**ISSN: 2346-3775** 



# Tropical Grasslands -Forrajes Tropicales Online Journal

Vol. 5 No. 2

May 2017

## **Published by:**

Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia In cooperation with:

- Chinese Academy of Tropical Agricultural Sciences (CATAS)
- Australian Centre for International Agricultural Research (ACIAR)

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#### **Research Paper**

# Complementary use of neotropical savanna and grass-legume pastures for early weaning and effects on growth and metabolic status of weaners and inter-calving intervals of dams

Uso complementario de sabana nativa neotropical y pasturas con asociaciones gramíneas-leguminosas para destete precoz y efectos en el crecimiento y metabolismo de los terneros y la fertilidad de las vacas

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#### Abstract

Extensive, rangeland-based beef production systems that predominate in the neotropical savannas of northern South America are low input-low output beef breeding systems, and their intensification faces major hurdles due to their location, soil, water and topographic constraints. Intensification requires the introduction of improved pastures strategically managed to complement the native savannas, to improve production and to allow opportunities for a wider range of associated ecosystem services. This study assessed the feasibility of early weaning of beef calves onto small areas of sown pastures to aid system intensification. Weaning calves at 90 days of age and grazing on Andropgon gayanus, A. gayanus-Pueraria phaseoloides, A. gayanus-Centrosema acutifolium and Brachiaria humidicola-Arachis *pintoi* sown tropical pastures stocked at 6 animals/ha was compared with conventional weaning on native savanna over 4 consecutive years. The reproductive performance of their Brahman (Bos indicus) and crossbred [Brahman x San Martinero (native; B. taurus)] dams grazing savanna (0.2 cows/ha) was monitored in comparison with control cow-calf systems, where calves were weaned onto savanna between 240 and 270 days of age. Post-weaning weight gains of early weaned calves on sown pasture (0.1-0.2 kg/d) were much lower than those of unweaned animals on savanna (0.31-0.35)kg/d), but compensatory gains realized after the end of the weaning experiments allowed early weaned calves to reach weights similar to those of control animals 400 days after the end of the pasture phase. As expected, early weaned cows achieved higher live weights and had shorter inter-calving intervals than their counterparts. Trade-offs between performance of calves and of cows are discussed, but it is suggested that the strategic use of small areas of sown pastures for early weaned calves may productively complement large areas of native savannas. It is hypothesized that the improved quality, frequency and intensity of management required by these intensified systems may place a burden on these low-input primary enterprises, which may also challenge the productive and environmental adaptive capacity of primary resource users.

Keywords: Cattle, extensive systems, intensification, rangelands, sustainability, tropical pastures.

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#### Resumen

Los sistemas de producción de carne extensivos basados en pasturas nativas, son frecuentemente considerados tradicionales y sustentables. Este tipo de sistema en las sabanas del norte de Sur América usa bajos insumos y su productividad también es baja. Su intensificación está limitada frecuentemente por su localización y por características de suelo, topografía y acceso a agua. La introducción de pasturas tropicales sembradas, usadas en forma estratégica como complemento de las sabanas, permite intensificar la producción animal y provee oportunidades para mejorar algunos servicios ecológicos. Durante 4 años consecutivos se experimentó el destete precoz de terneros de carne a los 90 días, en pasturas sembradas de Andropgon gayanus, A. gayanus-Pueraria phaseoloides, A. gayanus-Centrosema acutifolium y Brachiaria humidicola-Arachis pintoi. Simultáneamente se registró el desempeño reproductivo de hatos de la raza Brahman (Bos indicus) y cruzas con San Martinero (Bos taurus) en pastoreo en sabanas con carga de 0.2 vacas/ha, en comparación con sistemas control caracterizados por destete tradicional a los 240-270 días de edad. Las ganancias de peso vivo de los terneros destetados tempranamente fueron bajas en comparación con los animales control, pero se lograron ganancias compensatorias posteriormente, alcanzando pesos vivos a los 400 días después de la fase experimental sin diferencias significativas (P>0.05) entre ambos grupos de animales. Como se esperaba, las vacas destetadas precozmente lograron mayores pesos y mejor desempeño reproductivo que las del grupo control. Se discute el compromiso entre desempeño de terneros y de vacas, pero es aparente que áreas pequeñas de pasturas sembradas pueden complementar efectivamente grandes extensiones de sabana para intensificar la producción de hatos de carne. Se hipotetiza que estos usos complementarios requieren un mejoramiento en la cantidad, calidad y frecuencia de la gestión de dichos recursos. Este tipo de manejo puede ser una oportunidad para mejorar la productividad de los hatos por parte de los ganaderos, lo que requiere de ellos una mayor capacidad de adaptación y mejoramiento de la producción concomitante con las actuales necesidades ambientales.

Palabras clave: Ganado bovino, intensificación, pasturas tropicales, sabanas, sistemas extensivos, sostenibilidad.

#### Introduction

The continuous increase in demand for food requires intensification of land use systems in the agricultural sector and achieving a compromise between increases in production and ecological conservation constitutes an important challenge (Davies et al. 2010; van Vuuren and Chilibroste 2013). Additionally, climate change may further increase the demands on management of resources and the capacity of livestock farmers and their rural communities to adapt (Herrero et al. 2017).

The watershed of the Orinoco River covers 35 Mha in Colombia (33% of the land area) and includes mountains, foothills and a variety of seasonally flooded and well The savannas drained savannas. well drained ("Altillanura") extend over 13.5 Mha (CONPES 2014), 35% of which is plain ("Plains") and 54% is slopes and hills (Rippstein et al. 2001). The latter include small valleys suitable for cropping, surrounded by pronounced slopes that have shallow, stony soils. By 2007, 23% of the Plains had been converted to crops, sown pastures, palm oil plantations (Romero-Ruiz et al. 2012; Rausch 2013) and a variety of tree plantations and reforested areas amounting to close to 100,000 ha (MADR 2015). Palm oil plantations have experienced a significant and ongoing increase in the savannas, and have reached 112,186 ha (FEDEPALMA 2015), including large areas in the

Andean foothills. Overall, cropped areas represent 4.3–11.8%, whereas rangelands cover 72–89% of the Orinoco basin in Colombia (DANE 2016).

This expansion of crops and plantations has taken place at the expense of native savannas supporting beef cattle ranching. On areas closest to roads in the Plains, beef breeding herds have been replaced by yearlings brought in from the surrounding area for fattening, interspersed with crops and plantations (Romero-Ruiz et al. 2012; Huertas-Ramírez and Huertas-Herrera 2015). The cattle population of the region is estimated at 4.7 M head (DANE 2016), largely supported by native savannas that still constitute the main land use in the rest of the area, mainly dedicated to extensive beef breeding herds (Rausch 2013; Huertas-Ramírez and Huertas-Herrera 2015). The latter are low input-low output systems, frequently constrained by limited access, physiographic and water limitations, low soil fertility and often shallow soils that limit intensification (Seré and Vera 1983). On the other hand, these savannas are rich in plant and animal biodiversity (Rippstein et al. 2001; Lasso et al. 2011) and have varied landscapes and ecosystem services that attract rural tourism highlighted by educational and cultural values and traditions (Navas Ríos 1999; Molina and Triana 2011; Australian Government 2015).

Although the real impact of greenhouse gas emissions from cattle on neotropical savannas is still a challenge for

scientists, it is likely that plant dynamics may mitigate the demanding effects of climate variability, reflecting the capacity of these plant-animal evolutionary systems to adapt to genetic, environmental and management stressors (O'Neill et al. 2010; Herrero et al. 2015; Ramírez-Restrepo and Charmley 2015). In this complex scenario, primary producers have to contend with low seasonal biomass production and nutritive value (Paladines and Leal 1979; Rippstein et al. 1996; Durmic et al. 2017). Together, these factors negatively impact productivity in terms of slow growth and fertility rates that seldom exceed 50%, yielding no more than 3 or 4 calves weaned over a cow's lifetime (Kleinheisterkamp and Habich 1985; Plessow 1985; Squires and Vera 1992).

These traits contrast with the relatively high performance reported when Brahman (*Bos indicus*) beef cows are grazed year-round on well managed tropical sown pastures (Vera et al. 2002). Nevertheless, expensive sown pastures are mostly reserved for fattening yearlings and steers (Vera and Seré 1985), and their year-round use by the breeding herd may represent an economically suboptimal use of an expensive resource. It is possible that strategic and seasonal grazing of improved pastures by suckling cows to complement native savanna grazing may significantly increase reproductive indexes (Vera and Seré 1989).

Reproductive rates may be boosted further by early weaning of calves, a technology that is 50 years old. In principle, early weaning can be performed at 45 days of age (Rasby 2007) and it is particularly useful in drought situations and to contend with the negative effects of climate change (FAO 2013). Cow-calf research in the savannas of northern Australia has been amply documented in over 100 references and reports summarized by Tyler (2012) and Tyler et al. (2012). Similarly, a large amount of research was carried out in the USA (Arthington et al. 2005; Vendramini et al. 2006). Much of this research has addressed the consequences of early weaning on the dams' reproductive performance (Fordyce et al. 1988; Schlink et al. 1992; Short et al. 1996; Tyler et al. 2012). In the majority of cases, weaners have either been raised in feedlots (Arthington et al. 2005) or supplemented with concentrates or crop by-products on pasture (Vendramini et al. 2007; Vendramini and Arthington 2008). Consequently, Tyler (2012) indicates that the effect of tropical pastures on the performance of early weaners should be prioritized in future pastoral research.

Early weaning has rarely been investigated in the neotropics (Moore and da Rocha 1983; Betancourt-López et al. 2012) and, as elsewhere, the practice has generally been coupled with regular supplementation of cows and calves with different combinations of concentrates and/or other feedstuffs in different settings. However, to the authors' knowledge, raising early weaned beef calves exclusively on sown pastures in the neotropics has not been investigated.

The objectives of this study were to assess the effects on cow and calf performance of early weaning of beef calves onto sown tropical pastures, while their dams were maintained on savanna, in comparison with cow-calf pairs grazing native savanna with weaning at the conventional age.

#### **Materials and Methods**

#### Experimental design

The study was conducted during the late 1980s over 6 consecutive years at Carimagua Research Station, located 87 km northeast of Puerto Gaitán in the Meta Department on the eastern plains of Colombia (4°36'44.6" N, 74°08'42.2" W). Monthly rainfall, ambient temperature and their annual variations were recorded during 13 consecutive years (Table 1).

Care of animals and experimental procedures were performed by accredited Doctors of Veterinary Medicine (DVM), including the second author, following national husbandry and animal welfare regulations.

 Table 1. Monthly average climatic data over 13 years, and annual rainfall recorded during the 1984–1987 period at Carimagua Research Station, Meta Department, Colombia.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Mean rainfall 1979–1991 (mm)	10	25	76	236	292	368	274	260	292	203	116	50
Mean ambient temp 1979–1991 (°C) <sup>1</sup>	26.9	28.0	28.1	27.1	26.2	25.4	25.2	25.7	26.1	26.5	26.9	26.4
Annual rainfall (mm)												
1984	94	47	53	213	125	439	179	246	529	351	222	25
1985	0	0	33	196	544	445	327	250	351	266	180	0
1986	36	32	65	221	570	541	462	367	286	214	147	89
1987	n/a	n/a	n/a	n/a	558	528	445	354	275	204	138	82

n/a - records not available.

<sup>1</sup>Mean monthly temperature for 1984–1987 not included as there is less than 1 °C variation between years.

During the first 2 years (Years 1 and 2) exploratory trials were conducted to assess the feasibility of raising early weaned beef calves on sown pastures alone, and to monitor the associated health and mortality risks. At the end of the experimental phase of Year 2, the weaners were transferred to a savanna paddock and their weight was monitored until the savanna controls were weaned at the age of  $266 \pm 7$  days. A further 18 months were required to monitor growth of these weaners on savanna during 537 days after the early weaning date, and of the performance of their dams until the subsequent calving event. In the following 2 years (Years 3 and 4), trials compared the performance of early weaned calves on a number of sown tropical pastures with that of normally weaned calves on savanna.

In all cases Brahman and crossbred [Brahman x San Martinero (native; *B. taurus*)] cows and calves were used. Calves were born on savanna with a mean live weight (LW) of  $22.7 \pm 3.4$  kg. Body weight of calves during the experimental phase on sown pastures was recorded every 7 days. Cows remained on large savanna paddocks as part of a large herd and were weighed and rectally palpated at ~4-monthly intervals to determine if they were pregnant. Calving events were recorded daily, but mustering of the cows was avoided near calving time. Pregnancy rates and inter-calving intervals were calculated. Approximate date of conception was back calculated from the calving date. Internal and external parasite infestations were controlled throughout following commercial farming practices. Mortalities and incidents of ill health were recorded.

Cows and calves had free access to fresh water plus a commercial mineral supplement containing (as-fed): 17.5% Na, 26.9% Cl, 8.0% P, 13.7% Ca, 2.0% S, 0.104% Cu, 0.35% Zn, 0.001% Co and 0.008% I. Supplement consumption was recorded every 15 days.

#### Animals, forages and grazing management

In the first year (Year 1) 10 male calves  $[112 \pm 16 \text{ kg}]$ initial LW (ILW); mean  $\pm$  standard error of the mean (s.e.m.)] were weaned at 166  $\pm$  10 days old on 11 November 1984, coinciding with the end of the rainy season. They were rotationally grazed (6 calves/ha; 7 days grazing, 21 days rest) for 146 days during the dry season using 4 paddocks of equal size of a 6-year-old mixture of *Andropogon gayanus* cv. Carimagua-1 and commercial *Pueraria phaseoloides* (AgPp). Fifteen cow-calf pairs grazing savanna as part of a larger herd served as controls (Control 1) and were monitored at intervals of ~120 days. Their calves were weaned at 280  $\pm$  29 days of age, and the dams were monitored until the next calving event. Savanna cows were stocked at 0.2 cows/ha in large (>200 ha) paddocks managed with periodic fire following traditional and regional farming practices (Kleinheisterkamp and Habich 1985; Rippstein et al. 2001).

In Year 2, the experiment was methodologically similar to Year 1, but 15 calves were weaned at  $68 \pm 9$  kg ILW and  $110 \pm 8$  days of age on 30 May 1985 at the beginning of the rainy season. They were rotationally grazed on 15 paddocks of AgPp and Ag plus *Centrosema acutifolium* cv. Vichada (accession CIAT 5277; AgCa; 6 calves/ha with 7 days grazing, 21 days rest) for 147 days. In parallel, 15 similar calves (Control 2) continued to suckle their dams as part of a large herd grazing native savanna, and were weighed at intervals of 21 days during the same period but weaned at  $266 \pm 7$  days of age and  $144 \pm 20$  kg LW. Thereafter, weaned control and early weaned calves were grazed on a native savanna paddock as a single group at 0.25 head/ha for 18 months, and were weighed about every 120 days.

During Year 3, the experiment compared the performance of groups of 10 weaned calves ( $68 \pm 13$  kg ILW,  $93 \pm 4$  days of age) rotationally grazing 4 paddocks (6 calves/ha; 7 days grazing, 21 days rest) each of Ag, AgPp and AgCa for 123 days during the wet season, commencing on 25 June 1986 (mid rainy season).

Year 4 replicated the design of the third year, with the addition of a *Brachiaria humidicola* cv. Llanero (syn. *B. dictyoneura*)-*Arachis pintoi* (BhAp) pasture subjected to the same management and sampling practices previously described. The experiment began on 23 July 1987. Ten calves ( $81 \pm 9$  kg ILW,  $86 \pm 5$  days of age) were weaned and placed on each improved pasture, while 10 unweaned calves grazed on savanna with their dams until weaning at  $319 \pm 29$  days of age and  $155 \pm 25$  kg live weight.

In Year 3 a pilot test of weaning was carried out starting on 28 July 1986 with one commercial herd at Carimagua. Forty-six calves born in 2 different savanna paddocks were weaned at a mean age of  $186 \pm 62$  (range 54–285) days and LW  $131 \pm 26$  kg, and were transferred to a 1-year-old *A. gayanus-Stylosanthes capitata* cv. Capica pasture stocked at 3 calves/ha for 99 days until the end of the rainy season.

#### Sample collection and lab analyses

*Pasture*. Pre-grazing herbage mass and botanical composition of the introduced pastures were estimated by the BOTANAL method (Tothill 1978; data partially shown). Botanical composition, growth rate and nutritive value of the savanna have been previously described by Rivera Sánchez (1988).

*Blood.* Jugular blood samples were collected weekly from each of the calves into two 10 ml BD Vacutainers<sup>®</sup> (Becton Dickinson, Franklin Lakes, NJ, USA) for hematology [hematocrit (g/100 ml)], serum enzymes [aspartate aminotransferase (AST, U/ml) and gammaglutamyl transferase (GGT, U/ml)], total protein (g/100 ml), renal function [urea nitrogen (BUN, mg/100 ml)] and mineral [P, Ca and Mg (g/100 ml)] analyses. Hematology and serum biochemistry analyses were performed at the International Center for Tropical Agriculture, Cali, Colombia. Blood enzymes, protein and BUN were determined using standard kits (Sigma-Aldrich Corp., St. Louis, MO, USA), N by the micro-Kjeldahl method, P by colorimetry and the remaining minerals by atomic absorption.

*Feces.* Fecal grab samples were individually collected from the rectums of animals at weighing times to determine P, Ca, ash and N concentrations, with N expressed as percent of fecal organic matter.

#### Statistical analyses

Data were analyzed using the Statistical Analysis System, version 9.4 (SAS Institute, Cary, NC, USA). Results for the first 2 years were summarized using descriptive statistics (means  $\pm$  s.e.). In Years 3 and 4, calves were balanced for LW and randomly allocated to sown pastures considering in all cases individual animals as the experimental unit. Data distribution from all variables examined (i.e. blood, feces and LW) was reviewed prior to additional analyses. Repeated-measures of blood, feces and LW for the same calf were analyzed with the GLIMMIX procedure, using a linear mixed model that included the fixed effect of pasture (i.e. sown forages and savanna), and the interaction between pasture and the random effects of year. All interactions were initially included, and those that were not significant were discarded for the final analysis (Gbur et al. 2012).

Final analyses were preceded by a study of the covariance structure (Gbur et al. 2012) to adjust the model specification as required. Differences were considered significant when P $\leq$ 0.05, and there was tendency to significance if P $\leq$ 0.10. Denominator degrees of freedom for the test of fixed effects were specified by the Kenward-Roger procedure. Multiple comparisons of least squares means used the Tukey procedure, complemented with graphical interpretation using SAS diffograms (not

shown due to space limitations). Regressions were calculated with the GLMSELECT procedure.

#### Results

#### Forages and botanical composition

Pre-grazing herbage mass in all introduced pastures and across the experimental periods in Years 3 and 4 always exceeded 2,500 kg DM/ha, except in the Ag treatment in Year 3 for a brief period during temporary flooding of the paddock. Legume percentage in the forage on offer was higher for the AgPp (29%) mixture than for the BhAp (20%) association, with lower amounts (7–10%) in the AgCa sward. These legume percentages remained rela-tively stable throughout the experimental period as shown for Year 3 in Figure 1. The Ag pasture averaged 758 g/kg DM of neutral detergent fiber (NDF) and 80 g/kg DM crude protein (CP) during the rainy season, and 759 and 89 g/kg DM in the dry season, respectively. The AgPp and AgCa pastures had similar nutritive composition and averaged 756 g NDF and 115 g CP/kg DM and 757 g NDF and 110 g CP/kg DM for the rainy and dry seasons, respectively. Similarly, the BhAp pasture contained 716 g NDF and 84 g CP/kg DM in the wet season, and 691 g NDF and 21 g CP/kg DM in the dry season.

#### Mortalities

Over the 4 years of the study, 4 deaths out of 95 early weaned calves occurred in the AgPp paddocks in Year 3, which was related to a temporary flooding event. No calf mortality of control, suckling calves or their dams was recorded in the savanna paddocks.

#### Liveweight performance

*Years 1 and 2.* Data in Table 2 show the LWs and ages of calves during Years 1 and 2. Daily LW gains (LWG) of calves weaned at 166 days of age in Year 1 and grazed on the AgPp pasture averaged  $0.10 \pm 0.03$  kg/head, whereas contemporary suckling calves on savanna gained  $0.35 \pm 0.19$  kg/head. In Year 2, calves weaned at 110 days of age and grazed on AgPp and AgCa pastures gained  $0.19 \pm 0.06$  kg/day, and control suckling calves gained  $0.60 \pm 0.13$  kg/day.



**Figure 1**. Forage on offer (kg DM/ha) in 3 *Andropogon*-based pastures and their respective botanical compositions in Year 3. Grass DM stands for total grass DM on offer in the respective pastures; Green DM is green grass DM; Leg DM is total legume DM. Ag = *Andropogon gayanus*; Pp = Pueraria phaseoloides; Ca = *Centrosema acutifolium*.

Live weights of cows for Years 1 and 2 between consecutive calving seasons on savanna are shown in Figure 2. Year 1 dams of early weaned calves calved in the late rainy season, while control cows (normally weaned) calved at the end of the subsequent dry season (1985–1986). Early weaned dams in Year 2 calved at the end of the dry season, whereas control cows did so at the end of the following rainy season. Calculated LWs at conception for the 2 years were  $303 \pm 43$  and  $321 \pm 45$  kg, respectively.

**Table 2**. Mean ( $\pm$  s.e.) live weights and ages of early weaned Brahman and Brahman cross calves during the exploratory observations in Years 1 and 2.

		n	Initial	Final
Year 1	Live weight (kg)	10	$112 \pm 16$	$126 \pm 18$
	Age (d)	10	$166 \pm 10$	$312 \pm 10$
	Gain (kg/d)			0.10
Year 2	Live weight (kg)	15	$69 \pm 10$	$97 \pm 13$
	Age (d)	15	$109 \pm 8$	$257 \pm 8$
	Gain (kg/d)			0.19

n - number of animals.



**Figure 2**. Postpartum cows' live weights in relation to type of weaning. Early and normal refer to weaning treatment, followed by the calves' ages at weaning. Standard deviations are not shown for clarity, and ranged between 32 and 42 kg, and 24 and 55 kg for early and normal weaning, respectively. Vertical arrows indicate weaning times and ellipses indicate time and spread of calvings.

*Years 3 and 4.* Consistent with the preliminary observations, calves were weaned at average ages of 93 and 86 days in Years 3 and 4, respectively (Table 3). At the end of the 123 days of experimentation, LW was lower (P<0.0001) in calves on improved forages than in their suckling counterparts on savanna (Table 3). Differences in LW productivity amongst sown forages were small (P>0.05), with the exception of Ag in Year 3 (Table 3).

Table 3.	Least squares means	$(\pm$ s.e.) of ages	and live weight	ts (LW) of earl	y weaned calves	at weaning and their final	LW (FLW)
after 123	days of experimental	grazing on imp	proved pastures	(early weaned	) and of normally	y weaned calves off savann	a.

Pasture	n		Year 3			Year 4	
		Age (d)	LW (kg)	FLW (kg)	Age (d)	LW (kg)	FLW (kg)
Andropogon gayanus	10	$92 \pm 3$	$63 \pm 15$	$82a^1 \pm 4$	$85 \pm 4$	$77 \pm 9$	$84a \pm 4$
A. gayanus + Centrosema acutifolium	10	$93 \pm 5$	$69 \pm 9$	91ab ± 3	$89 \pm 5$	$77\pm 6$	$88a \pm 4$
A. gayanus + Pueraria phaseoloides	10	$92 \pm 4$	$72 \pm 13$	$95b \pm 4$	$83 \pm 5$	$89 \pm 10$	$92a \pm 4$
Brachiaria humidicola + Arachis pintoi	10	n.a.	n.a.	n.a.	$86 \pm 3$	$80\pm7$	$85a \pm 5$
Savanna <sup>2</sup>	10	$91 \pm 5$	$74\pm 6$	$117c \pm 3$	$96 \pm 10$	$89\pm14$	$129b \pm 5$
Р		NS	NS	< 0.0001	NS	NS	< 0.0001

n - number of animals.

n.a. - not applicable.

NS - not significant.

<sup>1</sup>Within columns values followed by different letters differ significantly (P<0.05).

<sup>2</sup>Weights and ages of savanna calves were obtained on the same date ( $\pm$  3 days) as those of the early weaners.

Data on daily LWG (DLWG, g/head) of calves for each individual grazing period and for each of the 3 Agbased pastures in 1986 were pooled and regressed on the amount of green grass leaf on offer (GGL; kg DM/ha). The resulting linear regression equation was: DLWG =  $0.702 \pm 0.156$  GGL -  $166.667 \pm 98.700$ ; r<sup>2</sup> = 0.63, P<0.01.

#### Pilot test

Despite the very low LWs of some of the early weaned calves, no deaths occurred. Weight gains during the sown pasture phase averaged  $0.18 \pm 0.10$  kg/day, while the correlation between weaning weight and subsequent weight gain on the sown pasture was not significant (r<sup>2</sup> = 0.24, P>0.05).

#### Blood and feces profiles

Blood data showed that the concentrations of total protein, urea nitrogen and the AST and GGT enzymes in weaned calves differed significantly (P<0.0001) between years, but there were much smaller differences in concentrations of protein and enzymes amongst the pastures (Table 4). In Year 3, blood urea nitrogen levels on the AgPp pasture were 8–10 times those on the other

pastures (P<0.0001), associated with a temporary increase in the percent legume. Over the same period, relative to calves grazing Ag as monoculture, hematocrit concentration was higher (P<0.001) in AgCa and AgPp mixtures by 31 and 19%, respectively. However, although within normal physiological values, a larger hematocrit difference (58%; P<0.001) was found with the unweaned calves on savanna (Table 4).

Concentrations of minerals in serum and feces (Table 5) varied considerably between years (P<0.0001). However, there were small and mostly non-significant differences between pastures. There were no effects of nutritional treatment upon ash or fecal N, on either a DM or an organic matter basis (Table 5).

#### Cow live weights and reproductive performance

Early weaned cows were significantly heavier at weaning than those weaned at normal times (Table 6; P<0.05). Inter-calving intervals increased significantly (P<0.05) with increasing calf weaning age, but were inversely related to cow weight at weaning (P<0.001), with the positive effect of cow weaning weight in reducing intercalving interval being greater with older than with younger weaning ages (regression equation in Table 6).

**Table 4.** Least squares means for total blood protein (TBP), hematocrit (HCT), urea nitrogen (BUN) and the body fluid enzymes aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) of early weaned calves grazing sown pastures and unweaned calves grazing savanna in Years 3 and 4.

	Pasture	TBP	HCT	BUN	AST	GGT
		(g/100 ml)	(g/100 ml)	(g/100 ml)	(IU/ml)	(IU/ml)
Reference values <sup>1</sup>		6.3-8.9	24–46	6–22	39–79	14–40
Year 3	A. gayanus	$5.9\pm0.14$	$26.1a \pm 1.56$	$7.4a\pm0.69$	$76.0\pm6.00$	$7.2\pm7.00$
	A. gayanus + C. acutifolium	$6.2\pm0.12$	$34.4b\pm1.38$	$5.8a\pm0.31$	$75.0\pm5.00$	$7.7\pm7.30$
	A. gayanus + P. phaseoloides	$6.3\pm0.13$	$31.1b\pm1.46$	$63.5b\pm41$	$69.0\pm5.00$	$3.3\pm7.40$
	Savanna	$6.8\pm0.29$	$41.3c\pm1.47$	$6.0a\pm0.35$	$83.0 \pm 14.00$	$42.9{\pm}8.90$
Year 4	A. gayanus	$6.7\pm0.53$	$41.3 \pm 1.47$	$6.6a\pm0.40$	$80.0\pm5.00$	$16.4\pm8.30$
	A. gayanus + C. acutifolium	$7.6\pm0.52$	n/a	$5.9a\pm0.31$	$82.0\pm5.00$	$5.2\pm8.10$
	A. gayanus + P. phaseoloides	$8.8\pm0.61$	n/a	$8.9b \pm 0.82$	$71.0\pm6.00$	$1.9\pm9.35$
	B. humidicola + A. pintoi	$8.0\pm0.52$	n/a	$8.8b\pm0.69$	$84.0\pm3.00$	$6.6\pm8.00$
	Savanna	$6.8\pm0.20$	n/a	$7.5b\pm0.81$	$83.0\pm8.00$	$15.0\pm7.80$
Year, Probability		< 0.0001		< 0.0001	< 0.0001	< 0.0001
Pasture (year), Probability		= 0.09	< 0.001	< 0.0001	= 0.09	= 0.25

n/a - not available.

<sup>1</sup>Aiello and Moses (2016).

Table 5. Least squares means for blood serum phosphorus and calcium (mg/dl), fecal phosphorus, calcium and ash (% fecal DM,
FDM) and fecal nitrogen (% fecal organic matter, FOM) concentrations (means ± s.e.) of early weaned calves grazing sown pastures
and un-weaned calves grazing savanna in Years 3 and 4.

	Pasture	Serum P	Serum Ca	Fecal P	Fecal Ca	Fecal ash	Fecal N
Year 3	A. gayanus	$4.28c^1\pm0.51$	$8.96 \pm 0.23$	$0.99a \pm 0.25$	$1.24a\pm0.46$	$13.57a\pm0.93$	$1.92\pm0.07$
	A.gayanus + C. acutifolium	$4.17c\pm0.43$	$8.83 \pm 0.21$	$0.76a \pm 0.13$	$1.03a\pm0.29$	$12.29a\pm0.85$	$1.69\pm0.07$
	A. gayanus + P. phaseoloides	$5.89b \pm 0.47$	$8.75\pm0.22$	$0.61a\pm0.08$	$2.38a \pm 1.54$	$13.08a\pm0.85$	$1.67\pm0.07$
	Savanna	$9.78a \pm 0.39$	$10.65\pm0.24$	n/a	n/a	n/a	n/a
Year 4	A. gayanus	$4.88a \pm 0.44$	$9.04\pm0.23$	$0.64a\pm0.45$	$0.77a \pm 0.84$	$9.43a\pm1.05$	$1.79\pm0.06$
	A. gayanus + C. acutifolium	$5.46a\pm0.42$	$9.52\pm0.22$	$0.61a\pm0.39$	0.75a ±0.77	$10.58a\pm1.06$	$1.75\pm0.05$
	A. gayanus + P. phaseoloides	$5.39a \pm 0.49$	$9.13 \pm 0.27$	$0.32b\pm0.14$	$0.76a \pm 1.07$	$7.33a \pm 1.67$	$1.87\pm0.06$
	B. humidicola + A. pintoi	$5.19a\pm0.42$	$9.71 \pm 0.22$	$0.46b\pm0.25$	$2.74b\pm5.0$	$9.20a\pm1.17$	$1.80\pm0.06$
	Savanna	$5.93a\pm0.75$	$10.25\pm0.30$	n/a	n/a	n/a	n/a
Year, Probability		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Pasture (year), Probability	7	< 0.0001	NS	< 0.05	< 0.0001	< 0.05*	NS

n/a - not analyzed.

NS - not significant.

<sup>1</sup>Letters compare pasture values within years. Differences were found between pastures in different years, but not within the same year.

Table 6.	Reproductive	performance a	and live	weight	(LW)	of cows	(n = 45)	in relation	to age and	weight o	f calves at	weaning.
		P			< · · · /		· · · ·					

Weaning age (d)	Inter-calving interval (d) <sup>1,2</sup>	Cow weaning LW (kg)	Calf weaning LW (kg)
110	472d <sup>3</sup> (454–491)	$339a \pm 8$	$68a \pm 4$
166	514c (504–557)	$308b \pm 11$	$112b \pm 5$
198	625a (594–656)	$304b \pm 11$	$114b \pm 5$
266	642a (616–669)	$306b \pm 9$	$143c \pm 4$

<sup>1</sup>Regression equation:

Inter-calving interval (d) =  $394 (\pm 38) + 2.76 (\pm 0.59) *$  calf weaning age  $- 0.00588 (\pm 0.0019) *$  calf weaning age \* cow weaning LW; adj R<sup>2</sup> = 0.45; P<0.001.

<sup>2</sup>Confidence interval in parentheses.

<sup>3</sup>Within columns values followed by different letters differ significantly (P<0.05).

#### Calf compensatory body growth

The possible carry-over effects of low calf weaning LW were examined by monitoring subsequent performance of early weaned calves compared with normally weaned calves, for a total of 414 days after the end of the early weaning experimental period (Table 7). At that time, there were no significant (P>0.05) differences in final LW between the early weaned and normally weaned calves (Table 7). Over the 414 days of common grazing in savanna, daily weight gains were inversely related to weaning weight, weight gains to weaning, and weight at end of the experimental phase ( $r^2 = 0.98$ , P<0.05 in all cases).

**Table 7.** Final live weights (LW) of contemporary calves (n) weaned early and grazed on various sown pastures for 123 days and normally weaned calves, following 414 days grazing savanna as a single group. Data are least squares means  $\pm$  s.e.m.

Weaning pasture	n	LW (kg)
A. gayanus	10	$187 \pm 19$
A. gayanus plus C. acutifolium	10	$187 \pm 16$
A. gayanus plus P. phaseoloides	10	$192\pm13$
Traditional weaning on savanna	10	$205\pm16$
Probability		NS

n - number of animals.

NS - not significant.

#### Discussion

The primary objective of this study was to assess the growth of early weaned calves on sown tropical pastures, while keeping the breeding cows on savanna. A secondary objective was to document the effect of early weaning (3-4 months) on inter-calving interval in breeders compared with traditional rangeland cow-calf breeding, where calves are weaned at about 9-10 months old. The low overall mortality rate of calves of 1% over 4 consecutive years was a significant finding when compared with commercial, extensive tropical herds that normally exhibit death rates of 7-9% (Kleinheisterkamp and Habich 1985; Rivera Sánchez 1988). Reduced calf mortality would then complement the improved reproductive performance of the breeding cows in terms of reduced inter-calving intervals associated with early weaning.

There has been limited research on management of early weaners on supplemented tropical pastures (Vendramini et al. 2008; Vendramini and Arthington 2008), and even less if unsupplemented. Post-weaning LW gains by early weaned calves were low and similar across the 4 years of experimentation (Tables 2 and 3) allowing for the differences between years in age of calves at weaning. Schottler and Williams (1975) compared the performance of Brahman-Shorthorn crossbred calves weaned at 4, 5, 6 or 7 months of age on a Para grass (Brachiaria mutica)-Siratro (Macroptilium atropurpureum) sward for 2 months, followed by a buffel grass (Cenchrus ciliaris)-Siratro pasture. Regardless of weaning age, LWG ranged between 0.20 and 0.32 kg/day. Holroyd et al. (1990) weaned calves in northern Australia at 5 and 8 months of age, and after a 10-day period of supplementation with good quality pasture hay they were transferred to a savanna paddock. The LW difference at 8 months between early and late weaners of 54 kg for males was reduced to 13 kg at the age of 3.5 years due to compensatory growth in younger weaned calves, and the research indicated that pasture quality was probably the limiting factor for better animal performance.

This hypothesis is supported by the observation that the LW of early weaners (100 days) placed on a higher quality annual ryegrass (*Lolium rigidum*) temperate pasture did not differ from those of late weaners at 365 days of age (Potter et al. 2004). The close correlation between green leaf and LWG found in the present research supports the hypothesis that performance of early weaners on pastures may be limited by forage quality. In this context, Aguiar et al. (2015) advocated limited creep feeding with soybean meal to improve the performance of early weaners on limpograss (*Hemarthria altissima*).

Casual visual observations showed highly selective grazing on the Ag-based pastures. In earlier studies (Böhnert et al. 1985; 1986), young steers grazing AgPp mixtures selected diets much higher in N than when grazing Ag as a monoculture but differences in in vitro DM digestibility of the diets selected were not significant. Although some caution is required in extrapolating selective grazing behaviors between weaners and 1-2 year old steers, the high BUN concentration on the AgPp pasture in Year 3 was likely due to temporary low availability of green leaf on Ag and high levels of Pp on offer, as Ag leaves were selectively grazed at the start of grazing in each paddock. However, as the availability of Ag leaves rapidly declined, calves were forced to consume the legume. The preliminary, positive relationship between green grass on offer on all Ag-based pastures and daily LWG points in the same general direction.

Overall, this study also showed that none of the early weaned calves demonstrated a deficiency of total protein, hematocrit, BUN and the sensitive enzyme marker of liver damage, AST (Table 4). However, compared with blood reference values, results from all pastures in Years 3 and 4 indicated low GGT activity, and high variability between animals (Table 4). Although the potential physiological effects of these values need to be clarified, it is reasonable to assume, as demonstrated by Stojević et al. (2005) with healthy dairy cattle, that the observed GGT concentrations reflected a temporary acute situation (i.e. circadian changes) associated with age of the calves, rather than long-term detrimental metabolic effects. Furthermore, it is unfortunately impossible to define whether the low enzyme concentrations suggested adverse metabolic effects of secondary compounds in the legumes. Nevertheless, it is worth noting that recent studies (Ramírez-Restrepo et al. 2016) demonstrated that supplementation of animals with plant-derived compounds increases GGT blood serum values in Brahman cattle, which is contrary to the present results.

Despite differences between pastures and years in mineral concentrations in serum and feces (Table 4), all values fell within normal ranges (Doornenbal et al. 1988; Aiello and Moses 2016). The fairly large between-year differences, and the between-animal variation indicated by the relative magnitude of the standard error terms, question the reliability of single samplings within years and pastures, and by inference, between farming systems as frequently carried out in survey studies. Several mineral deficiencies in unsupplemented adult beef cattle grazing savanna, accompanied by low breeding cow LWs, have been reported (Lebdosoekojo et al. 1980). Subsequent and detailed analyses by Rivera Sánchez (1988) in controlled savanna experiments over several years showed adequate serum, liver and fecal concentrations of all minerals in cows, when complete mineral mixes were provided. Furthermore, Rivera Sánchez (1988) found evidence of an interaction of mineral supplementation with management strategies that allowed access to improved grass-dominated pastures by the breeding herd.

Although fecal N as an indicator of nutrition has sometimes been questioned (Hobbs 1987), it is generally regarded as appropriate for free-ranging herbivores in the absence of better, simple indices (Leslie Jr. et al. 2008). Similarly, N in feces has been found to be broadly and linearly related to N intake with a variety of forage diets (Nunez-Hernandez et al. 1992). Allden and Jennings (1969) proposed that fecal N levels of 1.4–1.6% in sheep are indicative of N-limited diets. If these values are applicable to calves, data in Table 5 would suggest that dietary N would not have been the limiting nutritional variable. This view accords with that expressed by Lascano (1991), who showed that digestible energy intake is the most limiting nutrient for yearlings and adult cattle in neotropical savannas.

The low calf LWs at the end of the experimental periods, coupled with absence of indicators of specific nutritional deficiencies shown by the blood and fecal analyses, are indicative of a general condition of undernutrition, a hypothesis supported by the in-depth analyses of metabolic profiles of early weaned calves in tropical northeast Argentina. There, Coppo (2003; 2007a; 2007b) assessed the stress produced by early weaning at 60 days of age in crossbred Zebu cattle supplemented with concentrates, but could not relate it to a large set of blood parameters and suggested that there was no evidence of specific metabolic stresses. Arthington et al. (2005) studied the dynamics of acute-phase proteins in beef calves weaned at 89 days of age onto pasture and fed a supplement, and found them to rise in the first few days following separation from the dams, decreasing subsequently to normal values. While early weaned calves were lighter than contemporary suckling calves at 120 days of age, and had lower concentrations of BUN, total proteins, triglycerides, P, Mg, Fe and Cu, indicators of stress such as cortisol, aldosterone and AST did not differ between the 2 groups of calves. The authors concluded that early weaning does not produce clinical stress in crossbred Zebu calves, despite a general condition of under-nutrition.

One measure of the sustainability of beef herds is absence of animal stress, and the present results, supported by the literature, would suggest that stress in early weaned beef calves would have been short-term only. Furthermore, and despite the condition of generalized under-nutrition, there was no evidence of negative carry-over effects. In fact, by the end of the observation period, there were no significant differences in the final LWs (Table 7) despite the early advantage of animals allowed to suckle for the normal time, a finding confirmed by the longer-term on-farm study of Mejía et al. (2009), who weaned female and male calves at 4 months of age on a rotation of *B. decumbens*, B. humidicola and savanna pastures plus a medium quality concentrate until reaching 8 months of age. Thereafter these and normally weaned calves (8 months old) were raised on the same pastures as above without supplementation. No significant differences were found in age at first conception (35 vs. 33.4 months for early and traditional weaning, respectively). The inter-calving interval of early weaned dams was 141 days shorter than those weaned late. The corresponding males reached slaughter weight (450 kg) with a non-significant difference of 3.2 months and average monthly LWGs of 11.5 vs. 10.6 kg for late and early weaners, respectively. Under more severe pasture conditions, Holroyd et al. (1990) noted that the early weaners (5 months old at weaning) grazing savanna in northern Australia were still 13 kg lighter than late weaners (8 months old at weaning) at 3.5 years of age.

In view of the compensatory growth experienced by the early weaners in the present study, the low LWG recorded on pasture would be acceptable if, as is generally the case, weaners are not sold immediately, but are kept as young steers for an additional 12–18 months. This trade-off between calf LWG and cow reproductive performance is an important consideration for farm managers who need to balance different forage resources and the nutritional needs of stock and prioritize their use. The new 'crop' of improved, high-yielding and leafier *Brachiaria* cultivars (Pizarro et al. 2013), together with higher quality grasses such as *Panicum maximum*, may help resolve the above issues to some extent, if the *Brachiaria* cultivars do not lead to subclinical and clinical photosensitization (Lima et al. 2012).

Notwithstanding few to no weight differences in later life, possible negative effects of low early growth rate on lifetime beef production of early weaned females through epigenetic effects affecting them and their progeny cannot be ignored (Martin et al. 2007; Funston et al. 2012; Wathes et al. 2014), although this would probably be a minor concern in extensive systems.

In our study, early weaned male calves attained weights at 18 months of age similar to those of control

animals of equivalent ages. Vera (1991) and Vera et al. (1993) showed that, despite long periods of sustained under-nutrition, heifers and cows could achieve mature body sizes and inter-calving intervals similar to those of better-fed animals, when given the opportunity to make moderate compensatory gains. In the present study, weaning calves early removed lactation stress on cows, and allowed them to attain plateau weights of 350-400 kg, confounded with pregnancy, even during the dry season. While LWs of this magnitude are seldom observed in traditional savanna-based grazing systems (Rivera Sánchez 1988), weights of Zebu cows above 320 kg do not appear to limit re-conception (Mukasa-Mugerwa 1989; Vera et al. 1993). In fact, cows weaned at 166 days in the present research conceived after weaning with an average weight of  $303 \pm 43$  kg.

Early weaning is known to enhance reproductive performance of underfed beef cows (Moore and da Rocha 1983; Holroyd et al. 1990; Schlink et al. 1994; Coppo et al. 2002; Arthington et al. 2004), an effect shown also in Table 6 that demonstrates the trade-offs between weaning age and LWs of calves, LWs of their dams and intercalving intervals. Moore and da Rocha (1983) investigated the effects of 2 levels of nutrition and 5 weaning ages in Zebu Gyr breed cows fed hay of B. decumbens in the Brazilian Cerrados and found that, irrespective of the cows' nutritional level, early weaning of calves improved cow weights at different stages throughout the reproductive cycle and subsequent reproductive performance. Weights of cows at weaning were 313 kg and 325 kg on low and high supplementary energy treatments, respectively, and the authors suggested that Zebu cows rarely conceive if suckling cows weigh less than 300 kg. Weights of cows at weaning decreased linearly from 352 kg, when calves were weaned at 1 month of age, to 294 kg if calves were weaned at 6 months of age, with LW losses during lactation increasing from 21 to 102 kg for the respective weaning ages (Moore and da Rocha 1983).

Mukasa-Mugerwa (1989) reviewed the literature regarding reproduction of Zebu cattle in the tropics, and noted that tropical cattle dependent on natural pastures most often calve in alternate years, but animals with access to good quality sown pastures have improved reproductive performance. For example, Rivera Sánchez (1988) reported inter-calving intervals of 618 days on well managed savannas over 4 years, whereas Vera et al. (2002) found an average interval of 445 days on well managed *B. decumbens*. Equally large differences have been reported by other authors (Arthington et al. 2004). Hale (cited by Mukasa-Mugerwa 1989) found that, when the LW of suckling Zebu cows fell from 390 to 320 kg,

they stopped cycling, but they needed to reach weights in excess of 320 kg to start cycling again. As shown in Table 6, only cows, whose calves were weaned at 110 days, showed LWs above the purported lower critical weight of 320 kg. Inter-calving intervals increased significantly with increasing weaning age and were negatively related to cows' weights at weaning (P<0.001; footnote of Table 6), whereby the positive effect of cow weaning weight on inter-calving interval was greater with larger than with lower weaning ages (Table 5).

Lastly, early weaning of beef calves and the consequent changes in reproductive performance will likely lead to significant changes in herd dynamics (Turner et al. 2013), whose production, environmental and economic effects remain to be studied. These changes would significantly impact management decisions (Sullivan et al. 1997). The savannas of the Orinoco watershed are under transition as represented by: (i) increase in crop and tree plantation areas; (ii) oil exploration and extraction; (iii) mining; (iv) growing recognition of indigenous rights and lands; (v) and increasing appreciation of the savanna's relevance in terms of biodiversity and contribution to greenhouse gas emissions (Rausch 2013; CONPES 2014). This transition has been also noted in the Australian savannas (Holmes 2010). This implies that extensive beef cattle farming, even if it continues to represent an important land use system, will need to adapt and intensify to the extent possible. Further, farmers and their communities will need to modify their decision-making to take into account the multifunctional traits of these lands. The strategic use of areas of sown pasture could play an increasingly important role in management of breeding herds.

#### Conclusions

Results from this study suggest that there is considerable scope and flexibility in strategic use of small areas of sown pastures for weaners in combination with extensive savannas to improve productivity of beef breeding herds, if calves are given the opportunity to realize compensatory growth. Several authors have commented on the flexibility, adaptability and sustainability of extensive systems (Davies et al. 2010; Astigarraga and Ingrand 2011), while some of the environmental aspects have been also described (Ramírez-Restrepo and Charmley 2015) or are under scrutiny (Ramírez-Restrepo et al. unpublished data 2017). This study has demonstrated over 4 consecutive years, that early weaning of calves onto a variety of sown tropical pastures is technically feasible, resulting in improved LWGs and reproductive rates in their dams.

Adoption of such early weaning strategies in extensive systems, where mating generally extends over 6 or more months, would be constrained by the spread of the calvings over an extended period of time. A number of weaning events would be needed each year, resulting in increased labor and management requirements (Sullivan et al. 1997). On the other hand, with several batches of early weaned calves dispersed over several months, a smaller area of sown pastures would be needed than if all calvings were concentrated into a shorter period of time. From a management point of view, seasonal mating to facilitate early weaning would be desirable, but this would lead to a smaller calf crop in the initial year when the practice was first implemented. However, increased reproductive rates during the following years due to improved cow condition and LW, as demonstrated in this study, would soon compensate for the loss of production in the implementation year of seasonal mating. A possible added benefit would be a reduction in risks, which could be posed by climate change. Thus, productive and environmental trade-offs, as suggested above, would need better quality and intensity of animal and pasture management. Further savanna studies with larger numbers of calves and cows under commercial conditions would confirm these preliminary findings.

#### Acknowledgments

This research was financially supported by the International Center for Tropical Agriculture (CIAT)'s core funding in Colombia. The authors express our appreciation to Hernando Ayala, DVM (deceased), who supervised the first 2 years of the experiment. We further acknowledge Obed García Durán, DVM, who confirmed some of the views and opinions expressed here, and facilitated a recent trip by the senior author to the region to verify some of the assertions made in the paper. We thank staff at CIAT and Carimagua Research Station for their help and technical support with all aspects of this work. Finally, special thanks are extended to the Commonwealth Scientific and Industrial Research Organisation (CSIRO) for allowing the time to the second author to co-write the manuscript.

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(*Received for publication 27 October 2016; accepted 30 April 2017; published 31 May 2017*)

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#### **Research Paper**

# Dry matter accumulation and crude protein concentration in *Brachiaria* spp. cultivars in the humid tropics of Ecuador

Acumulación de materia seca y concentración de proteína cruda en cultivares de Brachiaria spp. en el trópico húmedo de Ecuador

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#### Abstract

Climatic conditions throughout the year and age of plants affect both yield and quality of forage grasses. In this research, we evaluated the effects of age of regrowth and seasonal conditions on dry matter accumulation and crude protein concentration in 5 cultivars of *Brachiaria* spp.: Señal, Xaraés, Marandú, Piatá and Mulato II, harvested at 2, 4, 6, 8 and 10 weeks after a uniformity cut, during the rainy and dry seasons. The variables were: total dry matter (TDM), leaf dry matter (LDM) and stem dry matter (SDM) yields, leaf area index (LAI), specific leaf area (SLA) and crude protein (CP) concentration. For TDM yield, in the rainy season there was no significant difference (P>0.05) among cultivars, with mean DM yield over 10 weeks of 6.34 t/ha; however, during the dry season Xaraés presented a higher (P<0.05) yield over 10 weeks than other cultivars (5.09 vs. 3.14–3.89 t/ha). Overall, mean DM yield in the dry season was only 62% of that in the wet season. In both periods, Señal tended to have the highest SDM yields, while Xaraés had the greatest (P<0.01) LDM yields in the dry season. Mulato II tended to have the highest CP concentrations throughout, especially in the dry season. This study was conducted in plots with plants only 12 weeks old at commencement. However, it indicated that all cultivars performed well and larger-scale studies of longer duration are warranted to test these cultivars under grazing, especially Mulato II, which showed both high dry matter yield and retention of high protein concentration throughout the study.

Keywords: Brachiaria decumbens, Brachiaria brizantha, Brachiaria hybrid cv. Mulato II, leaf area index, specific leaf area.

#### Resumen

En la finca experimental "El Oasis" de la Escuela de Ingeniería Agropecuaria, Universidad Tecnológica Equinoccial, Campus Santo Domingo, Ecuador, en un suelo Andosol se evaluaron los efectos de la edad de rebrote y la época del año sobre la acumulación de materia seca y la concentración de proteína bruta en 5 cultivares de *Brachiaria*: Señal (*B. decumbens*), Xaraés (*B. brizantha*), Marandú (*B. brizantha*), Piatá (*B. brizantha*) y Mulato II (*Brachiaria* híbrido), cosechados a 2, 4, 6, 8 y 10 semanas después de un corte de uniformidad, durante las estaciones lluviosa y seca. Las variables evaluadas fueron la materia seca (MS) total, MS de hoja y de tallo, el índice de área foliar, el área foliar específica y la concentración de proteína bruta. El rendimiento de MS total a 10 semanas de rebrote (6.34 t/ha en

Correspondence: E. G. Cienfuegos Rivas, Centro Universitario Adolfo López Mateos, Edificio de Gestión del Conocimiento 4º piso. Cd. Victoria, CP 87149, Tamaulipas, Mexico. Email: ecienfue@docentes.uat.edu.mx promedio) en la estación de lluvias no varió entre cultivares (P>0.05) mientras en la estación seca el cv. Xaraés presentó el mayor rendimiento (P<0.05) en comparación con los demás cultivares (5.09 vs. 3.14–3.89 t/ha). El rendimiento de MS total en la época seca fue sólo el 62% del obtenido en la época de lluvia. En ambos períodos, el cv. Señal tendió a tener los rendimientos de MS de tallos más altos, mientras que el cv. Xaraés presentó los mayores rendimientos de MS de hojas (P<0.01) en la estación seca. El cv. Mulato II tendió a tener las mayores concentraciones de proteína bruta en ambas épocas, especialmente en la seca. Este estudio se realizó en parcelas con plantas de sólo 12 semanas de edad al inicio. Sin embargo, indicó que todos los cultivares se comportaron satisfactoriamente y estudios a mayor escala y de mayor duración se justifican para probar estos cultivares bajo pastoreo, especialmente el cv. Mulato II que mostró tanto un alto rendimiento de MS como una retención de alta concentración de proteína a lo largo del estudio.

**Palabras clave:** Área foliar específica, *Brachiaria decumbens*, *Brachiaria brizantha*, *Brachiaria* híbrido cv. Mulato II, índice de área foliar.

#### Introduction

Poor grassland productivity is one of the most important limitations in the dual-purpose cattle system in the Ecuadorian tropics (Avellaneda et al. 2008), because most grazing areas are sown with forage species such as *Brachiaria humidicola*, *Brachiaria decumbens* and *Brachiaria brizantha* (Vera 2004). These grasses have limitations in productivity, adaptability and persistence in these environments; in addition, they are susceptible to the spittlebug of pastures caused by *Aeneolamia* spp. (Cardona et al. 2006) and foliar fungi such as *Rhizoctonia solani*, which significantly reduce productivity (Álvarez et al. 2013). However, new cultivars of the genus *Brachiaria* have been released to the market as options to overcome the problems observed in traditional forages, thus providing better fodder options (Pizarro et al. 2013).

In Ecuador, several cultivars of *Brachiaria* spp. are available that have potential for increasing the productivity of existing grass production systems (Faría Mármol 2006; Miles 2006). Jácome and Suquilanda (2014) indicated that cultivars Mulato I and Xaraés are well accepted by farmers because of their high nutritional value, adaptation to a range of soils and resistance to or tolerance of pests and diseases (Cardona et al. 2006; Argel 2008).

The productive capacity of forage species can be modified by changing various factors, such as: using different genotypes or cultivars; modifying the agronomic management and age of the plants at harvest; and by weather conditions throughout the year (Zaragoza et al. 2009; Lara et al. 2010). It is therefore important to assess the dynamics of herbage accumulation of different pasture species and to understand the impact of seasonal changes (Fagundes et al. 2005) on the pattern of growth, quality and chemical composition of pasture plants, in order to optimize their use and plan an appropriate agronomic management strategy for grasslands (Avellaneda et al. 2008). Based on the above, this research aimed to evaluate the effects of plant regrowth age and seasonal conditions on the accumulation of dry matter and crude protein concentration in 5 cultivars of *Brachiaria* spp. in the humid tropics of Ecuador.

#### **Materials and Methods**

The research was conducted under field conditions from December 2011 to November 2012, at the experimental farm "El Oasis", property of the Escuela de Ingeniería of the Agropecuaria Universidad Tecnológica Santo Domingo, Equinoccial, Campus Ecuador (00°13'20" S, 79°15'39" W; 406 masl). The experimental site has a predominantly humid tropical climate, with annual average temperature of 23.4 °C and annual rainfall of 2,600-3,500 mm with 2 seasons, defined as rainy and dry (SENPLADES 2015). The monthly rainfall and mean maximum and minimum temperatures during the evaluation period are reported in Figure 1.

In this research 5 *Brachiaria* cultivars were used: Señal (*B. decumbens*, considered as the control since it is most widely cultivated in the area); Marandú (*B. brizantha*), Piatá (*B. brizantha*), Mulato II (*Brachiaria* hybrid) and Xaraés (*B. brizantha*); and these were harvested at 5 growth stages (2, 4, 6, 8 and 10 weeks after an initial harvest) during the rainy (March–May) and dry (September–November) seasons to monitor the rate of DM accumulation over time and assess quality aspects.



**Figure 1**. Monthly precipitation and mean maximum and minimum temperatures during 2012 at the Climatological Station of the Universidad Tecnológica Equinoccial, Campus Santo Domingo, Ecuador.

A randomized complete block design in an arrangement in divided plots was used, where the large plot was the cultivar and the subplots the ages of regrowth. On 3 December 2011, seeds of each cultivar were placed in bags (3 seeds/bag) of black polyethylene with a capacity of approximately 2 kg of soil, to ensure one plant survived per bag and placed in a greenhouse. At 6 weeks after seedling emergence (21 January 2012; 7 weeks after sowing), the plants were transplanted to large plots of 5 x 5 m (25 m<sup>2</sup>), leaving an intra-row and interrow spacing of 0.50 m. The useful area within each large plot was 3 x 3 m, divided into 9 quadrants of 1 m<sup>2</sup> (subplots), of which 5 subplots were randomly selected to represent each age of regrowth (2, 4, 6, 8 and 10 weeks). The soil of the experimental site is of volcanic origin (Andosol) and a soil sample was taken using a 5-point method to 20 cm depth to provide the physicochemical analyses shown in Table 1. All plots were fertilized after transplanting with 120 kg N/ha (as urea), 60 kg P/ha (as Daphos), 70 kg K/ha (as KCl), 60 kg Mg/ha (as magnesium oxide) and 50 kg S/ha (as ammonium sulfate).

In the sixth week after transplantation the plants were 80–100 cm tall with 45–65 tillers/plant; senescent material was obvious in the lower stratum, so we applied an initial uniformity cut on 3 March 2012 to 15 cm above ground level. The evaluation during the rainy season then followed. At the end of this period (12 May 2012), the plots were allowed to stand for 15 weeks, when a second uniformity cut was applied on 1 September 2012. The dry season evaluation then followed. In each

season, observations commenced 2 weeks after each uniformity cut to measure: accumulation of total (TDM), leaf (LDM) and stem (SDM) dry matter; plant height (PH); leaf area index (LAI); specific leaf area (SLA); and crude protein concentration (CP). During each sampling for the relevant cutting treatment we measured the height of plants from ground level to ligule of the fully developed leaf and harvested the forage at 15 cm above ground level from the relevant 1 m<sup>2</sup> subplot (4 plants). The harvested forage (green matter) was weighed on a precision scale (Model PB3002-S, Mettler Toledo) and 2 subsamples were taken, the first (approximately 200 g) to define the proportions of leaf (leaf blade + sheath) and stem after separating these components. Leaves from this subsample were used for leaf area estimation as well. A multifunctional printer scanner (HP Photosmart D110) was employed using the methodology reported by Rincón et al. (2012). The second subsample (approximately 1 kg) was used to determine CP concentration by the Kjeldahl method (N x 6.25) (AOAC 2000); CP% was determined only for 4, 6 and 8 week cuttings. All samples were dried in a forcedair oven at 65 °C for 48 h to estimate the dry matter (DM) concentration and calculate DM yields.

The data were analyzed in time within season by using PROC GLM of SAS (SAS Institute 2010), in a complete randomized block design in an arrangement in divided plots. Tukey's Studentized range tests (P $\leq$ 0.05) were performed, when treatments were significantly different in each season.

**Table 1**. Chemical<sup>1</sup> characteristics of the soil used in the experiment<sup>2</sup>.

pН	OM <sup>3</sup>	NH <sub>4</sub>	Р	S	Fe	Cu	Zn	Mn	В	K	Ca	Mg
	(%)				(cmol/k	g)						
5.9	2.2	41.0	6.5	6.3	42.0	5.6	1.9	2.8	0.3	0.3	8.3	2.9

<sup>1</sup>Obtained in the Chemistry Laboratory, Universidad Tecnológica Equinoccial, Campus Santo Domingo.

<sup>2</sup>The analysis to determine the phosphorus concentration in the soil was performed by the method of Olsen modified with a solution of sodium bicarbonate and EDTA adjusted to pH 8.5 with 10N NaOH.

<sup>3</sup>The organic matter (OM) analysis was performed using the method of Walkley Black, suitable for the conditions of Ecuador.

#### Results

#### Total dry matter accumulation

Accumulation of total dry matter (TDM) followed an exponential pattern in both rainy and dry seasons (Table 2). There were no significant differences (P>0.05) in TDM accumulation among cultivars of *Brachiaria* during the rainy season, with yields ranging from 5.83 to 6.87 t DM/ha (mean 6.34 t DM/ha) over the 10 week period. However, in the dry season differences between cultivars (P<0.01) were observed in each of the regrowth periods. While there was little consistency in which cultivars were superior within particular cutting frequencies, cultivar Xaraés accumulated the greatest amount of TDM during the later part of the dry season and produced the highest DM yield during the dry season period (5.09 t DM/ha),

with the remaining cultivars producing 3.14–3.89 t DM/ha. Average accumulation of TDM during the dry season was 62% of that produced in the rainy season.

#### Leaf dry matter accumulation

Leaf dry matter (LDM) yield during the rainy season varied between cultivars at the various cutting ages but at the tenth week, there were no significant differences in leaf yield between cultivars (P>0.05; Table 3). On the other hand, during the dry season, significant differences (P<0.05) in leaf yield between cultivars at all ages of regrowth were observed. Towards the end of the dry season (8 and 10 week cuttings) Xaraés produced more leaf (P<0.05) than most other cultivars. Dry season leaf yield at the 10 week cutting varied from 4.28 t DM/ha (Xaraés) to 2.50 t DM/ha (Marandú) (P<0.001).

**Table 2**. Total dry matter yields at different ages of regrowth of 5 *Brachiaria* cultivars during the rainy (March–May) and dry (September–November) seasons in humid tropical conditions of Ecuador.

Cultivar					Total dry 1	matter (t/ha)						
		R	ainy seasor	ı		Dry season						
		Age of a	regrowth (v	weeks)			Age of 1	regrowth (w	weeks)			
	2	4	6	8	10	2	4	6	8	10		
Señal	0.24	0.96	2.96	4.02	6.87	0.16	0.39	0.75	1.98	3.69		
Marandú	0.24	0.85	2.54	4.00	5.83	0.23	0.42	0.73	1.59	3.14		
Mulato II	0.29	1.31	2.66	4.33	6.57	0.13	0.28	0.64	1.95	3.76		
Piatá	0.28	1.13	2.36	4.36	6.14	0.13	0.41	0.91	1.93	3.89		
Xaraés	0.32	1.28	3.16	4.49	6.27	0.13	0.41	0.87	2.49	5.09		
Mean	0.27	1.11	2.73	4.24	6.34	0.16	0.38	0.78	1.99	3.92		
$LSD^1$	0.09	0.29	1.05	1.59	2.38	0.04	0.1	0.19	0.54	1.09		
$LS^2$	0.058	< 0.001	0.177	0.812	0.686	< 0.001	0.005	0.004	0.003	0.001		

<sup>1</sup>Least Significant Difference (Tukey P≤0.05).

<sup>2</sup>Level of significance.

Cultivar					Leaf dry r	natter (t/ha)				
		R	ainy seasor	1			Ι	Dry season		
		Age of a	regrowth (v	veeks)			Age of 1	egrowth (v	veeks)	
	2	4	6	8	10	2	4	6	8	10
Señal	0.21	0.75	1.71	1.85	3.13	0.16	0.35	0.66	1.55	2.76
Marandú	0.24	0.79	1.76	2.49	3.46	0.23	0.42	0.72	1.48	2.50
Mulato II	0.29	1.26	1.90	2.66	3.85	0.13	0.28	0.63	1.88	3.19
Piatá	0.28	0.97	1.60	2.60	3.21	0.13	0.41	0.88	1.66	3.02
Xaraés	0.32	1.20	2.45	3.50	4.18	0.13	0.41	0.87	2.34	4.28
Mean	0.27	1.00	1.88	2.62	3.57	0.16	0.37	0.75	1.78	3.15
$LSD^1$	0.08	0.24	0.76	0.93	1.17	0.04	0.10	0.19	0.52	1.01
$LS^2$	0.017	< 0.001	0.029	0.002	0.072	< 0.001	0.004	0.002	0.001	0.001

**Table 3**. Leaf dry matter yields at different ages of regrowth of 5 *Brachiaria* cultivars during the rainy (March–May) and dry (September–November) seasons in humid tropical conditions of Ecuador.

<sup>1</sup>Least Significant Difference (Tukey P≤0.05).

<sup>2</sup>Level of significance.

#### Stem dry matter accumulation

As for leaf production, stem dry matter (SDM) accumulation during the rainy season varied between cultivars from the second to the sixth week (P<0.01). At the 8 and 10 week cuts cultivars Señal and Piatá tended to have the highest stem yields and Xaraés the lowest but differences were not significant (P>0.05; Table 4). During the dry season, SDM accumulation followed an exponential pattern, with greatest stem production between the 8 and 10 week cuts. At the 10 week cut, cultivars Señal and Piatá had the highest SDM (0.93 and 0.87 t DM/ha,

respectively), while Marandú and Mulato II had the least (0.64 and 0.57 t DM/ha) (P<0.001).

#### Plant height

During the rainy season plant height of the different cultivars varied but by the eighth and tenth weeks Señal was shorter than all other cultivars (P<0.001; Table 5). During the dry season Piatá, Señal and Xaraés progressively showed superiority in height over the other cultivars and by the tenth week Piatá and Xaraés were taller than all others (P<0.01).

**Table 4.** Stem dry matter yields at different ages of regrowth of 5 *Brachiaria* cultivars during the rainy (March–May) and dry (September–November) seasons in humid tropical conditions of Ecuador.

Cultivar					Stem dry r	matter (t/ha)							
		R	ainy seaso	n		Dry season							
		Age of	regrowth (	weeks)			Age of regrowth (weeks)						
	2	4	6	8	10	2	4	6	8	10			
Señal	0.03	0.20	1.22	1.69	2.81	-	0.04	0.09	0.41	0.93			
Marandú	-	0.06	0.78	1.39	1.95	-	-	0.02	0.11	0.64			
Mulato II	-	0.05	0.76	1.43	2.09	-	-	0.01	0.08	0.57			
Piatá	-	0.16	0.76	1.62	2.61	-	-	0.03	0.27	0.87			
Xaraés	-	0.08	0.71	1.00	1.86	-	-	0.01	0.15	0.80			
Mean	-	0.11	0.84	1.43	2.26	-	-	0.03	0.21	0.76			
$LSD^1$	-	0.07	0.37	0.82	0.95	-	-	0.02	0.10	0.22			
$LS^2$	-	< 0.001	0.005	0.127	0.068	-	-	< 0.001	< 0.001	0.001			

<sup>1</sup>Least Significant Difference (Tukey P $\leq$ 0.05).

<sup>2</sup>Level of significance.

Cultivar	Plant height (cm)													
			Rainy sea	son		Dry season								
		Age of	of regrowt	h (weeks)		Age of regrowth (weeks)								
	2	4	6	8	10	2	4	6	8	10				
Señal	43.1	53.5	81.8	95.4	80.2	29.6	42.5	70.4	91.3	77.5				
Marandú	40.3	48.8	77.2	117.1	129.8	32.4	30.0	41.5	55.0	67.8				
Mulato II	47.7	57.3	83.5	108.3	129.2	32.5	31.2	43.1	57.9	75.8				
Piatá	45.8	63.8	98.8	132.1	137.1	32.0	39.2	65.7	95.4	105.5				
Xaraés	54.5	71.6	91.2	126.3	137.8	32.3	48.3	71.0	91.3	103.5				
Mean	46.3	59.0	86.5	115.8	122.8	31.8	38.2	58.4	78.2	86.0				
$LSD^1$	9.4	10.6	14.3	10.1	19.8	10.1	9.1	16.5	16.8	23.8				
$LS^2$	0.005	0.001	0.003	< 0.001	< 0.001	0.879	< 0.001	< 0.001	< 0.001	< 0.001				

**Table 5.** Plant height at different ages of regrowth of 5 *Brachiaria* cultivars during the rainy (March–May) and dry (September–November) seasons in humid tropical conditions of Ecuador.

<sup>1</sup>Least Significant Difference (Tukey P≤0.05).

<sup>2</sup>Level of significance.

#### Leaf area index

During the rainy season, the leaf area index (LAI) followed a linear pattern for all cultivars, increasing from a mean of 0.6 at week 2 to 5.3 at week 10 (Table 6). The cultivars Mulato II and Xaraés reached the highest LAI at week 10 of 6.6 and 5.8, respectively. Throughout the wet season Piatá had lower LAI than most other cultivars (P<0.001). During the dry season, LAI followed an exponential pattern with the highest increase from the sixth week. As for the wet season, Piatá showed lower LAI than Xaraés and Mulato II by week 10 (P<0.05).

#### Specific leaf area

Specific leaf area (SLA) declined in all cultivars during both rainy and dry seasons (Table 7). Piatá tended to have

the lowest SLA throughout the wet season, while Señal, Mulato II and Marandú presented the highest values. In the dry season, cultivar differences again emerged with Mulato II, Marandú and Señal having the greatest SLA and Xaraés and Piatá the lowest (P<0.01).

#### Crude protein concentration

Data for weeks 4, 6 and 8 on whole plant samples are presented in Table 8. Differences in crude protein concentration (P<0.05) between cultivars were observed in both rainy and dry seasons, where Mulato II presented the highest concentration at most observations. In both seasons, mean crude protein concentration decreased as the regrowth age increased, declining from 14.1% to 9.1% in the wet season and from 12.6% to 7.6% in the dry season.

Table 6.	Leaf area index at diffe	rent ages of regrowth	of 5 Brachiaria culti	ivars during the rainy	(March-May) and	dry (September-
Novemb	er) seasons in humid trop	pical conditions of Ec	uador.			

Cultivar	Leaf area index										
		F	Rainy seaso	on			D	ry season			
		Age of	regrowth	(weeks)			Age of re	of regrowth (weeks)			
	2	4	6	8	10	2	4	6	8	10	
Señal	0.5	2.0	3.2	3.7	5.1	0.4	0.9	1.2	2.7	4.8	
Marandú	0.6	1.8	3.1	4.3	5.4	0.7	1.0	1.6	2.5	4.4	
Mulato II	0.8	2.6	3.8	4.7	6.6	0.3	0.8	1.4	3.6	5.8	
Piatá	0.5	1.3	2.1	3.4	3.8	0.3	0.7	1.4	2.2	3.6	
Xaraés	0.7	1.9	3.5	4.9	5.8	0.3	0.9	1.6	3.6	5.9	
Mean	0.6	1.9	3.1	4.2	5.3	0.4	0.9	1.4	2.9	4.9	
$LSD^1$	0.2	0.4	1.0	1.1	1.2	0.2	0.2	0.3	0.9	2.2	
$LS^2$	0.001	< 0.001	0.002	0.004	< 0.001	< 0.001	0.045	0.016	0.001	0.03	

<sup>1</sup>Least Significant Difference (Tukey P $\leq$ 0.05).

<sup>2</sup>Level of significance.

Cultivar	Specific leaf area (cm <sup>2</sup> /g)										
		Rai	ny season	l		_		Dry seaso	n		
		Age of re	growth (w	veeks)			Age	of regrowth	(weeks)		
	2	4	6	8	10	2	4	6	8	10	
Señal	229	246	181	190	151	248	3 232	173	163	164	
Marandú	242	216	168	163	145	273	3 221	213	158	162	
Mulato II	268	190	187	165	162	238	8 279	210	178	168	
Piatá	156	129	125	124	112	221	173	148	125	112	
Xaraés	207	148	134	130	132	237	213	170	142	129	
Mean	221	186	159	154	140	243	3 224	183	153	147	
$LSD^1$	29	30	41	40	34	85	39	25	28	27	
$LS^2$	< 0.001	< 0.001	0.001	0.001	0.005	0.43	3 <0.001	< 0.001	< 0.001	< 0.001	

**Table 7**. Specific leaf area at different ages of regrowth of 5 *Brachiaria* cultivars during the rainy (March–May) and dry (September–November) seasons in humid tropical conditions of Ecuador.

<sup>1</sup>Least Significant Difference (Tukey P≤0.05).

<sup>2</sup>Level of significance.

**Table 8.** Crude protein concentration at different ages of regrowth of 5 *Brachiaria* cultivars during the rainy (March–May) and dry (September–November) seasons in humid tropical conditions of Ecuador.

Cultivar			Crude protein o	oncentration (%)				
		Rainy season		Dry season				
	Age	of regrowth (we	eks)	Age o	of regrowth (week	ks)		
	4	6	8	4	6	8		
Señal	13.4	11.2	9.0	11.6	7.3	6.5		
Marandú	14.0	12.1	8.8	13.4	8.6	8.1		
Mulato II	14.6	12.5	10.1	14.3	10.1	10.2		
Piatá	13.8	10.9	8.9	12.2	8.3	6.8		
Xaraés	14.6	11.1	8.6	11.6	7.4	6.5		
Mean	14.1	11.6	9.1	12.6	8.3	7.6		
$LSD^1$	0.9	1.1	0.8	0.9	1.3	3.4		
$LS^2$	0.007	0.003	0.001	< 0.001	< 0.001	0.022		

<sup>1</sup>Least Significant Difference (Tukey P≤0.05).

<sup>2</sup>Level of significance.

#### Discussion

This plot study has provided useful information on the potential of the *Brachiaria* cultivars for use in pastures of humid tropical Ecuador. While the grasses were planted from seed, were only 12 weeks old at commencement of observations and the study was in only a single year, the excellent growth obtained and CP levels maintained indicate that these pastures can be very productive under these conditions. In fact, the DM yields obtained over only 10 weeks in pastures that were only 12 weeks old at commencement are quite remarkable. Further studies under field conditions would clarify how well these preliminary findings can be extrapolated to commercial situations.

The absence of cultivar differences in total DM production during the rainy season in this study was similar to the findings of Rojas-Hernández et al. (2011) for *B. decumbens*, *B. brizantha* cv. Libertad, *B.* hybrid cv. Mulato I and cultivars of *B. humidicola*. Those authors also failed to demonstrate any significant differences in total DM production. However, the differences we showed in proportions of leaf and stem in the different cultivars are important, as we showed higher production of leaves and higher CP concentration than Reyes-Purata et al. (2009). The reduction in growth in these cultivars during the dry season was not surprising, as the major climatic factors that determine forage production are precipitation and temperature (Gerdes et al. 2000; Cuadrado et al. 2004). Rainfall in the rainy season and

hence soil moisture was much higher than in the dry season and minimum temperatures were also lower in the dry season. The reduction in TDM yield in the dry season was somewhat lower than the 50% reduction reported by Benítez et al. (2007). This marked reduction in yield accompanied by a drop in CP concentration indicates the reduced carrying capacity of these pastures during the dry season.

The differences between cultivars in leaf DM yield we found in this study may be due to *B. decumbens* and *B. brizantha* having different rates of elongation in leaves (Dias-Filho and Carvalho 2000). In this regard, elongation rates in leaves of 2.5 and 3.5 cm/d and leaf percentages in total DM of 54 and 77% in *B. decumbens* and *B. brizantha*, respectively, have been reported by Guenni et al. (2005). In addition, Hare et al. (2009) reported that Xaraés and Mulato II have higher foliar DM yields than other *Brachiaria* cultivars, especially during the dry season. Similarly, Gerdes et al. (2000) reported a significant decrease in the accumulation of total DM during the period of minimum precipitation, with as much as 98% of the accumulation corresponding to leaf DM.

The pattern of stem DM accumulation during both periods would have been determined by environmental conditions that affected growth of the cultivars. Stem production during the dry season is reduced (Cab et al. 2008) due to stress caused by soil moisture deficiency, curbing the growth of the plant and reducing stem growth (Cruz et al. 2011a). Cruz et al. (2011b) reported that season has a marked effect on the relative growth of leaf and stem, and differences between stem elongation rates of 1.2, 0.8 and 0.6 mm/stem/d in Señal, Xaraés and Marandú, respectively, have been reported by Paciullo et al. (2011). The marked reduction in stem growth in the dry season results in increases in leaf:stem ratios in this season as found in this study. Leaf:stem ratios also declined significantly as plants matured and the proportion of stem in the forage increased.

Plant height was affected to a greater extent by seasonal conditions than by cultivar. Marandú has been shown to be quite susceptible to stress caused by soil water deficiency, presenting taller plants in the rainy season (67 cm) and shorter ones in the dry season (36 cm) at 35 d of regrowth (Gerdes et al. 2000). On the other hand, *B. decumbens* presented lower height, due to its decumbent growth habit, which is characteristic of this species (Pérez et al. 1999). In this study, *B. brizantha* cultivars were taller than the *Brachiaria* hybrid and *B. decumbens*, which agrees with what was reported by Gómez et al. (2000).

The differences among cultivars for LAI may be because some species such as *B. decumbens* are shorter than *B. brizantha*, and there is a positive correlation between plant height and LAI (Guenni et al. 2005). In addition, LAI increases as the plant grows (Gómez-Carabalí et al. 1999), and this increase is closely related to tillering of the crop and soil cover (Rincón et al. 2007; Ramírez-García et al. 2012). Therefore, as LAI of the pasture increases, the amount of light that reaches the ground will be reduced, which can prevent or retard weed growth.

Furthermore, it has been reported that SLA in *Brachiaria* cultivars varies according to species; Baruch and Guenni (2007) reported that at 4 weeks of age SLA in *B. decumbens* and *B. brizantha* were 300 and 270 cm<sup>2</sup>/g, respectively. Also, it has been reported that, as the height and age of the plant increase, SLA decreases (Gómez et al. 2012), as it did in our study, because there is an increment in the thickness of the leaves. Grasses with greater SLA have thinner leaves and higher concentrations of nitrogen (Pérez et al. 2004) and higher rates of photosynthesis (Reich et al. 1997), while a high SLA is related to greater palatability and consumption by animals (Lloyd et al. 2010; Zheng et al. 2014).

The CP values obtained in this study for Señal were higher than those reported by Alvarado et al. (1990) at 6 and 9 weeks of age (9.4 and 8.8%, respectively), while the values obtained in Mulato II were similar to those found by Castillo et al. (2006) at 3 weeks (15%). The values obtained in Xaraés, Marandú and Piatá were similar during the rainy season to, and higher in the dry season than, those obtained by Pérez et al. (1999) in B. brizantha at 6 and 9 weeks (11.9 and 8.6%, respectively). Water deficiency stress can have a negative effect on Brachiaria cultivars, decreasing the concentration of CP. In this regard, Cuadrado et al. (2004) reported that at 24 days of regrowth CP concentration in the wet season was higher than in the dry season for Marandú (10.5 vs. 9.3%), Señal (15.4 vs. 9.2%) and Xaraés (11.5 vs. 8.2%). Increasing age of the plant also decreases the concentration of CP in forage because of accumulation of dry matter (resulting in dilution of nutrients), increase in stem DM and decline in the proportion of leaf (Juárez-Hernández et al. 2004; Reyes-Purata et al. 2009), as shown in our study. All cultivars had CP concentrations above 7% which is considered the minimum, below which intake by ruminants could be suppressed (Lazzarini et al. 2009). The choice of cultivar to use could depend on a range of factors including DM yield of leaf, CP concentration, rate of regrowth, response to fertilizer etc.

#### Conclusions

While the dry season negatively affected the production and nutritional value of forage of all grasses evaluated, the most outstanding cultivars in terms of leaf yield, leaf area index and specific leaf area were: Mulato II, Marandú and Xaraés. Xaraés certainly showed the best tolerance of dry conditions, while performing well in terms of total DM and leaf production in the rainy season. However, Mulato II showed high DM yield and nutritional value over time, which supports its further evaluation in grass production systems in humid tropical conditions, especially as good CP levels (11-14%) were maintained for long periods in both rainy and dry seasons. This was a plot study and plants were only 12 weeks old when observations commenced, but the results do indicate that all cultivars performed well and further evaluation of the more promising cultivars under field conditions, particularly under grazing, is warranted.

#### Acknowledgments

We thank the Consejo Nacional de Ciencia y Tecnología (CONACYT) for the grant that made possible the stay of the first author in Ecuador. Our thanks to the Universidad Tecnológica Equinoccial, Campus Santo Domingo (Ecuador) and the Facultad de Ingeniería y Ciencias of the Universidad Autónoma de Tamaulipas (Mexico) for the facilities provided to make this research possible.

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#### (Received for publication 15 September 2016; accepted 02 May 2017; published 31 May 2017)

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#### **Research Paper**

# Effects of harvesting age and spacing on plant characteristics, chemical composition and yield of desho grass (*Pennisetum pedicellatum* Trin.) in the highlands of Ethiopia

*Efectos de la edad a la cosecha y del espaciamiento en las características de planta, composición química y rendimiento del pasto desho* (Pennisetum pedicellatum *Trin.*) *en las tierras altas de Etiopía* 

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#### Abstract

The study was conducted to evaluate effects of harvesting age and plant spacing on plant characteristics, composition and forage yield of desho grass (*Pennisetum pedicellatum* Trin.). A factorial experiment with 3 harvesting ages (75, 105 and 135 days after planting) and 3 plant spacings ( $10 \times 50$ ,  $30 \times 50$  and  $50 \times 50$  cm) with 3 replications was used. The data collected were morphological characteristics such as leaf length, plant height, number of tillers per plant and number of leaves per plant. Chemical analysis was conducted for crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL), and dry matter yield (DMY) was quantified. Results indicated that the only morphological characteristic significantly (P<0.05) affected by plant spacing was leaf length. However, harvesting age significantly (P<0.01) affected morphological characteristics and DMY as well as CP and NDF (P<0.05). Dry matter yield increased dramatically as harvesting dates were delayed but plant spacing had no significant effect on DMY. Crude protein concentration in forage declined as harvesting dates were delayed (10.9% at 75 d vs. 9.3% at 135 d). Factors such as weed control and amount of planting material required should be the criteria used by farmers to decide inter-row spacing as, within the conditions of our study, row spacing had minimal effect on yield. As only a single harvest at each age was conducted, the yields quoted in this study are not representative of the yields provided by multiple harvests at these intervals. Further studies are needed to quantify these differences.

Keywords: Biomass, harvesting day, morphological characteristics, nutritive value, plant spacing.

#### Resumen

En el distrito de Farta, Etiopía, se evaluaron los efectos de la edad a cosecha y la distancia de siembra de la hierba desho (*Pennisetum pedicellatum* Trin.) sobre las características morfológicas de la planta, la composición química y el rendimiento de forraje. Se utilizó un experimento factorial con 3 edades a la cosecha (75, 105 y 135 días después de la siembra), 3 espaciamientos de plantas ( $10 \times 50$ ,  $30 \times 50$  y  $50 \times 50$  cm) y 3 repeticiones. Se evaluaron las características morfológicas: longitud de hoja, altura de planta, número de tallos por planta y número de hojas por planta; se determinaron las concentraciones de proteína cruda (PC), fibra detergente neutra (FDN), fibra detergente ácida (FDA) y lignina detergente ácida; y se cuantificó el rendimiento de materia seca (MS). Los resultados mostraron que la longitud de hoja fue la única característica morfológica que fue afectada (P<0.05) por el espaciamiento de siembra de las plantas,

Correspondence: B. Asmare, Department of Animal Production and Technology, College of Agriculture and Environmental Sciences, Bahir Dar University, P.O. Box 5501, Bahir Dar, Ethiopia. E-mail: limasm2009@gmail.com mientras que la edad a la cosecha afectó tanto las características morfológicas y el rendimiento de MS (P<0.01) como la PC y la FDN (P<0.05). El rendimiento de MS aumentó marcadamente a medida que la edad a cosecha fue mayor, mientras que el espaciamiento de las plantas no tuvo un efecto significativo sobre los rendimientos obtenidos. La concentración de PC en el forraje disminuyó a medida que el intervalo de cosecha fue mayor (10.9% a 75 días vs. 9.3% a 135 días). Los resultados de este estudio sugieren que buenas prácticas de manejo como el control de malezas y la cantidad adecuada de material de siembra deben ser los criterios que deben utilizar los agricultores para seleccionar el espaciamiento entre hileras de siembra, ya que en las condiciones del estudio, la distancia no tuvo efecto sobre el rendimiento. Como se realizó una sola cosecha a cada edad, los rendimientos obtenidos en este estudio no son necesariamente representativos de los rendimientos a obtenerse por múltiples cosechas en estos intervalos. Se necesitan más estudios para cuantificar estas diferencias.

**Palabras clave:** Características morfológicas, día de cosecha, espaciamiento de plantas, producción de biomasa, valor nutritivo.

#### Introduction

Livestock production is an integral part of the subsistence crop-livestock systems in the Ethiopian highlands, as livestock provide draft power for land preparation and threshing, plus a source of cash income and assets and nutrition for the rural communities. In addition, livestock are considered as a mobile bank that can be hired, shared, inherited and contracted by rural households (Amede et al. 2005). However, the contribution of this subsector to date has been suboptimal (CSA 2015). One of the important constraints causing low productivity of livestock is low quality and insufficient supply of forage (FAO 2010). Overgrazing is common, resulting in land degradation and low carrying capacity. As a result, the decline in desirable plant species and nutritional value of the available feed resources, particularly protein, means most animals are unable to obtain their maintenance requirements from grazing (Mengistu 1987).

To combat this situation, the use of indigenous forage plants as a feed source, e.g. desho grass (Pennisetum pedicellatum Trin.), is recommended (Leta et al. 2013; Asmare 2016). Desho grass is a perennial grass from Chencha district in southern Ethiopia (Welle et al. 2006) and is currently utilized for soil conservation practices in the highlands of Ethiopia (Heuzé and Hassoun 2015). It is a highly popular, drought-tolerant species, and is used as one of the major feeds for ruminants (Bogdan 1977; FAO 2010; Asmare 2016) with high production potential under a multi-cut harvesting regime (MRDP 1990). However, the optimum plant spacing and intervals between harvests are not well known. The objective of this study was to assess the effects of harvesting age and plant spacing on morphological characteristics, dry matter vield and nutritive value of desho grass in the highlands of Ethiopia.

#### **Materials and Methods**

#### Description of the study area

The agronomic study was conducted in Farta district of northwestern Ethiopia located at 660 km northwest of Addis Ababa (11°32'–12°03' N, 37°31'–38°43' E; 2,720 masl). The topography of the district is 45% gentle slopes, 29% flat land and 26% steep slopes. In terms of land use, an estimated 65% of the area is cultivated and planted with annual and perennial crops, while the areas under grazing and browsing, forests and shrubs, settlements and wastelands account for about 10, 0.6, 8 and 17%, respectively. The total area of the district is estimated to be 1,118 km<sup>2</sup>. The average minimum, maximum and mean temperatures are 9.3, 22.3 and 15.8 °C, respectively. The rainfall pattern is uni-modal (May–September) and mean annual rainfall is 1,445 mm (FDOA 2015).

#### Treatments and experimental design

A factorial arrangement of treatments was employed using a randomized complete block design with 2 factors (plant spacing and harvesting age) with 3 replications. Three plant spacings within rows (10, 30 and 50 cm) were compared at 3 harvesting dates (75, 105 and 135 days). In all treatments inter-row spacing was 50 cm. The total experimental area was  $10 \times 19$  m (190 m<sup>2</sup>) with individual plot size of 3 m<sup>2</sup> and spacing between plots and replications of 50 and 100 cm, respectively. The land was prepared thoroughly by plowing at the start of the rainy season. Planting material of desho grass was collected from a nursery site at Farta District Office of Agriculture and planted on 15 July 2015. Urea fertilizer was applied at the rate of 100 kg/ha at planting and ammonium phosphate (DAP) was added at 25 kg/ha 21 days after establishment according to the local recommendations (Leta et al. 2013).

#### Methods of data collection

Data were recorded throughout the experimental period (June 2015-October 2015) on leaf length (LL), plant height (PH), leaf number per plant (LN) and number of tillers per plant (NT). Six plants in each plot were randomly selected for recording data at each harvesting date. The total herbage on each plot at the fixed dates was harvested leaving out border rows. From each plot, an area of 2.2 m<sup>2</sup> was used to calculate dry matter (DM) yield. Harvesting was done by hand using a sickle, leaving a stubble height of 10 cm, and the harvested herbage was weighed fresh in the field using a field balance. Random samples of fresh forage were taken and oven-dried at 60 °C for 72 h to determine DM concentration, before calculating dry matter yield (DMY). The dried desho grass samples were ground to pass through a 1 mm sieve (Wiley mill) and stored in airtight plastic bags until required for laboratory chemical analysis. Total ash concentration was determined according to AOAC (1990). Nitrogen was determined by the Kjeldahl method (AOAC 1990) and crude protein (CP) concentration was calculated as N%  $\times$  6.25. The neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) concentrations were determined according to Van Soest et al. (1991).

#### Methods of data analysis

All data were analyzed using the General Linear Model (GLM) procedure of SAS (2007) for least squares analysis of variance. Mean comparisons were done using Duncan's Multiple Range Test (DMRT) for variables whose F-values indicated significant difference. Differences were considered statistically significant at P<0.01 and P<0.05. The statistical model for the analysis of data was:  $Y_{ijk} = \mu + H_i + S_j + H_i^*S_j + e_{ijk}$  where:

yijk = all dependent variables (morphological data and chemical composition) collected

 $\mu = overall mean$ 

 $\begin{array}{l} H_i = the \; effect \; of \; i^{th} \; harvesting \; date \; (75, 105 \; and \; 135 \; days) \\ S_j = the \; effect \; of \; j^{th} \; spacing \; between \; plants \; (10, \; 30 \; and \; 50 \; cm) \end{array}$ 

 $H_i * S_j$  = the interaction of harvesting date and spacing  $e_{ijk}$  = random error.

For correlation analyses of parameters such as morphological characteristics, chemical composition and yield, simple bivariate Pearson correlation was employed.

#### Results

#### Morphological characteristics and dry matter yield of desho grass as affected by harvesting age and plant spacing

Overall, there were no significant interactions between the effects of the main treatment variables (plant spacing and harvesting age) so main effects only are presented. The effects of harvesting age and plant spacing on morphological characteristics and dry matter yield of desho grass are shown in Table 1. Mean leaf length at harvesting ages of 75 and 105 days was significantly (P<0.05) greater than at 135 days (18.1, 18.8 vs. 17.4 cm, respectively).

Similarly, mean length of leaves was significantly (P<0.05) greater at the narrow spacing (10 cm) than at the intermediate (30 cm) and wide spacings (50 cm) (19.0 vs. 18.2 and 17.7 cm, respectively). Harvesting age had a significant effect on plant height (P<0.01) with height increasing progressively from 46.2 cm at 75 days harvesting age to 69.8 cm at 105 days and 83.1 cm at 135 days (Table 1). Plant spacing had no significant (P>0.05) effect on plant height with a mean height overall of 66.4 cm.

Table 1. Morphological characteristics and dry matter yield of desho grass as affected by harvesting age and plant spacing.

Parameter		Harvesting	g age (days)			Plant spa	cing (cm)	
	75	105	135	Mean	10×50	30×50	50×50	Mean
Leaf length (cm)	19.0a	18.2b	17.7b	18.3	18.1a	18.8a	17.4b	18.1
Plant height (cm)	46.2c	69.8b	83.1a	63.4	67.3	66.2	65.7	66.4
Number of tillers/plant	36.4c	93.1b	106.4a	78.6	75.3b	76.7ab	83.9a	78.6
Number of leaves/plant	249c	554b	710a	508	497	498	517	504
Dry matter yield (t/ha)	7.1c	15.7b	25.5a	16.1	16.5	16.0	15.7	16.1

Mean values within rows followed by a different letter are significantly different at P<0.05.

Both harvesting age and plant spacing had significant effects on tiller numbers (Table 1). Mean tiller number per plant increased from 36.4 at 75 days growth to 106.4 at 135 days (P<0.01), while corresponding numbers for different plant spacings were 75.3 tillers/plant at 10 cm and 83.9 tillers/plant at 50 cm (P<0.05). Leaf number per plant, which, in part, determines the photosynthetic capacity of the plants, was significantly (P<0.01) affected by harvesting age, while plant spacing had no effect on this parameter (P>0.05) (Table 1). Number of leaves per plant increased from 249 leaves at 75 days to 410 leaves at 135 days.

The DM yield of desho grass was significantly (P<0.01) affected by harvesting age but not by plant spacing (P>0.05) (Table 1). Total DM harvested increased progressively from 7.1 t/ha at 75 days of age to 25.5 t/ha at 135 days of age. Mean DM yield overall for the different plant spacings was 16.1 t/ha.

# Chemical composition of desho grass as affected by harvesting age and plant spacing

The chemical composition of desho grass as affected by harvesting age and plant spacing is shown in Table 2. The DM concentration showed minimum variation and ranged from 88.2 to 89.1%. Crude protein (CP) concentration was significantly affected (P<0.05) by harvesting age, declining from 10.9% at 75 days to 9.3% at 135 days. Crude protein yields (CPY) increased progressively and significantly (P<0.01) as growth period increased (0.76 t/ha at 75 days to 2.36 t/ha at 135 days; Table 2). By contrast, plant spacing had no significant effect (P>0.05)

on CPY with an overall mean of 1.57 t/ha. Ash concentration declined significantly (P<0.01) as harvesting age increased with values at 75 days exceeding those at 105 and 135 days (Table 2). Progressive increases in plant spacing resulted in significant increases in ash concentration (Table 2).

Organic matter (OM) concentration increased progressively (P<0.01) as harvesting age increased and decreased progressively (P>0.05) as plant spacing increased (Table 2). While NDF concentration increased significantly as harvesting age (P<0.05) and plant spacing (P<0.05) increased, ADF concentration increased significantly (P<0.01) with increase in harvesting age but was unaffected (P>0.05) by plant spacing (Table 2). The highest ADF concentration (48.1%) was recorded in desho grass harvested at 135 days and grown at 50 cm plant spacing.

# Correlation among morphological characteristics and chemical composition of desho grass

The relationships among morphological parameters, nutritional parameters and yield of desho grass are shown in Table 3. The analysis showed that DM % and DMY were positively correlated (P<0.01). These parameters were also positively correlated with most chemical parameters, e.g. CPY plus NDF, ADF, ADL and OM concentrations. This indicated that, as the DM % increased, cell wall constituents also contributed to the increase in DMY. However, DM % and DMY were negatively correlated (P<0.01) with CP % and total ash %.

**Table 2.** Chemical composition of desho grass as affected by harvesting age and plant spacing.

Parameter		Harvesting	g age (days)			Plant space	cing (cm)	
	75	105	135	Mean	10×50	30×50	50×50	Mean
Dry matter (%)	88.2b	88.4b	89.0ab	88.5	89.1a	88.3b	88.2b	88.5
Ash (%)	9.16a	7.89b	7.0b	8.0	6.15c	8.15b	9.74a	8.1
Organic matter (%)	79.1c	80.6b	82.0a	80.6	83.0	80.2	78.5	80.9
Crude protein (%)	10.9a	10.2ab	9.3b	10.2	9.6	10.2	10.7	10.2
Crude protein yield (t/ha)	0.8c	1.6b	2.4a	1.57	1.5	1.6	1.6	1.6
Neutral detergent fiber (%)	45.2b	46.2b	51.7a	47.7	45.2c	47.8ab	50.1a	47.7
Acid detergent fiber (%)	33.1c	37.6b	42.6a	37.8	37.6	38.1	41.5	38.1
Acid detergent lignin (%)	17.3b	18.3b	20.7a	18.8	16.9c	18.8b	20.5a	18.7

Within parameters and treatments, means with different letters within rows are significantly different (P<0.05).

Table 3. Correlation coefficients among morphological parameters, nutritional parameters and yields of desho grass.

	DM	DMY	СР	CPY	Ash	ОМ	NDF	ADF	ADL	LL	NT	PH	NL
DM	1	0.55**	-0.43*	0.44*	-0.69**	0.81**	0.41*	-0.064	0.063	-0.02	0.31	0.44*	0.40*
DMY		1	-0.62**	0.95**	-0.45*	0.50**	0.49**	0.59**	0.59**	-0.42*	0.87**	0.94**	0.92**
CP			1	-0.29	0.26	-0.32	-0.26	-0.17	-0.08	0.18	-0.40*	-0.44*	-0.44*
CPY				1	-0.41*	0.44*	0.44*	0.63**	0.62**	-0.43*	0.87**	0.93**	0.91**
Ash					1	-0.98**	-0.41*	0.029	0.28	-0.23	-0.35	0.47*	0.44*
OM						1	0.44*	-0.40	-0.24	-0.18	0.36	0.49**	0.46*
NDF							1	0.12	-0.009	-0.27	0.03	0.47*	0.42*
ADF								1	0.6**	-0.46*	0.65**	0.58**	0.63**
ADL									1	0.62**	0.55**	0.53**	0.54**
LL										1	-0.29	-0.30	-0.30
NT											1	0.95**	0.97**
PH												1	0.97**
NL													1

Level of significance: \*\* = P < 0.01; \* = P < 0.05; DM = dry matter %; DMY = dry matter yield; CP = crude protein %; CPY = crude protein yield; Ash = ash %; OM = organic matter %; NDF = neutral detergent fiber %; ADF = acid detergent fiber %; ADL = acid detergent lignin %; LL = leaf length; NT = number of tillers per plant; PH = plant height; and NL = number of leaves per plant.

#### Discussion

#### Plant characteristics and their relation with DM yield

The absence of any significant effect of plant spacing on dry matter yield (DMY) (P>0.05) was at variance with the findings of Melkie (2005), who demonstrated the highest DMY at narrow spacing, which he attributed to the greater number of plants per unit area. In our study the higher plant population at narrow plant spacing was counteracted to some extent by the greater number of tillers per plant produced at wider plant spacing, although individual leaves were longer at narrow plant spacing. The finding that narrow plant spacing (10 and 30 cm) produced longer leaves than wider spacing (50 cm) supports the results of Yasin et al. (2003), who reported that narrow spacing in Napier grass increased interplant competition, causing individual plants to grow taller with longer internodes, plus slender, thin and weak stalks due to poor light exposure and hence poor photosynthetic output. However, Melkie (2005) and Alemu et al. (2007) reported the opposite effect for Bana grass (Pennisetum purpureum × Pennisetum americanum hybrid), where leaf length at relatively narrow plant spacing was shorter than at medium and wider plant spacings.

The higher dry matter yields at later stages of harvesting were to be expected as plants were taller, had more tillers per plant and more leaves per plant. All these characteristics would contribute to increased photosynthetic activity and hence higher DM production. Ansah et al. (2010) showed that total herbage yield in Napier grass increased with increase in harvesting age (60<90<120 days), which these authors attributed to the increase in tiller number, leaf formation, leaf elongation and stem development. Similarly, Melkie (2005) reported that yield of Bana grass increased as harvesting stage increased.

The observed high number of leaves per plant at later stages of harvesting reinforces the findings of Asmare (2016) with the same grass species, Butt et al. (1993) and Melkie (2005) with Bana grass and Zewdu et al. (2002) with Napier grass. Generally, the longer the vegetative phase and the taller the plant, the greater the number of leaves produced (Hunter 1980), a situation reflected in our study as the number of leaves from new tillers generally increased with increase in age at harvesting. The increase in plant height with harvesting age was not unexpected. In the same grass species, Asmare (2016) showed that plant height increased as plant age at harvest advanced to 120 days. Increments in plant height at later harvest stages could be due to massive root development and efficient nutrient uptake, allowing the plant to continue to increase in height as mentioned by Melkie (2005).

The current finding that the number of tillers per plant increased as plant spacing increased agrees with Melkie (2005), who reported similar results for Bana grass. At wider spacing, light can easily penetrate to the base of the plant and this may have stimulated tiller development. Moreover, under wider spacing competition for nutrients is less, so individual plants can support more tillers. For Napier grass, Yasin et al. (2003) reported that, when sufficient space is available to the individual plant, there is capacity to increase the number of tillers per plant with the variation among the different spacings being ascribed to variable nutritional areas and access to light. At narrow spacing, plants reach maturity before the achievement of optimal leaf area, which is important for estimating pasture productivity. Thus, the lower tiller counts at narrow plant spacing may be due to high plant competition for resources, namely light, space and nutrients. The increased competition for light causes reduced growth and tillering capacity. Interplant competition in grass causes rapid and exhaustive height increments, so that overcrowding results in neighboring plants producing weak tillers (Boonman 1993). Therefore, the competitor plants are forced to grow upright to dominate other tillers produced on the same plant rather than expanding laterally by bearing more tillers.

Desho grass harvested at young age in this study had excellent nutritional value, particularly high CP concentration, a limiting nutrient in tropical forages. Even forage cut at 135 days of age had CP concentrations well above 7.0%, which is the level below which voluntary intake of ruminants might be depressed. All of the forage produced would provide sufficient energy and protein to support some level of production above a maintenance level. However, harvesting at the early stage resulted in low DM yields at that harvest. Allowing the plants to grow until 135 days of age resulted in much higher yields without a great reduction in quality despite some reduction in CP concentration and increase in NDF. In any pasture situation, compromises between quality and yield must be made when deciding at what stage to harvest or graze a crop or pasture.

With regard to plant spacing and forage production, both narrow and wide plant spacings have implications for different aspects of forage production (Rao 1986) as the number of plants per unit area is the primary source of competition. Generally, narrow plant spacing suppresses the emergence of various weeds, but additional planting material is required. When density is maintained above optimum, there will be greater total demand for resources that results in stress in the plants (Trenbath 1986). Wider plant spacing requires less planting material and enables greater tillering capacity in forage grasses but the probability of weed invasion increases and may lead to extra cost of weeding. Again compromises must be made. Individual farmers may find that the optimum plant density and total population differ from those of others based on the resources at their disposal. Yasin et al. (2003) indicated that the correct use of relatively inexpensive and simple management practices such as correct plant spacing, regular weeding, appropriate cutting systems and application of fertilizers can help increase the level of fodder production.

#### Chemical composition and its relation to yield

Chemical analyses of forage in this study revealed that results conformed with other studies in terms of the effects of age at harvest on quality parameters. As would be expected, the highest CP concentration was obtained at the earliest stage of harvesting, with values declining as harvesting was delayed. This result agrees with the findings of Asmare (2016) for the same species. Similarly, Bayble et al. (2007) and Ansah et al. (2010) reported for Napier grass a decreasing trend of CP with increase in harvesting age (60>90>120 days). This phenomenon is referred to as a growth dilution effect with increase in structural carbohydrate content of forage materials harvested at late maturity reducing the percentage of protein in the forage.

Despite the reduction in CP percentage with time, crude protein yield (CPY) increased significantly as harvesting was delayed. Similar findings have been reported by Asmare (2016) for the same grass species and by Melkie (2005), who recorded mean CPYs at 60, 90 and 120 days of age of 0.47, 0.91 and 0.85 t/ha, respectively, in Bana grass. Obviously, decisions on the optimal time to harvest desho grass will depend on a compromise between yield and quality of forage.

However, plant spacing had no marked effect on CPY. Since CPY is the product of total DM yield and CP concentration in the plant and there were no significant effects of plant spacing on either of these parameters, one would not expect to record a significant outcome. Our results are at variance with those of Melkie (2005), who found lower CPY at a spacing of  $75 \times 75$  cm than at  $100 \times 50$  cm.

As would be expected, neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) concentrations all increased significantly (P<0.05) as harvesting time was delayed. Increase in plant spacing also resulted in higher NDF and ADL levels, although ADF was unaffected. While increase in stem percentage and increased lignification with maturity would account for the age effects, the increases with wider plant spacing would possibly reflect larger tiller development in the wider-spaced plants. Zewdu et al. (2002) and Bayble et al. (2007) reported that the predominant features of increasing plant density or narrow spacing were a marked reduction in leaf:stem ratio, which in turn resulted in an increase in cell wall and lignin concentrations in Napier grass. The increasing trend of NDF concentration with increase in harvesting age agrees with Asmare (2016) for the same grass species, where NDF concentration increased from 72.8% at 90 days to 77.7% at 150 days of

age. Bayble et al. (2007) recorded a similar trend when Napier grass was harvested at 60, 90 and 120 days.

#### Conclusions

This study has documented the increases in yield of desho grass as days to harvest are increased and has highlighted the reduction in quality, especially reduced CP concentrations and increased NDF, ADF and ADL concentrations, with advancing maturity. Farmers could use this information to assist in making decisions based on the relative importance of forage yield and quality in their operations. While delayed harvesting results in increased DMY, this is at the expense of a reduction in quality. However, these data do not present a complete picture as the pasture harvested early would regrow and the reduction in yield we observed would be much greater than actually would occur, where repeated cuttings would be made for the earlier ages of harvest.

The absence of any differences in yield with variation in plant spacing indicates that farmers can make their decisions on what spacing to use based on other factors, e.g. forage quality issues, weed control etc. Our findings suggest that plant spacing within rows can be varied quite markedly without any variation in forage yield and within the bounds of the spacings we used, farmers can choose a spacing to suit their conditions. An important limitation of this study was that the measurements of total forage yield at different harvest frequencies were not carried out. This information would be needed before a farmer could use these data effectively in decision making. Moreover, leaf:stem ratio was not measured in this study, a good indicator of forage quality. Both limitations of this study could be addressed in future studies.

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(Received for publication 26 December 2016; accepted 26 March 2017; published 31 May 2017)

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#### **Research Paper**

# Weeds alter the establishment of *Brachiaria brizantha* cv. Marandu Malezas afectan el establecimiento de Brachiaria brizantha cv. Marandu

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#### Abstract

The present study evaluated the effects of different periods of coexistence among the main weeds and Marandu brachiaria grass (*Brachiaria brizantha*, now *Urochloa brizantha*) in newly sown pasture. The experiment was conducted in a randomized block with 4 replications, with treatments being 8 coexistence periods: 0, 15, 30, 45, 60, 75, 90 and 120 days after emergence. A phytosociological assessment of the weed community was carried out at the end of the coexistence periods, and weeds were eliminated from the appropriate treatment using herbicide. Key morphogenic parameters of the forages were assessed at the end of the experimental period and dry matter production was determined. Results indicated that the presence of weeds had negative impacts on the main morphogenic components, such as plant height, number of tillers and production of leaf and stem dry matter. The presence of weeds reduced productivity in Marandu, with 15 days competition being sufficient to reduce forage production by approximately 50%, suggesting that weed control measures should be adopted within 15 days following emergence of seedlings of Marandu and weeds.

Keywords: Dry matter, interference, pasture renovation, weed competition.

#### Resumen

En un latosol localizado en Sinop, Mato Grosso, Brasil se evaluaron los efectos de la infestación de las principales malezas de la zona (*Hyptis suaveolens, Senna obtusifolia* y *Sida rhombifolia*), en diferentes edades después de la siembra, en la producción y algunas características morfogénicas de la gramínea forrajera *Brachiaria brizantha* (ahora: *Urochloa brizantha*) cv. Marandu. Los tratamientos fueron dispuestos en un delineamiento experimental de bloques al azar con 4 repeticiones y consistieron en los períodos de convivencia: 0, 15, 30, 45, 60, 75, 90 y 120 días después de la emergencia de la gramínea. Al finalizar cada uno de estos períodos se realizó una evaluación fitosociológica de la comunidad infestante y se eliminaron las malezas utilizando un herbicida. Al término del período experimental se evaluaron los principales parámetros morfogénicos del pasto, así como la producción de materia seca. Los resultados mostraron un efecto negativo de las malezas en la altura de planta y el número de rebrotes, así como en la producción de materia seca de hojas y tallos. La presencia de las malezas redujo la producción del pasto en todos los tratamientos, variando de 50% (competencia durante los primeros 15 días) hasta 74% (120 días). Por tanto las medidas de control de las malezas deben ser adoptadas durante las 2 primeras semanas de convivencia con el pasto Marandu.

Palabras clave: Competencia, interferencia, materia seca, renovación de pasturas.

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#### Introduction

Historically, the livestock production model practiced by most South American ranchers has a strong extractive aspect with little concern for protecting and renewing the natural resources. This absence of the use of technical criteria in the utilization of natural resources results in accelerated degradation of pasture areas, where degradation is mainly characterized by loss in the productive capacity of the forage grass due to the severe loss of soil fertility and the increase in weed infestation (Lima and Pozzobon 2005). Renewal of the area is the most rational solution when a pasture becomes degraded. Renewal consists basically of destroying the old vegetation, correcting soil fertility and planting the appropriate forage species for the local conditions, usually an exotic (introduced) species (Macedo 2009). The process of renovating the degraded pasture, however, is ineffective in removing the seeds left by weeds, so weed and pasture seeds germinate together, which initiates a new degradation cycle (Martins et al. 2007).

Brachiaria grass (*Brachiaria brizantha*, now *Urochloa brizantha*) is one of the most cultivated forages in the warm regions of South America and supports a large portion of the cattle herd. It has high forage yield, persistence, good capacity for regrowth and relative tolerance to attacks from spittlebugs such as *Deois* sp. The average annual productivity is 4,000–8,000 kg DM/ha

and can reach 20,000 kg DM/ha (Benett et al. 2008). It is considered an excellent source of good quality feed when appropriately fertilized and managed. However, nutritional value declines rapidly following flowering (Valle et al. 2000).

All studies we found in the literature on weed control in pastures focused on studying the effects of herbicides on controlling unwanted plants (Silva et al. 2005; Santos et al. 2007; Trigueiro et al. 2007). Practically no attention has been given to the study of interference relations between weeds and grasses, especially with regards to productivity and carrying capacity of the pasture.

This work sought to study the effects of increasing periods of weed presence on the initial development of *Brachiaria brizantha* cv. Marandu forage.

#### **Materials and Methods**

The experimental phase of this work was conducted in a pasture renewal area in the municipality of Sinop, Mato Grosso, Brazil, (11°11'29'' S, 55°15'13'' W), where, according to the Köppen (1948) classification, the climate is of type Aw. Rainfall data and average, minimum and maximum temperatures recorded during the experimental period are shown in Figure 1. The average annual temperature is 27 °C, varying between 17 and 40 °C. Average annual precipitation is 1,500 mm, varying from 1,200 to 1,800 mm.



Figure 1. Average monthly rainfall and temperature during the experimental period.

Representative soil samples (latosol) were collected and sent for laboratory analysis, which revealed the following chemical characteristics: pH (H<sub>2</sub>O): 3.9; soil organic matter: 18.45 g/dm<sup>3</sup>; P: 0.04 g/dm<sup>3</sup> (Mehlich-1); K<sup>+</sup>: 0.04 cmmol/dm<sup>3</sup>; Ca<sup>2+</sup>: 0.09 cmmol/dm<sup>3</sup>; Mg<sup>2+</sup>: 0.03 cmmol/dm<sup>3</sup>; Al<sup>3+</sup>: 0.3 cmmol/dm<sup>3</sup> (KCl 1 mol/L); base saturation: 0.16 cmmol/dm<sup>3</sup>; and effective CEC of 21%. Physical analysis indicated 866 g sand/kg, 39 g silt/kg and 91 g clay/kg, characterizing the soil as having a sandy texture. Based on these results, calcium lime was applied in the third week of September 2010 at rates of 2,000 kg/ha, while 62.5 kg P/ha (as single superphosphate), 25 kg N/ha (as urea) and 37.5 kg K/ha (as KCl) were broadcast on all areas in the first week of November 2010.

Existing forage was removed from the experimental area by herbicide application (glyphosate at 2.5 L/ha) followed by mechanical tillage of soil before the study commenced in November 2010. The area was fenced to exclude animals for the duration of the study. Seed of cv. Marandu with 70% maximum germination was broadcast on the area at a rate of 6.0 kg/ha. Seedling emergence commenced in 7 days and complete emergence occurred by 10 days. The experimental area was divided into 32 plots, each of 16 m<sup>2</sup> (4.0 x 4.0 m) with the central 9.0 m<sup>2</sup> of each plot used as the sampling area. The experimental design was a complete randomized block, with 8 treatments representing different periods of coexistence between forage and weed species (0, 15, 30, 45, 60, 75, 90 and 120 days after emergence) and 4 replications.

Assessments of the pasture communities for each treatment were performed at the end of the coexistence period for that treatment with the aid of a  $1.0 \text{ m}^2$  metal square randomly cast within the sampling area of each plot. All weed species within the metal square were separately identified, counted, cut at stem base and taken to the laboratory, where they were placed in properly labeled and perforated paper bags to be dried in a forced-air oven at 60–63 °C for 72 hours. After this procedure, the dry weight of the stems and leaves of each collected species was determined by using a 0.01 g precision balance.

The relative importance (RI) of the weeds was calculated by the formula:

 $RI = (IVIe/IVIt) \times 100$  (%), where: <u>IVIe</u> refers to the importance index of a determined species; and <u>IVIt</u> signifies the sum of the importance indices of all components of the community. The importance index of each species is estimated by the formula:

IVI = DeR + DoR + RF, where: DeR refers to the relative density of each species, estimated by the formula:

DeR = (De/Dt) x 100 (%), where: De is the density of each weed species and Dt is the total weed density; DoR refers to the relative dominance of each species and is estimated by the formula: DoR = (DMe/DMt) x 100 (%), where: DMe is the dry matter of each weed species and DMt is the total dry matter accumulated by the weed community; and RF refers to the relative frequency of each species and is estimated by the formula: RF = (FAe/FAt) x 100 (%), where: <u>FAe</u> is the absolute frequency of each species calculated by the expression: FAe = (NAe/NAt) x 100, where: <u>NAe</u> indicates the number of samples for a determined species; <u>NAt</u> is the total number of samples obtained; and <u>FAt</u> is the sum of the absolute frequencies of all species of the weed community (Mueller-Dombois and Ellenberg 1974).

All weeds were removed from the respective plots at the end of each period of coexistence by spraying with herbicide, and thereafter any emerging weeds were removed by applying 1.5 L/ha of herbicide formulated with 40 g acid equivalent/L of aminopyralid and 320 g acid equivalent/L of 2,4-D post-emergence.

The grass was evaluated only at the end of the experimental period, corresponding with 120 days after emergence of the seedlings, when the first grass inflorescences emerged. At this time plant height, number of tillers per plant and number of plants per square meter were measured. Forage samples were collected by cutting the plants to 10 cm from the ground within the area enclosed by the  $1.0 \text{ m}^2$  metal square, cast randomly in the sampling area of the experimental unit.

The samples were sent to the laboratory and sorted into green leaves, green stems and dead matter. The green inflorescences present were considered as part of the stem. The various fractions were duly packed in properly labeled and perforated paper bags and dried in a forcedair circulation oven at 60–63 °C for 72 hours. Dry matter (DM) yields for the different fractions [green leaf dry matter (GLDM), green stem dry matter (GSDM), dead material dry matter (DMDM) and total dry matter (TDM)] were calculated. Dry matter yields for the different treatments were compared with those for the 0 days treatment (control, = weed-free throughout) to determine the suppression in yield by exposure to weeds for the various times according to the formula:

 $ROF = [(MST - MSPC)/MST] \times 100$ , where: ROF is the reduction in forage offering in percent; MST is the total DM produced by the forage species that remained weed-free for 120 days; and MSPC is the DM produced by treatments with differing periods of weed infestation. The values obtained were submitted to analysis of variance by the F-test and the effects of the treatments were compared by the Scott-Knott test at 5% probability. The average values of total DM produced by Marandu and observed at 120 days were also adjusted according to the Boltzmann model for better understanding of the effects of coexistence, as used by Kuva et al. (2001). This model conforms to the following equation:

$$Y = \frac{(A_1 - A_2)}{1 + e^{(x - x_0)/d_x}} + A_2$$

where: Y is the estimated DM yield of the forage in  $g/m^2$ ; x is the upper limit of the coexistence period or control considered; A<sub>1</sub> is the estimated yield obtained in the plots maintained clean throughout the cycle; A<sub>2</sub> is the minimum estimated production obtained in the units maintained with weeds throughout the cycle; x<sub>o</sub> is the upper limit of the control or coexistence period corresponding to the intermediate value between maximum and minimum production; and d<sub>x</sub> is the parameter that indicates the velocity loss or production gain (tg  $\alpha$  at point x<sub>o</sub>).

#### Results

Only 3 weed species emerged during the experimental period: *Hyptis suaveolens* (L.) Poit. (Lamiaceae) (local name: cheirosa), *Senna obtusifolia* (L.) H.S. Irwin & Barneby (Leguminosae – Caesalpinioideae) (local name: fedegoso) and *Sida rhombifolia* L. (Malvaceae) (local name: guanxuma). *Senna obtusifolia* accumulated the greatest amount of DM up to 45 days after emergence (DAE), but was the only species that showed a reduction in DM accumulation at 120 DAE (Figure 2). Over the full 120 days, *H. suaveolens* showed the highest DM accumulation with rapid growth from 45 to 120 DAE; DM accumulation was almost 2.5 times that of *S. obtusifolia* (Figure 2).

Sida rhombifolia produced little growth up to 90 DAE, when compared with the other 2 species, but accumulation of DM increased considerably from 90 DAE. At 120 DAE total DM produced by the weeds was 288 g/m<sup>2</sup> (Figure 2).

The relative importance, obtained from density, dominance and frequency of each of the 3 species, was relatively equal throughout the experimental period (Figure 3).



Figure 2. Dry matter accumulated by the weeds in their coexistence periods.



Figure 3. Relative importance (%) of the weed species in their respective coexistence periods.

The height of Marandu was significantly altered by coexistence with the weeds; height of the control forage at 120 days was 62 cm compared with a mean of 38 cm for the remaining treatments (Table 1).

Similarly, exposure to weeds for periods of >15 days significantly reduced the number of tillers per plant (4.2 and 3.8 for 0 and 15 days, respectively, vs. a mean of 2.7 for the remaining treatments; P<0.05) (Table 1). Density

of Marandu plants did not differ between treatments (P>0.05) with a mean of 37.3 plants/m<sup>2</sup> at 120 days post emergence (Table 1). The production of green leaf, stem and total forage dry matter was significantly reduced by the presence of weeds in the sward, even for as little as 15 days (P<0.05; Table 2). After 120 days of growth, total DM production was suppressed by 50%, when weeds remained in the pasture for as little as 15 days

**Table 1.** Effects of duration of weed competition in a Marandu pasture on height, number of tillers per plant and density of Marandu plants at 120 days after emergence.

Days of coexistence	Height	Number of tillers	Density
-	(cm)	(No./plant)	(No. of plants/ $m^2$ )
0	61.7a <sup>1</sup>	4.2a	40.4
15	42.7b	3.8a	30.3
30	40.5b	3.0b	42.8
45	32.5b	3.0b	32.5
60	30.2b	2.7b	40.5
75	49.0b	2.5b	32.5
90	38.0b	2.3b	38.0
120	32.5b	3.0b	41.5
F Days	3.84*	4.80*	0.86 <sup>NS</sup>
F Block	0.19 <sup>NS</sup>	4.08*	0.14 <sup>NS</sup>
C.V. (%)	26.11	19.23	27.97

<sup>1</sup>Means followed by the same letter within columns do not differ by the Scott-Knott test at 5% probability.

Days of	GLDM	GSDM	DMDM	TDM	ROF <sup>1</sup> (%)
coexistence					
0	1,543a <sup>2</sup>	1,632a	256	3,431a	-
15	790b	710b	210	1,710b	50.2
30	610b	790b	165	1,565b	54.4
45	470b	480b	136	1,086b	68.3
60	460b	410b	193	1,063b	69.0
75	420b	490b	100	1,010b	70.6
90	530b	340b	111	981b	71.4
120	430b	350b	110	890b	74.1
F Days	5.35*	5.96*	1.41 <sup>NS</sup>	6.01*	-
F Block	0.32 <sup>NS</sup>	0.83 <sup>NS</sup>	0.84 <sup>NS</sup>	0.31*	-
C.V. (%)	49.82	54.02	58.68	47.06	-

**Table 2.** Effects of duration of weed competition on the production (kg/ha) of green leaf dry matter (GLDM), green stem dry matter (GSDM), dead material dry matter (DMDM) and total dry matter (TDM) of Marandu and reduction in forage offering (ROF) at 120 days after emergence.

<sup>1</sup>Reduction in forage offering relative to the control (0 days).

<sup>2</sup>Means followed by the same letter within columns do not statistically differ by the Scott-Knott test at 5% probability.

after emergence. This reduction in yield had increased to 74% when weeds remained in the pasture for the full 120 days (Table 2), but there were no significant differences between yields for the differing times that weeds remained in the pasture.

The relationship between total DM yield and number of days that weeds remained in the pasture is presented in Figure 4. Accordingly the suppressant effects of weeds on pasture growth had been expressed by 45 days after emergence.



Figure 4. Relationship between forage DM yield of Marandu and duration of weed growth in the pasture.

#### Discussion

This study has shown that weeds can provide severe competition for freshly sown stands of Marandu, which reduces DM production of the grass, even if weeds are present only for a short period initially, e.g. as little as 15 days. It could be concluded that this is due to competition for available resources, especially nutrients, sunlight and moisture.

Interference of the weeds was most evident on the height and number of tillers generated by Marandu plants. Nepomuceno et al. (2007) reported that plant height is not an adequate characteristic to evaluate the competition between species, since plants subjected to competition prioritize height growth in search of light rather than the accumulation of total DM. Vilela (2011) commented that tillering is a predominant feature in most grasses, and in the case of a pasture, the success of its production is related to good tillering and the consequent occupation of spaces between plants, thereby complicating the establishment of weed species. In this study the opposite was observed, i.e. the weeds dominated the spaces and adversely affected the emergence of tillers in the forage, even when present for only 15 days. In addition grass plants subjected to competition from weed plants for only 15 days showed lower vertical growth than those free of weed competition and failed to compensate when weeds were removed.

Both *S. obtusifolia* and *H. suaveolens* are annual weeds and grow rapidly early in life, growing much more rapidly than Marandu and the perennial *S. rhombifolia*. This may explain why the main suppressant effect of weeds on Marandu growth happened in the first 15 days following emergence. These weeds are native to the American continent with wide distribution in South America, especially in Brazil, and often infest areas of annual crops, perennials and pastures (Fleck et al. 2003; Souza et al. 2011). Besides the competition for environmental resources such as water, light and nutrients, they can also be toxic to animals if ingested during grazing (Pellegrini et al. 2007; Braga et al. 2012).

We found that *S. obtusifolia* grew most rapidly between 21 and 91 DAE, in agreement with the findings of Erasmo et al. (1997), after which growth rate decreased significantly. Those authors suggested that the reduction in growth was due to the natural senescence process of the species, with resources at that time being directed to reproduction. Studies performed by Gravena et al. (2002) demonstrated that *H. suaveolens* accumulated only 31% of total DM up to 45 DAE, with most rapid growth between 60 and 140 DAE. Our findings support these results with most rapid growth of this species between 75 and 120 DAE. It is significant that DM yields of this species at 120 days were equal to those of Marandu in the unweeded plots. Sida rhombifolia, despite the low DM accumulation during virtually the entire period, began to show greater vegetative growth only after 90 DAE, which is in agreement with findings of Bianco et al. (2014). When analyzing growth and mineral nutrition of S. rhombifolia, these authors found that the species actually has slow vegetative growth up to 80 DAE, with peak DM accumulation occurring after 140 DAE. However, the authors also found that, although initial growth is slow, this species has a high capacity for absorbing nutrients from about 35 DAE and has a comparatively higher capacity to absorb nutrients than other weeds. It is important to note that S. obtusifolia and H. suaveolens are annuals and S. rhombifolia is a perennial species. Annual species grow more quickly than perennials and this was probably the main factor suppressing growth of Marandu.

The different patterns of DM accumulation of the 3 weed species help to explain the similarity of the relative importance obtained for the various coexistence periods used in this study. This behavior may be correlated with competitiveness and the consequent coexistence of the 3 weed species in the area. In other words, the weed species complement one another, with *S. obtusifolia* having greater competitive ability in the early growth stages, while *H. suaveolens* and *S. rhombifolia* are able to grow slowly during this period, being more prominent as *Senna* matures (Gravena et al. 2002).

With this complementary growth, there was always at least one weed species exerting competitive pressure on Marandu, resulting in similar suppression of grass growth regardless of when weeds were removed. It must be remembered that the annual weeds are more adapted to the environmental conditions, have characteristics that make better use of the light, and are more efficient in the use of nutrients, since they are native in the region, while the majority of forage grasses are exotic in South America (Peron and Evangelista 2004; Benett et al. 2008). Most of them are also perennials with slow growth initially.

It is noteworthy that the main broadleaf weed control method in grazing areas is the application of specific selective herbicides. This represents a significant operating expense. Our study provides an indication of the possible reduction in pasture growth as a result of weed infestation, which may aid a farmer in making a decision to spray weeds or to let them grow. It seems that, if the weeds are not treated early after emergence, it is not worth treating them in terms of the effects on pasture growth in that season, as they will still have a depressant effect on pasture growth, even if removed. There is a small window of opportunity to take action. The decision then becomes one of preventing seed set to reduce the soil seed bank of the weeds for subsequent seasons. Our results show that the reduced growth of Marandu from weed competition was a function not of fewer grass plants but reduced growth of the same number of plants, through reduced numbers of tillers and reduced vertical growth. We are not aware of similar findings being reported in the scientific literature.

We consider that the regression equation obtained in this study should be of assistance to all professionals involved in the livestock production chain, especially the meat production chain, in which the productivity of a forage area is heavily dependent on the presence or absence of weeds. It should be used as a component when making decisions about weed control measures to ensure that the decision is soundly based economically. However, delaying a decision to treat weeds could have long-term consequences, especially in terms of additional seed added to the soil seed bank. As this study lasted for only 120 days, it is difficult to forecast the productivity of the pasture over subsequent years. Longer-term studies are needed to determine how the suppression of growth of the grass observed here is reflected over the complete life of the pasture.

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#### (Received for publication 12 May 2016; accepted 19 September 2016; published 31 May 2017)

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#### Short Communication

# A simple method for determining maize silage density on farms

Un método sencillo para determinar la densidad en ensilaje de maíz a nivel de finca

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#### Abstract

Several methodologies have been tested to evaluate silage density, with direct methods most popular, whereas indirect methods that can be used under field conditions are still in development and improvement stages. This study aimed to establish relationships between estimates of maize silage density determined using a direct and an indirect method, in an endeavor to provide an alternative to direct measurement for use in the field. Measurements were performed on maize silage in 14 silos. The direct method involved the use of a metal cylinder with a saw-tooth cutting edge attached to a chainsaw to extract a core of silage. Density of the silage was determined taking into consideration the cylinder volume and dry matter weight of silage removed at 5 points on the silage face. With the indirect method, a digital penetrometer was used to estimate silage density by measuring the penetration resistance at 2 points adjacent to the spots where the silage cores were taken, i.e. 10 readings per silo. Values of penetration resistance (measured in MPa) were correlated with the values of silage mass (kg/m<sup>3</sup>) obtained by direct measurement through polynomial regression analysis. A positive quadratic relationship was observed between penetration resistance and silage density for both natural matter and dry matter (R<sup>2</sup> = 0.57 and R<sup>2</sup> = 0.80, respectively), showing that the penetrometer was a reasonably reliable and simple indirect method to determine the density of dry matter in maize silage. Further testing of the machine on other silos is needed to verify these results.

Keywords: Ensiled matter, penetrometer, resistance, silos evaluation.

#### Resumen

Para determinar la densidad de ensilado, los métodos más usados son los directos mientras métodos indirectos, que se puedan usar a nivel de finca, están aún siendo desarrollados y mejorados. El objetivo de este estudio fue determinar la correlación entre las densidades de ensilado de maíz determinadas con un método directo, y las determinadas con un método indirecto. Las mediciones se hicieron en 14 silos de maíz de fincas lecheras en 5 municipios del estado de Paraná, Brasil. El método directo consistió en el uso de un cilindro metálico con un filo cortante de dientes serrados unido a una motosierra para extraer una muestra del ensilado; la densidad se determinó con base en el volumen del cilindro y el promedio del peso de las muestras extraídas en 5 puntos. El método indirecto consistió en el uso de un penetrómetro digital para medir la resistencia a la penetración en 2 puntos adyacentes a los sitios donde se tomaron las muestras del método directo (10 lecturas por silo). Los datos se sometieron a un análisis de regresión polinomial que mostró una relación cuadrática positiva entre la resistencia a la penetración (medida en MPa) y la densidad del ensilaje con base en los valores de la masa del ensilado (kg/m<sup>3</sup>) tanto para la materia natural como la materia seca (R<sup>2</sup> = 0.57 y R<sup>2</sup> = 0.80, respectivamente). Se concluye que el penetrómetro fue un método indirecto razonablemente confiable y sencillo para determinar la densidad de la materia seca en ensilado de maíz. Para verificar estos resultados se requieren pruebas adicionales con este equipo en otros silos.

Palabras clave: Evaluación de silos, materia ensilada, penetrómetro, resistencia.

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#### Introduction

Greater compaction of ensiled material provides greater specific mass (SM) by expelling air and providing anaerobic conditions for fermentation. This allows better conservation of soluble sugars, minor alteration of structural carbohydrates and reduced proteolysis in the resulting silage, aspects which increase acceptability and consumption by livestock (Velho et al. 2007).

Direct methods are used to evaluate SM of silages, mostly the determination of herbage mass for a known volume of silage, with values being expressed in kg of natural or dry matter per cubic meter. However, these methods involve measuring the volume of the sample, taking it to a suitable facility and drying it for at least 24 hours in an oven. More rapid, indirect methods, which however require sophisticated equipment, aim to facilitate the collection of such data under field conditions, such as: radiometric sensors that present a source and a receptor for gamma waves, a method based on microwave resonance; and the georadar system, also used to estimate SM of soils (Jobim et al. 2007).

However, these indirect methods are still in development and improvement stages and rely on strict calibration to produce reliable data. Among the various invasive tools to determine SM of silages, the penetrometer has specific advantages over other techniques because it requires a simple calibration procedure and can provide reliable data. Sun et al. (2010) suggested that this technique, when properly applied, has the potential to provide good information about silage storage conditions. In an on-station study under controlled conditions, Silva et al. (2011) correlated resistance values provided by maize silage to penetration by a penetrometer with SM values obtained by sampling with the use of a metal cylinder of known volume. Estimates of SM they obtained with this indirect method compared favorably with values obtained with direct measurement, causing them to conclude that the penetrometer could provide reliable estimates under field conditions both quickly and at low cost.

The objective of our study was to measure the SM of maize silage on farms by a direct method (core sampling in the silo panel) and an indirect measurement method (using a penetrometer), and to establish correlations between the estimates obtained, with the aim of establishing the penetrometer as a reliable tool for estimating the degree of compaction of stored forage in the field.

#### **Materials and Methods**

Specific mass measurements were made by a direct method in 14 bunker silos (treatments), employing methodologies described by Holmes and Muck (1999) and D'amours and Savoie (2005), in maize silages on dairy farms in Paraná State, Brazil, specifically in the Castro, Carambeí, Arapoti, Piraí do Sul and Ponta Grossa municipalities.

A metal cylinder, 20 cm length and 10 cm diameter, with a serrated cutting edge and attached to a chainsaw, was used, as described by Craig and Roth (2005) (Figure 1). The cylinder was screwed into the silage panel mechanically through the rotation exerted by the chainsaw. When the sample was withdrawn from the storage panel of the silo, the depth was measured with a rule to calculate the volume of the withdrawn sample. From the cylinder volume and the mass [both natural matter (NM) and dry matter (DM)] of the withdrawn sample, the SM of the silage in the silo was calculated. Whereas NM was the mass of the fresh silage, DM was determined conventionally (weighing after drying NM at 105 °C for 8 h in a forced-air oven).

Silage samples were withdrawn at 5 points (taken as replications) in the silo panel, 3 locations at the top and 2 at the bottom, forming a 'W' like figure. Before the sampling procedure commenced, a slice of silage had been removed manually from each silo panel in order to remove any loose silage from the silage 'face', so that the samples were collected from 'intact' (undisturbed) silage.

To estimate SM through the indirect method, a digital penetrometer (DLG, model PNT-2000-M), which follows the ASAE S313.3 rule that defines penetration resistance as the pressure over the area of a cone with a solid angle of 30°, was used. This equipment is used to determine the penetration resistance in soil compaction studies (Figueiredo et al. 2011; Storck et al. 2016). Penetration resistance was measured at the same time and using the same orientation as in the direct determination with the metal cylinder, with 2 measurements of resistance at each silo panel point, thus giving 10 measurements in each silo. Penetration resistance was measured at points adjacent to the spots where silage samples were taken for the direct measurements, at a distance of approximately 35 cm from those.

For resistance measurements, the penetrometer metal cone was manually pushed into the silage panel horizontally at a constant speed of approximately 2 cm/s up to the end of the cone length, a mandatory procedure according to the instruction manual for the device (Figure 2). Penetration depth into the silo panel was 0.9 m.



Figure 1. Cylinder and chainsaw in use. Source: Personal file.

Penetrometer resistance values (in megapascal, MPa) were correlated with the SM values (kg/m<sup>3</sup>) obtained with use of the cylinder coupled to the chainsaw by a polynomial regression study. The regression equations obtained were used to calculate the values of SM for natural matter (SMNM) and dry matter (SMDM) in each silo (in Table 1: estimated SMNM and estimated SMDM) and these were compared with the values obtained by the

direct method (in Table 1: observed SMNM and observed SMDM). Data were not statistically analyzed, considering that there was no replication (silo), since the silos were evaluated on different farms and factors other than the type of assessed silo and silage (maize) may present different characteristics. Therefore, the values obtained for the SMNM and SMDM were descriptively analyzed.



Figure 2. Penetrometer in use. Source: Personal file.

Silo	$DM^1$	Resistance	SMNM (kg NM/m <sup>3</sup> )		SMDM (kg DM/m <sup>3</sup> )	
	(g/kg)	(MPa)	Observed	Estimated	Observed	Estimated
1	327	2.20 <u>+</u> 0.50	839 <u>+</u> 80.5	767 <u>+</u> 45.3	274 <u>+</u> 26.3	260 <u>+</u> 11.6
2	350	1.92 <u>+</u> 0.35	748 <u>+</u> 48.8	837 <u>+</u> 14.9	262 <u>+</u> 17.1	282 <u>+</u> 4.8
3	292	0.24 <u>+</u> 0.05	469 <u>+</u> 90.8	571 <u>+</u> 17.2	137 <u>+</u> 26.5	146 <u>+</u> 7.5
4	277	0.54 <u>+</u> 0.10	751 <u>+</u> 108.7	704 <u>+</u> 25.7	208 <u>+</u> 30.1	204 <u>+</u> 11.6
5	294	1.07 <u>+</u> 0.40	876 <u>+</u> 49.5	850 <u>+</u> 47.9	258 <u>+</u> 14.6	271 <u>+</u> 24.3
6	274	1.00 <u>+</u> 0.14	904 <u>+</u> 73.0	837 <u>+</u> 22.1	247 <u>+</u> 20.0	265 <u>+</u> 10.7
7	336	0.99 <u>+</u> 0.35	787 <u>+</u> 62.7	836 <u>+</u> 49.1	265 <u>+</u> 21.1	264 <u>+</u> 24.0
8	287	0.68 <u>+</u> 0.22	787 <u>+</u> 87.4	755 <u>+</u> 48.4	226 <u>+</u> 25.1	227 <u>+</u> 22.1
9	279	0.74 <u>+</u> 0.25	801 <u>+</u> 22.9	773 <u>+</u> 50.8	223 <u>+</u> 6.4	235 <u>+</u> 23.5
10	325	1.15 <u>+</u> 0.29	854 <u>+</u> 126.2	861 <u>+</u> 39.2	277 <u>+</u> 41.0	277 <u>+</u> 19.3
11	391	1.06 <u>+</u> 0.14	710 <u>+</u> 103.2	847 <u>+</u> 23.1	277 <u>+</u> 40.3	270 <u>+</u> 11.1
12	345	0.85 <u>+</u> 0.37	867 <u>+</u> 67.4	803 <u>+</u> 73.7	299 <u>+</u> 23.2	248 <u>+</u> 34.1
13	278	0.48 <u>+</u> 0.24	776 <u>+</u> 59.8	684 <u>+</u> 62.6	216 <u>+</u> 16.6	195 <u>+</u> 28.0
14	270	0.53 <u>+</u> 0.18	660 <u>+</u> 50.6	702 <u>+</u> 47.2	178 <u>+</u> 13.6	203 <u>+</u> 21.2
Mean	309	0.96 <u>+</u> 0.58	773 <u>+</u> 128.5	773 <u>+</u> 71.0	239 <u>+</u> 48.4	239 <u>+</u> 34.3

**Table 1.** Dry matter concentration, resistance by ensiled mass to penetration of the metal cone ( $\pm$  SD), observed and estimated specific mass (natural matter and dry matter basis) ( $\pm$  SD) in 14 farm silos.

<sup>1</sup>DM – dry matter; NM –natural matter; Resistance – resistance to penetration of the metal cone; SMNM – specific mass of natural matter; SMDM – specific mass of dry matter.

#### Results

The average DM concentration found in the silages evaluated was 309 g DM/kg, ranging from 270 to 391 g DM/kg (Table 1). Density of silage as determined by the direct method ('observed') ranged from 469 to 904 kg/m<sup>3</sup> (mean 773  $\pm$  129 kg/m<sup>3</sup>) for NM and from 137 to 299 kg/m<sup>3</sup> (mean 239  $\pm$  48.4 kg/m<sup>3</sup>) for DM. The density measurements were compared with the range in penetration resistance in the silos, which varied from 0.24 to 2.20 MPa (mean 0.96  $\pm$  0.58 MPa). The results for this comparison are shown in Figures 3 and 4.



**Figure 3.** Relationship between SMNM (specific mass based on natural matter), expressed in kg/m<sup>3</sup> and resistance to penetrometer metallic cone, expressed in MPa.



**Figure 4.** Relationship between SMDM (specific mass based on dry matter), expressed in kg/m<sup>3</sup> and resistance to penetrometer metallic cone, expressed in MPa.

#### Discussion

The values observed for DM concentration of the silages evaluated are consistent with the recommendation of Nussio et al. (2001) that the optimal DM concentration of maize plants at ensiling should be 300–350 g DM/kg. According to these authors, DM concentrations below 300 g DM/kg are associated with lower DM yield, losses by leaching and low silage quality, factors that may lead to reduced intake by animals. The quality of silages was evidenced by parameters such as neutral detergent fiber, starch content and pH, which presented mean values of  $46.35 \pm 4.8 \%$ ,  $33.28 \pm 4.62 \%$  and  $3.81 \pm 0.07$ , respectively (A.M. Krüger unpublished data).

The values obtained using the direct method indicated that there was considerable variation in how well the material was compacted in the silos, which can be related to the method of compaction used, the stage of growth of the forage when ensiled, the moisture content of the forage at ensiling, etc. According to Jobim et al. (2007), although there is no optimal value for silage density, values in the range of 550-850 kg NM/m3 are most suitable, and these are obtained only under favorable conditions. Typically, appropriate compression for desirable fermentative characteristics and minimal losses in maize silage is obtained with minimum SMDM around 225 kg DM/m<sup>3</sup>. The majority of the silages sampled were above this minimum level. One might expect that the nutritional value and acceptability of the silage to livestock would also vary markedly.

The penetration resistance observed when employing the indirect method indicates that, while there was marked variation in density of the silage as measured directly, there was much greater variation in resistance as measured by the penetrometer. If one assumes that the density measurements were accurate, one might question the accuracy of the penetrometer readings for the same silages.

In an experiment in which 18 penetrometer measures were performed in one silage sample kept under controlled conditions in an experimental station, Silva et al. (2011) found a mean penetrometer resistance of  $1.09 \pm 0.23$  MPa and specific mass observed based on dry matter of  $170 \pm 36.5$  kg DM/m<sup>3</sup>. As in the present study, resistance values obtained were compared with direct measurements as well.

The results obtained for SM (Table 1) observed (direct method) and estimated (indirect method), both for NM and DM, are consistent with those typically observed in farm silos and the values found by the indirect method presented a smaller range of variation when compared

with the direct method, because regression equations determine the middle pathway and reduce the effect of outlying values. There was a positive relationship between the SM of silage and penetration resistance to the metal cone (Figures 3 and 4). This was a curvilinear relationship with silage SM increasing as the resistance to the cone penetration increased to a peak of about 900 kg NM/m<sup>3</sup> or 300 kg DM/m<sup>3</sup>.

Silva et al. (2011) observed a positive linear relationship between the SM of maize silage and penetration resistance to the metal cone in 2 experiments. However, in 1 of the experiments, the adjusted linear equation had a low coefficient of determination, explaining only 33% of the observed variation. This low coefficient was attributed to surface conformations of silos used in the second experiment, since they provided lower compression of the ensiled material, and altered the physical correlation of mass and volume, which may have influenced the relationship between SM of silage and penetration resistance to the metal cone. In order to correct this, they developed equations based on the stratum in which each measurement was taken.

Those authors observed that the SM estimation with the penetrometer had greater accuracy when expressed as SMNM rather than as SMDM, while in our study the SM estimated with the indirect method showed greater accuracy when expressed as SMDM ( $R^2 = 0.80$ ) rather than as SMNM ( $R^2 = 0.57$ ). The differences between the studies may be attributed to the range of uncontrolled factors that may affect silage in silos on different farms (the study of Silva et al. 2011 was conducted under controlled conditions on an experimental station); also, the penetrometer used in the above-mentioned study was a different model from the one we used.

After corrections considering silage stratum, Silva et al. (2011) found  $R^2 = 0.86$  between the observed and estimated SMNM and  $R^2 = 0.82$  between observed and estimated SMDM. Both Silva et al. (2011) and Vissers et al. (2007) concluded that the penetrometer is a reliable indirect method for determining the SM of maize silage.

Our work confirms this conclusion and in addition corroborates that the penetrometer method is a simple tool that can be used on farm. The regression equation  $y = -82.109x^2 + 257.9x + 89.182$  (R<sup>2</sup> = 0.80) we developed for estimating SMDM using this penetrometer provided good (80%) estimates of compaction of maize silage. However, how well this regression relates to other penetrometers and silage types needs further testing. Our data suggest that penetrometer measurements above 1.0 MPa in maize silage indicate compaction above 250 kg DM/m<sup>3</sup>. However, more testing would be needed to confirm this rule of thumb. It was of interest that all silages with SMDM above 260 kg DM/m<sup>3</sup> had DM % in the range 325–391 g DM/kg. All silages with penetrometer readings below 0.75 MPa had DM % below 300 g DM/kg and SMDM below 230 kg DM/m<sup>3</sup>. The relationship between DM concentration in silage and reliability of the machine needs to be investigated further to confirm these findings.

#### Acknowledgments

The authors thank CNPq (National Council for Research and Technological Development) for the financial support.

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(Received for publication 31 August 2016; accepted 22 February 2017; published 31 May 2017)

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#### **Book Review**

# Tropical forage legumes: Harnessing the potential of *Desmanthus* and other genera for heavy clay soils

Edited by JOHN R. LAZIER and NAZEER AHMAD. Published by CABI Publishing, Wallingford, UK, 2016. 480 pp. Price US\$ 225.00. ISBN 9781780646282. DOI: <u>10.1079/9781780646282.0000</u>



Over 200 years ago, Thomas Jefferson, principal author of the American Declaration of Independence and the third President of the USA, also a philosopher and farmer, wrote: "The greatest service which can be rendered any country is to add an useful plant to its culture". This book, "Tropical forage legumes", highlights the complexity of the process in domesticating species, and the remarkable work of one person in particular, Dr. Robert (Bob) L. Burt, in adding a useful plant to our culture.

The book itself has a somewhat poignant background, in that two of the initial authors, Dr. Burt and Professor Nazeer Ahmad, died before the book was published. Dr. Lazier, himself a scientist of note, and also a friend and colleague of both great professionals, persisted with the submission out of friendship and the recognition of the need to make the information available to others working in this general field. Dr. Lazier writes: "Since this research was undertaken there has been a marked decline in the funding for such research, and with the retirement of experienced researchers much of this information will be lost. This volume has been written in order that new scientists in this field will not be repeating work which has already been done, and can build upon the results. Recommendations are provided for further research." Recurrent themes are that: many areas of legume species diversity remain uncollected; potentially valuable genetic material is being lost through global warming and increasing agricultural and urban development; and there is an urgent need to conserve remaining material before it is lost completely. It was gratifying to note that the authors favored a responsible approach to plant introduction, particularly in relation to weediness. "Attempts to meet this demand (adapted legumes) have, in fact, been made for some time, with the due regard that must be paid to the potential of

any new species to be invasive, both within and outside the various regions in which they may be seen to have agronomic and economic merit (Williams and Burt 1982)".

The book describes the process followed by Dr. Burt, his co-authors and others to expand the range of legumes available for use on heavy clay soils in the tropics, using the range of soils and climates found in Belize and northern Australia as examples. Commendably, they did not feel constrained by the prevailing dogma that would have limited them to a particular group of already recognized forage legume genera, including *Desmodium*, *Macroptilium* and *Stylosanthes*, many of which had been selected for acidic soils in the subtropics. The book comprises a total of 19 chapters, largely written as journal papers with Abstract, Introduction, Materials and Methods, Results, Discussion and Conclusions.

The first 9 chapters by various combinations of Burt, Lazier and Ahmad covered all aspects of the work, from collection of wild-type legumes in the Yucatán Peninsula region of Belize, Guatemala and Mexico, as well as in the Caribbean islands, to the very involved methodology of identifying appropriate genotypes for evaluation, and ultimately to the evaluation programs in Belize and Australia. While much of the statistical methodology might have limited appeal to many readers, the actual philosophy and techniques involved in the distillation process, together with the range of genotypes investigated, will have broader appeal. The authors make the point: "It follows that if genetic material is to be selected for trials, for use as 'core collection', or 'representative range', it cannot be done solely on the basis of geographic or provenance data. A meaningful classification is required." Krull and Borlaug made a similar observation in 1970: "The major hurdle to unlocking the secrets (of our genetic resource collections) has been our inability to classify the variability." Various approaches to overcome this hurdle are proposed and discussed.

The research homes in on two species groups in particular, Stylosanthes hamata and Desmanthus spp., although a number of other species are put forward as being worthy of further evaluation. It should be remembered that the reported work was conducted in the 1970s and 80s, and results and discussion need to be interpreted in terms of the state of knowledge at that time. It is interesting to note that a number of species identified in the various studies reported have subsequently been absorbed into international tropical agricultural systems, often as a direct or indirect consequence of the work. As well as Stylosanthes hamata and Desmanthus spp. in grazing systems, Desmodium cinereum (long known incorrectly as Desmodium rensonii), which they flagged as a species of interest, is widely used in legume hedgerows in Southeast Asia for erosion control and livestock feed. Calopogonium caeruleum and Centrosema plumieri, considered as having forage potential, have been rejected in this role due to low palatability, but are successfully used as green manure cover crops under plantations. The Australian experience with the shrub, Codariocalyx gyroides, was similar to that in Belize; it is very palatable but brittle and fails to persist under grazing. The ubiquitous creeping legume, Desmodium incanum, has not been adopted commercially despite being promoted as a grazing legume (Kaimi clover) in Hawaii as early as the 1940s. The mimosoid shrub/small tree, Acacia angustissima, attracted some attention in Australia, but was found to be unpalatable and showed the potential to become an environmental weed.

Chapter 10 by Dr. Kendrick Cox from the Department of Agriculture and Fisheries, Queensland, summarizes the significant volume of work carried out in Queensland, Australia, seeking legumes to fulfil a range of roles on alkaline clay soils, mostly in the subhumid and semi-arid areas in central and southern parts of the State. Most of the previous work focused on selecting grazing legumes for the acidic infertile soils of the more humid coastal strip. The research covered in this chapter had two primary purposes: (1) to identify legumes to supplement the limited suite of species that could be used in pasture leys to build up the level of labile soil nitrogen in preparation for subsequent cropping; and (2) to identify legumes that could persist in pastures, particularly the large areas of buffel grass (Cenchrus ciliaris) that were becoming less productive as nitrogen became increasingly bound up in the extensive fibrous root system of the grass. Within each of these, additional research was

necessary to ensure seed production would not be a constraint to release and adoption, and to address other issues such as establishment, plant nutrition, nodulation and management. Once again, *Desmanthus* was identified as a source of adapted germplasm for pasture, along with another of the species discussed by Burt, initially known as *Stylosanthes* sp. aff. *scabra* and subsequently as *S. seabrana*. While traditional species such as *Lablab purpureus* were unsurprisingly successful for short-term leys, two species, which had been rejected from earlier evaluations in favor of more persistent species, found a significant role in ley pastures on clay soils – *Clitoria ternatea* and *Macroptilium bracteatum*.

The aim of plant evaluation is to bring new and useful germplasm into our culture, whether it be for immediate use by humans or livestock, or to inject a measure of sustainability into current management or production systems. On this basis, Chapter 11 provides an interesting case study of how Dr. Chris Gardiner from the James Cook University, Townsville, Queensland, capitalized on Dr. Burt's earlier work in the northern part of the State, by visiting the evaluation sites established in the 1980s, and collecting and identifying legume species, largely Desmanthus, persisting in this particularly testing environment – heavy clay soils and low, unreliable annual rainfall. This means he started with genotypes that had persisted for around 20 years under commercial management in the target environment. Drs. Burt and Lazier had already done the painstaking work of selecting genetic material that had a good chance of performing well in this environment. In the ensuing period, Dr. Gardiner and others have undertaken a range of studies to evaluate the most promising varieties, and to define aspects of their agronomy and productivity necessary to proceed to commercial release through a partnership with a private company. Accordingly, a mixture of 5 Desmanthus selections (three of D. virgatus and one each of D. leptophyllus and D. bicornutus) is being made available to producers under the Progardes trade mark. This chapter highlights the value of returning to discontinued evaluation sites if persistence is one of the key criteria of merit in selecting useful genotypes, and conversely demonstrates the folly of expecting to select persistent plants from short-term experiments.

In the final chapters, Dr. Lazier focuses again on the situation in Belize, a small country of only 23,000 km<sup>2</sup>, about 1/5 the size of neighbouring Guatemala. He initially outlines the development of the beef industry in the country, identifying constraints to its expansion, one being limited forage development. He follows up the industry review with an analysis of native pastures, in terms of both botanical and chemical composition, and

finally a series of experiments comparing the more productive native legumes with a range of exotic species. As in the earlier chapters, Dr. Lazier's work provides a roadmap of how researchers can approach a forage development program in an untested area. As with any roadmap, there are usually a number of ways to reach one's destination. It is now over 40 years since he commenced his work in Belize, in which time alternative methodologies have been developed for evaluating plants, which may have produced slightly different outcomes, partly through using different comparators, but also through using non-destructive sampling.

A note of caution – some of the species names used in the book are those that would have been applied in the 1970s. Although many appear to have been brought into line with the currently accepted taxonomy, some have not, e.g. the common centro mentioned in the book is listed as *Centrosema pubescens* but is now accepted as *C. molle*, while *C. pubescens* is now applied to cv. Belalto; many former *Cassia* spp. are now classified as *Chamaecrista* or *Senna*; *Macroptilium longepedunculatum* is now accepted as *M. gracile*; the *Arachis pusilla* referred to was actually misnamed in the Australian collection, and should be *Arachis triseminata*. There are misspelt names such as *Desmodium cinerium*, which should be *Desmodium cinereum*, *Stylosanthes sympodiales*, which should be *S. sympodialis*, and *Arachis pintoii*, which should be *A. pintoi*. There are other name changes and misspellings in the book, but it is not the role of a reviewer to act as proof reader. These are mentioned merely to draw the reader's attention to the issue, in order to prevent perpetuation of incorrectly spelled names.

This is not a text. It is a book that will appeal to people involved in the search for new species to play a role in the development of sustainable agricultural production systems. While it focuses on legumes for alkaline clay soils, potential readership should not be limited to those interested solely in legumes or alkaline clay soils. There are many facets to this publication – legume species, soil chemistry, plant ecology, field and statistical methodology, seed production and philosophy, not to mention a brilliant bibliography. Even the multitude of tables, that some may find tedious, provide detail for others that might not be available elsewhere. I cannot in all honesty say that this is a book that should grace the shelves of every student of leguminology, but it should be considered a must for libraries associated with agricultural R & D agencies around the world in both the tropics and subtropics.

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(Published 31 May 2017)

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