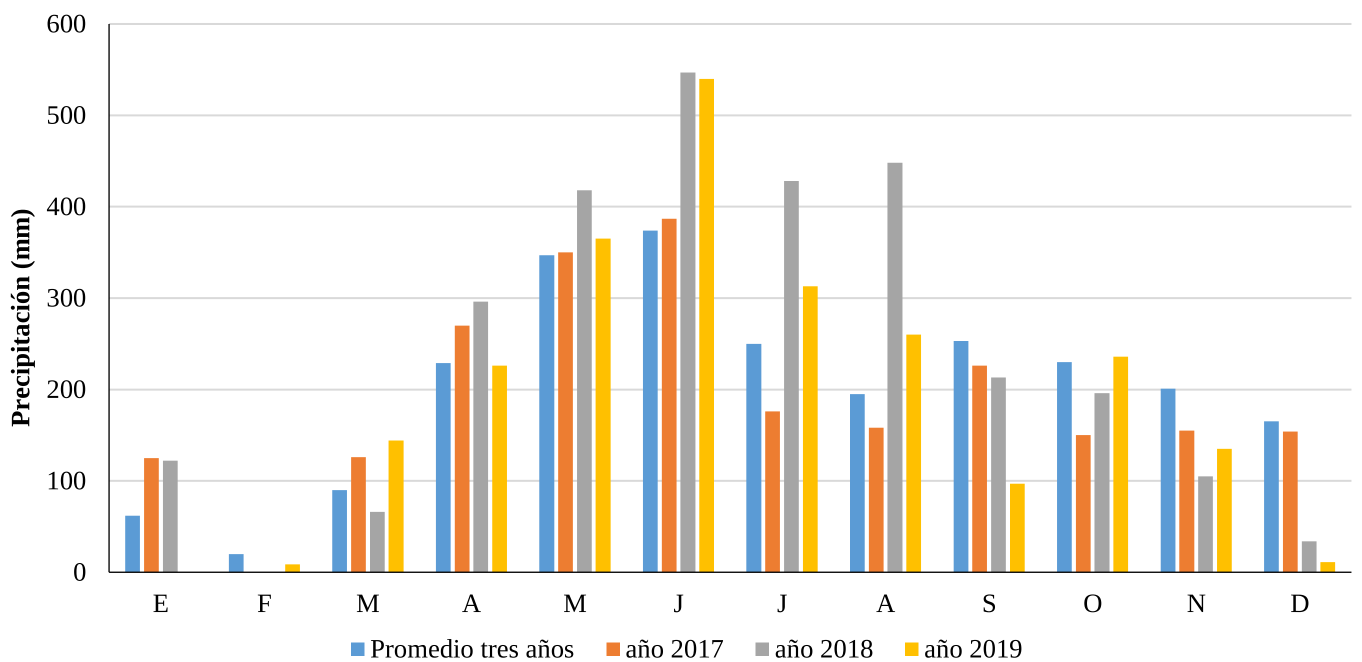


Figura 1. Precipitación mensual durante los años 2017, 2018 y 2019 comparada con el promedio de tres años anteriores (2014–2016). C.I. Carimagua, Altillanura colombiana.

Diseño experimental

Los tratamientos se distribuyeron en bloques completos al azar con tres repeticiones. El área de la unidad experimental fue de 0.56 ha (70 × 80 m) y el área de cada tratamiento con las tres repeticiones fue de 1.68 ha para un total de 6.72 ha.

Establecimiento del experimento

La preparación del suelo se realizó con dos pases de rastra y un pase pulidor. La densidad de siembra se ajustó al tamaño y la germinación de la semilla. Las leguminosas tuvieron una germinación de 70% (con una pureza de 90%) y se sembraron: *C. macrocarpum* – 12 kg de semilla/ha; *C. molle* – 8 kg/ha; cv. Maquenque – 0.5 kg/ha. La tasa de siembra de Decumbens (germinación y pureza de la semilla: 40 y 90%, respectivamente) fue de 4 kg/ha. La fertilización para la siembra estuvo constituida por (kg/ha): 620 Ca, 93 Mg, 51 P, 89 S, 45 N y 50 K. Las fuentes fueron cal dolomítica, escorias Thomas, yeso agrícola, fosfato diamónico (19% P, 18% N), cloruro de potasio (50% K), sulcamag (18% Ca, 9% Mg, 9% S) y kieserita (14% Mg, 20% S). Para la siembra se mezclaron la semilla y los fertilizantes y las mezclas se distribuyeron en sus respectivos lotes con una encaladora. Durante las dos primeras semanas se realizó control de hormiga cortadora de hoja (*Atta* sp. y *Acromyrmex landolti*), insuflando los hormigueros con un insecticida en polvo.

En el periodo de establecimiento de las pasturas se construyó la cerca perimetral con alambre de púas; la cerca divisoria interna de los potreros se hizo con cerca eléctrica. Se construyó la red hídrica para los bebederos en cada tratamiento, utilizando agua subterránea a partir de un pozo profundo accionado por un molino de viento.

Anualmente se aplicó una fertilización de mantenimiento compuesta por (kg/ha): 19 P, 18 K, 18 N, 22 S y 11 Mg, utilizando como fuentes fosfato diamónico y sulpomag (11% Mg, 22% S, 18% K).

Pastoreo

Para la evaluación de la producción animal, se utilizó inicialmente un grupo de cinco bovinos macho cebú Brahman con edades (aproximadamente 1 año y medio) y pesos similares en cada tratamiento. Por consiguiente, se tuvieron cuatro grupos de animales. Cada animal se tomó como una repetición. El pastoreo se realizó en forma rotacional en las tres repeticiones de cada tratamiento con un periodo de ocupación de 14 días y un periodo de descanso de 28 días. El pastoreo tuvo una duración total de 36 meses, tiempo durante el cual se realizó la ceba a dos grupos de animales. El primer grupo inició el pastoreo en enero de 2017 con un peso promedio inicial de 288 kg/animal y terminó en noviembre de 2018 para un total de 22 meses, con un peso final promedio de 509 kg/animal. Los cinco animales que iniciaron el pastoreo se ajustaron a tres cabezas en marzo de 2018 por baja disponibilidad de forraje (época seca). El segundo grupo (Grupo 2), compuesto también por cinco bovinos machos en cada tratamiento, inició el pastoreo en diciembre de 2018 con un peso inicial promedio de 240 kg/animal y finalizó en febrero de 2020 con un peso final promedio de 495 kg/animal, después de 14 meses de pastoreo. A los animales se le suministró sal mineralizada a voluntad y se cumplieron con los controles sanitarios y vacunas exigidos por el Instituto Colombiano Agropecuario (ICA).

Para determinar el aumento de peso de los animales y calcular las ganancias de peso vivo (PV) diarias, la carga animal y la productividad anual de las pasturas en términos de PV por hectárea, se pesaron los animales en lo posible cada 56 días utilizando una báscula Tru-Test EC 2000 (Tru-Test Ltd, Auckland, Nueva Zelanda).

Las cargas animales que se usaron fueron calculadas con base en: un consumo diario de forraje de 3.5 kg materia seca (MS)/100 kg de PV de bovinos de un peso aproximado de 300 kg/animal; una disponibilidad de forraje promedia de 806 kg MS/ha al inicio del pastoreo; y una pérdida de forraje de 12% por pisoteo. Por consiguiente, las cargas animales estuvieron en el rango de 753–1,117 kg PV/ha en el primer grupo (incluyendo un ajuste que resultó necesario en Marzo de 2018 por baja disponibilidad de forraje), y en el segundo grupo en el rango de 643 y 1,350 kg PV/ha (sin ajuste) (Anexos 3 y 4).

Evaluaciones

Durante el experimento se realizaron varias evaluaciones complementarias a nivel de suelo y pastura.

Suelo. Para determinar los efectos de los forrajes y la fertilización sobre la concentración de nutrientes en el suelo, se ejecutaron dos muestreos de suelo en el área experimental: antes de la labranza (10 submuestras) y a los dos años de realizada la siembra (10 submuestras). Los análisis se efectuaron en el laboratorio de suelos de Agrosavia y se determinaron: acidez intercambiable por extracción con KCl 1N, titulación con HCl y NaOH 0.01N; materia orgánica por el método de Walkley-Black; P por Bray II; Ca, Mg y K con extracción con acetato de amonio (NTC 5349 2008) y absorción atómica; y Fe, Mn, Zn, Cu y B con extracción con doble ácido (H_2S0_4, HCl) (NTC 5526 2007).

Pasturas. Para describir algunas características clave de las pasturas que pudieran ser útiles en la interpretación de los resultados de producción animal, se determinaron: la cobertura (%) y altura del pasto (cm); su composición botánica (%); y la disponibilidad de forraje (kg MS/ha). Estas evaluaciones fueron realizadas en transectos, con 20 observaciones en cada potrero con un marco de 0.50 × 0.50 m, al finalizar el periodo de descanso y antes de entrar los animales al pastoreo. En total se hicieron seis evaluaciones durante la época lluviosa y tres evaluaciones durante la época seca. La cobertura se estimó visualmente en cada marco y la altura del pasto se midió con una regla, sin estirar las hojas de las plantas. Debido a limitaciones logísticas y de disponibilidad de personal, también la composición botánica (proporción de gramínea, leguminosa, malezas, leguminosas nativas y material muerto) se estimó visualmente. Estas estimaciones fueron ejecutadas por la misma persona quien recibió un entrenamiento especial en esta técnica, obteniéndose en un ejercicio previo de validación con 50 muestras un coeficiente de determinación R^2 = 0.79 al comparar, mediante una regresión, los porcentajes estimados visualmente con los porcentajes basados en el peso verde de los diferentes componentes. Para determinar la disponibilidad de forraje (kg de MS/ha al final del periodo de 28 días de descanso), se cortó el material presente en el marco a una altura de 20–25 cm sobre el suelo o sea a la altura aproximada al final del pastoreo anterior. Se determinó el peso verde del forraje mediante una balanza electrónica y se secó en un horno secador de forraje a una temperatura de 70 ºC durante 3 días, para obtener el contenido de humedad y calcular materia seca de las muestras.

Una de las muestras de forraje enteras (0.50 × 0.50 m), tomada en las tres repeticiones de cada tratamiento en la época lluviosa de 2018, se utilizó para determinar las concentraciones de proteína cruda (PC) mediante el método micro-Kjeldahl (AOAC 1995); fibra en detergente ácido (FDA) (Van Soest 1963); fibra en detergente neutro (FDN) (Van Soest y Wine 1967); y degradabilidad in situ de la materia seca mediante el método descrito por Nocek y English (1986), utilizando bolsas de nylon con 5 g de forraje incubado por triplicado durante 48 horas en el rumen de un bovino cebú comercial de 680 kg fistulado que pastoreaba en una pradera de *U. decumbens* con acceso a sal mineralizada y agua a voluntad.

Análisis de datos

La información obtenida fue analizada mediante el paquete estadístico SAS 8.3. Los datos se sometieron a análisis de varianza y para la comparación de medias y para determinar la significancia se aplicó la prueba de Duncan con una probabilidad $P<0.05$.

Resultados

Suelo

Con excepción de la disminución de la concentración y saturación de aluminio, las características químicas del suelo cambiaron poco después de dos años como consecuencia de la fertilización y los tratamientos. Entre estos últimos no se observaron diferencias ni tendencias claras (Anexo 1).

Evaluaciones agronómicas

Los resultados de las evaluaciones de cobertura, altura y disponibilidad de forraje (Anexo 2) tampoco mostraron diferencias claras ni tendencias entre los tratamientos, tanto respecto a estos parámetros como a años. Las únicas diferencias claras se presentaron, como era de esperar, para la disponibilidad de forraje entre las épocas lluviosa y seca de cada año, sin que las diferencias entre los tratamientos fueran mayores. No obstante, se observó una leve ventaja de la pastura *C. molle* + Decumbens para la época seca (Figura 2).

Composición botánica

La proporción de la respectiva leguminosa en las pasturas asociadas varió significativamente entre los tratamientos (Cuadro 2). En todas las evaluaciones, *C. molle* en su asociación con Decumbens presentó los valores más altos, los cuales variaron de alrededor de 40% en la pastura durante la primera mitad del experimento (las primeras tres evaluaciones) hasta una estabilización alrededor de 25% en la segunda mitad (Figura 3). Esta disminución en la segunda parte del experimento se observó también en las pasturas de las otras dos leguminosas y se debió a las inundaciones que se presentaron en el experimento como consecuencia de las fuertes lluvias en el sitio (junio–agosto 2018). La participación de *C. macrocarpum* en su asociación fue muy baja, independiente de la época, y la leguminosa prácticamente desapareció. Respecto a la participación de los otros componentes [gramínea, material muerto, leguminosas nativas (*Aeschynomene* sp. y *Desmodium* sp.) y malezas], a pesar de algunas diferencias significativas en algunas evaluaciones, no se observaron tendencias claras (Cuadro 2).

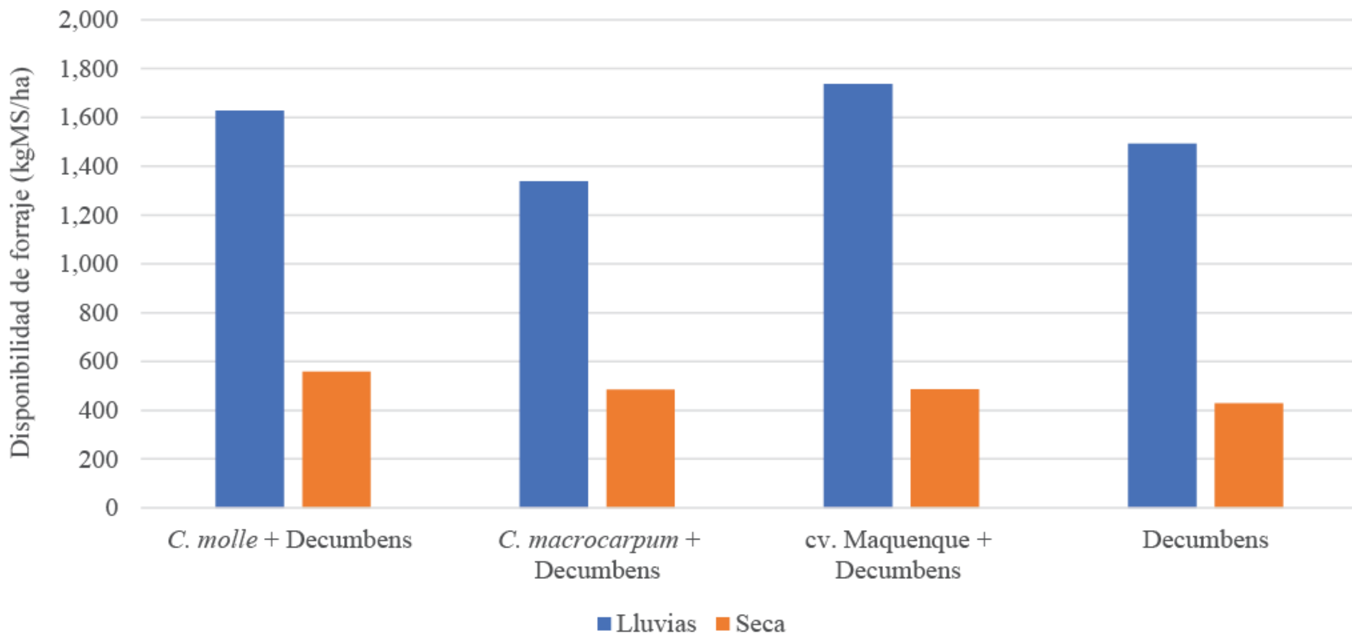


Figura 2. Disponibilidad promedio de forraje (rebrote de 28 días de edad) durante tres épocas lluviosas y secas en cuatro pasturas, 2017–2019. C.I. Carimagua, Altillanura colombiana.

Calidad nutritiva

En la época lluviosa del segundo año de pastoreo, se analizó la calidad nutritiva del forraje (Cuadro 3), destacándose la asociación de *C. molle* con Decumbens por la mayor (P<0.05) concentración de proteína cruda, lo cual era de esperar por la mayor presencia de la leguminosa en la mezcla. La fibra en detergente neutro (FDN) también fue superior en esta asociación, mientras que la fibra en detergente ácido (FDA) fue menor. La digestibilidad también fue más alta en esta misma asociación y en la pastura de Decumbens solo.

Producción animal

Ganancia de peso vivo diaria. En el Cuadro 4 se presentan las ganancias diarias de peso vivo (PV) del primer grupo de novillos con base en siete pesajes entre enero 2017 y noviembre 2018. En el primer periodo (enero a junio) de pastoreo no se presentaron diferencias significativas (P>0.05), pero en los pesajes posteriores los animales de la asociación de *C. molle* + Decumbens ganaron significativamente más peso. El efecto de las inundaciones presentadas entre junio y agosto 2018 por la alta precipitación es reflejado en las pérdidas de PV en casi todos los animales, con excepción de los que pastorearon la asociación *C. molle* + Decumbens.

En el Cuadro 5 se presentan las ganancias diarias de PV del segundo grupo de novillos con base en seis pesajes entre diciembre 2018 y febrero 2020. Se observa la misma tendencia que la del primer grupo pues en la mayor parte de los pesajes se pudo evidenciar más ganancia diaria de PV en la asociación de *C. molle* + Decumbens que en los otros tratamientos. Las diferencias, sin embargo, no fueron significativas. De igual forma al año 2018, en el periodo de mayo a septiembre de 2019, cuando ocurrieron inundaciones por alta precipitación y además se presentó fuerte ataque del insecto plaga 'salivazo' (*Aeneolamia* spp.), se observan ganancias de peso muy bajas (Cuadro 5).

En la época lluviosa, las ganancias de PV de los novillos en la asociación de *C. molle* con Decumbens fueron superiores (P<0.05) a las obtenidas en los otros tratamientos (Figura 4). Durante la época seca no se presentaron diferencias significativas entre los tratamientos.

Calculando la productividad por hectárea, con base en la ganancia diaria de peso y la carga animal expresada en kg peso vivo/ha, sobresale la asociación de *C. molle* con Decumbens, con una productividad de 611 y 595 kg/ha/año en el primer y segundo grupo, respectivamente (Figura 5). Las respectivas diferencias con el promedio de los otros tratamientos ascienden a 278 kg (80%) y 168 kg (39%) a favor de la pastura *C. molle* con Decumbens.

Cuadro 2. Composición botánica (%) de las pasturas durante las épocas lluviosas y secas 2017–2019. C.I. Carimagua, Altillanura colombiana.

Pastura	Leguminosa	Gramínea	Material muerto	Leguminosas nativas	Malezas
Época de lluvias 2017					
C. molle + Decumbens	38.0a	27.8b	24.0c	2.3b	6.3
C. macrocarpum + Decumbens	5.0c	39.8a	35.0ab	8.0a	3.3
cv. Maquenque + Decumbens	25.6b	33.8ab	28.0bc	3.6b	4.3
Decumbens solo	0.0c	43.1a	38.6a	5.6ab	5.6
Significancia	0.001	0.02	0.01	0.04	ns
C.V. (%)	19.3	14.1	13.4	41.9	31.0
Época seca 2017					
C. molle + Decumbens	37.3a	32.1b	26.0b	1.6	0.6
C. macrocarpum + Decumbens	7.1c	44.3a	39.0a	4.6	1.6
cv. Maquenque + Decumbens	22.9b	40.4ab	28.3b	3.3	1.6
Decumbens solo	0.0c	43.2a	43.3a	6.6	3.3
Significancia	0.001	0.06	0.006	ns	ns
C.V. (%)	24.9	12.4	14.1	51.4	87.3
Época de lluvias 2018					
C. molle + Decumbens	40.5a	34.6b	9.7b	5.3	3.6b
C. macrocarpum + Decumbens	5.3c	55.3a	18.3ab	4.3	13.0a
cv. Maquenque + Decumbens	20.6b	49.1a	19.0ab	3.0	4.6b
Decumbens solo	0.0c	58.6a	24.3a	4.3	7.0ab
Significancia	0.002	0.002	0.03	ns	0.06
C.V. (%)	26.9	10.5	26.7	35.3	54.1
Época seca 2018					
C. molle + Decumbens	26.7a	34.6	30.1	0.2c	0.0b
C. macrocarpum + Decumbens	1.6c	48.3	32.7	5.3a	2.3ab
cv. Maquenque + Decumbens	8.6b	40.4	49.3	1.3b	0.3b
Decumbens solo	0.0c	38.3	46.2	5.0a	4.6a
Significancia	0.003	ns	ns	0.04	0.04
C.V. (%)	56.7	22.6	30.6	56.8	72.4
Época de lluvias 2019					
C. molle + Decumbens	24.3a	42.4	28.3	0.6b	1.6
C. macrocarpum + Decumbens	0.3c	48.3	30.3	4.3ab	13.1
cv. Maquenque + Decumbens	17.0b	46.3	30.0	2.3b	2.0
Decumbens solo	0.0c	43.1	31.0	6.3a	12.3
Significancia	0.005	ns	ns	0.03	0.02
C.V. (%)	25.5	38.3	34.1	47.3	46.4
Época seca 2019					
C. molle + Decumbens	24.6a	47.0	20.6b	2.3b	3
C. macrocarpum + Decumbens	0.7c	51.6	28.1ab	5.3ab	4
cv. Maquenque + Decumbens	11.3b	48.0	29.0a	5.6ab	1
Decumbens solo	0.0c	50.4	31.0a	6.3a	5
Significancia	0.0001	ns	0.006	0.01	ns
C.V. (%)	28.8	5.5	14.8	37.1	56.7

Promedios con letras iguales en la misma columna no presentaron diferencias significativas (Duncan P>0.05).

Cuadro 3. Calidad nutritiva del forraje (rebrote de 28 días de edad) de las pasturas de tres asociaciones de leguminosa con *Urochloa decumbens* vs. *U. decumbens* solo, durante la época lluviosa 2018. C.I. Carimagua, Altillanura colombiana.

Pastura	Proteína cruda (%)	FDN (%)	FDA (%)	Digestibilidad (%)
C. molle + Decumbens	13.7a	66.6a	34.0b	66.7a
C. macrocarpum + Decumbens	9.8c	63.5b	36.3a	64.2b
cv. Maquenque + Decumbens	11.7b	64.7b	36.0a	62.4c
Decumbens solo	9.2c	68.2a	36.1a	67.6a
Significancia	0.006	0.009	0.08	0.0001
C.V. (%)	7.5	2.3	2.9	1.2

Promedios con letras iguales en la misma columna no presentaron diferencias significativas (Duncan P>0.05).

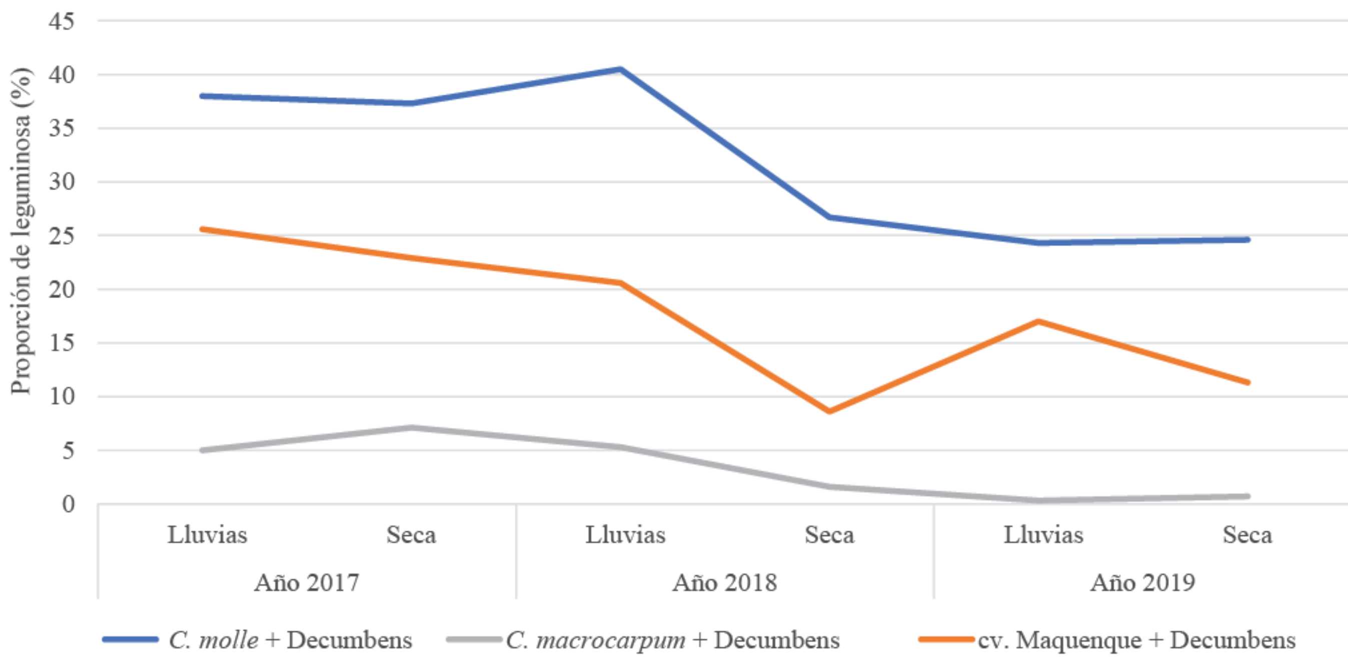


Figura 3. Evolución de la proporción de tres leguminosas asociadas con *Urochloa decumbens* cv. Decumbens, bajo pastoreo durante tres años. C.I. Carimagua, Altillanura colombiana.

Cuadro 4. Ganancia de peso vivo (kg/animal/día) del primer grupo de novillos durante 22 meses de pastoreo en pasturas de tres leguminosas asociadas con *Urochloa decumbens* y en la gramínea sola (enero 2017–noviembre 2018). C.I. Carimagua, Altillanura colombiana.

Pastura	Ene–Jun/17	Jun–Ago/17	Ago–Dic/17	Mar–Jun/18	Jun–Ago/18	Ago–Sep/18	Sep–Nov/18
C. molle + Decumbens	0.287	0.917a	0.780a	0.790	0.406a	0.553a	1.076
C. macrocarpum + Decumbens	0.255	0.620b	0.565b	0.511	-0.377b	0.106c	1.090
cv. Maquenque + Decumbens	0.234	0.660b	0.635b	0.670	-0.384b	0.284b	1.015
Decumbens solo	0.336	0.740ab	0.650b	0.740	-0.608b	0.276b	0.924
Significancia	ns	0.02	0.01	ns	0.007	0.001	Ns
C.V. (%)	38.3	24.7	30.2	35.7	58.8	35.9	25.6

Promedios con letras iguales en la misma columna no presentaron diferencias significativas (Duncan P>0.05). Por problemas de logística no se reportan datos para el período diciembre 2017–marzo 2018.

Cuadro 5. Ganancia de peso vivo (kg/animal/día) del segundo grupo de novillos durante 14 meses de pastoreo en pasturas de tres leguminosas asociadas con *Urochloa decumbens* y en la gramínea sola (diciembre 2018–febrero 2020). C.I. Carimagua, Altillanura colombiana.

Pastura	Dic–Abr/19	Abr–May/19	May–Sep/19	Sep–Oct/19	Oct–Dic/19	Dic–Feb/20
C. molle + Decumbens	0.550	1.140a	0.170	0.984a	0.947a	0.672
C. macrocarpum + Decumbens	0.486	0.570b	0.159	0.849ab	0.782ab	0.534
cv. Maquenque + Decumbens	0.397	0.770ab	0.082	0.783ab	0.817ab	0.586
Decumbens solo	0.420	0.610b	0.259	0.673b	0.716b	0.593
Significancia	ns	0.06	ns	0.01	0.05	ns
C.V. (%)	29.7	44.1	68.7	24.2	14.8	34.7

Promedios con letras iguales en la misma columna no presentaron diferencias significativas (Duncan P>0.05).

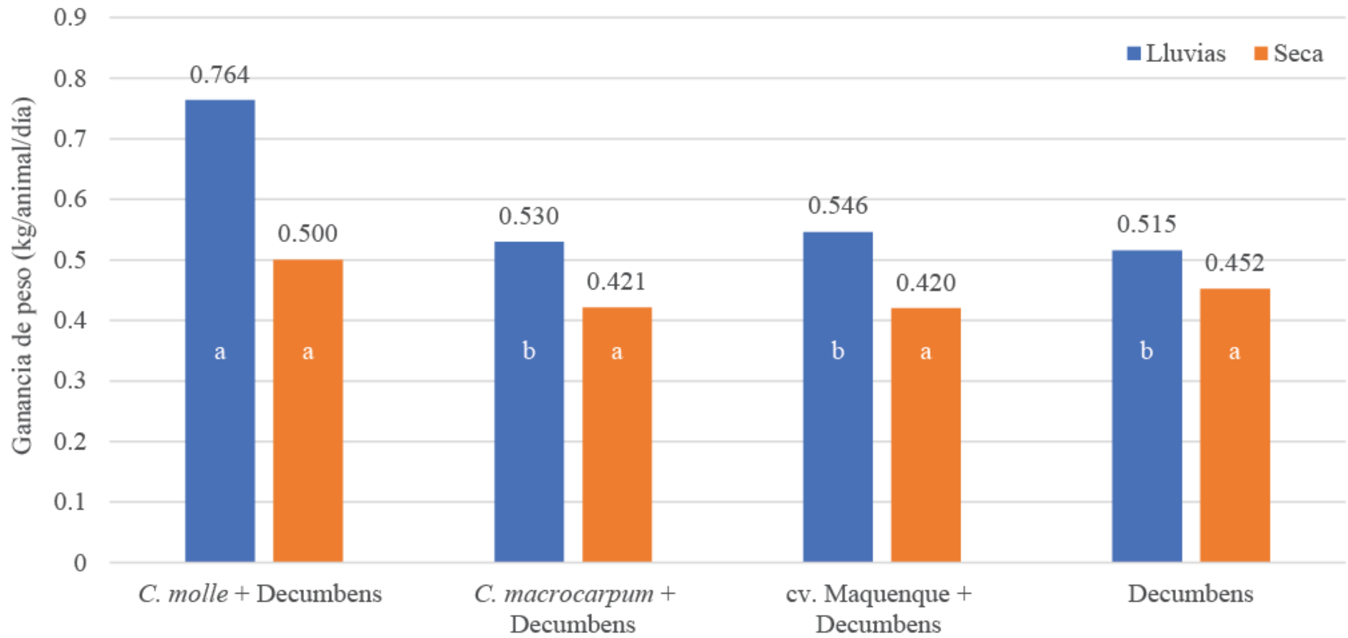


Figura 4. Ganancia de peso (kg/animal/día) de los novillos en pasturas de tres leguminosas asociadas con *Urochloa decumbens* y la gramínea sola durante las épocas lluviosa y seca (promedios de tres años). C.I. Carimagua, Altillanura colombiana. Columnas con el mismo color y letras iguales no difieren significativamente (Duncan P>0.05).

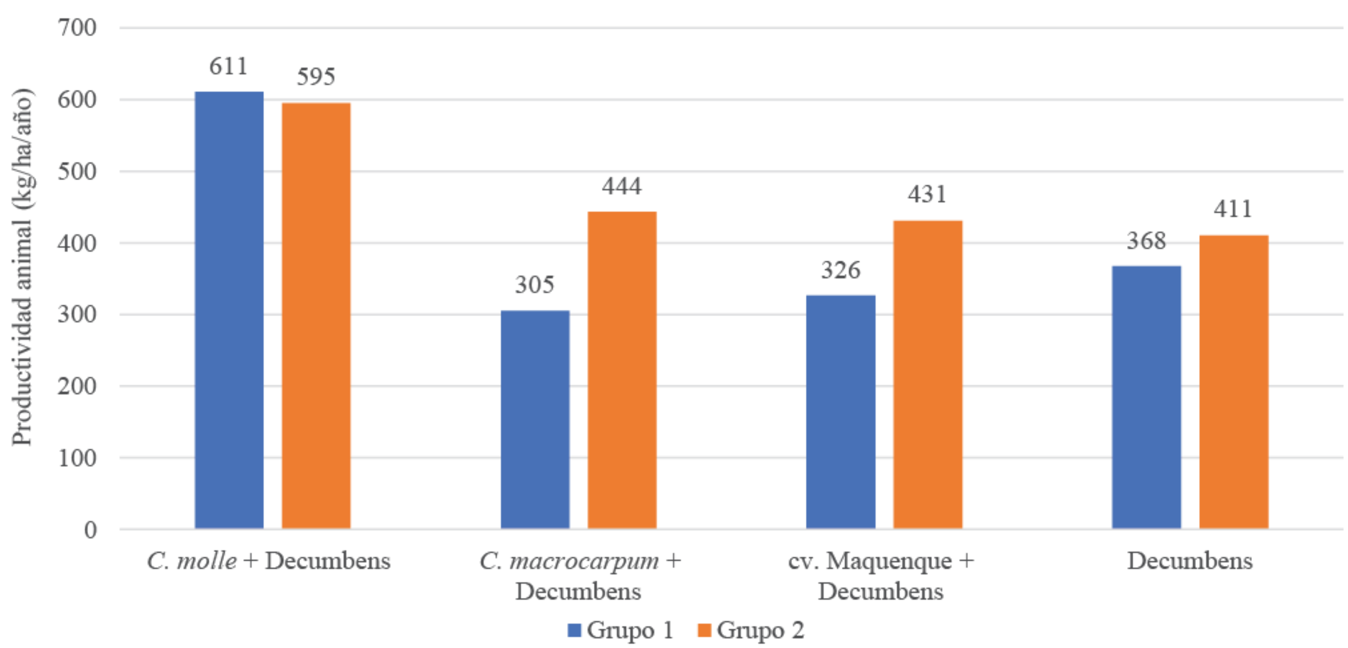


Figura 5. Productividad de peso vivo (PV) por hectárea y año en dos grupos de novillos en pasturas de tres leguminosas asociadas con *Urochloa decumbens* y gramínea sola durante tres años (2017–2019). C.I. Carimagua, Altillanura colombiana.

Discusión

Aunque no se presentó diferencia alguna entre los tratamientos, la disponibilidad promedia de forraje después del periodo de descanso cada 28 días, durante los tres años de evaluación fue de 1,500 kg MS/ha en época lluviosa, coincidiendo con lo encontrado por Rincón (2004) en asociaciones de *Brachiaria decumbens* (ahora: *Urochloa decumbens*) con *Pueraria phaseoloides* (kudzu; ahora: *Neustanthus phaseoloides*) y *Arachis pintoi* (maní forrajero) en la Altillanura, pero superior a lo encontrado por Pérez y Pérez (2006) en praderas de *Brachiaria brizantha* (ahora: *Urochloa brizantha*) solas y asociadas con kudzu, también en la Altillanura, con producción de forraje de 1,024 kg MS/ha con periodo de descanso de 28 días.

La alta proporción de *C. molle* en la asociación, desde el primer año de pastoreo, permite ver a este material como promisorio para los sistemas ganaderos de la región. Su capacidad de supervivencia después de más de dos meses bajo alta saturación de agua, ocurrida durante los periodos lluviosos de los años 2018 y 2019, permitió mantener un 24% de leguminosa en la composición botánica, demostrando la tolerancia de esta accesión de *C. molle* a los cambios del clima que se vienen percibiendo en la región en los últimos años. Humphreys et al. (1997) en Asia tropical, en la asociación de *C. pubescens* (ahora: *C. molle*) con *B. decumbens* (ahora: *U. decumbens*) reportaron una proporción de la leguminosa entre 21 y 24% que consideraron como adecuada frente a un mínimo deseable de 15%.

El *C. macrocarpum* asociado con Decumbens no alcanzó el 10% en la composición botánica en el primer año de pastoreo y después del segundo año disminuyó hasta casi desaparecer, mientras que el cv. Maquenque en el tercer año conservó una proporción de 14%. El factor que probablemente fue decisivo para la baja producción y persistencia de *C. macrocarpum*, en comparación con la buena producción y persistencia de *C. molle*, fue la falta de cepas efectivas de *Rhizobium* nativas en el área experimental con las que *C. macrocarpum* hubiera podido asociarse. Diferente a las evaluaciones agronómicas anteriores, en las que se identificó a *C. macrocarpum* como especie promisoria, las pasturas para producción animal se establecieron en sabana virgen donde no ha habido oportunidad de colonización por cepas específicas que *C. macrocarpum* parece requerir para una efectiva nodulación. Esta falta de asociación con rizobios aparentemente no pudo ser compensada por los 18 kg de N incluida en la fertilización de mantenimiento de todos los tratamientos. En contraste, *C. molle* sí noduló con rizobios nativos. Además, C. *molle* fue favorecido por dos características de la gramínea asociada: el hábito de crecimiento semierecto del Decumbens que permite el desarrollo de leguminosas de crecimiento voluble, y el debilitamiento de su vigor y capacidad de competencia con la leguminosa, debido al ataque del insecto plaga ‘salivazo’ (*Aeneolamia* spp.).

Al respecto, Kretschmer (1988) y Arcos Álvarez et al. (2018) hacen referencia a la falta de persistencia de las leguminosas forrajeras tropicales como el mayor obstáculo para su utilización en mezclas con gramíneas. Esta situación aún se mantiene y se agrava, por la falta de investigación que permita una mayor oferta de materiales e información sobre el adecuado manejo para asegurar la estabilidad de la leguminosa en las asociaciones. Sin embargo, Shelton et al. (2005) reportan un número considerable de casos de asociaciones estables a nivel del trópico y subtrópico. Similarmente, Lok Mejias et al. (2017), analizando una comparación de un sistema silvopastoril con una asociación gramíneas-múltiples leguminosas de 15 años en Cuba, concluyeron que asociaciones leguminosas-gramíneas pueden persistir durante largos periodos de tiempo con adecuada producción de biomasa e incremento de la fertilidad del suelo, siempre que se manejen con la correcta disciplina tecnológica.

El aporte de la leguminosa a la concentración de proteína cruda (PC) contribuyó al mejoramiento de la calidad del forraje disponible, especialmente en el caso de la pastura con *C. molle* (13.7% de PC en la mezcla). Esta especie es reconocida por su alta concentración de PC (17–25%; Lascano et al. 1997). También se pudo evidenciar buena concentración de PC en la pastura Decumbens solo con más de 9% y una digestibilidad de 67%, lo cual contrasta con la concentración de PC que con el manejo tradicional en la región y sin fertilización no alcanza 7% (Rincón 2004). Atribuimos la buena calidad del Decumbens a la edad del forraje disponible (28 días) y a la mayor concentración de materia orgánica en el suelo (2.9%) la cual, según los datos presentados por Rodríguez Borray y Bautista Cubillos (2019), debe considerarse como media a alta para la Altillanura. Esto indica que la fertilización aplicada en el establecimiento de las pasturas y la fertilización anual de mantenimiento, permitieron un buen desempeño de la gramínea (Rincón 2010; Pérez et al. 2018).

La mayor proporción de leguminosa se reflejó en las ganancias de PV en la asociación con *C. molle*, llegando a más de 700 g/animal/día en época lluviosa en comparación con las otras pasturas (algo más de 500 g/animal/día). Estas ganancias resultan ser superiores a las reportadas por Garza Treviño y Portugal (1978, citados por Argel et al. 1997), con aumentos diarios de 524 g/animal en una asociación de *C. pubescens* (ahora: *C. molle*) con pasto Pangola [*Digitaria decumbens* (ahora: *D. eriantha*)] frente a 385 g/animal en Pangola sola.

Las ganancias obtenidas en la época seca en todas las asociaciones, con un promedio de 448 g/animal/día, demuestran un impacto importante en la producción de peso vivo, si se tiene en cuenta que en la Altillanura se presentan en esta época, por lo general, pérdidas en la productividad de ganado de carne o leche (Castro et al. 2018). La mayor ganancia de peso animal en la asociación *C. molle* + Decumbens significó 222 kg/ha/año más PV con respecto a las demás pasturas estudiadas. La productividad en peso vivo por hectárea en esta asociación coincide con la producción de 500–600 kg

PV/ha/ año reportado por Lascano et al. (1997) para regiones tropicales húmedas con suelos de fertilidad natural intermedia siempre que las pasturas se manejan de forma adecuada y se les aplica la fertilización necesaria.

Conclusión

En el suelo franco arcilloso de la Altillanura colombiana, la accesión *C. molle* CIAT 15160 se destacó por su buena proporción en asociación con *U. decumbens* cv. Decumbens. Con una productividad de 600 kg PV/ha/año frente a 390 kg/ha/año producido por la gramínea sola, se demostró el beneficio de esta leguminosa en la alimentación de bovinos. Se recomienda revalidar su productividad y persistencia a nivel de productor, también en asociaciones con otras gramíneas. *C. molle* CIAT 15160 puede ser una buena opción para la deseada intensificación sostenible de las ganaderías en la Altillanura colombiana.

Agradecimientos

A la Corporación Colombiana de Investigación Agropecuaria – Agrosavia por el apoyo técnico y administrativo; al Ministerio de Agricultura y Desarrollo Rural de Colombia por la financiación del proyecto; a Jorge Lozano (q.e.p.d.), Investigador de Agrosavia, por su apoyo en el manejo animal; y a Luis Alberto Ciprián, Asistente de Agrosavia, por el apoyo en labores de campo y evaluaciones.

Referencias

(Nota de los editores: los enlaces se verificaron el 9 de marzo de 2021).

Andrade CMS; Ferreira AS; Casagrande DR. 2015. Uso de leguminosas em pastagens: Potencial para consórcio compatível com gramíneas tropicais e necessidades de manejo de pastejo. En: Anais, 27 Simpósio sobre Manejo de Pastagem. Sistemas de produção, intensificação e sustentabilidade da produção animal. FEALQ, Piracicaba, São Paulo, Brasil. p. 113–152. bit.ly/3cMNHCN

AOAC (Association of Official Agricultural Chemists). 1995. Official methods of analysis. 16th Edn. AOAC Inc., Arlington, VA, USA.

Arcos Álvarez CN; Lascano AP; Guevara Viera RV. 2018. Manejo de asociaciones gramíneas-leguminosas en pastoreo con rumiantes para mejorar su persistencia, la productividad animal y el impacto ambiental en los trópicos y regiones templadas. Revista Ecuatoriana de Ciencia Animal 2(2):1–31. bit.ly/3qQQEqM

Argel PJ; Peralta M; Pizarro EA. 1997. Experiencia regional con *Centrosema*: America Central y México. En: Schultze-Kraft R; Clements RJ; Keller-Grein G, eds. *Centrosema:* Biología, agronomía y utilización. CIAT, Cali, Colombia. p. 427–454. bit.ly/3mPBh1r

Barahona RR; Sánchez PS. 2005. Limitaciones físicas y químicas de la digestibilidad de pastos tropicales y estrategias para aumentarla. Revista Corpoica 6(1):69–82. doi: 10.21930/rcta.vol6_num1_art:39

Castro E; Mojica JE; Carulla JE; Lascano CE. 2018. Evaluación de leguminosas como abono verde en cultivos forrajeros para ganaderías en el Caribe seco colombiano. Agronomía Mesoamericana 29:597–617. doi: 10.15517/ma.v29i3.32350

Dean G.D.B. 2013. Importancia de las leguminosas en la alimentación de rumiantes. En: Perozo BA, ed. Manejo de pastos y forrajes tropicales. Cuadernos Científicos GIRARZ 13. Fundación GIRARZ. Ediciones Astro Data S.A., Maracaibo, Venezuela. p. 169–176. bit.ly/3vznY9i

Humphreys LR; Ivory DA; Wong Choi Chee; Topark-Ngarm A. 1997. Experiencia regional con *Centrosema*: Asia tropical y región del Pacífico. En: Schultze-Kraft R; Clements RJ; Keller-Grein G, eds. *Centrosema:* Biología, agronomía y utilización. CIAT, Cali, Colombia. p. 629–647. bit.ly/3gfKgHY

Kretschmer AE. 1988. Consideraciones sobre factores que afectan la persistencia de leguminosas forrajeras tropicales. Pasturas Tropicales 10(1):28–32. bit.ly/3foE8N3

Lascano CE; Teitzel JK; Eng Pei Kong. 1997. Valor nutritivo de *Centrosema* y producción animal. En: Schultze-Kraft R; Clements RJ; Keller-Grein G, eds. *Centrosema:* Biología, agronomía y utilización. CIAT, Cali, Colombia. p. 343–373. bit.ly/3twIjdK

Lazier JR; Clatworthy JN. 1997. Experiencia regional con *Centrosema*: África al sur del Sahara. En: Schultze-Kraft R; Clements RJ; Keller-Grein G, eds. *Centrosema:* Biología, agronomía y utilización. CIAT, Cali, Colombia. p. 649–684. bit.ly/3twIjdK

Lok Mejías S; Crespo G; Torres V. 2017. Influence of forage legumes on the soil-grass system. Cuban Journal of Agricultural Science 51(2):261–270. bit.ly/3sBjqgG

Nocek JE; English JE. 1986. In situ degradation kinetics: Evaluation of rate determination procedure. Journal of Dairy Science 69:77–87. doi: 10.3168/jds.s0022-0302(86)80372-1

NTC (Norma Técnica Colombiana) 5349. 2008. Determinación de las bases intercambiables: los cationes calcio, magnesio, sodio, potasio. Método de extracción con acetato de amonio 1 N y pH 7. ICONTEC. 6 p. bit.ly/30TPBvC

NTC (Norma Técnica Colombiana) 5526. 2007. Determinación de micronutrientes disponibles cobre, zinc, hierro, manganeso. ICONTEC. 6 p. bit.ly/2OAv8tv

Pérez O; Bueno GA; Baquero JE; Onofre HG; Cassalett E; Molina AM; Arango MA; Velázquez JG; Rodríguez NS; Moreno ML; Amaya CV; Bernal JH; Correal WA; Pabón DM; Villalobos MA; Velázquez JH; Fuentes JC. 2018. Modelo productivo. Tecnologías integrales para sistemas de producción de bovinos de cría en la altillanura colombiana. Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA), Mosquera, Colombia. p. 35–43. doi: 10.21930/agrosavia.model.7402681

Pérez R; Pérez O. 2006. Gramíneas forrajeras con potencial para sistemas de producción de ganadería bovina. MADR-CIAT-Corpoica. bit.ly/3qXXUB6

Rincón A. 2004. Rehabilitación de pasturas y producción animal en *Brachiaria decumbens* en la Altillanura plana de los Llanos Orientales de Colombia. Pasturas Tropicales 26(3):2–12. bit.ly/3rHnOtz

Rincón A. 2010. Concentración de minerales en suelos y fertilización de pastos en los Llanos Orientales de Colombia. En: Rincón A; Jaramillo, C, eds. Manejo de la nutrición mineral en sistemas ganaderos de los Llanos Orientales de Colombia. CORPOICA, Ministerio de Agricultura y Desarrollo Rural, Villavicencio, Meta, Colombia. p. 113–164. hdl.handle.net/20.500.12324/19506

Rodríguez Borray G; Bautista Cubillos R. 2019. Adopción e impacto de los sistemas agropecuarios introducidos en la altillanura plana del Meta. Corporación Colombiana de Investigación Agropecuaria de Colombia (AGROSAVIA). Mosquera. hdl.handle.net/20.500.12324/35451

Sánchez GAJ. 2013. Asociación de gramíneas y leguminosas. En: Perozo BA, ed. Manejo de pastos y forrajes tropicales. Cuadernos Científicos GIRARZ 13. Fundación GIRARZ. Ediciones Astro Data S.A., Maracaibo, Venezuela. p. 177–184. bit.ly/3ahvsF4

Schultze-Kraft R; Rao IM; Peters M; Clements RJ; Bai C; Liu G. 2018. Tropical forage legumes for environmental benefits: An overview. Tropical Grasslands-Forrajes Tropicales 6:1–14. doi: 10.17138/tgft(6)1-14

Shelton HM; Franzel S; Peters M. 2005. Adoption of tropical legume technology around the world: Analysis of success. Tropical Grasslands 39:198–209. bit.ly/3dmAyBD

Van Soest PJ. 1963. Use of detergent in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. Journal of the Association of Official Analytical Chemists 46:829–835. goo.gl/tAmbLm

Van Soest PJ; Wine RH. 1967. Use of detergent in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. Journal of the Association of Official Analytical Chemists 50:50–55. goo.gl/HhyMLS

Vázquez LD; Lara-Rodríguez DA; Sánchez AJ; Díaz PLA; Velázquez SMG. 2019. Tropical legumes as improvers of rangeland and agricultural soils. Tropical and Subtropical Agroecosystems 22(1):203–211. bit.ly/2QvRkoR

Anexo 1. Características químicas del suelo en sabana nativa (= antes del establecimiento de las pasturas) y después de dos años de pastoreo. C.I. Carimagua, Altillanura colombiana.

Pastura	pH	M.O.	P	S	Al	Ca	Mg	K	Sat. Al
		%	(mg/kg)		(cmol/kg)				(%)
Sabana nativa antes de siembra	5.0b	2.9ab	<3.87[1]	1.8b	2.2a	0.6b	0.20b	0.09	65a
C. molle + Decumbens	5.2a	3.0a	<3.87	1.5b	1.4b	1.1ab	0.28ab	0.09	44ab
C. macrocarpum + Decumbens	5.2a	2.8b	<3.87	5.1ab	1.3b	1.2ab	0.30ab	0.09	40ab
cv. Maquenque + Decumbens	5.2a	3.0a	<3.87	6.2a	1.0b	1.9a	0.43a	0.11	26b
Decumbens solo	5.3a	3.0a	<3.87	7.2a	1.1b	1.5ab	0.34ab	0.09	31b
Significancia	0.07	0.09		0.02	0.05	0.01	0.09	ns	0.06
C.V. (%)	1.3	3.2		30.2	22.1	30.2	19.6	16.7	24.3

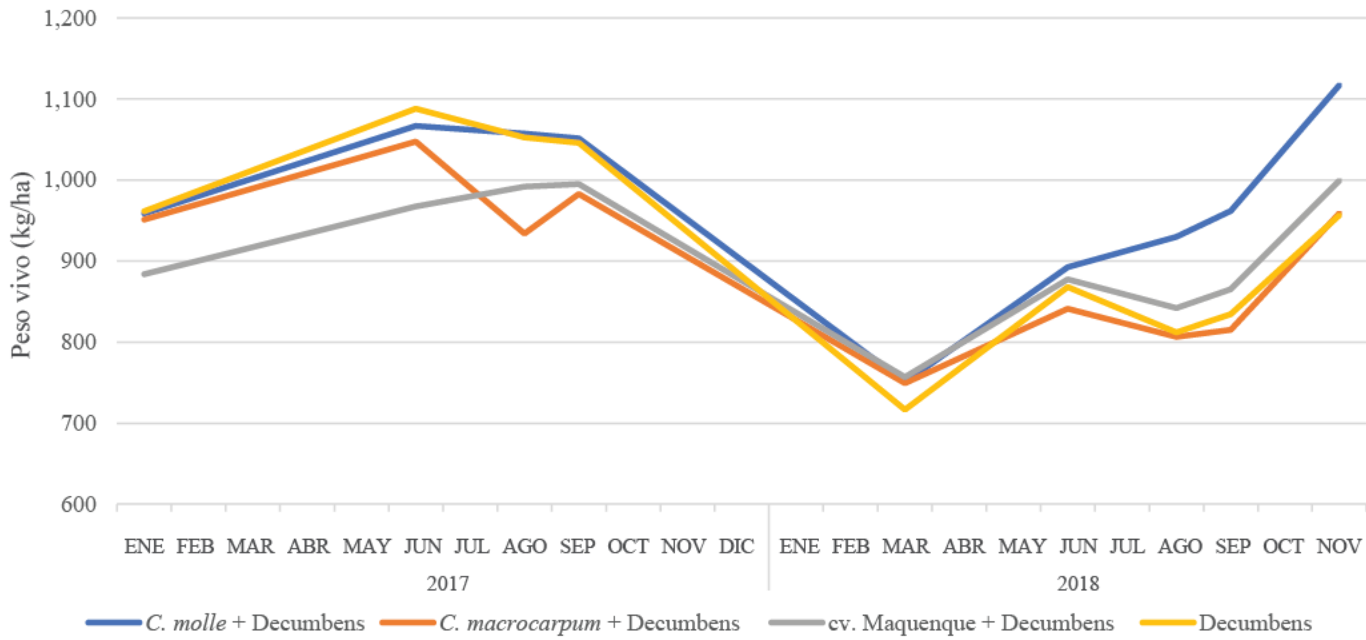
Promedios con letras iguales en la misma columna no presentaron diferencias significativas (Duncan P>0.05).
[1]El laboratorio que procesó las muestras no reporta valores de P menores de 3.87 mg/kg.

Anexo 2. Comportamiento agronómico de las pasturas durante las épocas lluviosa y seca, 2017–2019. C.I. Carimagua, Altillanura colombiana.

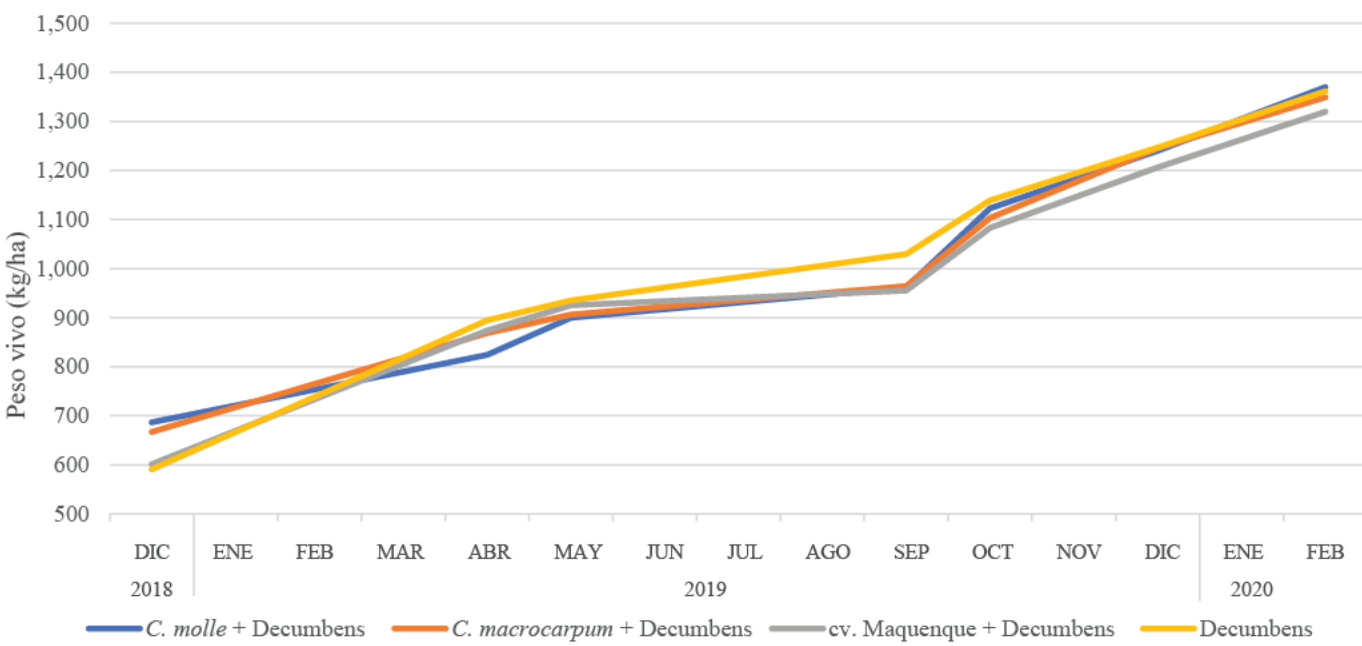
Pastura	Cobertura (%)	Altura (cm)	Disponibilidad de forraje (kg MS/ha)
Época de lluvias 2017			
C. molle + Decumbens	80.0	31.6	1,730
C. macrocarpum + Decumbens	76.0	30.6	1,491
cv. Maquenque + Decumbens	75.3	30.3	2,014
Decumbens solo	78.3	30.0	1,819
Significancia	ns	ns	ns
C.V. (%)	4.6	14.2	19.5
Época seca 2017			
C. molle + Decumbens	85.8a	35.0a	924
C. macrocarpum + Decumbens	77.1ab	34.1ab	821
cv. Maquenque + Decumbens	75.8b	31.0b	800
Decumbens solo	77.5ab	31.6b	678
Significancia	0.008	0.06	ns
C.V. (%)	5.6	5.2	20.1
Época de lluvias 2018			
C. molle + Decumbens	91.3a	33.0	1,971
C. macrocarpum + Decumbens	76.6c	31.6	1,652
cv. Maquenque + Decumbens	85.0ab	39.0	2,178
Decumbens solo	82.3b	36.6	1,855
Significancia	0.007	ns	ns
C.V. (%)	2.9	14.1	17.6
Época seca 2018			
C. molle + Decumbens	83.6a	32.1ab	193
C. macrocarpum + Decumbens	74.7b	31.7ab	159
cv. Maquenque + Decumbens	80.6ab	35.1a	181
Decumbens solo	79.0ab	30.0b	192
Significancia	0.01	0.01	ns
C.V. (%)	5.4	7.1	29.1
Época de lluvias 2019			
C. molle + Decumbens	77.3	42.0	1,180
C. macrocarpum + Decumbens	60.3	39.6	872
cv. Maquenque + Decumbens	69.3	42.6	1,020
Decumbens solo	55.0	39.6	803
Significancia	ns	ns	ns
C.V. (%)	28.6	16.8	32.3
Época seca 2019			
C. molle + Decumbens	80.0	29.0	560a
C. macrocarpum + Decumbens	75.3	28.7	477ab
cv. Maquenque + Decumbens	77.3	29.6	476ab
Decumbens solo	74.6	26.6	415b
Significancia	ns	ns	0.09
C.V. (%)	3.8	6.1	12,3

Promedios con letras iguales en la misma columna no presentaron diferencias significativas (Duncan P>0.05).

Anexo 3. Desarrollo de la carga animal (kg peso vivo/hectárea) en las pasturas. Primer grupo de animales, CI. Carimagua, Altillanura colombiana.



Anexo 4. Desarrollo de la carga animal (kg peso vivo/hectárea) en las pasturas. Segundo grupo de animales, CI. Carimagua, Altillanura colombiana.



(Recibido para publicación 17 de septiembre de 2020; aceptado 26 de febrero de 2021; publicado 31 mayo de 2021)

Tropical Grasslands-Forrajes Tropicales (2021) Vol. 9(2):206–215
doi: 10.17138/TGFT(9)206-215

Research Paper

Combinations of *Urochloa* hybrid Mulato II and natural pasture hays as a basal diet for growing Farta lambs in Ethiopia

Combinaciones del híbrido Urochloa *Mulato II y heno de pasto natural como dieta basal para el crecimiento de corderos Farta en Etiopía*

WUBETIE ADNEW[1], BERHANU ABRAHA TSEGAY[1], ASAMINEW TASSEW[2] AND BIMREW ASMARE[2]

[1]*Department of Biology, College of Science, Bahir Dar University, Bahir Dar, Ethiopia.*
[2]*Department of Animal Production and Technology, School of Animal Science and Veterinary Medicine, Bahir Dar University, Bahir Dar, Ethiopia.* www.bdu.edu.et

Abstract

The study was conducted to evaluate the feed intake, digestibility, bodyweight change and carcass characteristics of Farta lambs fed Brachiaria (now: *Urochloa*) hybrid Mulato II and natural pasture hays in various proportions as a basal diet supplemented with a local concentrate mixture (CM). Twenty-five yearling male Farta lambs with a mean initial body weight of 19.6±0.29 kg (mean±s.e.) were used in feeding (90 days) and digestibility (7 days) trials. The lambs were randomly allocated to the following 5 dietary treatments on the basis of stratified body weight: 100% natural pasture hay (NPH) (T1); 75% NPH+25% *Urochloa* hybrid Mulato II hay (MH) (T2); 50% NPH+50% MH (T3); 25% NPH+75% MH (T4); and 100% MH (T5). A local concentrate mixture [300 g dry matter (DM)/hd/d] was fed to all animals. Crude protein (CP) concentration of the basal diet increased as proportion of MH in the ration increased (P<0.05). Intake of DM and nutrients, and nutrient digestibility coefficients increased significantly (P<0.05) as proportion of MH in the roughage component of the ration increased. Final body weight, average daily bodyweight gain, feed conversion efficiency and most carcass parameters measured were significantly (P<0.05) higher as proportion of MH increased from 0 to 100% in the basal diet. Based on the biological performance of the experimental lambs, performance of sheep in the region could be enhanced significantly by incorporating MH with native pasture hay and concentrate supplement in feeding rations. Economic assessments would reveal the optimal combinations of native pasture and MH for feeding to achieve particular outcomes. Other improved grass and legume species may fill the same role and should be investigated in differing environments.

Keywords: Bodyweight gain, *Brachiaria*, carcass yield, digestibility, fattening sheep, tropical grasses.

Resumen

El estudio se realizó para evaluar la ingesta de alimento, la digestibilidad, el cambio de peso corporal y las características de la canal de los corderos Farta alimentados con Brachiaria (ahora: *Urochloa*) híbrido Mulato II y heno de pasto natural en diversas proporciones como dieta basal suplementada con una mezcla de concentrado local (CM). Se utilizaron veinticinco corderos Farta añojos con un peso corporal inicial medio de 19.6 ± 0.29 kg (media ± s.e.) en las pruebas de alimentación (90 días) y digestibilidad (7 días). Los corderos se asignaron aleatoriamente a los siguientes 5 tratamientos dietarios sobre la base del peso corporal estratificado: 100% heno de pasto natural (NPH) (T1); 75% de NPH + 25% de heno de Mulato II *Urochloa* híbrido (MH) (T2); 50% NPH + 50% MH (T3); 25% NPH + 75% MH (T4); y 100% MH (T5). Se alimentó a todos los animales con una mezcla de concentrado local [300 g de materia seca (DM)/hd/d]. La concentración de proteína cruda (CP) de la dieta basal aumentó a medida que aumentó la proporción de MH en la ración (P <0.05). La ingesta de DM y nutrientes, y los coeficientes de digestibilidad de los nutrientes aumentaron significativamente (P <0.05) a medida que aumentaba la proporción de MH en el componente forrajero de la ración. El

Correspondence: Bimrew Asmare, School of Animal Science and Veterinary Medicine, Bahir Dar University, P.O. Box 5501, Bahir Dar, Ethiopia. E-mail: limasm2009@gmail.com

peso corporal final, la ganancia de peso corporal diaria promedio, la eficiencia de conversión alimenticia y la mayoría de los parámetros de la canal medidos fueron significativamente (P <0.05) mayores a medida que la proporción de MH aumentó de 0 a 100% en la dieta basal. Basándose en el desempeño biológico de los corderos experimentales, el desempeño de los ovinos en la región podría mejorarse significativamente incorporando MH con heno de pasto nativo y suplemento concentrado en las raciones de alimentación. Las evaluaciones económicas revelarían las combinaciones óptimas de pastos nativos y MH para la alimentación para lograr resultados particulares. Otras especies mejoradas de gramíneas y leguminosas pueden desempeñar el mismo papel y deben investigarse en diferentes entornos.

Palabras clave: Aumento de peso corporal, *Brachiaria*, digestibilidad, engorde de ovejas, pastos tropicales, rendimiento en canal.

Introduction

Ethiopia has the largest livestock population among African countries (CSA 2017) and livestock production is an important component of farming systems in all parts of the country, playing a vital role in the livelihood of many people (Tekliye et al. 2018). Sheep production is a major component of the livestock sector in Ethiopia owing to the large population of 30.7 million head (CSA 2017). However, the productivity of livestock in general and sheep in particular is below the African average, mainly due to inadequate supply of feed and poor feeding practices (Mekuriaw and Asmare 2018). To combat the livestock feed shortage, use of improved forage plants as a feed source is recommended (Shapiro et al. 2015; FAO 2016).

Cultivars of *Urochloa* (new generic name of *Brachiaria* species that have provided grass cultivars) are possible candidates to alleviate ruminant feed shortage and thereby enable the country to exploit the potential of livestock resources. Cultivars of this genus are the most extensively grown tropical forages in Latin America, Asia, South Pacific and Australia, with an estimated 99 million hectares in Brazil alone (Jank et al. 2014). Recently, there has been considerable interest in *Urochloa* grasses in Africa, and several initiatives are ongoing to promote the genus to support the emerging livestock industry in the region, especially during the dry season (Maass et al. 2015). Some *Urochloa* species are reported to have several advantages over other tropical grasses including: adaptation to drought and infertile soils; ability to sequester carbon; increased efficiency of nitrogen use through biological nitrification inhibition; and arresting emissions of greenhouse gas, such as methane (Njarui et al. 2016). Currently, *Urochloa* hybrid cv. Mulato II (MH) grass is being used in dual-purpose 'push-pull' technologies (Khan et al. 2014) and as livestock feed in Ethiopia (Adnew et al. 2018). However, there is limited information on performance of animals consuming *Urochloa* grasses, particularly cv. Mulato II, in Ethiopia. Our research hypothesis was that feed value of Mulato II hay would be at least equal to that of natural pasture hay and feed intake, DM digestibility and resultant bodyweight change and carcass characteristics of Farta sheep would be superior for Mulato II hay. Therefore, this study was conducted to evaluate feed intake, digestibility, bodyweight change and carcass characteristics of Farta sheep fed cv. Mulato II and natural pasture hays in different proportions as a basal diet supplemented with a local concentrate mixture.

Materials and Methods

Description of the study area

The experiment was conducted at the Woreta Agricultural College in Ethiopia (11°58′ N, 37°41′ E; 1,774 masl). The experimental area has an annual average temperature of 29°C and annual rainfall ranges from 1,103 to 1,336 mm.

Experimental sheep and their management

Twenty-five yearling intact male Farta sheep with mean body weight (BW) of 19.6±0.29 kg (mean±s.e.) were housed in individual pens equipped with feed and water troughs. Before the commencement of the experiment, sheep were quarantined for 21 days and treated with Ivermectin to control internal parasites and Diazanon to control external parasites. They were also vaccinated against anthrax and pasteurellosis, which are the most common sheep diseases of the area.

Experimental design, treatments and feeding management

The experimental design employed in this study was a randomized complete block design with 5 treatments and 5 replications. Sheep were allocated to treatments by stratified randomization on the basis of initial BW. The treatments used in the study were: 100% native pasture hay (NPH; T1); 75% NPH+25% Mulato II hay (MH; T2); 50% NPH+50% MH (T3); 25% NPH+75% MH (T4); and

100% MH (T5) with equal amounts of concentrate supplement throughout [300 g dry matter (DM)/hd/d]. The concentrate mixture consisted of noug (*Guizotia abissynica*) seed cake and wheat bran in equal proportions. The sheep were offered the basal diet *adlibitum* with amount fed adjusted weekly to allow a 25% refusal. All sheep were fed in individual feed troughs. The natural pasture hay was obtained from farmers and consisted of a mixture of mainly *Andropogon* and *Cynodon* grasses with some contribution by native legumes (*Trifolium* and *Medicago*) (Yalew et al. 2020). The natural pasture was harvested at about 50% heading of the grass according to smallholder farmers' usage. Mulato II grass had been fertilized with diammonium phosphate (DAP) and nitrogen during establishment and was harvested at 4 months of age where it was fully mature and dried under shade.

Feed intake and body weight measurements

Daily feed offered and refusals were recorded for each lamb throughout the study and daily feed intake was calculated as the difference between the amounts offered and refused. Subsamples of feed offered and refusals were taken from each treatment and prepared for chemical analysis. The BW of each animal was measured every 10 days after overnight fasting (without feed only). Initial BW of experimental lambs was measured at the end of the quarantine/adaptation period, while final BW was measured at the end of the feeding trial. Daily BW gain was calculated as the difference between final BW and initial BW divided by the number of feeding days. Feed conversion efficiency (FCE) of experimental animals was determined by dividing the average daily feed consumed by daily BW gain of animals to give amount of feed consumed per unit of weight gained.

The digestibility trial was conducted at the end of the feeding trial. Each sheep was fitted with a fecal collection bag and a 4-day acclimatization period was followed by total collection of feces for 7 consecutive days. The parameters studied were: dry matter intake; dry matter digestibility; organic matter digestibility; and digestibility of individual nutrients. Feces voided were weighed and recorded each morning and thoroughly mixed, before a 20% representative sample was taken, frozen at -10°C and pooled over the collection period for each animal. At the end of the collection period, samples were pooled and mixed thoroughly for each sheep before drying at 60°C for 72 h. The digestibility of individual nutrients was determined as the difference between intake of the nutrient and that recovered in the feces expressed as a proportion of intake of the nutrient.

$$\text{Apparent digestibility \%} = \frac{\text{Nutrient intake} - \text{Nutrient in feces}}{\text{Nutrient intake}} \times 100$$

Carcass evaluation

At the end of the digestibility trial, all experimental lambs were slaughtered after overnight fasting for evaluation of carcass parameters using a standard slaughtering method. Pre-slaughter weight (PSW) of each lamb was recorded immediately before slaughtering. Hot carcass weight (HCW) was measured after removal of blood, skin, head, feet (legs below the hock and knee joints), gastrointestinal tract and internal organs. Based on feeding habits of people in the area, edible and non-edible offals were categorized and recorded. Blood, heart, liver, kidney, tongue, reticulo-rumen, omasum-abomasum, hind-gut, tail fat, kidney fat, pelvic fat, omental and mesenteric fat, testicles and pancreas were weighed and recorded as total edible offals (TEO), while head without tongue, skin, penis, feet, lungs with trachea, spleen, gall bladder with bile, oesophagus, bladder and gut contents were weighed and recorded as total non-edible offals. Total usable product was taken as the sum of HCW, skin and TEO. Empty body weight (EBW) was calculated as the difference between PSW and gut contents. Dressing percentage (DP) was calculated as HCW as a proportion of both PSW and EBW. To measure rib eye-area (REA) of the carcass, the loin part was partitioned into fore- and hindquarters between the 11th and 12th ribs. The cut ribs were chilled for 12 hours and the rib-eye area was measured at the 11–12th rib site (O'Rourke et al. 2005) by tracing first on transparent plastic paper before the traced paper was positioned on graph paper with 1×1mm squares. The number of squares included within the traced area was counted for left and right sides and rib-eye area was computed as the average of the two.

Chemical analysis

Samples of feed, refusals and feces were dried and ground using a laboratory mill to pass through a 1 mm sieve. The DM content was determined by oven-drying samples at 105 °C and organic matter (OM) percentage as the difference between DM percentage and ash percentage. Total nitrogen (N) was determined by the Kjeldahl method (AOAC 1990). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) concentrations were determined by the method of Van Soest and Robertson (1985).

Statistical analysis

The data obtained from the experiment were subjected to analysis of variance (ANOVA) using the general linear model procedure of SAS version 9.2 for least square ANOVA. Mean separations were done using polynomial contrasts for variables whose F-values declared a significant difference. Differences were considered statistically significant at $P<0.05$. The statistical model for data analysis was:

$$Y_{ij}= \mu+t_i+b_j+e_{ijk},$$

where:

Y_{ij} is the response variable;
μ is the overall mean;
t_i is the treatment effect;
b_j is the block effect; and
e_{ijk} is the random error.

Results

Chemical composition of feeds

The chemical composition of feeds used in the experiment is presented in Table 1. The natural pasture hay had crude protein (CP) concentration of 6.7%, while the Mulato II hay contained 12.1% CP. As percentage of Mulato II hay in the roughage increased, CP% increased progressively. With fiber and lignin concentrations, the opposite was the case with highest levels in 100% NPH and lowest in 100% MH. The concentrate mixture was a conventional supplement with 22.1% CP.

Dry matter and nutrient intake

The daily DM and nutrient intakes of sheep in the various treatments are presented in Table 2. Total DM and CP intakes increased progressively as proportion of Mulato II in the diet increased ($P<0.001$).

Bodyweight change and feed conversion efficiency

Bodyweight changes, average daily gain and FCE are presented in Table 3. Average daily gain (ADG) during the feeding period increased from 39.6 g/d for the 100% NPH ration to 82.7 g/d for the 100% MH ration ($P<0.001$). Correspondingly, FCE improved progressively from 18.5 g dry matter intake (DMI)/g gain for the NPH ration to 10.3 g DMI/g gain for the MH ration ($P<0.05$), indicating a marked improvement in efficiency of utilization of the rations as proportion of Mulato II in the ration increased.

Dry matter and nutrient digestibility

Table 4 presents the apparent-digestibility coefficients of DM and individual nutrients for the various rations. DM digestibility of the rations increased progressively as the proportion of MH in the rations increased from 62.0% for 100% NPH to 79.0% for 100% MH ($P<0.05$), while CP digestibility increased from 64.0 to 83.0% for the corresponding treatments ($P<0.05$). Similar increases in digestibility of NDF (64.0 to 82.0%) and ADF (63.0 to 79.0%) ($P<0.05$) were recorded for the relevant treatments.

Carcass evaluation

Pre-slaughter weight, empty body weight, hot carcass weight and dressing percentage. Final BW weights and carcass parameters of the experimental animals are presented in Table 5. Pre-slaughter body weight (PSW) mirrored the final BW for the various treatment groups, ranging from 22.5 kg for 100% NPH to 28.8 kg for 100% MH ($P<0.001$) as did EBW (18.0 to 23.1 kg; $P<0.01$). In a similar fashion, hot carcass weight (HCW) increased progressively as MH proportion in the ration increased from 9.00 kg for 100% NPH to 12.42 kg for 100% MH ($P<0.001$). Rib-eye area also increased from 6.06 cm^2 for 100% NPH to 8.28 cm^2 for 100% MH ($P<0.05$).

Edible and non-edible carcass offals

Total edible offal (TEO) also increased progressively from 3.70 kg for 100% NPH to 4.42 kg for 100% MH ($P<0.05$), as did total non-edible offal (9.22 to 10.04 kg) but differences between treatments were not significant ($P>0.05$). In this study, blood, heart, kidney, tongue, omasum-abomasum, hind-gut, tail fat, kidney fat, pelvic fat, omental and mesenteric fat, testicles, pancreas, skin, spleen, gall bladder with bile, bladder and gut contents showed increasing trends as proportion of MH in the ration increased but differences between treatments failed to reach significance ($P>0.05$; Table 6). However, increases in weight of liver and reticulo-rumen were significant ($P<0.05$). For non-edible offals, penis, feet and lungs with trachea increased linearly ($P<0.05$) with increasing proportion of Mulato II hay in the ration.

Table 1. Chemical composition of 4-month-old roughage ingested by Farta sheep fed natural pasture (NPH) and Mulato II (MH) hays alone and in various combinations along with a concentrate mixture (MH).

Treatment[1]	Parameter (%)						
	DM	Ash	OM	CP	NDF	ADF	ADL
T1	94	11.6	88.4	6.7	80.0	66.0	16.5
T2	92	11.6	88.4	7.9	75.3	63.5	15.7
T3	92	11.5	88.5	9.4	74.8	62.2	15.6
T4	93	12.4	87.6	10.5	70.1	59.2	14.9
T5	93	13.5	86.5	12.1	60.1	57.6	14.7
Conc.mix[2]	90.6	8.5	91.5	22.1	37.1	24.3	9.4

DM = dry matter; OM = organic matter; CP= crude protein; NDF= neutral detergent fiber; ADF= acid detergent fiber; ADL= acid detergent lignin; Conc. mix= concentrate mixture.
[1]T1 = 100% NPH; T2 = 75% NPH + 25% MH; T3 = 50% NPH + 50% MH; T4 = 25% NPH + 75% MH; T5 = 100% MH.
[2]A concentrate mixture of 50%noug seed cake: 50% wheat bran was fed at 300 g DM/hd/d on all treatments.

Table 2. Daily dry matter and nutrient intakes of Farta sheep fed natural pasture (NPH) and Mulato II (MH) hays alone and in various combinations along with a concentrate mixture.

Parameter	Treatment					s.e.m.	Sig.	P
	T1[1]	T2	T3	T4	T5			
Total DM intake (g/hd/d)	732d[2]	764c	774c	807b	851a	8.71	***	<0.001
DM intake (% BW)	3.08	3.19	3.31	3.25	3.31	0.00	NS	0.62
OM intake (g/hd/d)	384d	411c	417c	444b	476a	6.79	***	<0.001
CP intake (g/hd/d)	28.4e	36.7d	44.2c	53.1b	66.8a	2.72	***	<0.001
NDF intake (g/hd/d)	346	350	352	356	358	2.32	NS	0.08
ADF intake (g/hd/d)	285b	290b	293b	300ab	317a	3.02	**	0.001

DM = dry matter; OM = organic matter; CP= crude protein; NDF= neutral detergent fiber; ADF= acid detergent fiber; ADL= acid detergent lignin; Conc. mix= concentrate mixture.
[1]T1 = 100% NPH; T2 = 75% NPH + 25% MH; T3 = 50% NPH + 50% MH; T4 = 25% NPH + 75% MH; T5 = 100% MH. A concentrate mixture of 50% noug seed cake: 50% wheat bran was fed at 300 g DM/hd/d on all treatments.
[2]Means within rows with different letters are significantly different.

Table 3. Bodyweight parameters and feed conversion efficiency of Farta sheep fed natural pasture (NPH) and Mulato II (MH) hays alone and in various combinations along with a concentrate mixture.

Parameter	Treatment[1]					s.e.m.	Sig.	P
	T1	T2	T3	T4	T5			
Initial BW (kg)	19.0	19.8	19.2	19.6	20.2	0.29	NS	0.19
Final BW (kg)	22.6b[2]	24.5ab	25.6ab	26.2a	28.0a	0.51	***	<0.001
ADG (g/d/h)	39.6c	52.4bc	71.1ab	73.8ab	82.7a	4.09	**	<0.01
FCE	18.5a	14.6b	10.9c	10.9c	10.3c	0.005	***	<0.001

BW = body weight; ADG = average daily gain; FCE = feed conversion efficiency.
[1]T1 = 100% NPH; T2 = 75% NPH + 25% MH; T3 = 50% NPH + 50% MH; T4 = 25% NPH + 75% MH; T5 = 100% MH. A concentrate mixture of 50% noug seed cake: 50% wheat bran was fed at 300 g DM/hd/d on all treatments.
[2]Means within rows with different letters are significantly different. [3]FCE = DMI/ADG, i.e., g of DMI/g gain.

Table 4. Dry matter and nutrient digestibility coefficients for Farta sheep fed natural pasture (NPH) and Mulato II (MH) hays alone and in various combinations along with a concentrate mixture.

Digestibility coefficient	Treatment[1]					s.e.m.	Sig.	P
	T1	T2	T3	T4	T5			
DM (%)	62.0c[2]	65.0c	74.0abc	76.0ab	79.0a	0.02	***	<0.001
OM (%)	65.0b	69.0ab	74.0ab	76.0ab	79.0a	0.01	***	<0.001
CP (%)	64.0b	72.0ab	75.0ab	77.0ab	83.0a	0.02	***	<0.001
NDF (%)	64.0b	69.0ab	76.0ab	77.0ab	82.0a	0.02	***	<0.001
ADF (%)	63.0b	66.0b	75.0ab	73.0ab	79.0a	0.02	**	0.01

DM = dry matter; OM = organic matter; CP= crude protein; NDF= neutral detergent fiber; ADF= acid detergent fiber.
[1]T1 = 100% NPH; T2 = 75% NPH + 25% MH; T3 = 50% NPH + 50% MH; T4 = 25% NPH + 75% MH; T5 = 100% MH. A concentrate mixture of 50% noug seed cake: 50% wheat bran was fed at 300 g DM/hd/d on all treatments.
[2]Means within rows with different letters are significantly different (P<0.05).

Table 5. Carcass characteristics of Farta sheep fed natural pasture (NPH) and Mulato II (MH) hays alone and in various combinations along with a concentrate mixture.

Parameter	Treatment[1]					s.e.m.	Sig.	P
	T1	T2	T3	T4	T5			
PSW (kg)	22.50c[2]	23.76bc	24.50bc	26.60ab	28.83a	0. 65	***	<0.001
EBW (kg)	18.03c	20.27bc	20.17bc	21.50ab	23.70a	0.56	***	<0.001
HCW (kg)	9.00d	9.83cd	10.43bc	11.20b	12.42a	0.33	***	<0.001
Dressing percentage								
PSWB	40.0	41.4	42.6	42.1	43.2	0.41	*	<0.01
EBWB	49.9	48.7	51.7	49.2	52.5	0.84	NS	0.39
TEO (kg)	3.70b	3.94ab	3.94ab	4.22a	4.42a	0.08	***	<0.001
TNEO (kg)	9.22	9.00	9.32	9.93	10.04	0.21	NS	0.13
REA (cm^2)	6.06b	6.60ab	7.01ab	7.52ab	8.28a	0.25	***	<0.001

PSW = pre-slaughter weight; EBW =empty body weight; HCW =hot carcass weight; PSWB =pre-slaughter weight-basis; EBWB = empty body weight-basis; TEO =total edible offal; TNEO = total non-edible offal; REA = rib-eye area.

[1]T1 = 100% NPH; T2 = 75% NPH + 25% MH; T3 = 50% NPH + 50% MH; T4 = 25% NPH + 75% MH; T5 = 100% MH. A concentrate mixture of 50% noug seed cake: 50% wheat bran was fed at 300 g DM/hd/d on all treatments.

[2]Means within rows with the same letter are not significantly different (P>0.05).

Table 6. Edible and non-edible offal components of Farta sheep fed natural pasture (NPH) and Mulato II (MH) hays alone and in various combinations along with a concentrate mixture.

Parameter	Treatment[1]					s.e.m.	Sig.	P
	T1	T2	T3	T4	T5			
Edible offals								
Blood (mL)	933	1,033	900	1,100	1,000	35.8	NS	0.45
Heart (g)	86.1	83.3	97.7	108.1	105.5	3.9	*	0.02
Liver (g)	230b[2]	290a	293a	289ab	340a	10.5	**	0.00
Kidney(g)	54.1	68.0	65.1	70.0	74.1	2.47	*	0.01
Tongue (g)	141	128	127	111	113	4.7	*	0.04
Reticulo-rumen (g)	546b	522b	574ab	609ab	650a	14.4	**	0.00
Omasum-abomasum (g)	189	191	197	203	220	6.0	NS	0.51
Hind-gut (g)	719	775	812	825	943	27.8	*	0.01
Tail fat (g)	433	440	474	491	501	19.6	NS	0.25
Kidney fat (g)	27.9	26.7	21.3	30.7	27.8	3.64	NS	0.90
Pelvic fat (g)	32.0	25.7	28.9	30.7	36.3	2.04	NS	0.39
Omental and mesenteric fat (g)	57.2	55.1	62.2	62.8	79.3	6.04	NS	0.29
Testicles (g)	230	269	254	261	292	10.6	NS	0.16
Pancreas (g)	26.3	32.4	28.3	31.8	34.5	1.22	NS	0.07
Non-edible offals								
Head without tongue (g)	1,450b	1,495ab	1,401b	1,693a	1,485ab	32.0	NS	0.10
Skin (kg)	1.97	2.07	2.13	2.00	2.27	0.040	*	0.05
Penis (g)	38.1b	49.5ab	44.7ab	43.5ab	66.2a	3.26	*	0.01
Feet (g)	536b	617ab	634ab	621ab	666a	14.1	*	0.00
Lungs with trachea (g)	308b	368ab	317b	340b	466a	17.7	**	0.00
Spleen (g)	40.2	40.0	41.7	35.1	54.8	4.27	NS	0.47
Gall bladder with bile (g)	16.5	20.4	7.3	10.2	18.4	2.48	NS	0.72
Oesophagus (g)	36.5	40.6	45.1	39.6	40.5	1.79	NS	0.62
Bladder (g)	32.8	37.4	26.6	35.5	45.8	3.74	NS	0.41
Gut contents (kg)	4.80	4.27	4.67	5.10	4.93	0.20	NS	0.48

[1]T1 = 100% native pasture hay (NPH); T2 = 75% NPH + 25% Mulato II hay (MH); T3 = 50% NPH + 50% MH; T4 = 25% NPH + 75% MH; T5 = 100% MH. A concentrate mixture of 50% noug seed cake: 50% wheat bran was fed at 300 g DM/hd/d as well.

[2]Means within rows with the same letter are not significantly different (P>0.05).

Discussion

This study has shown the potential benefits of incorporating Mulato II hay with natural pasture hay in preparing rations fed to sheep in Ethiopia, in company with a concentrate supplement. Inclusion of MH in the ration increased performance from moderate weight gains (about 40 g/hd/d) to gains in excess of 80 g/hd/d. The increase in weight gains resulted in heavier sheep at slaughter with higher carcass and offal weights as well as higher levels of fatness of the sheep.

Chemical composition of feeds

The CP concentration of natural pasture hay alone was higher than the CP concentration (3.5% CP) in natural pasture hay reported by Asmare et al. (2016) in northwestern Ethiopia. This disparity in CP concentration could be a function of many factors, e.g., location, soil type, species composition, time of harvesting and post-harvest handling. Nevertheless, CP concentration in the natural pasture hay used in the current study was lower than the 7–7.5% required satisfying the maintenance requirement of ruminants (Van Soest 1982); thus, protein supplement is needed if weight gains are to be achieved.

In contrast, CP concentration in the Mulato II hay could be considered adequate to support weight gains in sheep, even in the absence of the concentrate supplement, as demonstrated by the increase in daily weight gains as proportion of MH in the ration increased. CP concentrations in the diet in excess of 7% ensure a sufficient supply of N for proper functioning of rumen microbes and to meet maintenance requirements of animals and support weight gains (Van Soest 1994), provided other factors such as lignification do not limit feed digestibility and nutrient utilization. The high NDF concentration in native pasture hay (80%) places it as a low-quality feed, since roughage with NDF concentration greater than 65% is categorized in that way (Van Saun 2006). According to McDonald et al. (2010), the NDF portion of feed is only partially digestible by any species of animals, but can be used to a greater extent by ruminants, which depend on microbial digestion for utilization of most fibrous plant components.

Dry matter and nutrient intake

Intakes of natural pasture hay and Mulato II hay obtained in the current study were higher than the values reported for natural pasture hay (Ephrem et al. 2015) plus concentrate supplement, natural pasture hay supplemented with graded levels of *Ficus thonningii* (local name: *Chibha*) leaves and a concentrate supplement (Mekuriaw and Asmare 2018) and Rhodes grass plus a concentrate supplement consumed by Washera sheep (Tefera et al. 2015). Higher intakes of natural pasture hay and Mulato II observed in the current study are probably related to the better overall quality of the roughage component of the ration. The increase in DM intake with increasing level of Mulato II hay in the rations would be a consequence of the higher DM digestibility of the Mulato II hay. As digestibility of DM increases, the rate of passage of ingesta through the digestive tract increases, allowing the animal to consume more feed. Hence mean total DM intake increased significantly ($P<0.05$) from T1 to T5 as the proportion of the more digestible forage, Mulato II hay, in the roughage component of the ration increased. The higher CP concentration of the Mulato II hay would ensure adequate N supply for the microbial population. As Negesse et al. (2001) confirmed, DM intake of sheep increases as the level of CP in the diet increases. The total DM intake expressed as percent of BW obtained from the current study agrees with the results of different authors (Mekuriaw and Asmare 2018; Asmare et al. 2016; Tekliye et al. 2018) and fell within the range of recommended DM intakes of ruminants (2 to 6%) (ARC 1980; Devendra and Burns 1983). DM intake is an important factor in the utilization of roughage by ruminant livestock and is a critical determinant of nutrient and energy intake and performance in small ruminants (ARC 1980). Intake of available energy is a vital factor determining performance of livestock and high DM intakes are essential to achieve high rates of weight gain.

Nutrient digestibility

The significant increase in apparent digestibility of DM and of nutrients as level of Mulato II hay increased from 0 to 100% would be related to the higher CP concentration in Mulato II hay relative to natural pasture hay as well as the lower levels of fiber in the Mulato II hay. The CP digestibility of the various rations in the current study increased significantly as the proportion of Mulato II hay in the ration increased as a reflection of the superior quality of the Mulato II hay and the more active rumen microflora in the presence of additional nutrients, especially N. McDonald et al. (2010) and Negesse et al. (2001) reported that feed which is rich in protein promotes high microbial population, which in turn facilitates rumen fermentation. The increase in digestibility of nutrients with increasing levels of Mulato II in the current study agrees with the findings of Asmare et al. (2016) for Washera sheep fed increasing proportions of desho grass in a native pasture basal diet supplemented with local concentrate mixtures, where digestibility of nutrients increased as proportion of desho grass hay in the basal diet increased.

Body weight and feed conversion efficiency

Better performance of sheep in terms of higher average daily gain as the proportion of Mulato II in the ration increased is a function of a higher intake of a better-quality ration, hence greater nutrient intake. The results of this study agree with the report of Asmare et al. (2016) in which sheep fed on a high protein (208 g/kg DM) diet showed high liveweight gain (36.6 g/d), whereas those fed on a low protein (106 g/kg DM) diet gained only 10.7 g/d. The improvement in FCE as proportion of Mulato II in the ration increased in the current study merely reflects the increasing nutrient concentration in the diet, the higher feed intakes and the consequent increase in bodyweight gains which result. The poor FCE of sheep in the Control treatment (T1) was probably the result of low protein intake and high fiber concentration of the native pasture hay diet, resulting in a greater proportion of feed consumed being required for maintenance, leaving fewer nutrients to support weight gains.

Slaughter weight, hot carcass weight and dressing percentage

In the current study, increases in bodyweight change as proportion of Mulato II in the ration increased were reflected in increases in carcass weight as well. The highest pre-slaughter body weight was recorded in T5 (28.83kg) as was the highest carcass weight (12.42 kg). Dressing percentage also increased as the level of Mulato II in the ration increased despite the weight of some offal components also being directly related to the proportion of Mulato II in the ration. One contributing factor was the absence of any significant difference in weight of gut contents between the different treatments despite animals in T5 eating 16% more dry matter daily than the 100% native pasture hay group and being 28% heavier pre-slaughter. The absence of a greater quantity of gut contents despite greater intake would be a function of faster rate of passage of ingesta in treatments with higher levels of MH. The increase in rib-eye area with increase in level of Mulato II in the ration in the present study (from 6.06 to 8.28 cm^2) was not unexpected as rib-eye area is affected by the weight and muscularity of the live animal (O'Rourke et al. 2005) and increases with carcass weight (Park et al. 2002).

Edible and non-edible carcass offals

In different parts of the country, different proportions of the internal offal including blood are both edible and saleable and are a source of additional income that could add value to the carcass. Due to differences in the eating habits of people in different areas, edible and saleable portions of the offal scan vary depending on the location (Legesse 2001). In the current study, among the offal components classed as edible, the weights of liver and reticulo-rumen increased with increase in Mulato II inclusion in the ration. Similarly, Alemu et al. (2014) and Gebreyohannes et al. (2003) observed significant increase in liver weight with concentrate supplementation. In addition, Lawrence and Fowler (1998) reported an increase in liver weight following supplementation, which they attributed to the storage of reserve substances such as glycogen in the liver in supplemented sheep.

Conclusion

The high CP and relatively lower fiber fractions of Mulato II as produced for this study make it a valuable source of nutrients for feeding to local sheep in Ethiopia. Incorporating it with native pasture hay in conjunction with a concentrate supplement can allow a given amount of this valuable feed to be used to improve the performance of many more sheep than if fed as the sole diet. Other improved species of grass and legumes could provide a similar result and should be evaluated in this and other regions to ensure a wide range of potential sources of fodder.

Conflict of Interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Acknowledgments

The first author is grateful to Bahir Dar University for funding the research work under grant code BDU/RCS/SC02/2010. He also extends his thanks to Fogera National Rice Research Institute, Farta and Tach Gayint district agricultural offices for their permission to use the respective experimental sites, and appreciates the contribution of Mr Bamlaku Getie, Getachew Molla and Yilak Hulgezie for their assistance during data collection in the field.

References

(Note of the editors: All hyperlinks were verified 28 April 2021.)

Adnew W; Tsegay BA; Tassew A; Asmare B. 2018. Assessments of farmers′ perception and utilization status of *Brachiaria* grass in selected areas of Ethiopia. Biodiversitas Journal of Biological Diversity 19:951–966. doi: [10.13057/biodiv/d190326](https://doi.org/10.13057/biodiv/d190326)

Alemu B; Animut G; Tolera A. 2014. Effect of *Millettia ferruginea* (Birbra) foliage supplementation on feed intake, digestibility, body weight change and carcass characteristics of Washera sheep fed natural pasture grass hay basal diet. Springer Plus 3:50. doi: [10.1186/2193-1801-3-50](https://doi.org/10.1186/2193-1801-3-50)

AOAC (Association of Analytical Chemists). 1990. Official methods of analysis. 15th Edn. AOAC Inc., Arlington, VA, USA.

ARC (Agricultural Research Council). 1980. The nutrient requirements of ruminant livestock. Technical Review by an Agricultural Research Council Working Party.

Asmare B; Demeke S; Tolemariam T; Tegegne F; Wamatu J; Rischkowsky B. 2016. Evaluation of desho grass (*Pennisetum pedicellatum*) hay as a basal diet for growing local sheep in Ethiopia. Tropical Animal Health and Production 48:801–806. doi: [10.1007/s11250-016-1031-8](https://doi.org/10.1007/s11250-016-1031-8)

CSA (Central Statistical Agency). 2017. Agricultural Sample Survey. Livestock and Livestock Characteristics (Private Peasant Holdings).Central Statistical Agency, Addis Ababa, Ethiopia. Statistical bulletin 585:33–35.

Devendra C; Burns M.1983. Goat production in the tropics. Commonwealth Agricultural Bureaux, Farnham Royal, UK.

Ephrem N; Tegegne F; Mekuriaw Y; Yiheyis L. 2015. Nutrient intake, digestibility and growth performance of Washera lambs supplemented with graded levels of sweet blue lupin (*Lupinus angustifolius* L.) seed. Small Ruminant Research 130:101–107. doi: [10.1016/j.smallrumres.2015.07.019](https://doi.org/10.1016/j.smallrumres.2015.07.019)

FAO (Food and Agriculture Organization). 2016. In: Jirata M; Grey S;Kilawe E, eds. Ethiopia Climate-Smart Agriculture Scoping Study. FAO, Addis Ababa, Ethiopia.

Gebreyohannes G; Geleti D; Gizachew L; Gojam Y; Gemeda D. 2003. Effect of noug cake and *Sesbania sesban* supplementation on the growth performance and carcass characteristics of Horro rams. In: Proceedings of the 10th Annual Conference of the Ethiopian Society of Animal Production (ESAP) held in Addis Ababa, Ethiopia. p. 335–339.

Jank L; Barrios SC; Valle CB do; Simeão RM; Alves GF. 2014. The value of improved pastures to Brazilian beef production. Crop Pasture Science 65: 1132–1137. doi: [10.1071/CP13319](https://doi.org/10.1071/CP13319)

Khan ZR; Midega CAO; Pittchar JO; Alice WM; Michael AB; Toby JA; Bruce TJA; Picket JA. 2014. Achieving food security for one million sub-Saharan African poor through push-pull innovation by 2020. Philosophical Transactions of the Royal Biological Society 369: 20120284. doi: [10.1098/rstb.2012.0284](https://doi.org/10.1098/rstb.2012.0284)

Lawrence TJ; Fowler VR. 1998. Growth of farm animals. CABI, Wallingford, UK.

Legesse G. 2001. Growth pattern and carcass characteristics of Somali and Mid-Rift valley goats. M.Sc. Thesis. Haramaya University, Haramaya, Ethiopia.

Maass BL; Midega AO; Mutimura M; Volatsara BR; Paulo S; Jolly MK; Zeyaur R; Sita RG; Idupulapati MR. 2015. Homecoming of *Brachiaria*: Improved hybrids prove useful for African animal agriculture. East African Agriculture and Forestry Journal 81:71–78. doi: [10.1080/00128325.2015.1041263](https://doi.org/10.1080/00128325.2015.1041263)

McDonald P; Edwards RA; Greenhalgh JD; Morgan CA; Sinclair LA; Wilkinson RG. 2010. Animal Nutrition.7th Edn. Longman Group UK Ltd, Harlow, UK.

Mekuriaw Y; Asmare B. 2018. Nutrient intake, digestibility and growth performance of Washera lambs fed natural pasture hay supplemented with graded levels of *Ficus thonningii* (*Chibha*) leaves as replacement for concentrate mixture. Agriculture Food Security 7:30. doi: [10.1186/s40066-018-0182-4](https://doi.org/10.1186/s40066-018-0182-4)

Negesse T; Rodehutscord M; Pfetter E. 2001. The effect of dietary crude protein level on intake, growth, protein retention and utilization of growing male Saanen kids. Small Ruminant Research 39:243–251. doi: [10.1016/S0921-4488(00)00193-0](https://doi.org/10.1016/S0921-4488(00)00193-0)

Njarui DMG; Gichangi EM; Ghimire SR; Muinga RW. 2016. Climate Smart Brachiaria Grasses for Improving Livestock Production in East Africa – Kenya Experience. Proceedings of the workshop held in Naivasha, Kenya, 14 –15 September 2016.

O'Rourke B; Russell R; Buege D. 2005. Lamb carcass evaluation. UW- Madison, Department of Animal Sciences. [Accessed on 21 March 2018]. Fact sheet.

Park GB; Moon SS; Ko YD; Ha JK; Lee JG; Chang HH; Joo ST. 2002. Influence of slaughter weight and sex on yield and quality grades of Hanwoo (Korean native cattle) carcasses. Journal of Animal Science 80:129–136. doi: [10.2527/2002.801129x](https://doi.org/10.2527/2002.801129x)

Shapiro BI; Gebru G; Desta S; Negassa A; Negussie K; Aboset G;Mechal H. 2015. Ethiopia livestock master plan, 2015. ILRI Project Report. International Livestock Research Institute (ILRI), Nairobi, Kenya.

Tefera G; Tegegne F; Mekuriaw Y; Melaku S; Tsunekawa A. 2015. Effects of different forms of white lupin (*Lupinus albus*) grain supplementation on feed intake, digestibility, growth performance and carcass characteristics of Washera sheep fed Rhodes grass (*Chloris gayana*) hay-based diets. Tropical Animal Health and Production 47:1581–1590. doi: [10.1007/s11250-015-0901-9](https://doi.org/10.1007/s11250-015-0901-9)

Tekliye L; Mekuriaw Y; Asmare B; Mehret F. 2018. Nutrient intake, digestibility, growth performance and carcass characteristics of Farta sheep fed urea treated rice straw supplemented with graded levels of dried *Sesbania sesban* leaves. Agricultural Food Security 7(77)1–10. doi: [10.1186/s40066-018-0226-9](https://doi.org/10.1186/s40066-018-0226-9)

Van Saun RJ. 2006. Determining forage quality: understanding feed analysis. Lamalink.com 3(8):18–19.

Van Soest PJ. 1982. Nutritional ecology of the ruminant. O and B Books Inc., Corvallis, OR, USA.

Van Soest PJ. 1994. Nutritional ecology of the ruminant. Cornell University Press, Ithaca, NY, USA and London, UK.

Van Soest PJ; Robertson JB. 1985. Analysis of forage and fibrous food. A laboratory manual for Animal Science 613. Cornell University, Ithaca, NY, USA.

Yalew S; Asmare B; Mekuriaw Y. 2020. Effects of fertilizer type and harvesting age on species composition, yield and chemical composition of natural pasture in the highlands of Ethiopia. Biodiversitas Journal of Biological Diversity 21(11):4009–5007. doi: 10.13057/biodiv/d211103

(Received for publication 15 September 2020; accepted 27 March 2021; published 31 May 2021)

doi: 10.17138/TGFT(9)216-224

Research Paper

Effects of inter-cropping lablab (*Lablab purpureus*) with selected sorghum (*Sorghum bicolor*) varieties on plant morphology, sorghum grain yield, forage yield and quality in Kalu District, South Wollo, Ethiopia

*Efectos de intercalar lablab (*Lablab purpureus*) con variedades seleccionadas de sorgo (*Sorghum bicolor*) en la morfología de las plantas y el rendimiento y calidad del forraje en Kalu District, South Wollo, Etiopía*

RAHEL KAHSAY[1], YESHAMBEL MEKURIAW[2] AND BIMREW ASMARE[2]

[1]*South Wollo Zone Technical, Vocational and Enterprises Development Office, Dessie, Ethiopia.*
[2]*School of Animal Science and Veterinary Medicine, Bahir Dar University, Bahir Dar, Ethiopia.* bdu.edu/caes

Abstract

An experiment was conducted to determine effects of inter-cropping lablab (*Lablab purpureus*) with 3 selected early-maturing sorghum (*Sorghum bicolor*) varieties (Teshale, Girana-1 and Misikir) on plant morphology, sorghum grain and forage yield and quality plus yield and quality of lablab forage, and to assess farmers' perceptions of the crops in Kalu District, South Wollo, Ethiopia. Seven treatments, namely: T1 - sole lablab (SL); T2 - Teshale + lablab (TL); T3 - Girana-1 + lablab (GL); T4 - Misikir + lablab (ML); T5 - sole Teshale (ST); T6 - sole Girana-1 (SG); and T7 - sole Misikir (SM), were used with 3 replications in a randomized complete block design. The data collected from sorghum varieties were: plant height, number of leaves per plant, leaf area, dry biomass yield and grain yield; and for lablab was: plant height, number of leaves per plant, leaf area, number of branches per plant, number of nodules per plant and dry biomass yield. Grain yield was determined on sorghum at maturity, while lablab was harvested at 50% flowering. Inter-cropped Girana-1 produced yields of both grain and stover and lablab forage similar to those for pure stands of the 2 crops, while inter-cropping of Teshale and Misikir with lablab reduced height, grain and stover yields of sorghum and yields of lablab forage ($P<0.05$). However, crude protein concentration in sorghum stover was enhanced when grown as an inter-crop with lablab ($P<0.05$). Land equivalent ratios for inter-crop treatments were 54–87% higher than those for pure stands. Farmers readily identified the combination Girana-1 + lablab as superior to the other associations. While farmers can improve productivity of their farms by inter-cropping these sorghum varieties, preferably Girana-1, with lablab, more studies should be conducted to determine benefits from sowing other legumes with sorghum. Any improvements in soil N levels from planting the legumes should be quantified.

Keywords: Cropping system, forage biomass, forage cropping, farmers' perceptions.

Resumen

Se realizó un experimento para determinar los efectos de intercalar lablab (*Lablab purpureus*) con 3 variedades seleccionadas de sorgo (*Sorghum bicolor*) de maduración temprana (Teshale, Girana-1 y Misikir) sobre la morfología de la planta, el

Correspondence: Bimrew Asmare, School of Animal Science and Veterinary Medicine, Bahir Dar University, PO Box 5501, Bahir Dar, Ethiopia. Email: limasm2009@gmail.com

rendimiento y la calidad del grano, calidad del forraje, más el rendimiento. y la calidad del forraje de lablab, y evaluar las percepciones de los agricultores sobre los cultivos en el distrito de Kalu, South Wollo, Etiopía. Siete tratamientos, a saber: T1 - lablab solo (SL); T2 - Teshale + lablab (TL); T3 - Girana-1 + lablab (GL); T4 - Misikir + lablab (ML); T5 - solo Teshale (ST); T6 - solo Girana-1 (SG); y T7 - solo Misikir (SM), se utilizaron con 3 repeticiones en un diseño de bloques completos al azar. Los datos recolectados de las variedades de sorgo fueron: altura de planta, número de hojas por planta, área foliar, rendimiento de biomasa seca y rendimiento de grano; y para lablab fue: altura de planta, número de hojas por planta, área foliar, número de ramas por planta, número de nódulos por planta y rendimiento de biomasa seca. El rendimiento de grano se determinó en sorgo en la madurez, mientras que el lablab se cosechó al 50% de floración. El cultivo intercalado de Girana-1 produjo rendimientos tanto de grano como de rastrojo y forraje de lablab similares a los de los rodales puros de los 2 cultivos, mientras que el cultivo intercalado de Teshale y Misikir con lablab redujo la altura, los rendimientos de grano y rastrojo de sorgo y los rendimientos de forraje (P <0.05). Sin embargo, la concentración de proteína cruda en el rastrojo de sorgo aumentó cuando se cultivó como una siembra intercalada con lablab (P <0.05). Las proporciones de equivalentes de tierra para los tratamientos entre cultivos fueron 54–87% más altas que las de los rodales puros. Los agricultores identificaron fácilmente la combinación Girana-1 + lablab como superior a las otras asociaciones. Si bien los agricultores pueden mejorar la productividad de sus fincas intercalando estas variedades de sorgo, preferiblemente Girana-1, con lablab, se deben realizar más estudios para determinar los beneficios de sembrar otras leguminosas con sorgo. Se debe cuantificar cualquier mejora en los niveles de N del suelo por la siembra de leguminosas

Palabras clave: Biomasa de forrajes, cultivo de forrajes, percepción de los agricultores, sistema de cultivo.

Introduction

In Ethiopia, the dominant farming system is a crop-livestock system (Assefa et al. 2016), in which both crop and livestock production are economically important. In the country, natural pasture is the primary feed source, which has low biomass yield and nutritional value because of mismanagement (CSA 2018). In addition, grazing land is being converted to crop production to provide food for the rapidly increasing human population in the nation. Consequently, the major feed resource for livestock during the dry season is crop residues (CSA 2018), which have low nutritive value, resulting in poor animal performance unless concentrates or conserved hay is fed (Tolera 2008). The average land holding of households is less than a hectare in different areas of Ethiopia (Gedefaw et al. 2019), so integrated usage of land by inter-cropping of food and forage crops would provide efficient resource utilization (Tarekegn and Zelalem 2014). Inter-cropping legumes with cereal crops has multiple advantages in terms of improving biomass yield, nutritive value and land equivalent ratio (Jensen et al. 2020). In an investigation of the diverse potential of a multi-purpose legume, lablab [*Lablab purpureus* (L.) Sweet], for smallholder production systems, increased biomass yield and nutritive value of forage were recorded (Nord et al. 2020). Shehu et al. (2001) reported that inter-cropping of sorghum with lablab improved the protein concentration in cereal stem as well as leaf yield.

Lablab is adapted to most tropical environments (Grotelüschen 2014), producing high yields of fodder with crude protein (CP) in whole plants of 15–21% (Murphy and Colucci 1999). It is resistant to drought, diseases and pests and improves soil fertility; shade tolerance allows it to be grown successfully with tall cereal crops, including maize (Grotelüschen 2014).

However, the local sorghum varieties are becoming less relevant because of unprecedented climatic variability. Moreover, these local varieties are not highly productive and are failing to meet the alarmingly increasing human population´s food demand. The improved early-maturing sorghum varieties, Teshale, Girana-1 and Misikir, are adapted to the study area as reported by several authors (Tesfaye 2013) and are commonly grown in farming systems.This early-maturing feature of sorghum leads to the possibility of sustainable production in moisture-deficient areas. To the best of our knowledge, no studies have been conducted where these sorghum varieties have been inter-cropped with lablab. Among the commonly available improved lablab accessions is cultivar Highworth (lablab accession ILRI 147). A report by Grotelüschen (2014) showed that ILRI 147 had higher protein concentration than 3 other lablab accessions. The growth habit of ILRI 147 is shorter and more horizontally spreading than cultivar Rongai Noir (ILRI 11609) and cultivar Jhansi (ILRI 6529) (Hunegnaw et al. 2016). This growth habit may makes ILRI 147 more compatible than other accessions for inter-cropping with Teshale, Girana-1 and Misikir sorghum varieties, which have shorter plant height than local sorghum landraces. Hence, we initiated this study to determine the outcomes from inter-cropping these sorghum varieties with lablab to improve productivity of cropping land on farms in the area.

Materials and Methods

Climate

The field experiment was conducted in Kalu district of South Wollo Zone at Harbu Agricultural TVET (Technical Vocational Education and Training) College (10°55' N, 39°46' E; 1,484 masl). The daily mean minimum and maximum temperatures are 8.1 and 23.2 °C, respectively, and average annual rainfall is 1,091 mm (National Meteorology Agency, Kombolcha Branch 2018). The average monthly minimum and maximum temperatures and monthly rainfall for the experimental site during the study are presented in Figure 1.

Soil characteristics

To evaluate the physico-chemical properties of the soil, a Vertisol, at the experimental site, soil samples were taken randomly before planting from 5 spots diagonally across the site to a depth of 20 cm and mixed to make a composite sample. The composite soil sample was air-dried, lightly crushed with a wooden pestle and mortar and screened through a 2 mm sieve for analysis of physical and chemical properties, i.e. soil texture, electrical conductivity, organic carbon, soil pH, cation exchange capacity, total nitrogen and available phosphorus (Olsen) at Sirinka Agricultural Research Centre Soil Laboratory (Table 1).

Trial design

A randomized complete block design was used to conduct the field experiment with 3 replications. Each plot measured 3.75 m long and 3 m wide (11.25 m^2) and the gross area used in the experiment was 370.5 m^2. Each plot of inter-cropping consisted of 5 rows of sorghum and 4 rows of lablab.

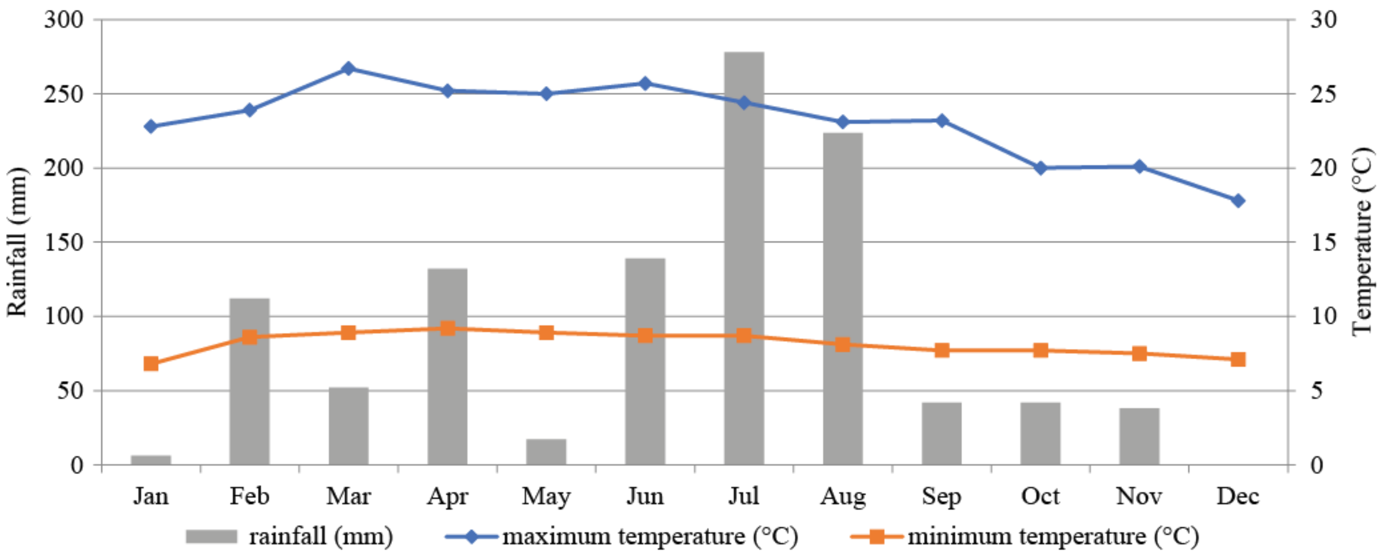


Figure 1. Mean monthly minimum and maximum temperature (°C) and rainfall distribution (mm/month) during 2018 in Kalu district at Harbu. Source: Annual report, National Meteorology Agency, Kombolcha Branch 2018.

Table 1. Physico-chemical properties of the soil at the experimental site before sowing.

Parameter	Value	Rating
Particle size distribution		
Sand (%)	28.3	
Silt (%)	39.2	
Clay (%)	32.5	
Textural class	Silty clay loam	
pH	6	Slightly acidic
Cation exchange capacity ($cmol_{(+)}$/kg soil)	23.2	High
Electrical conductivity (dS/m)	2.33	Low
Total N (%)	0.16	Medium
Available P (ppm)	23.6	High
Organic carbon (%)	1.16	Medium
Organic matter (%)	2	Medium

Spacing between plots and blocks were 1 and 1.5 m, respectively. Three sorghum varieties (Teshale, Girana-1 and Misikir) were inter-cropped with lablab accession ILRI 147 (which was adapted to and recommended for the area), while pure stands of the sorghum varieties and lablab were also grown. This provided 7 treatment combinations consisting of: lablab (LL) only (T1); Teshale + lablab (TL) (T2); Girana-1 + lablab (GL) (T3); Misikir + lablab (ML) (T4); Teshale (TT) only (T5); Girana-1 (GG) only (T6); and Misikir (MM) only (T7).

The land was ploughed 3 times to provide a good seedbed and divided into plots. Sorghum varieties were sown into holes within rows with 75 cm between rows and 25 cm between plants within rows; pure lablab was also sown at the same spacing (Mpairwe et al. 2002). Fifteen days after emergence sorghum plants were thinned to a single healthy plant per hole. Inter-cropped lablab was sown down the center of the sorghum inter-row spaces, i.e. 37.5 cm from the rows of sorghum; with 20 cm between plants, at 18 days after sorghum was sown. All plots of sorghum received a basal application of 100 kg N:P:S fertilizer (19:38:7) per ha at sowing and were top-dressed with 50 kg urea/ha when knee-high (40 days after sowing), while all plots were weeded at 45 days after sowing. Sole lablab plots received a basal application of N:P:S (19:38:7) at 46 kg/ha at sowing according to FAO (2012) recommendations.

Plant height, leaf area and number of leaves, branches and nodules of lablab were assessed on 10 randomly selected plants from the middle 2 rows of each plot at 50% flowering stage (at about 3 months after sowing) (ILRI 2013). Plant height, leaf area and number of leaves of sorghum varieties were taken from 10 randomly selected plants from the middle 3 rows of each plot at milk stage (about 15 days after flowering). Leaf area was calculated as Leaf area = leaf length × maximum width × 0.75 using the method described by Stickler et al. (1961).

When sorghum seed-heads were mature, 10 plants were selected at random from the middle rows. Seed-heads were harvested by hand using sickles, were sun-dried and threshed and the grain was weighed to estimate grain yields. After grain harvesting sorghum stove was harvested by cutting at 10 cm above ground level. Similarly, the whole plot of lablab was harvested at 5 cm above ground level for biomass yield determination. Harvested material was weighed fresh using a balance with sensitivity of 0.1 g. Sub-samples (500 g) were taken from each plot, chopped into small pieces and air-dried until constant weight was recorded.

Dried forage samples from each plot were ground and subjected to chemical analyses at Debre Birhan Agricultural Research Center, Animal Nutrition Laboratory to determine: ash concentration by combusting in a muffle furnace at 500 °C for 6 hours; N concentration by Kjeldahl method; and neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) by the procedures of Van Soest et al. (1991).

The land equivalent ratio (LER) for inter-cropping was calculated, using the formula:

LER = (IYS/SYS) + (IYL/SYL) (Mead and Willey1980),

where:

SYS = yield of pure sorghum; IYS = inter-cropped yield of sorghum; IYL = inter-cropped yield of lablab; and SYL = yield of pure lablab.

Farmers were invited to visit the experimental area during the vegetative stage to form their views and perceptions about the forages when inter-cropped. The perceptions of 8 male and 4 female farmers were recorded through participation using 6 evaluation criteria set by farmers themselves. The criteria were: biomass yield; speed of growth; compatibility of sorghum with lablab; drought tolerance; grain yield; and ability to stay green.

Data analysis

Analysis of variance was used to analyze data on morphological characteristics, forage dry matter yield and chemical composition, grain yield and LER. Fisher's LSD test ($P<0.05$) was employed for separation of means carried out using the procedure of SAS (2002).

The model used for data analysis was:

$Y_{ij} = \mu + B_i + T_j + e_{ij}$,

where:

Y_{ij} = all dependent variables;

μ = overall mean;

B_i = effect of i^{th} block;

T_j = effect of j^{th} treatment; and

e_{ij} = the random error.

Results

With the exception of Girana-1, sorghum varieties and lablab were affected morphologically by inter-cropping (Table 2).

Girana-1 outperformed Teshale and Misikir in terms of plant height, number of leaves/plant and total leaf area, whether grown as a pure stand or when inter-cropped with lablab ($P<0.01$; Table 2). Similarly, height and number of leaves per plant for Girana-1 were not affected by inter-cropping with lablab ($P>0.05$), while both parameters for Teshale and Misikir were reduced by growing in association with lablab ($P<0.01$). Surprisingly leaf area of Girana-1 was not affected when grown with lablab ($P>0.05$), while leaf area was reduced in the case of Teshale and Misikir (P<0.01). For lablab, inter-cropping with Teshale or Misikir reduced plant height and total leaf area per plant (P<0.01). Number of branches per plant and number of nodules per plant for lablab were reduced when lablab was grown with any of the sorghum cultivars (P<0.01).

Both grain and stover yields of Teshale and Misikir were reduced (P<0.001) by inter-cropping with lablab (Table 3; P<0.001). Sowing lablab with sorghum reduced forage yields of lablab but differences were significant (P<0.05) only for Teshale and Misikir.

Inter-cropping of sorghum varieties with lablab increased both ash and CP concentrations in sorghum stover but lowered NDF, ADF and ADL concentrations (P<0.001; Table 4). Crude protein levels in stover of Girana-1 reached 7% when grown with lablab.

Inter-cropping lablab with sorghum varieties significantly (P<0.001) increased ash concentration but reduced CP, NDF, ADF and ADL concentrations of lablab (Table 5).

While land equivalent ratios (LERs) for biomass yield (non-grain component) for inter-cropping lablab with sorghum varieties were less than unity for each species, the combined LERs were significantly (P<0.001) greater than unity, with increases of 54–87% (Table 6). Girana-1 + lablab produced the greatest yield advantage over pure stands.

Farmers' perception of the inter-cropping technology was assessed during a field day. Farmers preferred inter-cropping to planting pure stands of sorghum and lablab and ranked Girana-1+ lablab as the preferred option (data not presented). They said that: Girana-1 inter-cropped with lablab had largest heads and seed size; its superior plant height provided physical support for climbing lablab; and it was more suitable for lablab growth and development as compared with Teshale and Misikir.

Table 2. Plant morphology of 3 sorghum varieties and lablab sown alone or inter-cropped.

Treatment	PH (cm)		NLPP		LA (cm^2)		NBPP	NNPP
	Sorghum	Lablab	Sorghum	Lablab	Sorghum	Lablab	Lablab	Lablab
SL	-	238a[1]	-	58.4a	-	138a	11.9a	17.4a
TL	189c	198b	10.5c	45.5c	602c	118b	6.4bc	13.8b
GL	245a	231a	11.3a	52. 0b	631a	127ab	8.4b	14.2b
ML	176e	172c	9.6e	41.8c	579e	103c	4.4c	10. 0c
ST	192b	-	10.9b	-	608b	-	-	-
SG	247a	-	11.4a	-	633a	-	-	-
SM	183d	-	10.0d	-	595d	-	-	-
LSD	3.5	26.0	0.16	6.35	6.4	8.22	1.85	1.96
Prob.	***	**	***	**	***	**	**	***

[1]Within columns means followed by different letters differ significantly (P<0.01; P<0.001). SL = sole lablab (T1); T L = Teshale + lablab (T2); GL = Girana-1 + lablab (T3); ML = Misikir+ lablab (T4); ST = sole Teshale (T5); SG = sole Girana-1 (T6); SM = sole Misikir (T7); PH = plant height; NLPP = number of leaves per plant; LA = leaf area; NBPP = number of branches per plant; and NNPP = number of nodules per plant.

Table 3. Forage yields of lablab plus stover and grain yields of sorghum sown alone or inter-cropped.

Treatment	Dry biomass (t DM/ha)		Grain yield (quintal/ha)
	Sorghum stover	Lablab	Sorghum
SL	-	5.65a	-
TL	7.75c[1]	4.08b	54.6c
GL	9.08a	5.01a	59.5a
ML	5.84e	3.47b	49.1 e
ST	7.90b	-	56.6b
SG	9.08a	-	59.5a
SM	6.24d	-	52.5d
LSD	0.005	0.635	0.365
Prob.	***	**	***

[1]Means within columns followed by different letters differ significantly (P<0.01; P<0.001). 1 quintal = 100 kg. SL = sole lablab (T1); TL = Teshale + lablab (T2); GL = Girana-1 + lablab (T3); ML = Misikir + lablab (T4); ST = sole Teshale (T5); SG = sole Girana-1 (T6); SM =sole Misikir (T7).

Table 4. Chemical composition (%) of stover from sorghum varieties sown alone or inter-cropped with lablab.

Treatment	Ash	CP	NDF	ADF	ADL
TL	12.1a	5.8b	52.5d	39.1d	10.1d
GL	12.1a	7.0a	51.0e	37.6e	7.9e
ML	11.2b	5.8c	53.2c	40.9c	10.2c
ST	9.1d	3.5e	68.8a	50.5a	12.1b
SG	9.4c	5.6d	63.4b	47.2b	12.1b
SM	8.0e	2.2f	68.8a	50.5a	12.2a
LSD	0.0048	0.0041	0.0217	0.0079	0.0043
Prob.	***	***	***	***	***

Means within columns with different letters differ significantly (P<0.001). CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; SL = sole lablab (T1); TL =Teshale + lablab (T2); GL = Girana-1 + lablab (T3); ML = Misikir + lablab (T4); ST = sole Teshale (T5); SG =sole Girana-1 (T6); SM = sole Misikir (T7).

Table 5. Chemical composition (%) of lablab forage sown alone or inter-cropped with sorghum varieties.

Treatment	Ash	CP	NDF	ADF	ADL
SL	8.7b	15.9a	35.4a	27.8a	6.4a
TL	9.8a	14.8c	32.2c	24.3c	6.1c
GL	9.8a	15.6b	32.2c	24.3c	6.1c
ML	9.8a	14.5d	33.3b	25.8b	6.3b
LSD	0.012	0.2	0.0067	0.0046	0.01
Prob.	***	***	***	***	***

Means within columns with different letters differ significantly (P<0.001). CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; SL = sole lablab (T1); TL = Teshale + lablab (T2); GL = Girana-1 + lablab (T3); ML = Misikir + lablab (T4); ST = sole Teshale (T5); SG = sole Girana-1 (T6); SM = sole Misikir (T7).

Table 6. Land equivalent ratio for biomass yield (non-grain component) of inter-cropping sorghum varieties with lablab.

Treatment	Land equivalent ratio of dry biomass			Yield advantage (%)
	Sorghum	Lablab	Total	
TL	0.98b	0.72b	1.70b	70
GL	0.99a	0.88a	1.87a	87
ML	0.93c	0.61b	1.54c	54
LSD	0.0014	0.143	0.1417	-
CV	0.0623	8.5101	3.6486	-
Prob.	***	*	**	-

Means within columns with different superscripts differ significantly (P<0.05). TL = Teshale + lablab; GL = Girana-1 + lablab; ML = Misikir + lablab; SL = sole lablab (T1); TL =Teshale + lablab (T2); GL = Girana-1 + lablab (T3); ML = Misikir + lablab (T4); ST = sole Teshale (T5); SG = sole Girana-1 (T6); SM = sole Misikir (T7).

Discussion

This study has shown the substantial benefits in terms of total yields of forage to be obtained from inter-cropping lablab and sorghum in comparison with sowing the 2 crops as pure stands. Not only was total DM yield of forage increased but also the CP concentration in the sorghum stover was increased. It was of interest that growth of Girana-1 was not significantly affected by being grown in association with lablab, while both Teshale and Misikir suffered depression in yields of both stover and grain. This finding was in spite of the fact that Girana-1 was the tallest sorghum accession but did not significantly suppress the growth of lablab in terms of plant height or total leaf area, although it did reduce the number of branches per plant. Musa et al. (2012) reported that dry forage and grain yields of sorghum varieties (S1007, Pioneer and local Shahlaa) varied between varieties when inter-cropped with cowpea.

This finding of an overall lack of response of Girana-1 to inter-cropping with lablab is in contrast to the results with Teshale and Misikir, which behaved according to results from other published studies. Shehu et al. (2001) found that inter-cropping of lablab with sorghum caused a marked reduction in yields of sorghum stem, leaves and grain, while Isaacs et al. (2018) found a reduction in height of maize following inter-cropping with lablab. Similarly, significant reduction of number of leaves and leaf area of sorghum was shown when inter-cropped with legumes (Arshad et al. 2014). This suppresion in growth would normally be expected when two crops are inter-planted because of increased competition for light, nutrients and moisture.

The increased CP concentration in sorghum stover when inter-cropped with lablab is in agreement with the findings of Akhtar et al. (2013) and Mbahi et al. (2017), who reported increases in CP concentration in sorghum when inter-cropped with lablab and groundnut, respectively. This response is probably due to increased soil N availability resulting from atmospheric N fixation by rhizobia on lablab root nodules. Significantly higher CP concentration in Girana-1 than in Teshale and Misikir, regardless of whether pure stands or inter-cropped with lablab, suggests that Girana-1 had better ability to extract nitrogen from soil than the other varieties, as it also presented higher grain and stover yields.

The lower NDF, ADF and ADL concentrations in inter-cropped sorghum stover were in line with reports that showed inter-cropping sorghum with lablab significantly reduced NDF concentration in forage produced (Mpairwe et al. 2002; Amole et al. 2015), while ADF concentration in sorghum was lower when inter-cropped with groundnut and lablab than when sorghum was grown as pure stands (Zhang et al. 2015). Zhang et al (2015) and Amole et al. (2015) reported lower fiber levels in sorghum inter-cropped with legumes than in pure sorghum.

The consistently lower concentrations of NDF and ADF in Girana-1 than in Teshale and Misikir, regardless of whether grown as pure stands or inter-cropped with lablab, is further evidence of the superiority of this variety over Teshale and Misikir.

The reduction in number of leaves per plant and number of branches per plant for lablab when inter-cropped with sorghum conforms with the findings of Redfearn et al. (1999) and Iqbal et al. (2018) that inter-cropping sorghum with soybean reduces the number of leaves and branches of soybean, respectively. Similarly, Ngongoni (2007) found that inter-cropping maize with lablab and cowpea significantly reduced number of nodules per plant of both legumes.

The reduction in biomass yield of inter-cropped lablab relative to pure lablab might be a function of competition for light, soil moisture and nutrients. Growth of lablab was depressed more by competition with sorghum than sorghum growth was depressed by competition with lablab, indicating that sorghum was more competitive than lablab under the conditions of the study. Since sorghum was much taller than lablab, one might suspect that competition for light played a significant role in differences which occurred.

Inter-cropping lablab with the 3 sorghum varieties was more advantageous than sole cropping, since 54–87% more land would be required with sole cropping to produce a similar quantity of dry forage as obtained with inter-cropping. This finding is in line with reports that LER values greater than 1 occurred when sorghum was inter-cropped with cowpea and rice bean (Singh Pal et al. 2014). The highest LER value obtained with inter-cropped Girana-1 + lablab is further evidence of the benefits of planting sorghum and lablab under an inter-cropping system in this environment. In a study of inter-cropping of maize with vetch, berseem clover and beans, LER exceeded 1 in combinations of both maize hybrids studied (704 and 301) and vetch (Ozpinar 2009).

Girana-1 was so superior to other sorghum varieties, in terms of greater height, larger seed heads, largest seed size and compatibility with lablab, that it was not surprising that most participating farmers identified Girana-1 + lablab as the preferred combination.

Conclusion and recommendations

The findings showed that of the 3 sorghum varieties inter-cropped with lablab, Girana-1 was superior to Teshale and Misikir in overall performance. Even when planted as a pure crop Girana-1 was superior to the other 2 varieties and continued to produce at the same level when intercropped with sorghum, while Teshale and Misikir had reduced performance relative to their performance as pure stands. It is obvious that farmers in the area can improve productivity of their land by inter-cropping Girana-1 with lablab. Further studies should be conducted to test findings with other legume species and to quantify any improvements in soil N produced by the legume.

Acknowledgments

The first author acknowledges Bahir Dar University (BDU) for allowing the study to be conducted, and Amhara National Regional State Technical, Vocational and Enterprises Development Bureau for financial support.

References

(Note of the editors: All hyperlinks were verified 16 April 2021.)

Akhtar MF; Ahmad AH; Zamir MSI; Khalid F; Mohsin AU; Afzal M. 2013. Agro-qualitative studies on forage sorghum (*Sorghum bicolor* L.) sown alone and in mixture with forage legumes. Pakistan Journal of Science 65(2):179–185. bit.ly/3xHUPK3

Amole TA; Oduguwa BO; Onifade SO; Jolaosho AO; Amodu JT; Arigbede MO. 2015. Effect of planting patterns and age at harvest of two cultivars of *Lablab purpureus* in *Andropogon gayanus* on agronomic characteristic and quality of grass / legume mixtures. Tropical Agricultural Science 38(3):329–346. bit.ly/3xEgeUq

Arshad M; Nawaz R; Shahnawaz M; Sarfaraz MS; Ahmad S; Ranamukhaarachi SL. 2014. Effect of legume type, nitrogen dose and air quality on biomass and bioethanol production in sweet sorghum-legume Intercropping. Agriculture & Forestry 60(3):257–274. bit.ly/3t55mf3

Assefa G; Mengistu S; Feyissa F; Bediye S. 2016. Animal feed resources research in Ethiopia: Achievements, challenges and future directions. In: Ethiopian Institute of Agricultural Research 50 years of service for ethiopian agriculture. Ethiopian Journal of Agricultural Sciences, Special issue, Addis Ababa, Ethiopia. p. 141–156.

CSA (Central Statistical Agency). 2018. Federal Democratic Republic of Ethiopia Central Statistical Agency, Agricultural sample survey. Volume II. Report on Livestock and Livestock Characteristics. Addis Ababa, Ethiopia.

FAO (Food and Agriculture Organization). 2012. Grassland species index. *Lablab purpureus*.

Gedefaw AA; Atzberger C; Seher W; Mansberger R. 2019. Farmers' willingness to participate in voluntary land Consolidation in Gozamin District, Ethiopia. Land 8(148):1–21.doi: 10.3390/land8100148

Grotelüschen K. 2014. *Lablab Purpureus* (L) Sweet: a promising multipurpose legume for enhanced drought resistance in smallholder farming-systems of eastern Kenya. M.Sc. Thesis. University of Göttingen, Germany.

Hunegnaw B; Mekonen W; Walie M; Amanie A; Tamir S. 2016. Participatory selection of different lablab accessions for under sowing on maize in Western Gojjam, Ethiopia. In: Abegaz S; Yeheyis L, eds. Proceedings of the 9th Annual Regional Conference on Livestock Completed Research Activities, 9–20 March 2015, Bahir Dar, Ethiopia. p. 46–58.

Isaacs KB; Snapp SS; Kelly JD; Chung KR. 2016. Farmer knowledge identifies a competitive bean ideotype for maize–bean intercrop systems in Rwanda. Agriculture & Food Security 5:15. doi: 10.1186/s40066-016-0062-8

ILRI (International Livestock Research Institute). 2013. Lablab (*Lablab purpureus* cultivar Rongai) for livestock feed on small-scale farms (English Version). ILRI, Nairobi, Kenya. hdl.handle.net/10568/1815

Iqbal MA; Iqbal A; Abbas RN. 2018. Spatio-temporal reconciliation to lessen losses in yield and quality of forage soybean (*Glycine max* L.) in soybean-sorghum intercropping systems. Bragantia 77(2):283–291. doi: 10.1590/1678-4499.2017043

Jensen ES; Carlsson G; Hauggaard-Nielsen H. 2020. Intercropping of grain legumes and cereals improves the use of soil N resources and reduces the requirement for synthetic fertilizer N: A global-scale analysis. Agronomy for Sustainable Development 40(5):1–9. doi: 10.1007/s13593-020-0607-x

Mead R; Willey RW. 1980. The concept of a land equivalent ratio and advantages in yields from intercropping. Experimental Agriculture 16:217–228. doi: 10.1017/S0014479700010978

Mpairwe DR; Sabiiti EN; Ummuna NN; Tegegne A; Osuji P. 2002. Effect of intercropping cereal crops with forage legumes and source of nutrients on cereal grain yield and fodder dry matter yields. African Crop Science Journal 10(1):81–97.doi: 10.4314/acsj.v10i1.27559

Murphy AM; Colucci PE. 1999. A tropical forage solution to poor quality ruminant diets: A review of *Lablab purpureus*. Livestock Research for Rural Development 11:Article #21. bit.ly/3cbhy8F

Musa EM; Elsheikh EAE; Ahmed IAM; Babiker EE. 2012. Intercropping sorghum (*Sorghum bicolor* L.) and cowpea (*Vigna unguiculata* L.): Effect of *Bradyrhizobium* inoculation and fertilization on minerals composition of sorghum seeds. International Scholarly Research Notices 2012:1–9. doi: 10.5402/2012/356183

National Meteorology Agency, Kombolcha Branch. 2018. Annual Report, Kalu District 2018. Meteorological data.

Ngongoni NT; Mwale M; Mapiye C; Moyo MT; Hamudikuwanda H; Titterton M. 2007. Evaluation of cereal-legume intercropped forages for smallholder dairy production in Zimbabwe. Livestock Research for Rural Development. Volume 19:129. bit.ly/2PR8xta

Nord A; Miller NR; Mariki W; Drinkwater L; Snapp S. 2020. Investigating the diverse potential of a multi-purpose legume, *Lablab purpureus* (L.) Sweet, for smallholder production in East Africa. PLoS ONE 15(1):e0227739. doi: 10.1371/journal.pone.0227739

Ozpinar S. 2009. Tillage and cover crop effects on maize yield and soil nitrogen. Bulgarian Journal of Agricultural Science. 15(6):533–543. bit.ly/33j5QDy

Redfearn DD; Buxton DR; Devine TE. 1999. Sorghum intercropping effects on yield, morphology and quality of forage soybean. Crop Science 39(5):1380–1384. doi: 10.2135/cropsci1999.3951380x

SAS (Statistical Analysis Systems). 2002. SAS Version 9.1. SAS Institute Inc., Cary NC, USA.

Shehu Y;Alhassan WS; Pal UR; Phillips CJC. 2001. The effect of intercropping *Lablab purpureus* L. with sorghum on yield and chemical composition of fodder. Journal of Agronomy and Crop Science 183(2):73–79. doi: 10.1046/j.1439-037x.1999.00338.x

Singh Pal M; Reza A; Joshi YP; Panwa UBS. 2014. Production potential of forage sorghum (*Sorghum bicolor* L) under different intercropping systems. Agriculture for Sustainable Development 2(1):87–91. bit.ly/2Pj64qI

Stickler FC; Wearden S; Pauli AW. 1961. Leaf area determination in grain sorghum. Agronomy Journal 53:187–188. doi: 10.2134/agronj1961.00021962005300030018x

Tarekegn A; Zelalem T. 2014. Evaluation of the performance of herbaceous forage legumes under sown with maize under irrigation condition of Megech, North Gondar, Ethiopia. Livestock Research for Rural Development 26: Article #102. lrrd.org/lrrd26/6/tare26102.html

Tesfaye E. 2013. Adoption of improved sorghum varieties and farmers' varietal trait preference in Kobo District, North Wollo Zone, Ethiopia. M.Sc. Thesis. Haramaya University, Haramaya, Ethiopia.

Tolera A. 2008. Feed resources and feeding management: A manual for feedlot operators and development workers. Ethiopia Sanitary and Phytosanitary Standards and Livestock & Meat Marketing Program (SPS-LMM), USAID, Addis Ababa, Ethiopia.

Van Soest P; Robertson J; Lewis B. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74(10):3583–3597. doi: 10.3168/jds.S0022-0302(91)78551-2

Zhang J; Yin B; Xie Y; Li J;Yang Z; Zhang, G. 2015. Legume-cereal intercropping improves forage yield, quality and degradability. PloS ONE 10(12):e0144813. doi: 10.1371/journal.pone.0144813

(Received for publication 3 May 2020; accepted 13 March 2021; published 31 May 2021)

doi: 10.17138/TGFT(9)225-234

Research Paper

Effects of seeding rate, fertilizing time and fertilizer type on yield, nutritive value and silage quality of whole-crop wheat

Efectos de tasa de siembra, momento de aplicación y tipo de fertilizante en el rendimiento, el valor nutritivo y la calidad del ensilaje de trigo integral

LIUXING XU[1], ZHAOHONG XU[1], MINGXIA CHEN[2] AND JIANGUO ZHANG[1]

[1]*Department of Grassland Science, South China Agricultural University, Guangzhou, China.* english.scau.edu.cn
[2]*Qingyuan Agricultural Science and Technology Promotion Service Center, Qingyuan, China.*

Abstract

Whole-crop wheat (WCW) is rich in nutrients and is widely used as a forage crop. This study consisted of 2 experiments: Experiment 1 studied the yield, nutritive value and silage quality of WCW at 3 seeding rates (320 kg/ha, S_{320}; 385 kg/ha, S_{385}; and 450 kg/ha, S_{450}) and different fertilizing times, i.e. 60% at seedling stage and the remaining 40% at the jointing stage vs. heading stage; and Experiment 2 examined the yield, nutritive value and silage quality of WCW receiving different fertilizer types, i.e. urea, compound fertilizer (N:P:K) and urea + compound fertilizer (all iso-nitrogenous). With the increased seeding rate, dry matter (DM) and crude protein (CP) yields tended to increase, but relative feed value tended to decrease. Experiment 1: there was no significant interaction between time of applying the second fertilizer dose and seeding rate in terms of concentrations of CP, crude fiber, ether extract, crude ash, nitrogen-free extract, neutral detergent fiber (NDF) and acid detergent fiber (ADF) in wheat (P>0.05). However, a significant interaction between fertilizing time and seeding rate was observed in terms of silage fermentation quality (pH, lactic acid, butyric acid and NH_3-N concentrations) (P<0.05). Experiment 2: DM yield, CP yield and concentrations of CP, ADF and water-soluble carbohydrate were not affected by fertilizer type (P>0.05). Fertilizer type had significant effects on pH of silage and concentrations of organic acids (except propionic acid) and NH_3-N in WCW silage (P<0.05). Under the present study conditions, considering DM yield, nutrient composition and silage fermentation quality, an optimal seeding rate of wheat for forage appears to be about 385 kg/ha. N fertilizer should be applied at the seedling stage and jointing stage. Although applying a mixture of urea and compound fertilizer had no significant effects on yield and nutritive value of WCW relative to applying urea alone, it did improve silage fermentation quality. Results may differ on different soils.

Keywords: Fertilizer application, nutritional composition, seeding rate, whole-crop wheat, yield.

Resumen

El trigo integral (WCW) es rico en nutrientes y se usa ampliamente como cultivo forrajero. Este estudio consistió en 2 experimentos: el Experimento 1 estudió el rendimiento, valor nutritivo y calidad del ensilaje de WCW a 3 tasas de siembra (320 kg/ha, S_{320}; 385 kg/ha, S_{385}; y 450 kg/ha, S_{450}) y diferentes tiempos de fertilización: el 60% en la etapa de plántula y el 40% restante entre las etapas de unión y encabezado. El Experimento 2 examinó el rendimiento, el valor nutritivo y la calidad del ensilaje de WCW que recibieron diferentes tipos de fertilizantes: urea, fertilizante compuesto (N:P:K) y urea + fertilizante compuesto (todos iso-nitrogenados). Con el aumento de la tasa de siembra, los rendimientos de materia seca (DM) y proteína cruda (CP) tendieron a aumentar, pero el valor relativo del alimento tendió a disminuir. Experimento 1: no hubo interacción significativa entre el tiempo de aplicación de la segunda dosis de fertilizante y la tasa de siembra en términos de concentraciones de CP, fibra bruta, extracto de éter, ceniza bruta, extracto libre de nitrógeno, fibra detergente neutra (NDF) y fibra detergente ácida (ADF) en trigo (P>0.05). Sin embargo, se observó una

Correspondence: Jianguo Zhang, Department of Grassland Science, South China Agricultural University, Guangzhou, 510642, China.
Email: zhangjg@scau.edu.cn

significativa interacción entre el tiempo de fertilización y la tasa de siembra en términos de la calidad de fermentación del ensilaje (pH, ácido láctico, ácido butírico y concentraciones de NH_3-N) (P <0.05). Experimento 2: El rendimiento de DM, el rendimiento de PC y las concentraciones de PC, ADF y carbohidratos solubles en agua no se vieron afectados por el tipo de fertilizante (P>0.05). El tipo de fertilizante tuvo efectos significativos sobre el pH del ensilaje y las concentraciones de ácidos orgánicos (excepto ácido propiónico) y NH_3-N en el ensilaje WCW (P<0.05). En las condiciones del presente estudio, considerando el rendimiento de DM, la composición de nutrientes y la calidad de la fermentación del ensilaje, una tasa óptima de siembra de trigo para forraje parece ser de unos 385 kg / ha. El fertilizante N debe aplicarse en la etapa de plántula y en la etapa de unión. Aunque la aplicación de una mezcla de urea y fertilizante compuesto no tuvo efectos significativos sobre el rendimiento y el valor nutritivo de WCW en relación con la aplicación de urea sola, sí mejoró la calidad de la fermentación del ensilado. Los resultados pueden diferir en diferentes suelos.

Palabras clave: Aplicación de fertilizantes, composición nutricional, rendimiento, tasa de siembra, trigo integral.

Introduction

The demand for animal proteins in China is rapidly growing with the improvement of living standards and the change of food consumption habits. However, the development of animal husbandry is usually restricted by shortage of herbage supply (Liu et al. 2012). Therefore, there is increasing need to develop and utilize new herbage resources or find new lands to grow herbage crops. Whole-crop wheat (*Triticum aestivum*) (WCW) has relatively high nutritional value (Sprague et al. 2015) and total DM intake of WCW diets exceeded that of grass diets (Günal et al. 2018). In order to improve the economic returns from farms, more and more WCW is being planted instead of grass (Huuskonen et al. 2017), often as a specialized forage wheat or as an addition within forage production systems. For example, WCW is processed into hay and silage to feed beef cattle in Finland (Huuskonen et al. 2017), while in Australia, dual-purpose wheat is often planted and used for grazing to alleviate winter feed shortages (Sprague et al. 2015). In Oklahoma, USA, in response to the lack of forage in winter, large areas are used to grow wheat for animal forage in the form of whole plants at maturity (Kim and Anderson 2015). Although wheat has been widely used as forage, few studies have focused on optimal planting techniques. Previous studies on forage wheat have concentrated on variety screening (Li 2015), nitrogen (N) application rate, harvest time (Xie 2012) and silage utilization (Filya 2003; Shaani et al. 2017).

Among cultivation measures, the factors that have the greatest impact on forage yield and nutritive value are seeding rate and N fertilizer management (Guo et al. 2017). Li (2015) found that, at a seeding rate of 260 kg/ha, there was still potential for dry matter yield (DMY) of forage wheat to increase if seeding rate was increased. However, the number of wheat spikes tended to decrease with increases in seeding rate (Yang 2011). In order to obtain data on optimal seeding rates to achieve a desirable balance between yield and quality of forage wheat, further research is needed. While Pan et al. (1999) found that, in terms of DMY, the Law of Diminishing Returns operated with increase in N application rate and yield even declined past a certain application rate, application of N increased crude protein (CP) concentration, in vitro dry matter digestibility and silage fermentation quality of forage wheat (Li et al. 2016).

Not only is amount of N applied important but also timing of the application is critical. Accumulation of DM in wheat occurs mainly during the period from jointing to maturity, accounting for 70% of the total DM yield (Wu and Cui 2000). Applying N fertilizer at the jointing stage increases the leaf area index of wheat, accumulates more DM during the vegetative period and increases the number of tillers (Ravier et al. 2017). However, little is known of the efficiency of fertilizer use when applied to wheat close to flowering (heading stage).

In winter, fields are fallowed after the harvest of late rice in Southern China, which would allow the planting of a winter-forage crop (Cinar et al. 2020). However, frequent cultivation leads to low nutrient levels in the soil, so producers often use compound fertilizer to meet the needs of winter-forage crops. The effects of seeding rate, fertilizing time and fertilizer type on yield, nutritive value and silage quality of WCW have not been explored. Therefore, in this study, we aimed to compare the effects of different seeding rates and timing of fertilizer application on yield and nutritive value of forage produced. We hypothesized that: (i) high seeding rate would increase both yield and nutritive value of forage; (ii) applying part of the fertilizer at jointing stage is better than applying all at heading stage; and (iii) applying urea with compound fertilizer would increase yield and nutritive value of WCW to higher levels than urea or compound fertilizer alone.

Materials and Methods

Experimental sites

Experiment 1 was carried out at Meitan Experimental Field of Agricultural Science Institute of Qingyuan (23°42′ N, 115°50′ E), Guangdong Province, China. The site is located within a subtropical monsoon humid climate zone with an annual average temperature of 22.3 °C. The hottest month is July with a monthly average temperature of 31.4 °C, while the coldest month is January with a monthly average temperature of 14.0 °C. The annual average rainfall and sunshine time are 1,842 mm and 2,245 hours, respectively.

Experiment 2 was carried out at Ningxi Experimental Field of South China Agricultural University (23°14′ N, 113°38′ E), Zengcheng, Guangzhou, Guangdong Province, China. This site is also located within a subtropical monsoon humid climate zone with an annual average temperature of 21.6 °C. The hottest month is July with a monthly average temperature of 29.4 °C, while the coldest month is January with a monthly average temperature of 13.3 °C. The annual average rainfall and sunshine time are 1,968 mm and 2,107 hours, respectively.

Meteorological data for the 2 sites during the study plus the medium-term mean data are presented in Table 1.

For the two experimental sites, the general cropping systems are early rice in spring (summer harvest) and late rice in summer (autumn harvest), then either fallowing or planting winter forage crops (to be harvested in spring of the following year). Soil types are cinnamon soil for Meitan Experimental Field and paddy soil for Ningxi Experimental Field (Zhang et al. 2014). Before sowing the forage wheat, 5 soil cores (each 2.5 cm diameter) were randomly excavated and mixed to give a composite sample for determining soil chemical properties. The soil chemical composition was similar at both sites (Table 2).

Wheat planting and management

Wheat phenology was regularly monitored based upon the Decimal Code (DC) (Zadoks et al. 1974). In Experiment 1, a factorial arrangement of timing of N application (jointing vs. heading) × seeding rate was utilized. A compound fertilizer (N:P:K, 15:6:8) was applied at 150 kg/ha with 60% at the seedling stage (DC13) and 40% at the jointing (DC31)or heading stage (DC41), with 3 seeding rates, i.e. the recommended rate of 320 kg/ha (S_{100}) (Li 2015) and increased rates of +20% (384 kg/ha; S_{120}) and +40% (448 kg/ha; S_{140}) (Table 3). In Experiment 2, urea, compound fertilizer (N:P:K, 15:6:8) and a combination of urea and compound fertilizer (5:5) were compared. All treatments were designed to apply 150 kg N/ha in total. A standard seeding rate of 385 kg/ha was used. Sixty percent of the fertilizer was applied at the seedling stage (DC13) and the remaining 40% at the jointing stage (DC31).

In Experiment 1, the planting and harvesting dates of wheat were 8 November 2014 and 10 March 2015, respectively, while in Experiment 2, the planting and harvesting dates were 10 November 2014 and 25 March 2015, respectively. The wheat variety was Shimai No.1 (seed germination rate 98%, 53 mg per seed). In both experiments there were 3 replicates of the above treatments, arranged as a randomized block, and each plot was 12 m^2 (3 × 4 m).

Table 2. Soil data for trial sites.

Parameter	Meitan EF[1]	Ningxi EF
Organic matter (g/kg)	4.20	2.75
Total nitrogen (g/kg)	1.35	1.38
Total phosphorus (g/kg)	1.58	2.89
Total potassium (g/kg)	16.6	18.7
Available nitrogen (mg/kg)	92.4	84.1
Available phosphorus (mg/kg)	63.4	67.6
Available potassium (mg/kg)	132	150
pH	5.27	5.15

[1]EF: Experimental field

Table 1. Meteorological data during growing period of whole-crop wheat plus medium-term mean data at the experimental fields.

Location	Parameter	Nov	Dec	Jan	Feb	Mar
Meitan experimental field	Mean temperature (°C)	19.6	16.7	16.2	15.6	18.3
	Rainfall (mm)	64	14	10	18	125
	No. of days with rainfall (d)	9	5	9	8	17
Ningxi experimental field	Mean temperature (°C)	19.9	16.6	14.1	14.5	19.9
	Rainfall (mm)	59	10	136	12	69
	No. of days with rainfall (d)	8	1	8	5	8
Meitan experimental field – 20-year mean	Mean temperature (°C)	21.1	17.3	13.6	16.0	19.8
	Rainfall (mm)	38	44	41	20	103
	No. of days with rainfall (np-)	5	9	8	4	12
Ningxi experimental field – 20-year mean	Mean temperature (°C)	20.4	15.6	14.4	15.2	18.7
	Rainfall (mm)	46	50	56	22	116
	No. of days with rainfall (no.)	7	8	6	7	13

Table 3. Agricultural operation dates and Decimal Code of wheat development stages.

Experiment	Planting date	Fertilizing date and Decimal Code						Harvesting date and Decimal Code		Crop growth time (No. of days)
		Seedling stage	Decimal Code	Jointing stage	Decimal Code	Heading stage	Decimal Code	Date	Decimal Code	
Experiment 1 (Seeding rate and fertilizing time)	08/11/2014	16/12/2014	DC13	18/02/2015	DC31	27/02/2015	DC41	10/03/2015	DC77	122
Experiment 2 (Fertilizer type)	10/11/2014	24/12/2014	DC13	22/02/2015	DC31	-	-	25/03/2015	DC87	135

Field investigation and sampling

In Experiment 1, wheat was harvested at the milk stage (DC77), while in Experiment 2 harvesting was at the soft dough stage (DC87). Fifteen wheat plants per plot were randomly selected to determine plant height and tiller number, and the average value was calculated. In each plot a 1 m^2 (1 × 1 m) site was selected at random and forage was harvested at 5 cm from ground level to measure yield. All harvested material was taken back to the laboratory and cut into 2–3 cm pieces by a forage chopper. Fresh material was used to determine microorganisms present and to make silage.

Silage making

After being cut into pieces, fresh material from each plot was mixed uniformly and a 200 g sample was packed into a 30 × 20 cm polyethylene silage bag, air was removed and the bag was sealed with a vacuum packer (Sinbo Vacuum Sealer, Hong Tai Home Electrical Appliance Co. Ltd, Hong Kong, China) (Xie et al. 2012). Silage packs were stored in the dark at room temperature for 60 d, before being analyzed for silage fermentation quality.

Chemical and microbial analyses

Crop material was dried at 70 °C for 48 hours in an oven with forced-air circulation for determination of DM concentration. N concentration was determined by the Kjeldahl method (Nitrogen analyzer KN680, Shandong Jinan Alva Instrument Co. Ltd, Jinan, China), and ammonia nitrogen (NH_3-N) was directly distilled by an automatic Kjeldahl nitrogen analyzer. Determination of ether extract concentration was by the ether extraction method (AOAC 2011). Crude ash concentration was determined by burning at 550 °C for 3 h and water-soluble carbohydrate (WSC) concentration by the anthrone-sulfuric acid method (Murphy 1958). Buffering capacity was determined by hydrochloric acid and sodium hydroxide titration (Playne and McDonald 2010), while crude fiber, neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the filter bag method (Van Soest et al. 1991). Nitrogen free extract was calculated based upon the concentrations of CP, crude fiber, ether extract and crude ash. Relative feed value (RFV) was calculated based on concentrations of ADF and NDF (Rohweder et al. 1978).

The numbers of lactic acid bacteria (LAB), aerobic bacteria, yeasts and molds were counted by culturing on de Man Rogosa Sharpe agar, nutrient agar and potato dextrose agar, respectively. The lactic acid bacteria were cultured for 2–3 d at 37 °C under anaerobic conditions (YQX II anaerobics box, Shanghai Xinmiao Medical Device Manufacturing Co. Ltd, Shanghai, China). Aerobic bacteria, yeasts and molds were cultured under aerobic conditions at 37 °C for 3–4 days (Liu et al. 2013).

After the silage bags were opened, 20 g of the mixed silage was placed in a polyethylene plastic bag, to which 80 mL of distilled water was added before sealing. After soaking at 4 °C for 18 h, the contents were filtered and the pH of the extract was determined using a pH meter. The concentrations of lactic acid, acetic acid, propionic acid and butyric acid were determined by high performance liquid chromatography (column: Sodex RS Pak KC-811, Showa Denko KK, Kawasaki, Japan), and the operating conditions were the same as in the study by Xie et al. (2012).

Statistical analysis

Data from Experiment 1 were analyzed by 2-way analysis of variance to evaluate the effects of seeding rate, fertilizing time and their interaction on the yield, nutrient composition and silage fermentation characteristics of WCW. In Experiment 2, data were analyzed by a one-way analysis of variance. The means were compared for significance by Duncan's multiple range method (SPSS 17.0 for Windows; SPSS Inc., Chicago, IL, USA).

Results

Experiment 1

Plant height, tiller number, yield and relative feed value. Seeding rate, fertilizing time and their interaction had no significant effects on plant height or tiller number per plant ($P>0.05$) (Table 4). However, increasing seeding rate significantly ($P<0.01$) increased DM and CP yields of wheat forage but reduced relative feed value ($P<0.05$). Timing of the second application of fertilizer significantly ($P<0.05$) affected only CP yield with yields from application at jointing stage exceeding that at heading.

Chemical composition. Mean DM concentration in fresh wheat forage was 235 g/kg fresh material. There was no significant interaction between time of fertilizer application and seeding rate for concentrations of CP, crude fiber, ether extract, crude ash, nitrogen-free extract, NDF and ADF ($P>0.05$), but there was significant interaction for WSC concentration and buffering capacity ($P<0.05$) (Table 5). In general, WSC concentration and buffering capacity were higher ($P<0.05$) when the second fertilizer application was made at jointing rather than at heading. With the increase in seeding rate, NDF and ADF concentrations in WCW tended to increase, but CP concentration tended to decrease. Regardless of whether the second fertilizer application was made at the jointing or heading stage, seeding rate had no significant effect on CP (range 88.4-97.4 g/kg DM), ether extract and NDF (range 608-660 g/kg DM) concentrations ($P>0.05$). While populations of yeast, molds and aerobic bacteria were unaffected by treatment, LAB populations were consistently higher at the intermediate fertilizer level ($P<0.05$) (Table 5).

Silage fermentation characteristics. All silages had pH between 3.64 and 3.94 with no consistent pattern between the treatments (Table 6). Concentrations of organic acids in the silages had the following ranges: lactic acid – 14.1–21.4 g/kg DM; acetic acid – 1.08–1.56 g/kg DM; butyric acid – 0.88–2.14 g/kg DM; and propionic acid – 1.04–2.68 g/kg DM, with significant differences between treatments but no consistent pattern over the various treatments. NH_3-N concentration ranged from 141 to 172 g N/kg total N, again with differences between treatments but no consistent pattern.

Experiment 2

Plant height, tiller number, yield and relative feed value. Plant height was significantly ($P<0.05$) affected by fertilizer type with urea>compound fertilizer>urea + compound fertilizer (Table 7). However, tiller number/plant (mean 1.75), DM yield (mean 9.25 t/ha) and CP yield (mean 1.0 t/ha) were not affected by fertilizer type ($P>0.05$). Relative feed value varied with fertilizer type, being higher with compound fertilizer alone than with the other fertilizers (113 vs. 103; Table 7) ($P<0.05$).

Chemical and microbial composition. DM concentration of fresh forage was affected by fertilizer type, being highest for urea (391 g/kg FM) and lowest for compound fertilizer alone (338 g/kg FM) ($P<0.05$) (Table 8). However, fertilizer type had no effect ($P>0.05$) on concentrations of CP (mean 108 g/kg DM), ADF (mean 313 g/kg DM) and WSC (mean 105 g/kg DM) in forage or pH (mean 5.55). On the other hand, fertilizer type affected NDF concentration (urea and urea + compound fertilizer>compound fertilizer) and buffering capacity (compound fertilizer>urea>urea + compound fertilizer). Fertilizer type had no effect on numbers of lactic acid bacteria, aerobic bacteria, yeasts or molds in fresh forage ($P>0.05$).

Silage fermentation characteristics. Fertilizer type had significant effects on the pH value and concentrations of organic acids (except propionic acid) and NH_3-N in WCW silage ($P<0.05$) (Table 9). The pH value for silages from both urea and compound fertilizer alone exceeded that from the combined fertilizer (4.21 vs. 4.05; Table 9). While lactic acid concentration for silage from the urea + compound fertilizer treatment was greater than that from the other treatments, acetic acid concentration was higher for silage from the urea treatment than from the 2 treatments containing compound fertilizer ($P<0.05$). Propionic acid concentration in the various silages did not differ ($P>0.05$), while butyric acid concentration in silages followed the order: urea>compound fertilizer>urea + compound fertilizer ($P<0.05$). NH_3-N concentration was greater for silage from the urea treatment than from the other 2 treatments ($P<0.05$).

Table 4. Effects of seeding rate and fertilizing time on forage and crude protein yields, plant height and tiller number (± SD) of wheat and relative feed value.

Parameter	Jointing stage			Heading stage			Significance		
	S_{320}	S_{385}	S_{450}	S_{320}	S_{385}	S_{450}	Fertilizing time	Seeding rate	Interaction
Plant height (cm)	85.2 ± 2.63	87.8 ± 2.36	91.6 ± 1.72	87.5 ± 1.28	86.6 ± 1.12	87.3 ± 1.33	NS	NS	NS
Tiller number/plant	2.13 ± 0.20	1.96 ± 0.14	1.63 ± 0.12	1.88 ± 0.15	1.67 ± 0.13	1.83 ± 0.14	NS	NS	NS
DM yield (t/ha)	7.87 ± 0.37c	9.41 ± 0.39ab	10.69 ± 0.21a	8.21 ± 0.37bc	8.04 ± 0.67bc	10.30 ± 0.56a	NS	**	NS
CP yield (t/ha)	0.77 ± 0.04bc	0.88 ± 0.04ab	0.98 ± 0.04a	0.75 ± 0.03bc	0.71 ± 0.06c	0.92 ± 0.04a	*	**	NS
Relative feed value	93.3 ± 1.42ab	91.0 ± 2.21b	89.8 ± 1.08bc	97.3 ± 2.62a	92.5 ± 2.36ab	84.7 ± 0.83c	NS	*	NS

Means within a row with different letters differ at $P<0.05$. S_{320}, seeding rate of 320 kg/ha; S_{385}, seeding rate of 385 kg/ha; S_{450}, seeding rate of 450 kg/ha.

Table 5. Effects of seeding rate and fertilizing time on chemical and microbial composition (± SD) of wheat forage.

Items	Jointing stage			Heading stage			Significance		
	S_{320}	S_{385}	S_{450}	S_{320}	S_{385}	S_{450}	Fertilizing time	Seeding rate	Interaction
Crude protein (g/kg DM)	97.4 ± 0.53	93.4 ± 0.56	91.5 ± 1.79	91.5 ± 0.28	88.4 ± 0.42	89.2 ± 1.13	NS	NS	NS
Crude fiber (g/kg DM)	330 ± 7.3	347 ± 3.2	358 ± 12.0	318 ± 6.1	329 ± 12.7	336 ± 11.1	NS	NS	NS
Ether extract (g/kg DM)	26.2 ± 0.43	25.8 ± 0.07	26.0 ± 0.74	29.6 ± 0.71	27.1 ± 0.33	29.6 ± 1.01	NS	NS	NS
Crude ash (g/kg DM)	82.9 ± 1.71a	72.7 ± 3.00b	70.6 ± 1.94b	80.2 ± 1.23a	66.9 ± 0.69b	73.49 ± 2.32b	NS	**	NS
Nitrogen free extract (g/kg DM)	464 ± 9.9abc	462 ± 8.3bc	452 ± 17.9c	481 ± 17.6ab	488 ± 6.1a	476 ± 11.4ab	**	NS	NS
Neutral detergent fiber (g/kg DM)	616 ± 8.2b	627 ± 8.1b	629 ± 0.4b	608 ± 8.9b	633 ± 10.3b	660 ± 2.5a	NS	**	NS
Acid detergent fiber (g/kg DM)	349 ± 2.5abc	354 ± 9.9ab	362 ± 9.9ab	325 ± 12.1c	334 ± 7.8bc	370 ± 4.7a	NS	*	NS
Water-soluble carbohydrate (g/kg DM)	114 ± 0.5a	110 ± 0.8ab	111 ± 2.0ab	99.6 ± 2.51d	106 ± 1.8bc	101 ± 2.5cd	**	NS	**
pH	5.96 ± 0.06a	5.92 ± 0.02a	5.95 ± 0.05a	5.64 ± 0.06b	5.92 ± 0.02a	5.87 ± 0.03a	NS	**	NS
Buffering capacity (mE/kg DM)	271 ± 2.6b	320 ± 3.0a	258 ± 5.0b	224 ± 2.7d	242 ± 276c	241 ± 7.7c	**	**	**
Lactic acid bacteria (lg cfu/g FM)	5.98 ± 0.24b	6.87 ± 0.07a	5.92 ± 0.17b	5.45 ± 0.15b	6.68 ± 0.03a	5.57 ± 0.20b	NS	**	NS
Aerobic bacteria (lg cfu/g FM)	8.45 ± 0.03	8.49 ± 0.02	8.43 ± 0.03	8.37 ± 0.09	8.46 ± 0.07	8.43 ± 0.05	NS	NS	NS
Yeasts (lg cfu/g FM)	6.49 ± 0.11	6.51 ± 0.12	6.47 ± 0.18	6.55 ± 0.04	6.40 ± 0.07	6.42 ± 0.11	NS	NS	NS
Molds (lg cfu/g FM)	4.96 ± 0.14	4.90 ± 0.10	4.90 ± 0.10	4.70 ± 0.01	4.80 ± 0.10	4.86 ± 0.16	NS	NS	NS

Means within a row with different letters differ at $P<0.05$. S_{320}, seeding rate of 320 kg/ha; S_{385}, seeding rate of 385 kg/ha; S_{450}, seeding rate of 450 kg/ha. FM, fresh matter; DM, dry matter; lg, denary logarithm of the numbers; cfu, colony-forming unit.

Table 6. Effects of seeding rate and fertilizing time on the fermentation quality of wheat silage.

Items	Jointing stage			Heading stage			Significance		
	S_{320}	S_{385}	S_{450}	S_{320}	S_{385}	S_{450}	Fertilizing time	Seeding rate	Interaction
pH	3.75 ± 0.00c	3.85 ± 0.02b	3.64 ± 0.01d	3.68 ± 0.01d	3.83 ± 0.01b	3.94 ± 0.03a	**	**	**
Lactic acid (g/kg DM)	19.4 ± 0.14b	16.6 ± 0.30c	21.4 ± 0.87a	18.5 ± 0.23b	15.5 ± 0.28cd	14.1 ± 0.45d	**	**	**
Acetic acid (g/kg DM)	1.18 ± 0.01bc	1.56 ± 0.04a	1.33 ± 0.12b	1.09 ± 0.01d	1.24 ± 0.02bc	1.08 ± 0.11cd	**	**	NS
Propionic acid (g/kg DM)	1.69 ± 0.09b	2.68 ± 0.04a	2.58 ± 0.17a	1.04 ± 0.16c	2.38 ± 0.19a	2.51 ± 0.22a	*	**	NS
Butyric acid (g/kg DM)	1.28 ± 0.11b	1.31 ± 0.09b	1.47 ± 0.12b	0.88 ± 0.05c	1.57 ± 0.07b	2.14 ± 0.03a	NS	**	**
NH_3-N (g/kg TN)	172 ± 1.18a	163± 3.03b	141 ± 1.22d	133 ± 2.24e	153 ± 2.10c	157 ± 3.18bc	**	**	**

Means within a row with different letters differ at $P<0.05$. S_{320}, seeding rate of 320 kg/ha; S_{385}, seeding rate of 385 kg/ha; S_{450}, seeding rate of 450 kg/ha. TN, total nitrogen.

Table 7. Effects of fertilizer type on yield and relative feed value of wheat forage.

Parameter	Urea	Compound fertilizer	Urea + compound fertilizer
Plant height (cm)	86.8 ± 1.04a	83.8 ± 1.14b	82.3 ± 1.08c
Tiller number per plant	1.67 ± 0.14	1.79 ± 0.15	1.79 ± 0.17
Dry matter yield (t/ha)	9.34 ± 0.69	9.22 ± 0.74	9.19 ± 0.88
Crude protein yield (t/ha)	0.99 ± 0.07	1.00 ± 0.80	1.00 ± 0.10
Relative feed value	103 ± 1.45b	113 ± 0.25a	103 ± 0.82b

Means within a row with different letters differ at P<0.05

Table 8. Effects of fertilizer type on chemical and microorganism composition (± SD) of wheat forage.

Parameter	Urea	Compound fertilizer	Urea + compound fertilizer
Dry matter (g/kg FM)	393 ± 0.5a	338 ± 3.8c	351 ± 4.3b
Crude protein (g/kg DM)	106.5 ± 1.25	108.1 ± 2.70	109.1 ± 0.40
Crude fiber (g/kg DM)	301 ± 6.4b	340 ± 2.8a	324 ± 6.7a
Ether extract (g/kg DM)	25.1 ± 1.50b	32.1 ± 0.70a	26.6 ± 0.75b
Crude ash (g/kg DM)	50.6 ± 1.39	54.8 ± 0.87	53.4 ± 1.29
Nitrogen free extract (g/kg DM)	517 ± 6.0a	465 ± 16.9b	487 ± 6.4b
Neutral detergent fiber (g/kg DM)	579 ± 5.3a	537 ± 10.7b	580 ± 1.5a
Acid detergent fiber (g/kg DM)	322 ± 5.3	301 ± 8.6	315 ± 7.5
Water-soluble carbohydrate (g/kg DM)	103 ± 1.6	111 ± 6.0	102 ± 1.1
pH	5.51 ± 0.11	5.45 ± 0.03	5.70 ± 0.21
Buffering capacity (mE/kg DM)	183 ± 2.9b	219 ± 3.4a	161 ± 9.5c
Lactic acid bacteria (lg cfu/g FM)	5.94 ± 0.13	6.05 ± 0.03	6.23 ± 0.05
Aerobic bacteria (lg cfu/g FM)	8.23 ± 0.02	8.35 ± 0.01	8.37 ± 0.05
Yeasts (lg cfu/g FM)	6.54 ± 0.06	6.79 ± 0.08	6.74 ± 0.09
Molds (lg cfu/g FM)	5.00 ± 0.17	4.80 ± 0.10	4.90 ± 0.10

Means within a row with different letters differ at P<0.05. FM, fresh matter; lg, denary logarithm of the numbers; cfu, colony-forming units.

Table 9. Effects of fertilizer type on the fermentation quality (± SD) of wheat silage.

Parameter	Urea	Compound fertilizer	Urea + compound fertilizer
pH	4.24 ± 0.03a	4.18 ± 0.05a	4.05 ± 0.01b
Lactic acid (g/kg DM)	12.03 ± 0.41b	11.88 ± 0.27b	14.09 ± 0.70a
Acetic acid (g/kg DM)	1.62 ± 0.17a	1.17 ± 0.14b	1.15 ± 0.02b
Propionic acid (g/kg DM)	2.61 ± 0.33	2.36 ± 0.22	2.26 ± 0.03
Butyric acid (g/kg DM)	2.87 ± 0.11a	2.48 ± 0.12b	1.01 ± 0.02c
NH_3-N (g/kg TN)	150 ± 14.2a	124 ± 3.4b	132 ± 11.9b

Means within a row with different letters differ at P<0.05. TN, total nitrogen.

Discussion

Both seeding rate and timing of fertilizer application are considered important management strategies affecting crop production, and an optimal seeding rate can achieve a balance between yield of wheat forage and cost of seed. In general, increasing seeding rates results in higher yield (Counce et al. 1992; Jia et al. 2018).

In this study, both DM and CP yields of forage planted at 450 kg seed/ha (S_{450}) were significantly higher than that of S_{320} (P<0.05), which supports the statement above. In general, high seeding rate of crops exacerbates the competition among plants for critical resources such as water, nutrients and light (Xue et al. 2016), so accumulation of DM per plant can be reduced, but the higher plant population more than makes up for the reduction in DM yield per plant, thus increasing yield (Liu et al. 2011). Plants at the higher seeding rate in our study possibly intercepted more incident light, thus resulting in greater DM accumulation, which is consistent with the results of Arduini et al. (2006).

Tran and Tremblay (2000) found that applying fertilizer at the heading stage promoted the growth of wheat during the reproductive period, reduced the effects of inefficient tillering and increased the nitrogen concentration in grain. In our study, time of applying the second application of fertilizer had no significant effect on most of the parameters measured, suggesting that timing of fertilizer application in this case was not critical.

With the increase in seeding rate, concentrations of crude fiber, NDF and ADF in wheat forage tended to increase in this study but differences failed to reach significance. This suggests that the nutritive value of WCW would tend to decrease at higher seeding rates as was shown by a trend of lowering relative feed value as seeding rate increased.

When wheat was fertilized at the heading stage, the WSC concentration in the forage tended to decrease, compared with wheat fertilized at the jointing stage. From the perspective of silage production, higher WSC concentration can promote lactic acid fermentation and improve silage fermentation quality. Lactic acid and acetic acid production in silages in Experiment 1 showed no consistent pattern across treatments but pH of all silages was in the range 3.64–3.94, indicating good quality silage, which was reinforced by the low concentrations of butyric acid (0.88–2.14 g/kg DM). When fertilizer application occurred at the jointing stage, the NH_3-N concentration in the silage decreased significantly with increase of seeding rate, indicating that protein decomposition of silage was low under high seeding rate. However, when fertilizer was applied at the heading stage, the silage fermentation quality of wheat forage decreased with increasing seeding rate. Generally, the production of acetic acid is dominated by *Enterobacter*, *Enterococcus* and *Clostridium*, which are also the bacteria that decompose amino acids to produce NH_3-N. *Enterobacter* also dominates the production of NPN, degrading protein by secreting carboxypeptidase (Li 2018).

Nitrogen from urea is released rapidly in the early stages after application to the soil when the release rate can exceed the crop demand, which can result in insufficient N supply in the later stages of crop growth. In this study, substituting compound fertilizer for urea or combining urea and compound fertilizer, resulted in no significant change in DM yield of WCW, indicating that the N component was the over-riding factor determining growth of the wheat and losses of N from volatilization of urea were not a significant issue. Increased quantities of phosphate (P) and potassium (K) obviously had no effect on growth of the wheat. Beauregard et al. (2010) suggested that applying P_2O_5 could directly or indirectly change the chemical, physical and biological characteristics of soil, increase soil P availability and increase the CP concentration of forage without having any significant effect on forage yield. Given the available P and K levels in the soil where the study was conducted, it is not surprising that there were no DM yield responses to compound fertilizer over that with urea application. The application of compound fertilizer improved the relative feed value of wheat, which was a function of a significant increase in ether extract and a significant reduction in NDF concentration in forage from this treatment. Berg et al. (2007) found that application of phosphate fertilizer reduced NDF and ADF concentrations in forage.

NH_3-N, acetic acid and butyric acid concentrations in silage from the urea treatment were higher than those in silages from compound fertilizer and urea + compound fertilizer treatments, which supported the results reported by Namihira et al. (2011). The wheat silage from the urea + compound fertilizer treatment had the highest lactic acid concentration and the lowest butyric acid concentration in the 3 fertilizer treatments, possibly because the lower buffering capacity accelerated the decrease in pH and promoted the fermentation of lactic acid.

Conclusions

This study has shown that WCW has the propensity for high yields of forage of high feeding value. Under the conditions of this study, considering DM yield, nutrient composition and silage fermentation quality, a seeding rate of wheat for forage of 385 kg/ha would seem appropriate. If fertilizer application to wheat is to be split, applying a part at jointing stage would be more beneficial than that at heading stage. Compared with urea and compound fertilizer alone, applying urea with compound fertilizer did not affect the yield and nutritive value of WCW, but did improve the silage fermentation quality. These results need verification on different soil types.

Acknowledgments

This work was supported by the National Key Research and Development Program of China (2017YFD0502102-02) and Science and Technology Project of Guangdong Province, China (2016A020210065).

References

(Note of the editors: All hyperlinks were verified 16 April 2021.)

AOAC International. 2011. Official methods of analysis. 18th Edn. AOAC International, Gaithersburg, MD, USA.

Arduini I; Masoni A; Ercoli L; Mariotti M. 2006. Grain yield, and dry matter and nitrogen accumulation and remobilization in durum wheat as affected by variety and seeding rate. European Journal of Agronomy 25(4):309–318. doi: 10.1016/j.eja.2006.06.009

Beauregard MS; Hamel C; Atul-Nayyar; St-Arnaud M. 2010. Long-term phosphorus fertilization impacts soil fungal and bacterial diversity but not AM fungal community in alfalfa. Microbial Ecology 59(2):379–389. doi: 10.1007/s00248-009-9583-z

Berg WK; Cunningham SM; Brouder SM; Joern BC; Johnson KD; Santini JB; Volenec JJ. 2007. The long-term impact of phosphorus and potassium fertilization on alfalfa yield and yield components. Crop Science 47(5):2198–2209. doi: 10.2135/cropsci2006.09.0576

Cinar S; Özkurt M; Cetin R. 2020. Effects of nitrogen fertilization rates on forage yield and quality of annual ryegrass (*Lolium multiflorum* L.) in central black sea climatic zone in Turkey. Applied Ecology and Environmental Research 18(1):417–432. doi: 10.15666/aeer/1801_417432

Counce PA; Wells BR; Gravois KA. 1992. Yield and harvest-index responses to preflood nitrogen fertilization at low rice plant populations. Journal of Production Agriculture 5(4):492–497. doi: 10.2134/jpa1992.0492

Filya I. 2003. Nutritive value of whole-crop wheat silage harvested at three stages of maturity. Animal Feed Science and Technology 103:85-95. doi: 10.1016/S0377-8401(02)00284-5

Günal M; McCourt A; Zhao Y; Yan ZG; Yan T. 2018. The effect of silage type on animal performance, energy utilisation and enteric methane emission in lactating dairy cows. Animal Production Science 59(3):499–505. doi: 10.1071/AN16435

Guo J; Thapa S; Voigt T; Owens V; Boe A; Lee DK. 2017. Biomass yield and feedstock quality of prairie cordgrass in response to seeding rate, row spacing, and nitrogen fertilization. Agronomy Journal 109:2474–2485. doi: 10.2134/agronj2017.03.0179

Huuskonen A; Pesonen M; Joki-Tokola E. 2017. Feed intake and live weight gain of Hereford bulls offered diets based on whole-crop barley and whole-crop wheat silages relative to moderately digestible grass silage with or without protein supplementation. Annals of Animal Science 17(4):1123–1134. doi: 10.1515/aoas-2017-0007

Jia QM; Sun LF; Ali S; Zhang Y; Liu DH; Kamran M; Zhang P; Jia Z; Ren XL. 2018. Effect of planting density and pattern on maize yield and rainwater use efficiency in the Loess Plateau in China. Agricultural Water Management 202:19–32. doi: 10.1016/j.agwat.2018.02.011

Kim KS; Anderson JD. 2015. Forage yield and nutritive value of winter wheat varieties in the southern Great Plains. Euphytica 202(3):445–457. doi:10.1007/s10681-014-1325-8

Li CJ. 2015. Effects of varieties, N applying and seeding rates on nutritive value and fermentation quality of whole-crop wheat. M.A.Sc. Thesis. South China Agricultural University, China.

Li CJ; Xu ZH; Dong ZX; Shi SL; Zhang JG. 2016. Effects of nitrogen application rate on the yields, nutritive value and silage fermentation quality of whole-crop wheat. Asian-Australasian Journal of Animal Sciences 29(8):1129–1135. doi: 10.5713/ajas.15.0737

Li XJ. 2018. Research on mechanism and modification of protein degradation in alfalfa silage. Ph.D. Thesis. China Agricultural University, China.

Liu QH; Shao T; Zhang JG. 2013. Determination of aerobic deterioration of corn stalk silage caused by aerobic bacteria. Animal Feed Science and Technology 183:124–131. doi: 10.1016/j.anifeedsci.2013.05.012

Liu SM; Cai YB; Zhu HY; Tan ZL. 2012. Potential and constraints in the development of animal industries in China. Journal of the Science of Food and Agriculture 92(5):1025–1030. doi: 10.1002/jsfa.4534

Liu W; Zhang JW; Lyu P; Yang JS; Liu P; Dong ST; Sun QQ. 2011. Effect of plant density on grain yield, dry matter accumulation and partitioning in summer maize cultivar Denghai 661. Acta Agronomica Sinica 37(7):1301–1307. (in Chinese)

Murphy RP. 1958. A method for the extraction of plant samples and the determination of total soluble carbohydrates. Journal of the Science of Food and Agriculture 9(11):714–717. doi: 10.1002/jsfa.2740091104

Namihira T; Shinzato N; Akamine H; Nakamura I; Maekawa H; Kawamoto Y; Matsui T. 2011. The effect of nitrogen fertilization to the sward on guineagrass (*Panicum maximum* Jacq cv. Gatton) silage fermentation. Asian-Australasian Journal of Animal Sciences 24(3):358–363. doi: 10.5713/ajas.2011.10191

Pan QM; Yu ZW; Wang YF; Tian QZ. 1999. Studies on uptake and distribution of nitrogen in wheat at the level of 9000 kg per hectare. Acta Agronomica Sinica 25(5):541–547. (in Chinese)

Playne MJ; McDonald P. 2010. The buffering constituents of herbage and of silage. Journal of the Science of Food and Agriculture 17(6):264–268. doi: 10.1002/jsfa.2740170609

Ravier C; Jean-Marc M; Cohan JP; Gate P. 2017. Early nitrogen deficiencies favor high yield, grain protein content and N use efficiency in wheat. European Journal of Agronomy 89:16-24. doi: 10.1016/j.eja.2017.06.002

Rohweder DA; Barnes RF; Jorgensen N. 1978. Proposed hay grading standards based on laboratory analyses for evaluating quality. Journal of Animal Science 47(3):747–759. doi: 10.2527/jas1978.473747x

Shaani Y; Nikbachat M; Yosef E; Ben-Meir Y; Mizrahi I; Miron J. 2017. Effect of feeding long or short wheat hay *v* wheat silage in the ration of lactating cows on intake, milk production and digestibility. Animal 11:2203–2210. doi: 10.1017/S1751731117001100

Sprague SJ; Kirkegaard JA; Dove H; Graham JM; McDonald E; Kelman WM. 2015. Integrating dual-purpose wheat and canola into high-rainfall livestock systems in south-eastern Australia. 1. Crop forage and grain yield. Crop and Pasture Science 66(4):365–376. doi: 10.1071/CP14200

Tran TS; Tremblay G. 2000. Recovery of ^{15}N-labeled fertilizer by spring bread wheat at different N rates and application times. Canadian Journal of Soil Science 80(4):533–539. doi: 10.4141/S99-098

Van Soest PJ; Robertson JB; Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74(10):3583–3597. doi: 10.3168/jds.S0022-0302(91)78551-2

Wu GL; Cui XZ. 2000. Study on nutrient mechanism of N P K fertilizer and its absorbed law of winter wheat with high yield. Chinese Agricultural Science Bulletin 16(2):8–11. (in Chinese)

Xie ZL; Zhang TF; Chen XZ; Li GD; Zhang JG. 2012. Effects of maturity stages on the nutritive composition and silage quality of whole-crop wheat. Asian-

Australasian Journal of Animal Sciences 25(10):1374–1380. doi: 10.5713/ajas.2012.12084

Xue J; Gou L; Zhao YS; Yao MN; Yao HS; Tian JS; Zhang WF. 2016. Effects of light intensity within the canopy on maize lodging. Field Crops Research 188:133–141. doi: 10.1016/j.fcr.2016.01.003

Yang J. 2011. Effect of sow dates and densities on wheat growth characteristics and yield. M.A.Sc. Thesis. Northwest A & F University, Yangling, China.

Zadoks JC; Chang TT; Konzak CF. 1974. A decimal code for the growth stages of cereals. Weed Research 14(6):415–421. doi: 10.1111/j.1365-3180.1974.tb01084.x

Zhang WL; Xu AG; Zhang RL; Ji HJ. 2014. Review of soil classification and revision of China soil classification system. Scientia Agricultura Sinica 47(16):3214–3230. doi: 10.3864/j.issn.0578-1752.2014.16.009 (in Chinese).

(Received for publication 15 March 2020; accepted 7 January 2021; published 31 May 2021)

doi: 10.17138/TGFT(9)235-242

Research Paper

Evaluation of cactus pear clones subjected to salt stress

Evaluación de clones de nopal sometidos a estrés de salinidad

JOELMA DE LIRA FREIRE[1], MÉRCIA VIRGINIA FERREIRA DOS SANTOS[1*], JOSÉ CARLOS BATISTA DUBEUX JÚNIOR[2], EGIDIO BEZERRA NETO[1], THE LATE MÁRIO DE ANDRADE LIRA[1], MÁRCIO VIEIRA DA CUNHA[1*], DJALMA CORDEIRO DOS SANTOS[3], ALEXANDRE CARNEIRO LEÃO DE MELLO[1*] AND CRISTIANE GOMES DA SILVA OLIVEIRA[1]

[1]*Universidade Federal Rural de Pernambuco, Dois Irmãos, Recife-PE, Brasil.* ufrpe.br/
[2]*University of Florida, North Florida Research & Education Center, Marianna-FL, United States of America.* nfrec.ifas.ufl.edu/
[3]*Instituto Agronômico de Pernambuco, Bongi, Recife-PE, Brasil.* ipa.br/novo/
[*]*CNPq fellow*

Abstract

Cactus pear is an important source of fodder for herbivores, though its production is affected by soil salinity. This work aimed to evaluate the development of cactus pear clones subjected to salt stress as a measure of their salinity tolerance. The experimental design was a randomized block with 6 replications. Twenty genotypes of cactus pear were used from the Active Germplasm Bank, with variability in resistance to carmine cochineal [*Dactylopius opuntiae* (Cockerell)] as this insect species destroys many stands of cactus on farms. One cladode was planted per pot containing 12 kg of sandy soil. Water with a salinity of 3.6 dS/m was applied at 14-day intervals. The clones were scored at 28-day intervals for degree of damage to cladodes (1–5) and were harvested when they reached a score of 5, showing 100% damage (chlorosis and dehydration) to the whole plant. The Liso Forrageiro clone took 419 days to reach Score 5, indicating its greater tolerance to salinity. Highest dry matter yields were achieved by Orelha de Elefante Mexicana and Orelha de Elefante Africana clones with 51.5 and 50.8 g/plant, respectively, while Liso Forrageiro yielded only 36.1 g/plant, despite having similar surface area and weight of roots to these 2 clones. Further studies in the field involving these better-performing clones seem warranted to determine how the pot trial results are repeated in the field, especially in areas where carmine cochineal insect is prevalent.

Keywords: Cactus forage, *Nopalea cochenillifera*, *Opuntia ficus-indica*, root, salinity damage.

Resumen

El nopal es una fuente importante de forraje para los herbívoros, aunque su producción se ve afectada por la salinidad del suelo. Este trabajo tuvo como objetivo evaluar el desarrollo de clones de nopal sometidos a estrés salino como medida de su tolerancia a la salinidad. El diseño experimental fue en bloques al azar con 6 repeticiones. Se utilizaron veinte genotipos de nopal del Banco de Germoplasma Activo, con variabilidad en la resistencia a la cochinilla silvestre [*Dactylopius opuntiae* (Cockerell)], ya que esta especie de insecto destruye gran cantidad de plantaciones de nopal en áreas agrícolas. Se plantó un cladodio por maceta que contenía 12 kg de suelo arenoso. Se aplicó agua con una salinidad de 3.6 dS/m a intervalos de 14 días. Cada 28 días, puntajes fueron otorgados a los clones en relación al grado de daño a los cladodios (1–5) y se cosecharon cuando alcanzaron una puntuación de 5, mostrando un 100% de daño (clorosis y deshidratación) en toda la planta. El clon Liso Forrageiro tardó 419 días en alcanzar el puntaje 5, lo que indica su mayor tolerancia a la salinidad. Los clones Orelha de Elefante Mexicana y Orelha de Elefante Africana obtuvieron los mayores rendimientos de materia seca con 51.5 y 50.8 g/planta, respectivamente, mientras que Liso Forrageiro rindió solo 36.1 g/planta, a pesar de tener una superficie y peso de raíces similares a estos 2 clones. Estudios adicionales en campo que

Correspondence: M.V.F. dos Santos, Federal Rural University of Pernambuco, Rua Manoel de Medeiros, s/n, Dois Irmãos, CEP 52171-900, Recife-PE, Brazil. Email: mercia.vfsantos@ufrpe.br

involucran a estos clones de mejor desempeño parece justificado, para determinar cómo se repiten los resultados obtenidos en macetas en campo, especialmente en áreas donde prevalece el insecto cochinilla silvestre.

Palabras clave: Daño por salinidad, *Nopalea cochenillifera*, Nopal forrajero, *Opuntia ficus-indica*, raíz.

Introduction

The cactus pear (*Opuntia* spp. and *Nopalea* spp.) is an important fodder in semi-arid regions due to its adaptation to rainfall irregularities. According to Fonseca et al. (2019), cactus pear is considered a xerophilic plant, so it is adapted to harsh semi-arid conditions and is an important forage resource for animal production in regions that are subject to feed shortage and long dry periods. The growth rate and low-input requirements of prickly pear cactus [*Opuntia ficus-indica* (L.) Mill.] make it an excellent forage or fodder supplement/replacement in semi-arid and arid regions (Mayer and Cushman 2019). It is regarded as a good and inexpensive source of energy, which may reduce the use of concentrate feeds and expensive fodder crops in dry areas (Rodrigues et al. 2016).

Saline water is used in agriculture in arid and semi-arid zones because potable water is scarce, especially in the dry season, but its application contributes to soil salinization (Katerji et al. 2003). To obtain economically viable and environmentally sustainable production, farmers are compelled to substitute salt-tolerant species for more sensitive crops in agricultural areas affected by salinity (Oliveira et al. 2005).

According to Inglese et al. (2017), *Opuntia* spp. are relatively tolerant of water stress, but sensitive to salinity. These authors also mention that the variation in salinity tolerance between cactus pear accessions is not well known. According to Jacobo and Chessa (2017), genetic variability in cacti must be documented if their full potential is to be exploited. Lallouche et al. (2017) completed a Biplot-based species analysis which revealed that *O. engelmannii* was the most salt-tolerant and *O. amyclaea* and *O. robusta* were the most sensitive ones. Freire et al. (2018) observed that water salinity of 3.6 dS/m and an irrigation frequency of 7 days resulted in significant damage to and reduced productivity of cactus pear cv. Miúda relative to other salinity levels and irrigation frequencies, suggesting high sensitivity of this clone to salt stress.

The carmine cochineal insect [*Dactylopius opuntiae* (Cockerell)] (Hemiptera: Dactylopiidae) has affected large areas of cactus pear in northeastern Brazil, so evaluation of cactus pear clones resistant to carmine cochineal under different environmental conditions is extremely important if cactus is to be used commercially as a fodder.

In the literature, information on the effect of salinity on the development of cactus pear clones with different levels of resistance to carmine cochineal is rare. This strengthens the need for these evaluations to improve the understanding of the morphological and productive responses of the clones to saline conditions.

The hypothesis was that cactus pear species and clones differ in sensitivity to abiotic factors, which may affect their development. Thus, the objective of this work was to evaluate the development of 20 cactus pear clones with different levels of resistance to carmine cochineal when submitted to saline stress.

Materials and Methods

The experiment was conducted in a greenhouse, with a maximum temperature of 45 ºC, a minimum of 30 ºC, maximum relative humidity of 85% and a minimum of 75%.

Fresh cladodes were used as planting material, weighing between 175 and 1,950 g (fresh weight). One cladode was planted in each polyethylene pot containing 12 kg of soil with a sandy texture, collected in São Bento do Una, a semi-arid region of Pernambuco, Brazil. Chemical attributes of the soil were as follows: pH – 5.80; phosphorus (Mehlich I) – 41 g/dm^3; sodium – 0.03 cmolc/dm^3; potassium – 0.17 cmolc/dm^3; calcium (1 mol/L KCl) – 0.95 cmolc/dm^3; magnesium – 0.75 cmolc/dm^3; aluminum (1 mol/L KCl) – 0.10 cmolc/dm^3; hydrogen + aluminum (0.5 mol/L calcium acetate) – 0.49 cmolc/dm^3; and organic matter 8.52 g/kg; and physical attributes, determined by hydrometer method (error = ± 5%), were: 88% sand; 2.14% silt; and 9.86% clay with a silt:clay ratio of 0.21:1.

Twenty clones of cactus pear (Table 1) were used from the Active Germplasm Bank – BAG, with variability in their resistance to carmine cochineal. The resistance level was classified according to Vasconcelos et al. (2009), as well as previous evaluations carried out at the Germplasm Bank (Table 1).

From 35 days after planting, each pot was irrigated with saline water with an electrical conductivity (EC) of 3.6 dS/m at 14-day intervals. The criteria for irrigation frequency and salinity level of irrigation water were chosen according to Freire et al (2018). Those authors indicated that this salinity level caused the greatest damage to cactus pear.

Table 1. Scientific names, common names and resistance classification of cactus pear species and clones used in the experiment.

Species	Common name of clones/genotypes/cultivars	Resistance to carmine cochineal
Nopalea cochenillifera (L.) Salm-Dyck	F-21	High
Nopalea cochenillifera(L.) Salm-Dyck	Miúda	High
Nopalea cochenillifera(L.) Salm-Dyck	Ipa Sertânia	High
Nopalea cochenillifera (L.) Salm-Dyck	Orelha de Onça	High
Opuntia fícus-indica (L.) Mill.	Copena F1	Low
Opuntia fícus-indica (L.) Mill.	Copena V1	Low
Opuntia fícus-indica (L.) Mill.	Gigante	Low
Opuntia fícus-indica (L.) Mill.	Ipa Clone 20	Low
Opuntia fícus-indica (L.) Mill.	Redonda	Low
Opuntia fícus-indica (L.) Mill.	IPA 90-156	Low
Opuntia fícus-indica (L.) Mill.	IPA 90-111	Low
Opuntia fícus-indica (L.) Mill.	Algerian	Low
Opuntia fícus-indica (L.) Mill.	Liso Forrageiro	Low
Opuntia fícus-indica (L.) Mill.	Chile Fruit/1317	Low
Opuntia undulata Griffiths	Orelha de Elefante Africana	High
Opuntia stricta (Haw.) Haw.	Orelha de Elefante Mexicana	High
Opuntia larreyi F.A.C. Weber ex J.M. Coult.	V-16	Low
Opuntia larreyi F.A.C. Weber ex J.M. Coult.	F-13	High
Opuntia atropes Rose	F-24	Low
Opuntia atropes Rose	F-8	Low

Each pot received 8 mL/pot of micronutrient solution as described by Epstein and Bloom (2006). Nitrogen fertilizer was applied at 3 g N/pot in the form of 33% ammonium nitrate (NH_4NO_3). The amount of irrigation water applied per pot on each occasion was quantified, and irrigation ceased as soon as drainage began. At the end of the experiment, the total volume of water applied per treatment during the experimental period was calculated.

The experimental design was a randomized block, adopting the initial weight of the cladodes as the blocking criterion, with 6 replications.

The EC of drainage water from each pot was determined each 28 days, and then the drained pot was returned to its position. The EC of the soil saturation extract at the beginning of the experiment was 0.34 dS/m.

A scale ranging from 1 to 5 was used to assess the damage to the plants, and the entire plant and cladodes were individually evaluated each 14 days. Cladodes showing dehydration and yellowish coloration were considered to be damaged. Scores of 1, 2, 3, 4 and 5, respectively, were allocated where 0, 1–25, 26–50, 51–75 and 76–100% of plant damage was visible. Plants that obtained a maximum score for damage (5) at 3 consecutive evaluations were harvested by removing the shoots and roots. As the plants were harvested, the soil was collected, and EC of the saturation extract was determined. The whole root system was washed in a tray separating the roots aggregated to the soil, washing them with the aid of a sieve and a water jet until the whole root system was free of soil particles. After the washing procedure, a morphological analysis of the root system was performed using the WinRHIZO Pro 2009 system (Regent Instrument Inc., Québec, Canada) coupled to an Epson XL 10000 professional scanner. For this purpose, the roots were placed in acrylic trays (20 cm wide × 30 cm long) containing water. Use of this accessory allowed 3-dimensional images to be obtained as well as length, surface area and average diameter of roots, avoiding them from overlapping.

Samples of cladodes and roots were taken to determine the chemical composition, analyzing dry matter (DM) content and sodium (Na) concentration according to the methodology described by AOAC (2005) and chlorophyll concentration according to Bezerra Neto and Barreto (2011).

Analysis of variance was performed using the Proc mixed procedure of the SAS Software, and the experimental treatment means were compared using Scott Knott at 5% probability, when significant.

Results

There were significant ($P<0.05$) differences between cactus pear clones in terms of the total water supplied (Table 2). The Liso Forrageiro clone (25.8 L/plant) and F-13 (22.0 L/plant) received the greatest amounts of water, while F-8 (14.0 L) and Chile Fruit (15.2 L) received the least (Table 2).

Significant ($P<0.05$) differences between clones emerged in the number of days required to reach Scores 2, 3, 4 and 5, which represent the different damage levels sustained by the plants (Table 2). Chile Fruit incurred 25% damage (Score 2) in the shortest time (41.5 days), while Liso Forrageiro (137.4 days), F-21 (125.3 days), and Algerian (118.5 days) took the longest time (P<0.05) (Table 3). Damage scores had increased to 3 for Chile Fruit, Gigante, Copena V1, V-16 and F-8 in 43.3–75.4 days, while Ipa Sertânia, Ipa 90-156, F-21, Orelha de Elefante Mexicana, Orelha de Onça, Algerian and Liso Forrageiro clones took 135–172 days to do so (P<0.05). The same pattern continued with Chile Fruit, F-8 and Gigante reaching Score 4 in 111–121 days, while Liso Forrageiro took 265 days (P<0.05). The gap between clones increased with time with F-8 reaching Score 5 in 131 days and Liso Forrageiro in 419 days (P<0.05) (Table 2).

Electrical conductivity of drainage water from the pots increased with time with significant differences between clones throughout (Table 2). At Score 2, lowest EC levels occurred for F-24, F-8, Copena V1 and Ipa Clone 20 (31.7–33.0 dS/m) and the highest EC levels for Algerian, Liso Forrageiro, F-21, and Ipa Sertânia (62.7–69.9 dS/m) (P<0.05). By Score 3, EC levels had increased, with lowest values for Copena V1, Gigante and F-8 (32.8–38.3 dS/m) and the highest for Ipa Sertânia, Liso Forrageiro, Algerian, F-21, Ipa 90-111 and Redonda (69.0–75.0 dS/m) (P<0.05). By Score 4 values had increased further with the lowest values for F-8 (52.7 dS/m) and highest for Ipa Sertânia, Ipa 90-156, Algerian, Liso Forrageiro, V16 and Orelha de Elefante Africana (80.8–88.9 dS/m) (P<0.05). Electrical conductivity of soil at harvest time varied from 66.5 dS/m for F-8 to 91.0-95.8 dS/m for Liso Forrageiro, Ipa Sertânia, Ipa 90-156, F-13, Algerian and V-16 (P<0.05).

There were significant differences (P<0.05) in DM production of cactus pear clones (Table 3).

The lowest yields were observed in F-8, F-21 and Algerian clones (19.2–28.2 g/plant), while highest yields occurred for Orelha de Elefante Africana, Ipa Clone 20, Orelha de Elefante Mexicana, Ipa 90-156 and F-13 (41.3–50.8 g/plant) (P<0.05).

Sodium concentration in plants ranged from 5.7 to 10.7 g/kg, being greater (P<0.05) for Chile Fruit, Ipa Sertânia, F-24, F-21, Orelha de Elefante Mexicana, Redonda, Algerian, Gigante and V-16 clones than for the remainder (Table 3). The lowest values occurred in Liso Forrageiro, Copena V1 and Ipa 90-156 (5.7–6.6 g/kg DM).

Similarly, there were significant differences (P<0.05) in chlorophyll concentrations in the various clones with values varying between 0.54 (Copena V1) and 0.82 mg/g (Liso Forrageiro).

Table 2. Total amount of water, number of days required for cactus pear clones to record damage scores of 2, 3, 4 and 5 and electrical conductivity of drainage water at these stages and electrical conductivity of soil at harvest.

Clone	Total amount of water (L/plant)	Days to reach score				EC drain (dS/m)[1]			Soil EC (dS/m)[2]
		Score				Score			
		2	3	4	5	2	3	4	
F-21	19.2b	125.3a	137.5a	158.2b	227.3c	67.9a	69.7a	79.7b	81.8b
Miúda	18.1b	75.3b	117.5b	150.0c	204.0c	46.7b	62.2b	70.2b	83.3b
Ipa Sertânia	19.6b	90.8b	143.8a	200.2b	242.2c	62.7a	75.0a	82.6a	91.0a
Orelha de Onça	16.4c	69.5c	137.3a	158.3b	228.8c	42.3b	65.7b	70.0b	74.1b
Copena F1	17.0c	66.7c	122.8b	169.7b	213.5c	39.8b	44.3b	72.4b	75.2b
Copena V1	19.1b	61.7c	67.7c	140.8c	236.5c	31.7c	32.9c	66.0b	81.0b
Gigante	17.1c	57.0c	58.7c	120.7c	209.0c	36.7b	38.3c	62.3b	76.1b
Ipa Clone 20	19.2b	64.3c	114.7b	153.8b	229.7c	32.8c	63.0b	74.7b	80.6b
Redonda	17.4b	59.8c	102.3b	173.5b	232.5c	38.5b	69.0a	78.4b	80.3b
Ipa 90-156	20.6c	64.0c	144.0a	203.0b	261.3b	42.3b	62.7b	82.1a	92.7a
Ipa 90-111	19.4b	62.7c	104.2b	186.0b	259.3b	45.0b	69.6a	73.1b	83.5b
Orelha de Elefante Africana	19.9b	66.7c	109.7b	186.7b	251.0b	36.5b	60.2b	80.8a	83.1b
Orelha de Elefante Mexicana	19.1b	60.0c	135.0a	168.2b	247.6b	44.0b	54.4b	72.7b	82.2b
Algerian	20.9b	118.5a	172.0a	205.0b	297.5b	69.9a	70.3a	82.8a	91.3a
Liso Forrageiro	25.8a	137.4a	137.6a	265.2a	419.2a	69.7a	71.7a	88.9a	95.8a
Chile Fruit	15.2c	41.5d	43.3c	111.2c	175.3c	41.2b	52.8b	70.3b	75.0b
V-16	21.4b	71.7b	74.7c	206.5b	323.5b	42.4b	57.3b	83.0a	90.5a
F-13	22.0a	69.2c	113.7b	180.8b	310.8b	37.8b	56.7b	70.0b	93.0a
F-24	18.3b	61.8c	122.0b	145.3c	216.5c	31.8c	64.3b	68.2b	81.4b
F-8	14.0c	60.0c	75.4c	120.8c	130.5d	33.0c	34.9c	52.7c	66.5c
CV (%)	7.8	15.7	10.3	12.2	14.7	9.1	16.3	15.8	13.3

[1]EC: Electrical conductivity in drainage on the day the score was reached. [2]Soil EC: Electrical conductivity in soil. Means followed by different letters within each column differ significantly (P<0.05) by the Scott Knott test.

Significant differences (P<0.05) in length, mean diameter, surface area and dry weight of roots were recorded (Table 4). The longest roots were recorded for Miúda, Orelha de Elefante Africana, Ipa Clone 20, Orelha de Elefante Mexicana, Redonda, Gigante and Liso Forrageiro (32.76–45.89 cm), while shortest roots occurred in Chile Fruit, F-8, Orelha de Onça, Algerian, V-16 and Copena V1 (1.60–3.26 cm) (P<0.05). Similarly, roots of the various clones had differing diameters with thickest roots for F-8, Miúda, Ipa Clone 20, Orelha de Elefante Mexicana, Redonda, Algerian, Gigante and Liso Forrageiro (0.619–0.939 mm), and thinnest roots

Table 3. Dry matter yield and sodium and total chlorophyll concentrations in cactus pear clones grown under saline conditions.

Clone	DM (g/plant)	Na concentration (g/kg DM)	Chlorophyll concentration (mg/g DM)
F-21	28.2 c	10.2 a	0.70 a
Miúda	34.2 b	7.6 b	0.62 b
Ipa Sertânia	39.4 b	9.1 a	0.57 b
Orelha de Onça	38.3 b	8.3 b	0.62 b
Copena F1	35.2 b	7.8 b	0.64 b
Copena V1	32.4 b	6.2 b	0.54 b
Gigante	35.3 b	10.2 a	0.65 a
Ipa Clone 20	50.6 a	8.1 b	0.73 a
Redonda	33.4 b	9.1 a	0.76 a
Ipa 90-156	45.1 a	6.6 b	0.61 b
Ipa 90-111	35.3 b	7.0 b	0.75 a
Orelha de Elefante Africana	50.8 a	7.9 b	0.79 a
Orelha de Elefante Mexicana	51.5 a	9.4 a	0.78 a
Algerian	19.2 c	10.7 a	0.59 b
Liso Forrageiro	36.1 b	5.7 b	0.82 a
Chile Fruit	33.2 b	9.0 a	0.62 b
V-16	32.2 b	9.1 a	0.72 a
F-13	41.3 a	7.4 b	0.68 a
F-24	34.1 b	10.5 a	0.55 b
F-8	25.3 c	7.9 b	0.60 b
CV (%)	6.22	5.67	7.82

Means followed by different letters within each column differ significantly (P<0.05) by the Scott Knott test

Table 4. Length, mean diameter, surface area and dry biomass of roots of cactus pear clones irrigated with saline solution.

Clone	Length (cm)	Mean diameter(mm)	Surface area (cm^2)	Dry weight(g/plant)
F-21	13.25 c	0.587 b	1.45 c	15.2 b
Miúda	39.73 a	0.736 a	3.04 a	15.4 b
Ipa Sertânia	14.72 b	0.372 b	1.30 c	10.3 b
Orelha de Onça	3.26 d	0.297 c	0.89 c	5.1 c
Copena F1	15.89 b	0.589 b	2.12 b	12.8 b
Copena V1	3.00 d	0.229 c	1.02 c	3.1 c
Gigante	43.78 a	0.743 a	3.92 a	29.5 a
IPA Clone 20	38.72 a	0.879 a	2.03 b	22.3 a
Redonda	32.76 a	0.715 a	4.10 a	33.7 a
IPA 90-156	17.89 b	0.249 c	1.23 c	14.7 b
IPA 90-111	26.54 b	0.329 c	1.46 c	15.6 b
Orelha de Elefante Africana	32.91 a	0.515 b	3.42 a	16.1 b
Orelha de Elefante Mexicana	42.09 a	0.829 a	3.15 a	20.2 a
Algerian	1.99 d	0.619 a	2.12 b	3.0 c
Liso Forrageiro	45.89 a	0.939 a	3.51 a	34.7 a
Chile Fruit/1317	2.97 d	0.362 b	0.99 c	5.2 c
V-16	1.60 d	0.216 c	1.03 c	3.2 c
F-13	25.33 b	0.479 b	2.43 b	19.1 b
F-24	18.24 b	0.426 b	1.36 c	13.1 b
F-8	1.96 d	0.682 a	0.76 c	3.1 c
CV (%)	47.1	33.3	52.37	13.12

Means without a common letter within each column differ significantly (P<0.05) by the Scott Knott test.

for Ipa 90-156, Ipa 90-111, Orelha de Onça, V-16 and Copena V1 (0.216–0.297 mm) ($P<0.05$). Consequently, surface area of roots varied widely among the clones with greatest areas for Miúda, Orelha de Elefante Africana, Redonda, Gigante and Liso Forrageiro (3.04–4.10 cm^2) and smallest areas for F-8, Orelha de Onça, Chile Fruit, V-16 and Copena V1 (0.76–1.03 cm^2) (Table 4; $P<0.05$).

The greatest values for root biomass were reached by Liso Forrageiro, Gigante, Redonda, Orelha de Elefante Mexicana and IPA Clone 20 (20.2–34.7 g/plant), while lowest yields were for Chile Fruit, F-8, Orelha de Onça, Algerian, V-16 and Copena V1 (3.0–5.2 g/plant) ($P<0.05$).

Discussion

This study has shown that cactus pear clones display marked variation in adaptation to salinity under greenhouse conditions, indicating that there are options for selecting more productive clones to grow on saline soils.

The amount of water supplied to the cactus pear clones directly influenced the accumulation of Na in the soil and the EC of the soil saturation extract (Table 2). Consequently, chlorosis and wilting symptoms appeared in the clones at different ages due to the accumulation of Na in the soil or inside the plants, making the water or nutrients required unavailable.

While Liso Forrageiro took far longer than other species to reach Score 5 for harvest, this clone has low resistance to carmine cochineal, which is an undesirable characteristic for cactus pear in areas where this insect pest occurs. In addition, this clone was not as productive as some other clones studied.

The clone that reached Score 5 most quickly was F-8 which did so in 130 days (Table 2), possibly indicating lower tolerance to saline stress. The soil, in which this clone was grown, had lower EC (66.5 dS/m) at harvest, probably influenced by the smaller amount of water received. Freire et al. (2018) observed that irrigating cv. Miúda every 7 days with saline water (3.6 dS/m) resulted in an increase in soil EC and a high percentage of damage to cladodes, suggesting high sensitivity of this clone to salt stress.

Franco-Salazar and Veliz (2008) demonstrated that *O. fícus-indica* could survive 10 weeks of treatment with 100 mM NaCl, but the shoot showed symptoms of chlorosis and dehydration at harvest. In our study, most clones had barely reached Score 2 after 70 days of irrigation with saline water. Values for soil EC we recorded are considered high because, according to Bor et al. (2003), most plants can develop when the EC of soil is less than 8 dS/m, while some species grow only in soils with EC $\leq$3 dS/m. Osmotic or ionic stress occurs above this value, causing the plants to die. According to Dias et al. (2016), good quality water for irrigation should have EC <0.75 dS/m, while plant growth generally is not affected below 2.0 dS/m, although differences can be found between species and cultivars. EC values in soil at the end of the study were much higher than these levels.

Accumulation of soluble salts in the root zone in saline environments causes a decrease in cellular expansion, thus reducing the overall development of the plant (Khalid and Silva 2010). Franco-Salazar and Véliz (2008) observed that, when NaCl concentration in the root medium is increased, there is a reduction in the formation of new organs, which causes negative effects in the development of *Opuntia* spp. According to Munns and Tester (2008), the survival of plants subjected to saline stress depends on the ability to restrict ion absorption. It is probable that the Liso Forrageiro clone used tolerance mechanisms to survive for 419 days in a saline environment.

The highest dry matter yields observed in Ipa 90-156, Orelha de Elefante Africana, Ipa Clone 20, F-13 and Orelha de Elefante Mexicana clones (Table 3) may result from the storage of ions in large vacuoles of the cactus pear as well as the accumulation of organic solutes in these vacuoles (Fonseca et al. 2019). Although they were harvested after a shorter growing period than Liso Forrageiro, they showed greater production, and are considered to possess some resistance to carmine cochineal (low, high, low, high and high, respectively).

Amador et al. (2001) observed a productivity of 46 g DM/plant for the Copena V1 clone after 147 days of growth in salinity of 2 dS/m, which was considered low productivity, even though the clone is cultivated and considered adapted to the conditions of the region of Mexico, where mean soil salinity is 2–5 dS/m. Fonseca et al. (2019) irrigated Gigante with saline (3.6 dS/m) water and 33% reference evapotranspiration every 3 days and it grew, increasing in height, number of cladodes, cladode area index, green mass and DM yield. The tolerance of crops to salinity is generally displayed as phytomass production, plant growth, plant number/unit area and ability to survive in saline environments (Dias et al. 2016).

The low yields for F-8, F-21 and Algerian clones (Table 3) indicated that these clones did not grow well in saline soils. Gajender et al. (2014) observed that *O. ficus-indica* was moderately tolerant of salinity (52 mM NaCl), but sensitive to pH, with negligible growth at pH 9.8. Liso Forrageiro was the last clone to be harvested and contained 5.7 g Na/kg DM (Table 3). Thus, it is probable that Na exclusion and/or ion absorption restriction are more efficient in this clone than in the others, indicating its greater adaptation to salt stress, since the soil of this clone presented greater EC. According to Lastiri-Hernández et al. (2018), due to transpiration Na^+ is deposited and accumulates in leaves rather than in roots. In perennial species, Na^+ accumulation in leaves results mainly from their longer lifespan and thus longer duration of transpiration. Silva et al. (2014) observed that Orelha de Elefante Mexicana presented greater water use efficiency, followed by IPA Sertânia. Miúda was the least efficient clone for the Brazilian semi-arid region. Orelha de Elefante Mexicana and Miúda presented greater Na use efficiency.

In a study assessing *Nopalea cochenillifera*, Freire et al. (2018) observed that increasing the salinity level of irrigation water increased Na concentration in the cactus, and there was a significant interaction between irrigation frequency and irrigation water salinity. Amador et al. (2001) observed that Na concentration in shoot DM of Copena F1 increased from 0.51 to 1.51% when grown for 21 weeks in a greenhouse and salinity level of irrigation water was increased from 2 to 21 dS/m. At the salinity level of 5 dS/m, Na concentration in shoots was 0.62%. Schuch and Kelly (2008) irrigated species of cacti (*Echinocactus grusonii*, *Carnegiea gigantea*, *Fouquieria splendens* and *Agave parryi* var. *truncata*) for 5 months and observed that Na concentration in plants varied from 15.52 to 18.30 g/kg DM, as the EC of the irrigation water rose from 0.6 to 15 dS/m.

Franco-Salazar and Veliz (2008) grew *O. fícus-indica* hydroponically for 10 weeks with concentrations of 0, 50 and 150 mM NaCl and showed that chlorophyll concentration ranged from 0.5 to 1.0 mg/g fresh tissue weight. As salinity increased, titratable acidity in apical cladodes also increased, while no significant effect was detected for either the number of apical or basal cladodes or for chlorophyll concentration in either type of cladodes, suggesting some kind of osmotic adjustment or osmoprotection.

The significant differences in lengths of roots in the various cacti (Table 4) may indicate differences in tolerance to salinity of the various clones. Amador et al. (2001) showed that the length of roots of *O. ficus-indica* cv. Copena V1 was 10 cm when grown for 21 weeks and irrigated with saline water, while roots of the Control plants were 21.7 cm long. Corresponding DM yields of plants were 2.7 and 13 g/plant, respectively. In that study, the salinity of water applied increased gradually from 2 to 21 dS/m, and the authors concluded that inhibition of root growth in treated plants was due to Na toxicity and that the clone under study was not tolerant of salinity.

In cultivating *O. ficus-indica* and *O. robusta* in South Africa, Snyman (2004) found that root growth decreased significantly under water stress. According to the author, cactus pear roots do not develop well in saline environments since they produce little dry mass and tend to die under dry or ionic stress. Working with *O. ficus-indica* in hydroponic culture with different concentrations of NaCl, Franco-Salazar and Veliz (2007) observed that increased salinity reduced growth of both shoots and roots.

As expected, the largest surface areas of roots occurred in clones with greater root length and larger mean root diameter (Table 4); mean diameter and root surface area are directly related to nutrient uptake. The genotype Liso Forrageiro was harvested at the oldest age and presented the largest mean diameter and largest root surface area but surprisingly did not produce the highest DM yield.

Since highest yields occurred for Orelha de Elefante Africana, Ipa Clone 20, Orelha de Elefante Mexicana, Ipa 90-156 and F-13, it would seem that these clones would be the most promising candidates for further study to identify most appropriate cacti for growing as a source of fodder. As 3 of the clones (Africana, Mexicana and F-13) are regarded as highly resistant to carmine cochineal insect, while Clone 20 and Ipa 90-156 are only lowly resistant, further evaluations should be carried out in the field where these insects are prevalent.

Acknowledgments

We are grateful for the support provided by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Ciência e Tecnologia de Pernambuco, Brazil. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

References

(Note of the editors: All hyperlinks were verified 16 April 2021.)

Amador MB; Avila AC; Diegues ET; Garibay NA; Jones HG. 2001. Effects of NaCl salinity on growth and production of young cladodes of *Opuntia fícus indica*. Journal of Agronomy and Crop Science 187(4):269–279. doi: 10.1046/j.1439-037X.2001.00529.x

AOAC (Association of Official Analytical Chemists). 2005. Official methods of analysis. 18th Edn. AOAC, Gaithersburg, MD, USA.

Bezerra Neto E; Barreto LP. 2011. Análises químicas e bioquímicas em plantas. UFRPE, Recife, Brasil.

Bor M; Özdemir F; Türkan I. 2003. The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* and wild beet *Beta maritima*. Plant Science 164(1):77–84. doi: 10.1016/S0168-9452(02)00338-2

Dias NS; Blanco FF; Souza ER; Ferreira JFS; Souza Neto ON; Queiroz ISR. 2016. Efeitos dos sais na planta e tolerância das culturas à salinidade. In: Gheyi HR; Dias NS; Lacerda CF; Gomes Filho E, eds. Manejo da

salinidade na agricultura: estudos básicos e aplicados. 2nd Edn. INCTSal, Fortaleza, Brasil. p. 51–63.
Epstein E; Bloom AJ. 2006. Nutrição mineral de plantas: princípios e perspectivas. 2nd Edn. Planta, Londrina.
Fonseca VA; Santos MR; Silva JÁ; Donato SLR; Rodrigues CS; Brito CFB. 2019. Morpho-physiology, yield, and water-use efficiency of *Opuntia ficus-indica* irrigated with saline water. Acta Scientiarum. Agronomy 41:1–11. doi: 10.4025/actasciagron.v41i1.42631
Franco-Salazar VA; Veliz JA. 2007. Respuestas de La tuna (*Opuntia fícus-indica* (I) Mill) al NaCl. Interciencia 32(2):125–130. bit.ly/3c2gxhW
Franco-Salazar VA; Veliz JA. 2008. Efectos de la salinidad sobre el crecimiento, acidez titulable y concentracion de clorofila em *Opuntia ficus-indica* (L.) Mill**.** Revista Multidisciplinaria del Consejo de Investigación de la Universidad de Oriente 20(1):12–17. bit.ly/3rjqFcI
Freire JL; Santos MVF; Dubeux Júnior JCB; Bezerra Neto E; Lira MA; Cunha MV; Santos DC; Amorim SO; Mello ACL. 2018. Growth of cactus pear cv. Miúda under different salinity levels and irrigation frequencies. Anais da Academia Brasileira de Ciências 90:3893–3900. doi: 10.1590/0001-3765201820171033
Gajender GS; Dagar JC; Lal K; Yadav RK. 2014. Performance of edible cactus (*Opuntia ficus-indica*) in saline environments. Indian Journal of Agricultural Sciences 84:509–513. bit.ly/3bgJpE6
Inglese P; Liguori G; Barrera E. 2017. Ecophysiology and reproductive biology of cultivated cacti. In: Inglese P; Mondragon C; Nefzaoui A; Sáenz C, eds. Crop ecology, cultivation and uses of cactus pear crop ecology, cultivation. FAO and ICARDA, Rome, Italy. p. 29–41.
Jacobo CM; Chessa I. 2017. Nopalitos or vegetable cactus production and utilization. In: Inglese P; Mondragon C; Nefzaoui A; Sáenz C, eds. Crop ecology, cultivation and uses of cactus pear crop ecology, cultivation. FAO and ICARDA, Rome, Italy. p. 93–104.
Katerji N; Van Hoorn JW; Hamdy A; Mastrorilli M. 2003. Salinity effect on crop development and yield, analysis of salt tolerance according to several classification methods. Agricultural Water Management 62(1):37–66. doi: 10.1016/S0378-3774(03)00005-2
Khalid A; Silva JAT. 2010. Yield, essential oil and pigment content of *Calendula officinalis* L. flower heads cultivated under salt stress conditions. Scientia Horticulturae 126(2):297–305. doi: 10.1016/j.scienta.2010.07.023
Lallouche B; Boutekrabt A; Hadjkouider A; Riahi L; Lamine S; Zoghlami N. 2017. Use of physio-biochemical traits to evaluate the salt tolerance of five *Opuntia* species in the Algerian steppes. Pakistan Journal of Botany 49:837–845. bit.ly/3uWoSMF
Lastiri-Hernández MA; Alvarez-Bernal D; Cárdenas GC; Estrada SO. 2018. Improvement of saline soils with *Vicia sativa* L. from a semiarid region. Bragantia 77(3):501–509. doi: 10.1590/1678-4499.2017275
Mayer JA; Cushman JC. 2019. Nutritional and mineral content of prickly pear cactus: a highly water-use efficient forage, fodder and food species. Journal of Agronomy and Crop Science 205(6):625–634. doi: 10.1111/jac.12353
Munns R; Tester M. 2008. Mechanisms of salinity tolerance. Annual Review of Plant Biology 59:651–681. doi: 10.1146/annurev.arplant.59.032607.092911
Oliveira FG; Ferreira PA; Santos DB; Garcia GO. 2005. Índice de estresse hídrico diário do feijoeiro irrigado com água salina. Revista Brasileira de Engenharia Agrícola e Ambiental 9:6–10. bit.ly/3qlu9tE
Rodrigues AM; Pitacas FI; Reis MG; Blasco M. 2016. Nutritional value of *Opuntia ficus-indica* cladodes from Portuguese ecotypes. Bulgarian Journal of Agricultural Science 22(1):40–45. bit.ly/3v02L82
Schuch UKS; Kelly JJ. 2008. Salinity tolerance of cacti and succulents. Turfgrass, Landscape and Urban IPM Research Summary 155:61–66. bit.ly/3sPYBxQ
Silva TGF; Primo JTA; Silva SMS; Moura MSB; Santos DC; Silva MC; Araújo JEM. 2014. Indicadores de eficiência do uso da água e de nutrientes de clones de palma forrageira em condições de sequeiro no semiárido brasileiro. Bragantia 73(2):184–191 doi: 10.1590/brag.2014.017
Snyman HA. 2004. Effect of water stress on root growth of *Opuntia fícus-indica* and *Opuntia robusta*. South African Journal of Animal Science 34(6):101–103. bit.ly/3rgm7ng
Vasconcelos AGV; Lira MA; Cavalcanti ULB; Santos MVF; Willadino L. 2009. Seleção de clones de palma forrageira resistentes a cochonilha-do-carmim. Revista Brasileira de Zootecnia 38(5):827–831. doi: 10.1590/S1516-35982009000500007

(Received for publication 7 April 2020; accepted 31 January 2021; published 31 May 2021)

doi: 10.17138/TGFT(9)243-248

Short Communication

Studies with *Urochloa brizantha* cv. MG5 Vitória in Okinawa, Japan: Vegetative propagation and a tractor tyre stress test

Estudios con Urochloa brizantha *cv. MG5 Vitória en Okinawa, Japón: Propagación vegetativa y una prueba de estrés por presión de llantas de tractor*

TAKASHI HANAGASAKI

Okinawa Livestock Research Center, Nakijin, Okinawa, Japan. pref.okinawa.jp/site/norin/chikuken/

Abstract

Feeding of high quality grass is critical to ensure breeding cows remain healthy with high reproductive rates and growing and fattening cattle achieve good growth rates. The Brazilian grass cultivar, *Urochloa brizantha* cv. MG5 Vitória, is highly nutritious and is known for its drought tolerance. In view of its low seed production potential in subtropical Japan and of phytosanitary problems (contamination with soil particles) of imported seed, a study was conducted in Okinawa to assess 2 methods of propagating this cultivar vegetatively. Cutting stems (culms) at about 10 cm from ground level and inserting them 3 cm into a 50:50 compost:soil mixture produced a 77% success rate in terms of rooted plantlets in a glasshouse compared with 67% for cutting the culm at 3 nodes from the base, subsequently allowing 2 weeks for adventitious roots to form on the lowest node, then cutting below the node where roots emerged and planting the rooted propagule in the same mixture. It seems that the simple process of cutting stems at about 10 cm from ground level and inserting them into a suitable mixture of soil and compost should result in an acceptable yield of plantlets for establishment of an MG5 forage crop. However, locating a source of high-quality seed free of phytosanitary problems seed would seem to be a better solution to increase the areas in Okinawa planted to MG5.

In the tractor tyre stress trial conducted over 2 years, an MG5 forage crop established from seed showed depressed yields on the treatment subjected to tractor tyre pressure but performed as well as *Chloris gayana*, a much-used forage grass in Okinawa.

Keywords: *Urochloa brizantha*, dry matter yield, pasture establishment, plantlet production, tropical pasture.

Resumen

La alimentación con pasto de alta calidad es fundamental para garantizar que las vacas reproductoras se mantengan sanas con altas tasas de reproducción y que el ganado en crecimiento y engorde alcance buenas tasas de crecimiento. El cultivar de pasto brasileño, *Urochloa brizantha cv*. MG5 Vitória, es muy nutritivo y es conocido por su tolerancia a la sequía. En vista de su bajo potencial de producción de semillas en el Japón subtropical y de los problemas fitosanitarios (contaminación con partículas del suelo) de las semillas importadas, se realizó un estudio en Okinawa para evaluar 2 métodos de propagación vegetativa de este cultivar. Cortar tallos (culmos) a unos 10 cm del nivel del suelo e insertarlos 3 cm en una mezcla 50:50 de compost y suelo produjo una tasa de éxito del 77% en términos de plántulas enraizadas en un invernadero en comparación con el 67% al cortar el culmo a 3 nudos de la base, luego dejando 2 semanas para que se formen raíces adventicias en el nudo más bajo, luego cortando debajo del nudo donde emergieron las raíces y plantando el propágulo enraizado en la misma mezcla. Parece que el simple proceso de cortar los tallos a unos 10 cm del nivel del suelo e insertarlos en una mezcla adecuada de tierra y compost debería dar como resultado un rendimiento aceptable de plántulas para el establecimiento de un cultivo forrajero MG5. Sin embargo, localizar una fuente de semilla

Correspondence: T. Hanagasaki, Okinawa Agricultural Research Center, Itoman, Okinawa 901-0336, Japan.
E-mail: hangskit@yahoo.co.jp

de alta calidad libre de problemas fitosanitarios parece ser una mejor solución para aumentar las áreas en Okinawa sembradas con MG5.

En la prueba de estrés de los neumáticos del tractor realizada durante 2 años, un cultivo forrajero MG5 establecido a partir de semillas mostró rendimientos reducidos en el tratamiento sometido a la presión de los neumáticos del tractor, pero tuvo un rendimiento tan bueno como *Chloris gayana*, una pastura forrajera muy utilizada en Okinawa.

Palabras clave: *Urochloa brizantha*, establecimiento de pastos, producción de plántulas, pastos tropicales, rendimiento de materia seca.

Introduction

In Okinawa, the southernmost part of Japan, rate of increase in gross agricultural production between 2011 and 2016 was the highest in Japan. In particular, the beef industry accounted for 22.3% of gross agricultural production in 2017 and sales of calves during the past 10 years ranked fourth throughout Japan. In addition, more than 3 million foreign tourists, mainly from Taiwan, Hong Kong, South Korea and China, visited Okinawa in 2018, which was the highest number recorded in any year. In view of the resulting increased demand for livestock products, there is an urgent need to boost the production of Okinawa's famous unique beef brands such as 'Ishigaki Gyu', 'Yamashiro Gyu' and 'Miyako Gyu'. Feeding high quality grass is necessary for breeding cows to be healthy with high reproductive rates and for growing and fattening animals to achieve high levels of production. Okinawa has a subtropical climate and warm season perennial grasses can be grown successfully. The many small islands comprising the Prefecture of Okinawa where cattle are raised often suffer from drought, so introduced forage species should be drought-tolerant.

Some species of the genus *Brachiaria*, which are now recognized as species of the genus *Urochloa*, introduced from Africa, are of considerable economic importance in the tropics due to their adaptation to low-fertility soils (Rao et al. 1996), their drought-tolerance (Gayalin et al. 1994; Guenni et al. 2002) and good nutritive value (Lascano et al. 1996). In Brazil, *Urochloa* pastures extend over almost 100 million hectares (Jank et al. 2014).

A cultivar of one species, *Urochloa brizantha* (syn. *Brachiaria brizantha*) cv. MG5 Vitória (referred to subsequently as MG5), in tropical America also known under the cultivar names 'Toledo' and 'Xaraés' (Cook et al. 2020), has been shown to have high nutritive value in studies with growing cattle in Okinawa and has performed comparably with *Chloris gayana* cv. Katambora and *Digitaria eriantha* cv. Transvala (Nakanishi et al. 2006, 2008). This cultivar also proved to be more tolerant of drought than all other *Urochloa* species and cultivars tested (Kudaka et al. 2010). Based on these and other regional research results, MG5 was recommended for use in Okinawa Prefecture in 2016.

However, seed production and seed viability of this species in Okinawa are low (Kouki et al. 2007; 2009) and due to phytosanitary considerations (contamination of commercial seed lots with soil particles), it is difficult to import seeds of MG5 from other countries such as Brazil (Kouki and Ebina 2009). As a result, MG5 is still uncommon in Okinawa and mechanisms and strategies for increased usage should be developed.

While cattle are grazed in some areas of the Prefecture, forage is usually used for hay production which involves mowing, aerating and baling using heavy tractors, 5 or 6 times per year. Consequently, a forage cultivar such as MG5 must be resistant to tractor tyre stress and produce acceptable growth under this regime.

To address these issues we investigated methods of vegetative propagation of MG5 and production of MG5 for 2 years, while being harvested by tractors. Some of the data reported here have also been reported in Japanese language in the Okinawa Livestock Research Center´s bulletin series.

Materials and Methods

The research was conducted at Okinawa Livestock Research Center (Nakijin, Okinawa, Japan) (26º41' N, 127º56' E; 90 masl).

Study 1: Vegetative propagation

Raising plantlets. A tray comprising 55 cells, each 4.5 × 4.5 × 4.5 cm, was filled with a 50:50 mixture of potting compost (TAKII & Co. Ltd, Kyoto, Japan) and red ball earth[1] (TAKII & Co. Ltd). For raising plantlets, soil in the trays was kept moist by sprinkling with 3.7 mm water per day in a glass-house.

Cuttings were taken from a mature pasture stand of MG5 of 70–90 cm height. Two methods were compared to obtain material for planting. For the first method (Method 1: higher cutting and root formation), grass stems (culms) were cut above the third joint (node) from

[1]Granular clay-like mineral of volcanic origin.

the base. While the cut plant portion was removed and discarded, the uncut stem portion stayed in the field for two weeks (Figure 1). During this time adventitious roots start to develop from the lowest node. The stem was then cut to retain 2 nodes above the rooting node and was inserted to a depth of 3 cm into soil in the trays. For the second method (Method 2: lower cutting and direct planting), MG5 stems were cut at about 10 cm from ground level (Figure 1) and the lowest joint was inserted immediately into the soil in the trays to a depth of 3 cm.

Acceptably formed plantlets were identified about 2 months later by counting those rooted cuttings where, if lifted by the stem, soil did not fall away from the stem as roots were completely attached to the soil.

Transplanting plantlets into the ground. Plantlets obtained by vegetative propagation and about 21 weeks of age, were transplanted into the field of Kunigami merge[2]. Two transplanting methods were compared in terms of time necessary for planting a given area: using a vegetable transplanter machine (Yanmer, Osaka, Japan); and by means of a manual planting tool with 2 handles (Figure 2). In the former, plantlets were fed into a hopper on the machine and were drawn down into the ground while in the latter, a plantlet was placed in the bottom of the tool, the jaws at the bottom were inserted into the soil, and the soil was opened by forcing the levers at the top apart. While press wheels compacted the soil around the plantlet for the vegetable planter, soil was pressed down with the foot for the planting tool method. Both methods involved 3 people and their working time was recorded. For each method, 110 plantlets were planted 18 cm apart in 8 furrows 36 cm apart, giving an area of about 50 m^2 for each method.

Figure 2. Machine planting and manual planting of *Urochloa brizantha* plantlets.

Test for tractor tyre stress

Seeds of *Chloris gayana* cv. Katambora and MG5 were each sown at 30 kg/ha on 27 September 2005. For each grass, an area of 47.6 m^2 (14 × 3.4 m) replicated 4 times

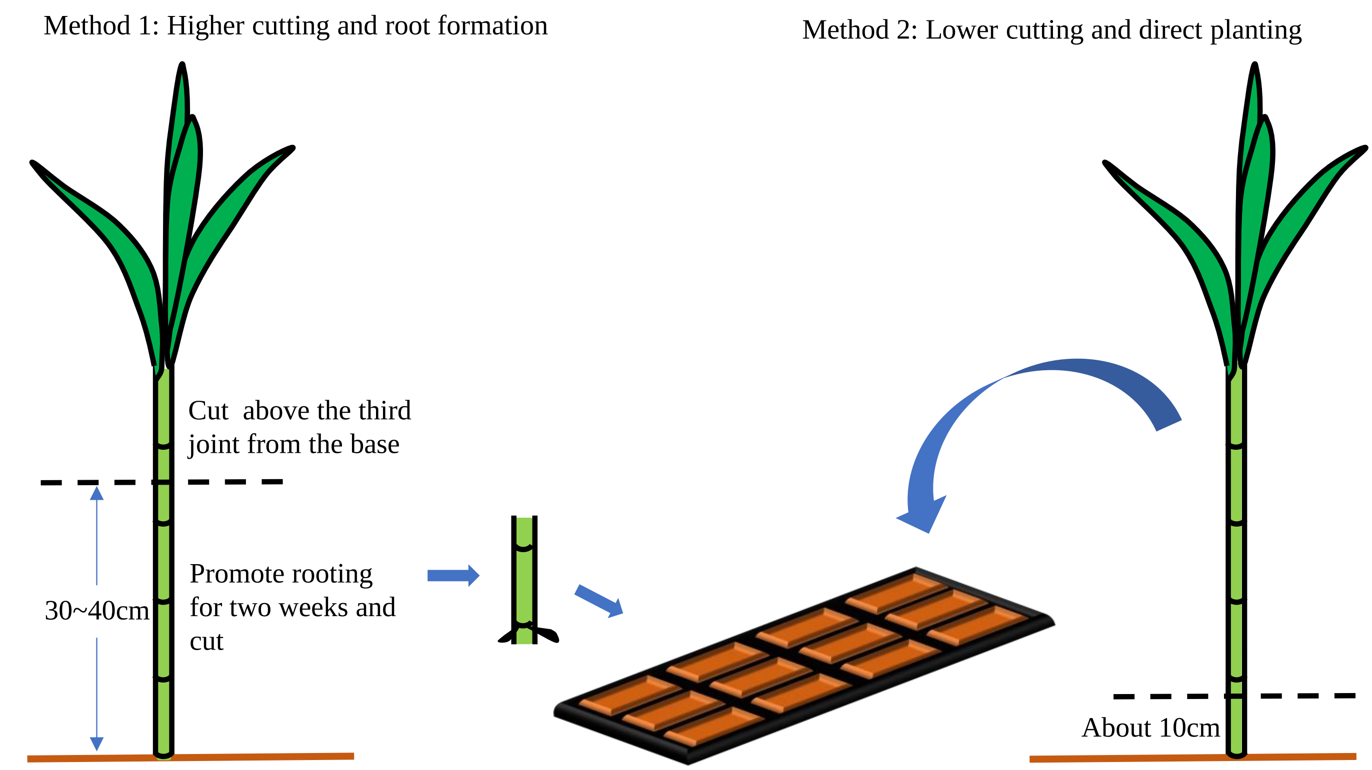


Figure 1. Method 1 – Higher cutting and root formation; and Method 2 – Lower cutting and direct planting.

[2]A fine-grained red soil sometimes containing gravel but with low organic matter concentration.

was used to assess the impacts of tractor tyre stress or no tractor stress (Control) on plant growth. Both grasses were mown by hand 3 times, the last one on 27 June 2006, before the investigation commenced. To impose tractor tyre stress on appropriate plots, a tractor (98 PS, about 3 tonnes weight, gear M-2, Iseki Co. Ltd, Ehime, Japan) was driven over the whole of each plot 3 times evenly every harvest: 16 August, 05 October and 19 December in 2006, and 21 May, 15 August and 29 October in 2007, to simulate mowing, aerating for drying and collecting in round bales as for a conventional harvest. To determine dry matter yield, 3 different fixed areas (1 × 1 m) in each plot were cut by hand for each treatment and the harvested material was dried for 48 h at 72 ºC.

Statistical analysis

Statistical treatment of the tyre stress test was by two-way analysis of variance (ANOVA) with a Fisher's least significant difference test for the 2 factors, grass species and tractor tyre stress, regarding dry matter yield for each date and total yield.

Results and Discussion

Raising and transplanting plantlets

The standard method for vegetative propagation of grasses in Okinawa is to cut stems (culms) to retain 2 joints (nodes), the cuttings then being inserted into a mixture of soil and potting mix (Mochizuki et al. 2005). With MG5 this method normally results in a success rate of about 10% of plantlets being produced (T. Hanagasaki unpublished data). Results have been similar when a commercial rooting accelerator (TGG010S or TGG020S, both from the auxin group; Tokai Global Greening Co. Ltd, Gifu, Japan) was used, indicating that treatment with that plant hormone had no effect on rooting of MG5 cuttings. In a comparison trial, success rate for MG5 (18%) has been lower than those for other *Urochloa* species and cultivars (*U. brizantha* cv. Marandu at 31%, *U. decumbens* at 28%, *U. ruziziensis* at 52% and *U. humidicola* at 56%) (T. Hanagasaki unpublished data).

However, in the current study both methods to produce rooted cuttings resulted in a satisfactory percentage of plantlets (Table 1). With about 77% of plantlets produced, the lower cutting of stems followed by direct insertion into soil is a successful and practical method for vegetative propagation of MG5.

In a complementary study, time involved in transplanting plantlets showed that machine planting took 107 seconds per 20 m and 39 seconds for a change of direction. In contrast, manual planting took 287 seconds per 20 m. Thus, manual planting of 1,000 m^2 took twice as much time as machine planting (Table 1). MG5 generally grew rapidly with both methods of transplanting.

Tractor tyre stress test

There was no major difference between the 2 grasses regarding Grand total DMY over 2 years (Table 2). However, tyre stress depressed ($P<0.05$) Total DMY in 2007 and Grand total DMY over 2 years in MG5, while Katambora was generally unaffected by tyre stress. Considering that MG 5 is a fairly erect-growing tussock grass with short rhizomes and Katambora is stoloniferous (although it can attain an erect growth habit in a dense pasture), this finding is in general agreement with the observations of Honda and Yamanobe (1958), who reported that tractor tyre stress generally markedly suppressed growth of erect grasses but could have favorable impact on sod-forming grasses, if subjected to stress on only few occasions separated by reasonable intervals. Hosono et al. (1965) reported that forage yield of Italian ryegrass decreased as the number of transits increased (0, 1, 3 and 5 times).

Table 1. Vegetative propagation and transplanting of *Urochloa brizantha* cv. MG5: **A**) Percentage of rooted cuttings (plantlets) produced with 2 methods of selecting and planting of cuttings. **B**) Time for transplanting rooted cuttings in a 1,000 m^2 plot by 2 methods.

Method	A) Percentage of rooted plantlets after 2 months	B) Time for transplanting
Higher cut to promote rooting	66.7% (1,155)[1]	Not applicable
Lower cut with direct insertion	76.7% (648)	Not applicable
Machine planting	Not applicable	6.3 hours
Manual planting	Not applicable	12.9 hours

[1]Numbers in parenthesis represent the number of stems inserted in a compost-soil mixture.

Table 2. Effects of tractor tyre stress on dry matter yield of 2 tropical grasses during 2 years (kg/10a).

Date		*Urochloa brizantha* cv. Vitória MG5		*Chloris gayana* cv. Katambora	
		Control	Tyre stress	Control	Tyre stress
2006	16 Aug	750ab	660bc	792a	643c
	05 Oct	484a	483a	503a	476a
	19 Dec	330a	312b	423a	405a
Total 2006		1,564ab	1,455b	1,718a	1,524ab
2007	21 May	735a	420b	893a	828a
	15 Aug	1,255a	871b	812b	796b
	29 Oct	723a	436b	556b	491b
Total 2007		2,683a	1,727	2,261b	2,115bc
Grand total		4,247a	3,182c	3,979ab	3,639bc

Means followed by different letters within each row differ significantly according to Fisher's least significant difference test (P<0.05).

Conclusion

In conclusion, it appears that under the conditions of Okinawa, cutting stems of MG5 low to the ground and inserting them immediately into a soil-compost mixture in a glass-house will result in successful production of plantlets. While this methodology is acceptable for small areas, for planting large areas there is a need to locate a source of commercial seed free of contamination by soil particles and thus can be safely imported.

While tractor tyre stress did not influence DM yields of Katambora severely, impact of tractor tyres markedly lowered yields of MG5, especially in the second year. However, total DM yield of MG5 under tractor tyre stress for the 2 years was not significantly different from that of Katambora, which indicates the production capacity of MG5. Tractor tyre stress could be a concern where material is harvested as hay using heavy tractors and balers but it would not be a significant issue under cut-and-carry or grazing systems. Furthermore, in a practical situation the impact would probably be reduced as the total area of pasture is not normally affected by each operation in the haymaking process. However, soil compaction, which was not considered in this study, should also be taken into account.

Acknowledgments

I am grateful to Mr Yokota, Mr Morikawa and Dr Nakanishi for providing technical advice; Mr Asato, Mr Nagatoshi and Mrs Kouki for providing technical support; Mr Ebina for providing the opportunity to research this study; and Mr Kohama, Mr Teruya and Mrs Takeuchi for supporting this research.

References

(Note of the editors: All hyperlinks were verified 9 March 2020).

Cook BG; Pengelly BC; Schultze-Kraft R; Taylor M; Burkart S; Cardoso Arango JA; González Guzmán JJ; Cox K; Jones C; Peters M. 2020. Tropical Forages: An interactive selection tool. 2nd and Revised Edn. International Center for Tropical Agriculture (CIAT), Cali, Colombia and International Livestock Research Institute (ILRI), Nairobi, Kenya. tropicalforages.info

Gayalin I. 1994. Stockpiling of tropical forage for the dry season in Martinique. In: Proceedings of the XVII International Grassland Congress, 8-21 Febrary 1993, Vol. 1. NZGA, TGSA, NZSAP, ASAP-Qld., and NZIAS, Palmerston North, New Zealand. p. 518–519.

Guenni O; Marín D; Baruch Z. 2002. Responses to drought of five Brachiaria species. I. Biomass production, leaf growth, root distribution, water use and forage quality. Plant and Soil 243:229–241.

Honda H; Yamanobe M. 1958. Influence of treading pressure exerted on growth of Japanese lawn grass. Japanese Journal of Landscape and Architecture 22(4):16–20. (in Japanese). bit.ly/3qwMJ2a

Hosono K; Tamura T; Ogino S. 1965. Effect of farm machine tread on pasture grasses. Japanese Journal of Grassland Science 11(3):123–131. (in Japanese). bit.ly/3v8IxZS

Jank L; Barrios SC; Valle CB do; Simeão RM; Alves GF. 2014. The value of improved pastures to Brazilian beef production. Crop and Pasture Science 65(11):1132–1137.

Kouki K; Ebina M. 2009. Breeding and forage utilization of *Brachiaria* grass (*Brachiaria* spp.). Japanese Journal of Grassland Science 55(2):179-187. (in Japanese). bit.ly/3kWyVwI

Kouki K; Ebina M; Nakanishi Y; Morikawa N; Hanagasaki T. 2007. Development of grassland of the genus Brachiaria: Investigation of possibility of breeding seeds. Bulletin of The Okinawa Prefectural Livestock and Grassland Research Center 44:119-121. (in Japanese).

Kouki K; Ebina M; Suenaga K; Nakanishi Y; Yokota M; Inafuku M; Hanagasaki T. 2009. Seed production properties of the genus Brachiaria. Bulletin of The Okinawa Prefectural Livestock and Grassland Research Center 46:75-79. (in Japanese).

Kudaka M; Shioyama A; Nitta M. 2010. Productivity and drought tolerance of new introduced *Brachiaria* grasses. Bulletin of The Okinawa Prefectural Livestock and Grassland Research Center 48:71-78. (in Japanese). bit.ly/3slSgJO

Lascano CE; Euclides VPB. 1996. Nutritional quality and animal production of Brachiaria pastures. In: *Brachiaria*: Biology, agronomy and improvement (Eds Miles JW, Maass BL, do Valle CB, CIAT, Cali, Colombia and CNPGC/EMBRAPA， Campo Grande, MS, Brazil, p 106-123. bit.ly/3uXbTth

Mochizuki T; Morikawa N; Nagatoshi M; Toma S. 2005. Grassland establishment using tropical grass through vegetative reproduction. Bulletin of The Okinawa Prefectural Livestock and Grassland Research Center 42: 42-45. (in Japanese).

Nakanishi Y; Hanagasaki T; Kouki K; Yokota M; Hirano K; Kouji A. 2008. Dry matter yield and nutritive yield of tropical grass Brachiaria brizantha cv. MG5. NARO Research result information No. 7. (in Japanese). bit.ly/2OwNmLS

Nakanishi Y; Hirano K; Kouji A. 2006. Nutritive quality and feeding amount of tropical grass Brachiaria brizantha cv. MG5 for breeding cow. NARO Research result information No. 5. (in Japanese). bit.ly/3sVyLs8

Rao IM; Kerridge PC; Macedo MCM. 1996. Nutritional requirements of Brachiaria and adaptation to acid soils. In: Miles JW; Maass BL, Valle CB do, eds. Brachiaria: Biology, agronomy and improvement. CIAT Publication no. 259. CIAT, Cali, Colombia and EMBRAPA-CNPGC, Campo Grande, MS, Brazil. p. 53–71.

(Received for publication 20 September 2020; accepted 4 February 2021; published 31 May 2021)

Tropical Grasslands
-Forrajes Tropicales
Online Journal

TGFT Editorial Team

A.A. 6713, Km 17 Recta Cali-Palmira, Cali, Valle del Cauca, Colombia.

Phone: +57 2 4450100 Ext. 3084

Email: CIAT-TGFT-Journal@cgiar.org