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This inaugural issue of *Tropical Grasslands−Forrajes Tropicales* is the result of a co-publication agreement with the Organizing Committee of the 22nd International Grassland Congress, Sydney, 15-19 September 2013 (IGC 2013). It contains those papers presented at the Congress in Session 1.2.1 ("Development and Impact of Sown Tropical Species"), including 2 invited Keynote papers, 2 oral papers and 15 poster papers.

The content of papers is essentially the same as that published in the Conference Proceedings. Minor changes are the result of additional reviewing and adapting to the journal style.

Further contributions on tropical themes, presented in sessions other than 1.2.1 at the Congress, will also be co-published in *Tropical Grasslands−Forrajes Tropicales*, in the near future, in a second "Special Issue IGC 2013".

This issue is dedicated to the memory of Professor **Derrick Thomas** (1944-2013), British pasture agronomist, who made outstanding contributions to the science of tropical pastures and forages in South America and Africa, and a member of the first Editorial Board of *Tropical Grasslands−Forrajes Tropicales.*

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Leucaena toxicity: a new perspective on the most widely used forage tree legume

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Abstract

The tree legume *Leucaena leucocephala* (leucaena) is a high quality ruminant feed, vitally important for livestock production in the tropics, despite the presence of mimosine in the leaves. This toxic non-protein amino acid has the potential to limit productivity and adversely affect the health of animals. In the 1980s, the ruminal bacterium *Synergistes jonesii* was discovered and subsequently distributed in Australia as an oral inoculum to overcome these toxic effects. However, in recent times, a number of factors, including: surveys of the status of toxicity worldwide; improved understanding of the chemistry and mode of action of the toxins; new techniques for molecular sequencing; and concerns about the efficacy of the in vitro inoculum; have cast doubt on some past understanding of leucaena toxicity and provide new insights into the geographical spread of *S. jonesii*. There is also confusion and ignorance regarding the occurrence and significance of toxicity in many countries worldwide. Ongoing research into the taxonomy and ecology of the *Synergistetes* phylum, improved methods of inoculation, and improved management solutions, along with awareness-raising extension activities, are vital for the future success of leucaena feeding systems.

Resumen

La leguminosa arbórea *Leucaena leucocephala*, conocida comúnmente como leucaena, es un alimento para rumiantes de alta calidad y de vital importancia para la producción pecuaria en el trópico, a pesar de la presencia de mimosina en sus hojas. Este tóxico aminoácido no proteico tiene el potencial de limitar la productividad y afectar negativamente la salud de los animales. En la década de 1980, se descubrió la bacteria ruminal *Synergistes jonesii*, la cual fue luego distribuida en Australia como inóculo oral para superar estos efectos tóxicos. Sin embargo, en los últimos tiempos un número de factores no solo han puesto en duda lo que se conocía acerca de la toxicidad de leucaena, sino que también han proporcionado nuevos conocimientos acerca de la distribución geográfica de *S. jonesii*. Estos factores incluyen encuestas sobre la situación de toxicidad a nivel mundial, una mejor comprensión de la química y el modo de acción de las toxinas, nuevas técnicas de secuenciación molecular y el cuestionamiento de la eficacia del inóculo in vitro. También existe confusión e ignorancia respecto a la ocurrencia e importancia de toxicidad en muchos países en todo el mundo. Las investigaciones en curso sobre la taxonomía y ecología del phylum *Synergistetes* y el desarrollo de mejores métodos de inoculación y de mejores soluciones de manejo, junto con actividades de extensión de sensibilización, desempeñarán un papel fundamental en el éxito futuro de los sistemas de alimentación ganadera a base de leucaena.

Introduction

Leucaena is a multipurpose forage tree legume widely used in the tropical world (Shelton and Brewbaker 1994) with estimates of up to 5 Mha planted worldwide (Brewbaker and Sorensson 1990). There are no current estimates but this area will have increased substantially (Dalzell et al. 2012). While first domesticated for human use 7000 years ago (Hughes 1998), its primary value is now as a feed for ruminants. Leucaena is high in crude protein (Jones 1979), highly palatable (Andrew et al. 2000), long-lived, and tolerant of frequent severe defoliation and drought. This latter feature is especially im-

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portant, as it provides edible forage long into the dry season (Shelton and Brewbaker 1994). Leucaena is a vitally important source of protein for ruminants throughout south-east Asia, China, India, Hawaii, the Pacific islands, Mexico, Central America, South America and Australia (Shelton and Brewbaker 1994). In tropical Australia, *L. leucocephala* ssp. *glabrata* cultivars were first released in the 1960s, and there are currently more than 200,000 ha of leucaena-grass pastures (Dalzell et al. 2012), with more plantings each year.

Factors such as the rapid increase in international demand for animal products, high cost of protein concentrates, and shortage of nitrogen for tropical grass pastures will increase the need for alternative high protein feed sources in the tropics. This is leading to increased plantings of leucaena globally. The eastern islands of Indonesia are just one of many examples, where leucaena is fulfilling an important role in ruminant production (Panjaitan 2012) and where its wider use is being promoted in Government programs.

However, while it has many positive nutritional benefits, leucaena possesses the toxic non-protein free amino acid mimosine in relatively high concentrations in leaves and young pods [up to 9% dry matter (DM) in young leaves and 4−7% DM in seeds] (Hegarty et al. 1964b). Pioneering work on leucaena toxicity was conducted between 1976 and 1994 by R. J. Jones and colleagues, who published widely on the symptoms, chemistry, microbiology and management of toxicity (Hegarty et al. 1964b; Jones et al. 1976; Allison et al. 1992; Jones 1994). They found that mimosine was rapidly converted to 3,4-dihydroxypyridine (3,4-DHP) post-ingestion, resulting in reduced feed intake, decreased liveweight gain (LWG), and poor animal performance on otherwise high quality pasture (Jones and Hegarty 1984; Pattanaik et al. 2007). Jones and Hegarty (1984) reported that these symptoms occurred at intakes of leucaena >30% of diet.

Research conducted in the 1980s led to the isolation and identification of a previously unknown, and at the time unusual species of bacterium from the rumen of a Hawaiian goat; it was shown to be a gram-negative obligatory anaerobic bacterium and was subsequently named *Synergistes jonesii*. Strains from this species of bacterium were introduced into Australian cattle herds in 1983 and provided protection against the toxic effects of leucaena by degrading DHP to harmless by-products (Jones and Megarrity 1986). This success was followed by the development of a commercial inoculum in Australia, comprised initially of rumen fluid (in vivo) and subsequently of fermenter-produced (in vitro) mixed inoculum containing *S. jonesii* (Klieve et al. 2002). Since animals were reported to readily transfer the 'bug' within a herd (Quirk et al. 1988; Pratchett et al. 1991) (the exact methods of which are unknown, but likely encapsulated in saliva or faeces), it was recommended that only 10% of the herd need be inoculated. Protection of herds from toxicity in this way has led to annual LWGs of up to 300 kg in inoculated beef cattle grazing leucaena-grass pastures in northern Australia (Wildin 1994).

For these reasons, the problem of leucaena toxicity was considered resolved in Australia; however, a survey of the protection status of herds in Queensland on high leucaena diets has shown that almost 50% appeared to be unprotected, including previously inoculated herds (Dalzell et al. 2012). There was also poor understanding and awareness of leucaena toxicity by farmers, with ignorance of both methods of detection of toxicity and management to limit adverse effects. This was of great concern as undiagnosed subclinical toxicity leads to reduced animal production (Dalzell et al. 2012).

The aim of this paper is to clarify the many issues regarding leucaena toxicity and to update readers regarding recent findings and future directions.

Occurrence of toxicity in tropical countries

During 1984−94, a survey of leucaena toxicity status, based on assay of urinary DHP excretions, was conducted in many countries, where leucaena was being fed to ruminants, to determine the presence or absence of DHP-degrading bacteria (Jones 1994). R. J. Jones concluded that countries protected from toxicity by the presence of *S. jonesii* included: Indonesia, Vanuatu, Thailand, Malaysia, India, Seychelles, Mauritius and Mexico, while at least 13 other countries were not protected. He hypothesized that lack of protection in the latter countries may have caused an aversion to intensive feeding of leucaena and become a barrier to adoption, while countries protected from toxicity had a long history of use of leucaena as forage for ruminant production (Jones 1981; 1986).

However, detailed recent monitoring of ruminant leucaena toxicity has demonstrated a more complex situation. Using the criterion that mean urinary DHP levels in protected herds should be <100 mg/L (Dalzell et al. 2012), a survey of 20 villages within 4 islands of eastern Indonesia found that village herds within close proximity (<40 km and in some cases <1 km) differed in their protection status (Halliday et al. 2013b). It is therefore unwise to classify the protection status of whole countries. Furthermore, as leucaena feeding in the tropics increases, with movement of animals with DHP-degrading bacteria both into and out of villages where leucaena is fed, some ruminants may lose protection, while others in neighboring villages may gain protection, if their animals are maintained on leucaena.

Thailand's inclusion as a protected country (Jones 1994) was based on the absence of urinary DHP in a single goat farm in 1983. However, a comprehensive survey of goat farms within 4 Thai provinces in 2009 (Phaikaew et al. 2012) found that all 63 goats sampled had exceedingly high levels of urinary DHP, often >1,000 mg/L. Similarly, urine samples collected from goats consuming 100% leucaena in Mexico, also previously listed as a protected country, within the Yucatan region where leucaena is thought to have originated, had exceedingly high levels of DHP, in one case >10,000 mg/L (Graham Kerven, unpublished data). Thus, it is not appropriate to refer to 'protected or unprotected' countries, as capability to degrade leucaena toxins varies among ruminants at the village and even farm level.

Confusion regarding toxicity symptoms

When leucaena was first introduced to Australia, the clinical symptoms of toxicity were quite serious, presenting as emaciated animals, with some animal mortalities (Jones et al. 1978). However, severe clinical symptoms are now rarely seen, and most farmers feeding leucaena are ignorant of the visible symptoms of toxicity. While some Australian graziers suspect subclinical toxicity by monitoring reductions in LWGs, livestock raisers in most other countries have no way of knowing if their animals are suffering from toxicity, as data are not available. Since their animals fed leucaena may still be performing better than those without legume, there is understandable confusion over the toxicity issue. Another highly anomalous situation is that many ruminants in the tropical world are consuming large amounts of leucaena for prolonged periods and excreting high concentrations of urinary DHP without any apparent clinical symptoms of toxicity (Phaikaew et al. 2012; Graham et al. 2013; Halliday et al. 2013b), including no indication of goiter (Palmer et al. 2010). The reasons for this are not understood, but may be due to chemical conjugation of the toxins, reducing their toxicity (mentioned later in this paper).

The symptoms associated with leucaena toxicity are now discussed and clarified. They are ascribed to both mimosine and its primary metabolites: 3,4-DHP and 2,3-DHP (Figure 1). While structurally similar, these toxins have different modes of toxicity. Although there is some overlap, they are responsible for different clinical and subclinical symptoms. It is therefore essential that toxicity symptoms are understood and the causes correctly identified for proper management of leucaena-based feeding systems.

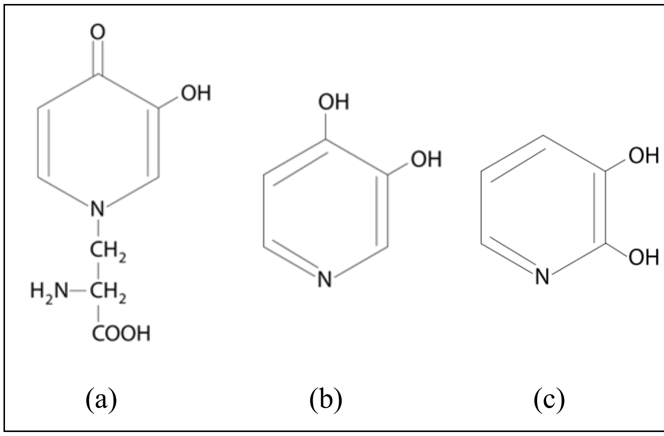

Figure 1. Mimosine (a) and its ruminal degradation products 3,4-DHP (b) and 2,3-DHP (c); adapted from Hammond (1995).

Mimosine toxicity

Mimosine [β-[*N*-(3-hydroxy-4-oxopyridyl)]-α-amino-propionic acid] (Hegarty et al. 1976) is acutely antimitotic, inhibiting the synthesis of DNA (Perry et al. 2005; Pandey and Dwivedi 2007), particularly in rapidly dividing cells (Tsai and Ling 1971; Jones and Hegarty 1984), and can cause damage to internal organs (Prasad and Paliwal 1989). The symptoms ascribed to mimosine include alopecia (Hegarty et al. 1964a; Ram et al. 1994), oesophageal lesions (Jones et al. 1978), foetal abortions (Holmes 1980), low bull fertility (Holmes 1981) and death (Jones et al. 1978; Prasad and Paliwal 1989; Dalzell et al. 2012). It should be noted that mimosine itself is not responsible for the symptoms of goiter (Hegarty et al. 1979).

Structurally, mimosine is a tyrosine analogue (Hegarty et al. 1964a; Hashiguchi and Takahashi 1977), capable of inhibiting enzyme functions such as tyrosine decarboxylase and tyrosinase (Crounse et al. 1962). The inhibition of these enzymes, especially of [3+]thymidine in the follicle bulbs of hair cells, along with the incorporation of mimosine into biologically vital proteins (Tsai and Ling 1971), can result in depilation of actively growing hairs.

For this reason, alopecia is one of the most commonly reported symptoms, when animals are first introduced to leucaena, and can occur within 7 days on 100% leucaena (Hegarty et al. 1964a). Hair loss is commonly observed from the penis sheath and tail areas, where growth is more continuous; mimosine affects the follicle bulb only in the active phase of growth (Hegarty et al. 1964a). This is best demonstrated in sheep as wool growth is largely continuous and therefore sensitive to alopecia from mimosine toxicity (Hegarty et al. 1964a). Alopecia is evident when levels of mimosine in diets are >0.015%

body weight (BW) (Szyszka and Termeulen 1985). As a diet of 100% fresh leucaena leaves may result in consumption of as high as 0.031% BW mimosine (Tangendjaja et al. 1985), it would seem that leucaena feeding at levels greater than 50% would lead to alopecia in unprotected animals.

While mimosine toxicity can be potentially severe, it is relatively short-term and appears only when animals are first introduced to high leucaena diets (Ghosh et al. 2007; O'Reagain 2009). Ruminants typically acquire the ability to fully degrade mimosine in high leucaena diets (>50%) within 2 weeks from initial introduction (Ghosh et al. 2007); and excretion of mimosine ceases within 24 hours of removal of leucaena from the diet (O'Reagain 2009). For this reason, symptoms of mimosine toxicity rarely persist within an animal, especially if introduced to leucaena slowly (Jones 1979).

The degradation of mimosine to 3,4-dihydroxypyridine (3,4-DHP) occurs via many endogenous rumen bacteria (Hegarty et al. 1964a) and by plant hydrolase activity of endogenous enzymes within leaves (Smith and Fowden 1966; Lowry et al. 1983). Up to 30% of the mimosine is converted to 3,4-DHP in the initial process of mastication (Ram et al. 1994) within 1 hour of ingestion (Jones and Megarrity 1983). Although mimosine is readily degraded to 3,4-DHP, this does not result in detoxification.

Mimosine also forms strong complexes with metal ions (Hashiguchi and Takahashi 1977). While the chelation of mimosine antagonizes its antimitotic ability (Hashiguchi and Takahashi 1977), the process of chelation with high affinity metal ions from animal cells, such as Fe, Cu and Zn, inhibits many enzymatic pathways; these metals are also required for normal hair growth (Hegarty et al. 1964a; Hashiguchi and Takahashi 1977; Paul 2000). Ultimately, the chelatory effects of mimosine exacerbate the overall depilatory effects, resulting in overlap of some symptoms with DHP toxicity (Puchala et al. 1995).

DHP toxicity

The primary metabolite of mimosine is the compound 3-hydroxy-4(1H)-pyridine (3,4-DHP) (Hegarty et al. 1976), which, in the presence of certain ruminal microbes, can be further converted to its isomer 2,3-dihydroxypyridine (2,3-DHP) (D'Mello 1992). DHP acts as a potent goitrogen, due to its antiperoxidase activity. By inhibiting essential peroxidase- and lactoperoxidase-catalyzed reactions (Christie et al. 1979; Lee et al. 1980), the iodination of tyrosine in the binding step of the thyroid is inhibited. This step is crucial for the synthesis of thyroid hormones, such as thyroxin (T_4) and triiodothyronine (T_3), resulting in depressed serum T_4 levels, which causes overstimulation and enlargement (up to 4 times) of the thyroid glands (goiter) (Hegarty et al. 1979; Jones 1979; Megarrity and Jones 1983). Studies have also shown that persistent administrations of DHP increase the uptake of iodine in the hyperplastic thyroid, confirming the anti-thyroid effects of DHP (Hegarty et al. 1979). One effect of lowered serum T_4 and T_3 levels is reduced appetite, and ultimately a decreased LWG. Serum T_4 levels below 13 nmol/L may even result in death (Jones et al. 1978).

Reduced iodine availability due to DHP can also affect the salivary glands. Iodine is incorporated into salivary glands via a different method and inhibits the trapping step rather than the binding step, as in the thyroid (Koutras et al. 1967; Harden et al. 1968), leading to excessive salivation after prolonged exposure to leucaena (Jones et al. 1976, 1978; Holmes 1981; Megarrity and Jones 1983; Ram et al. 1994).

Compounding the goitrogenic effects of DHP, is the fact that both isomers also strongly chelate with metal ions (Tsai and Ling 1971), forming complexes with Zn, Cu, and Fe in particular, leading to excretion and depletion of these minerals (Ghosh and Samiran 2007). A deficiency in Zn was shown to be responsible for skin lesions (Mills 1978; Paul 2000), increased salivation (Mills 1978; Puchala et al. 1996) and abnormal hair growth (Hashiguchi and Takahashi 1977). Zn deficiency can also be responsible for inhibiting DNA replication (Perry et al. 2005) and can adversely affect spermatogenesis (Yamaguchi et al. 2009). These all suggest that the chelation of essential minerals is a major toxic effect of DHP. The presence of existing deficiencies of essential minerals in the diet may hasten the manifestation of clinical toxicity symptoms.

The effects of DHP toxicity are a function of both amount of leucaena in the diet and time on leucaena. Clinical symptoms may take up to 8 weeks to become evident (Quirk et al. 1988). This can manifest itself in scenarios where new animals on a leucaena-grass paddock can gain up to 1 kg/hd/d, while existing animals grazing that same paddock for longer periods can be gaining as little as 0-0.2 kg/hd/d (Jones and Bray 1983). These lowered LWGs were found to be directly related to lowered levels of serum T_4, with a threshold of 25 nmol/L before clinical symptoms such as drooling and hair loss were observed (Jones and Winter 1982). It is this chronic nature of DHP, the dual modes of toxicity, and the long periods when toxicity can go unnoticed that contribute to the complexity of recognition of potential toxicity.

However, as mentioned, inexplicably, goats in Thailand and Indonesia on long-term high leucaena diets were shown to be excreting high concentrations of 2,3-DHP in urine without indication of goiter (Phaikaew et al. 2012; Halliday et al. 2013b).

The dominance of 2,3-DHP

The isomer 2,3-DHP was originally thought to be transitory, and indicative of incipient ruminal colonization of *S. jonesii* (Ford et al. 1984; Jones et al. 2009). Much recent data from Australia, Thailand and Indonesia contradict this notion of 2,3-DHP being transitory (Dalzell et al. 2012; Phaikaew et al. 2012; Graham et al. 2013; Halliday et al. 2013b) and indicate that it is usually the dominant isomer in ruminants fed leucaena long-term. Phaikaew et al. (2012) reported exceedingly high levels (>1,000 mg/L) of 2,3-DHP in the urine of ruminants fed over extended periods (>3 months). It is generally considered that the isomers 3,4-DHP and 2,3-DHP are equally harmful (Lee et al. 1980; Ghosh et al. 2008). The latter has been shown to reduce intake (suppress appetite) (McSweeney et al. 1984), reduce milk production in dairy cows (Ghosh et al. 2007), and be fatal given intraruminally in pure form (Puchala et al. 1995).

In a controlled cattle feeding trial in India (Ghosh et al. 2007), 2,3-DHP was the dominant isomer 21 days after commencement of leucaena feeding in cattle naïve to *S. jonesii*, as was the case for animals offered 25-100% leucaena in a controlled trial in Queensland (Halliday et al. 2013a). In the latter trial, all steers on diets of 25−100% leucaena were excreting 2,3-DHP at levels above 100 mg/L after only 2 weeks on leucaena, increasing through to week 7, when it formed the majority of the DHP excreted.

The accumulation of high levels and proportions of 2,3-DHP in ruminants, both previously exposed to *S. jonesii* and those naïve to it, may suggest that: (a) rumen microbes other than *S. jonesii* are capable of degrading 3,4-DHP to 2,3-DHP; (b) the in vitro *S. jonesii* inoculum released in Australia may have lost/mutated the strains effective at complete DHP degradation; or (c) there are other environmental factors (including regulation of genes involved in DHP metabolism) affecting optimal ability of *S. jonesii* to completely degrade all DHP.

Taxonomy and distribution of *S. jonesii*

Recent advances in molecular techniques for the detection and sequencing of *S. jonesii* have allowed greater insights into the geographic spread and genetic variability of the bacterium. From the culture originally imported into Australia from a Hawaiian goat in 1982, 4 strains were later isolated, including the designated type-strain 78.1 (ATCC 49833) and 3 others (100-6, 113-4, 147-1) (Allison et al. 1992). The strains were shown to have differential specificity for degrading mimosine, and/or 3,4-DHP and 2,3-DHP (Jones 1994).

PCR amplification using *S. jonesii* specific primers of the 16S rDNA (small sub-unit of the ribosome-ssRNA) was used as a molecular phylogenetic classifier, and has even identified *S. jonesii* 16S rDNA sequences in the gut of disparate animals such as the horse, Tamar wallaby, baboon and the emu (Chris McSweeney, unpublished data). The theoretical limit of detection of *S. jonesii* by PCR is 10^3 cells/mL; however, a realistic PCR amplification of genomic DNA from digesta is 10^4−10^5 cells/mL due to the co-precipitation of contaminating inhibitors. This problem was overcome by a second round of PCR (nested PCR) on the initial PCR products, which increased the sensitivity of detection to near theoretical limits. Sequencing of the amplified (nested) products not only confirmed the identity of *S. jonesii* but also detected mutations in that segment of the 16S gene. These changes appeared as discrete mutations or 'single nucleotide polymorphisms' (SNPs) that may be correlated with their ability to degrade DHP, relative to the type strain. SNPs can be random or occur consistently at the same locus, termed 'hot-spots'.

A survey of animals consuming diets of 0−100% leucaena was conducted to understand the distribution and changes at the molecular level of *S. jonesii* (Chris McSweeney, unpublished data). Rumen fluid or faeces was collected from cattle in Queensland and from cattle, sheep, goats, buffalo and yak from Indonesia, Thailand, Vietnam, China and Brazil. In a number of these sites, animals were naïve to leucaena. In general, faecal samples failed to generate PCR products for *S. jonesii* 16S rDNA from either Australian or international samples. The survey revealed that *S. jonesii* is far more widespread and indigenous to the rumen than originally realized and that many molecular variants of the type strain exist. The molecular detection of this bacterium internationally in ruminants, irrespective of their leucaena feeding status, corroborates some of the findings of Jones (1994), reporting on the inconsistent DHP-degrading capability of rumen fluid from animals from cross-continental sites.

Yet, this information on its ubiquity has come with a proviso, that our sequence analysis from Australia and other countries showed SNPs, across ~800 of the 1500 bases of the 16S gene of the bacterium. These SNPs were distributed primarily at 'hot-spots' in bases corresponding to *E. coli* nucleotide positions 268 (C→T), 306(A→G), 328 (G→A) and 870 (A→C) between bases 200-900 [~700 base pairs (bp)] of the *S. jonesii* ATCC

16S rDNA. All 4 SNPs were identified at varying frequencies in all farms surveyed in Queensland. Of these loci, '306' and '870' were almost always mutated when SNPs were detected. Surprisingly, these two SNPs were present consistently in the inoculum provided to the farmers. In about 50% of these sequences, all 4 SNPs were present.

In animals overseas, the very same SNPs were also distributed, ranging from frequencies of 15% (for '870' in Brazilian cattle) to 100% (all 4 SNPs in Vietnamese cattle and goats). Among all the international samples analyzed, those from Jinnan cattle, Tibetan yak and Indonesian buffalo were found to have *S. jonesii*, 100% identical to the type strain. Interestingly, the buffalo in Indonesia were on 100% leucaena for 0.5−1 years and had high clearance of both 3,4-DHP and 2,3-DHP. The Jinnan cattle and Tibetan yak were naïve to leucaena. Other SNPs were detected, whose frequencies were not consistent across animals, geographical regions or loci.

It remains to be determined if these molecular changes also modify the ability of these strains to degrade DHP. A detailed molecular and physiologic analysis of these 'strains' is, thus, highly desirable in order to elucidate the link between genetic changes and DHP-degrading potential.

Attempts at molecular enumeration of *S. jonesii* from rumen digesta are similarly fraught with ambiguity, relating to the sensitivity and specificity of the quantitative real-time PCR (qPCR) technique. Compared with the 2-step nested PCR which produces ~800 bp long products, the qPCR typically uses a 100−200 bp PCR product for real-time amplification and analysis. Although this method has a statistically higher chance of amplifying a shorter target (100−200 bp) than the nested PCR (700−800 bp), it still has to overcome PCR inhibition and thus a lack or reduction of sensitivity, unlike the nested PCR. Any specific amplification, nevertheless, can accurately enumerate the target molecule (16S rDNA of *S. jonesii*) from a rumen sample. Several primer sets targeting the *S. jonesii* 16S DNA, designed and tested during the past 4−5 years, have indicated *S. jonesii* exists in the rumen at very low numbers, even in animals foraging on leucaena and where degradation of DHP was apparently occurring (Graham et al. 2013).

This extensive molecular analysis of animals consuming leucaena demonstrates that *S. jonesii* is widespread with variants of the ATCC type strain. These variants may potentially be responsible for the partial DHP degradation results.

Management of toxicity

Methods for detecting DHP toxicity

A full understanding of DHP toxicity and of effective methods to detect subclinical toxicity is vital for productive management of animals on leucaena. There are several possible approaches to detection of toxicity, but the most effective method is assay of urine for DHP (Lowry et al. 1985; Phaikaew et al. 2012). Unmetabolized mimosine, 3,4-DHP and 2,3-DHP are readily absorbed into the blood stream and voided in the urine via glomerular filtration at the kidneys (Hammond 1995). While small amounts (up to 15%) may be voided in the faeces (Hegarty et al. 1979; Jones and Hegarty 1984; Hammond 1995), most is readily absorbed from the gut and excreted via the kidneys (Lowry et al. 1985).

While HPLC analysis of urine (Dalzell et al. 2012) is currently the most accurate method of measuring DHP toxicity, sampling many animals for analysis by HPLC is too expensive for graziers, and prohibitive for farmers in developing countries. In response to the need for a rapid, accurate and inexpensive urine assay, a colorimetric test kit, modified from earlier work (Lowry et al. 1985), has been developed and refined as the most cost-effective and immediate assessment of toxicity (Graham et al. 2013). This is now available to Australian graziers uncertain about the toxicity status of their herds. This improved urine test gives reliable, although not quantitatively precise, indications of the concentration and nature of the toxin.

DHP can be excreted in both the free form and as a conjugate with a glucose molecule. HPLC analysis with a reduced flow rate has been able to separate free DHP from conjugated DHP-glucoside. However, preservation of urine prior to analysis requires strong acidification, which hydrolyzes all conjugated DHP (Tangendjaja and Wills 1980), unless analysis is conducted immediately.

While assay of serum T_4 and T_3 can be used as an indicator of DHP toxicity (Jones et al. 1978; Megarrity and Jones 1983; Jones and Hegarty 1984; Ghosh et al. 2007), changes in thyroxin levels occur after longer-term exposure with considerable variation among animals (Michael Halliday, unpublished data). Likewise, the development of goiter in response to depressed thyroid hormones is also a cumulative longer-term effect. As noted, ruminants do not always develop enlarged thyroids on high leucaena diets. Accordingly, these methods are not a reliable indicator of current toxicity status.

Inoculation

Following the initial discovery of *S. jonesii*, inoculation with rumen fluid collected from protected animals was the preferred method for transferring protection between herds, and even between countries (Jones et al. 1985). This seminal work on inoculation demonstrated that the capability to completely degrade DHP was transferred within 5 days following direct inoculation with rumen fluid (Jones and Lowry 1984; Jones and Megarrity 1986). In 1982, 10 cultures of rumen fluid from a single goat in Hawaii were imported to Australia and, in 1983, dosed into rumen-fistulated steers at the CSIRO Lansdown Research Station near Townsville (Jones and Megarrity 1986). Rumen fluid from these steers was then used as the source of inoculum, given via direct rumen injection, to ~10% of the remaining herd at Lansdown, spreading passively to the entire herd. In 1984, strained rumen fluid from these cattle was administered to steers at the Queensland Government Brian Pastures Research Station near Gayndah (Quirk et al. 1988). It was from these 2 locations that rumen fluid was collected and distributed to Australian graziers and also to livestock raisers in both Ethiopia and China, with successful transfer of protection.

In 1995, production of the inoculum in an in vitro fermenter began, using rumen fluid containing the mixed bacterial inoculum sourced from cattle at Brian Pastures (Klieve et al. 2002). While originally successful in transferring protection, the efficacy of the current in vitro inoculum appears in doubt, as it is neither rapid nor completely successful in its degradation of DHP. It was thus postulated that the continually cultured oral inoculum may have lost some strains, and/or have undergone genetic modification and/or contained other strains with an altered DHP-degrading potential (Graham et al. 2013; Halliday et al. 2013a).

In Indonesia, animals identified as having *S. jonesii* by PCR analysis and confirmed by very low urinary DHP excretions, were selected as inoculum donors for direct rumen fluid transfer. To test the efficacy of transfer, animals known to be excreting high levels of DHP were inoculated with rumen fluid, which had been maintained in a strict anaerobic state at an appropriate temperature. However, the recipient goats continued excreting 2,3-DHP at levels estimated at >1,000 mg/L based on colorimetric assay 10 days after inoculation, indicating a lack of rapid successful response to inoculation. This occurred during August 2012 and was repeated in November 2012 with cattle and goats. Donors were established as high degraders of DHP and the presence of *S. jonesii* was confirmed by PCR analysis. Again, the ability to degrade DHP (or increased degradation) was not transferred within 10 days. These recent findings (Michael Halliday, unpublished data) are in stark contrast with earlier work, where rumen fluid transfer resulted in complete degradation of DHP within 5 days in Indonesia (Jones and Lowry 1984) and within 3 days in Thailand (Palmer et al. 2010).They also raise questions regarding the ability of *S. jonesii* to be easily transferred, and therefore the capacity to degrade DHP.

Other microbial control options

Other microbial solutions apart from *S. jonesii* have been investigated as alternative biological control methods. Domínguez-Bello and Stewart (1991) reported the isolation of a DHP-degrading *Clostridium* bacterium, which was subsequently lost. In China, 4 strains were isolated, which together were able to degrade up to 60% of DHP in vitro within 3 days. These gram-positive facultative and obligate anaerobes were identified as *Lactobacillus* spp., *Streptococcus bovis* and *Clostridium sporogenes* (Tan et al. 1994) and were quite different from *S. jonesii*. Strains of obligate aerobic *Streptococci*, capable of degrading DHP, were reported by Chhabra et al. (1998). Researchers in Germany isolated a mimosine- (and DHP-) degrading bacterium from the rumen fluid of steers naïve to leucaena (Aung et al. 2011). After continuous culture with mimosine for 16 days, their work reported an aero-tolerant gram-negative coccobacillus was isolated, belonging to the genus *Klebsiella*. While capable of growing under anaerobic conditions, it grew best in aerobic conditions. As such, it may not readily persist in high numbers in the anaerobic conditions of the rumen. The researchers also described a method for stabilizing the inoculum in alginate beads, increasing its shelf-life at room temperature by up to 8 weeks. Methods such as this, and freeze-drying, may be incorporated into the production in vitro of *S. jonesii* inoculum in the future, preventing loss of bacterial numbers in transit. However, further research is required.

Supplementation and conjugation

As mentioned, one of the major toxic effects of DHP is to strongly chelate essential minerals such as Fe and Zn (Stunzi et al. 1980). Methods for increasing conjugation and/or chelation of DHP by modifying the diet have been postulated as a way to reduce the toxic effects of leucaena.

While Christie et al. (1979) reported DHP conjugated with a glucose molecule to have reduced antiperoxidase activity in vitro, Hegarty et al. (1979) showed that the conjugate was of the same order of chronic goitrogenicity in vivo. However, chelation and conjugation increase the polarity of DHP, resulting in a more water-soluble molecule, which can be voided more efficiently. In addition, by attaching a sugar molecule or metal ion at the active binding hydroxyl site, its toxic chelating ability is reduced (Lowry et al. 1985). There is other evidence that the toxic effects of DHP are reduced due to conjugation; recent tests on highly productive animals consuming high leucaena diets in eastern Indonesia showed that they were excreting high levels (estimated at >1,000 mg/L using colorimetric analysis) of conjugated DHP (Michael Halliday, unpublished data). These animals may have developed a coping mechanism of being able to conjugate DHP, without ruminal degradation, thus reducing its toxicity. This may explain the absence of symptoms.

While DHP can be excreted in the free form, it is normal for up to 33% to be found conjugated as a glucuronide in urine (Hegarty et al. 1964a; 1976). Supplementation with molasses has previously been shown to increase both the conjugation of DHP, and the amount being excreted in unprotected animals (Elliott et al. 1985). Animals receiving a high molasses supplement did not exhibit a decline in T_4 levels. However, the level of molasses required to achieve this effect was ~40% dry matter intake, making it an impractically large component of the diet.

Supplementation with minerals to chelate mimosine and DHP has been suggested as a method of detoxification (Hashiguchi and Takahashi 1977). Supplemental mineral ions were shown to have prevented hair loss and skin lesions (Jones et al. 1978), doubled excretion of intra-ruminally infused 2,3-DHP, lowered the levels of 2,3-DHP in the rumen and plasma, and prevented clinical toxicity symptoms from developing (Puchala et al. 1995), suggesting that kidney clearance of DHP is more efficient when chelated or conjugated (Puchala et al. 1995). While DHP is more efficiently voided in the chelated form, if essential metal ions are not replaced, deficiencies in these elements will result in toxicity symptoms (Puchala et al. 1996).

Feeding management

Management strategies that control exposure to leucaena have been effective in limiting the extent of toxicity. Prior to the discovery of *S. jonesii*, Jones and Hegarty (1984) suggested that leucaena should not exceed 30% of the diet. As mimosine toxicity rarely occurs in animals regularly consuming leucaena and since DHP toxicity is a factor of both time on leucaena and amount of leucaena in the diet (Hammond 1995), moderate levels of leucaena in the diet for short periods (≤2−3 months) can limit the toxic effects. Quirk et al. (1988) showed that it can take up to 8 weeks for clinical symptoms to become evident. Alternating time on leucaena with time on other feed sources can reduce the toxic effects associated with DHP. However, reducing consumption of leucaena limits the LWG potential of a fattening system. Given that leucaena is often one of the only feedstuffs available during the extended dry season in many tropical smallholder feeding systems, an effective biological control mechanism is the most practical solution to leucaena toxicity.

An example of limiting exposure is found in fattening systems in Sumbawa, eastern Indonesia, many of which feed high levels of leucaena to Bali bulls (up to 100% of the diet), and typically fatten over a 4−6-month period (Panjaitan et al. 2013). Farmers report that initial hair loss is common (probably due to mimosine toxicity), followed by complete recovery. They also report that an initial aversion of naïve animals to high leucaena diets can be overcome by using a mixture of fresh faeces from an older bull and fresh leucaena leaves as a source of 'inoculum' (Panjaitan et al. 2013).

Other less effective management strategies include drying leucaena. While this converts most of the mimosine to DHP (Hegarty et al. 1964b; Wee and Wang 1987), it does not overcome the toxic effects of DHP itself.

Future research, development and extension directions

Leucaena toxicity, as indicated by high levels of urinary DHP, is still common through tropical countries, where leucaena is fed to ruminants.

Farmers and Government agencies promoting the use of leucaena are often ignorant of the effects of leucaena toxicity, especially since there are usually no clinical symptoms. It is vital that research and education are continued to improve understanding and management of this extremely productive legume, in order to optimize animal production and eliminate negative impacts on animal health.

Bacterial control offers the most beneficial and practical solution, provided easy transfer mechanisms are developed, and long-term persistence of efficacy of DHP degradation can be assured. Future research goals include: (a) improved molecular methods for the identification and understanding of the functionality of *S. jonesii* and the *Synergistetes* phylum; (b) alternative microbial control options; (c) improved inoculation protocols within Australia and developing countries; and (d) alternative methods of detoxifying DHP.

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Global impacts from improved tropical forages: A meta-analysis revealing overlooked benefits and costs, evolving values and new priorities

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Abstract

The wider use and improved performance of planted tropical forages can substantially change social, economic and environmental landscapes. By reviewing impact-related studies published in the past two decades, this paper shows how evolving development priorities have influenced the types of impacts being documented. A meta-analysis was used to examine 98 studies according to: (i) breadth of reported effects, as related to development goals of social equity, economic growth and environmental sustainability; (ii) extent of effects, ranging from intermediate to longer-term impacts; and (iii) measurement precision (identification, description or quantification) of impacts.

Impacts have been assessed for fewer than half of the documented 118 Mha with improved forages. Although Brazil accounts for 86% of the known planted area, widespread irregular reporting of technology adoption affects accuracy of global estimates. Over 80% of the impact-related studies reported economic effects, while fewer than 20% were quantitative estimates of longer-term economic impacts. Inconsistent valuation methods and assumptions prevented valid summation of total economic impacts. Social effects were reported in fewer than 60% of studies and emphasized household-level outcomes on gender and labor, with most reported effects being non-quantitative. Environmental effects were reported slightly more often than social effects, with recent increases in quantitative estimates of carbon accumulation. Few studies analyzed tradeoffs. Independent reviewers conducted approximately 15% of the studies. Newer development priorities of environmental sustainability, system intensification, organizational participation and innovation capacities require broader approaches to assess impacts. Increased marketing and coordination with development and environmental organizations can generate greater demands for improved forages.

Resumen

El uso más amplio y el desempeño mejorado de forrajes tropicales sembrados pueden cambiar sustancialmente los paisajes social, económico y ambiental. Mediante la revisión de estudios relacionados con el impacto de forrajes tropicales publicados en las últimas dos décadas, este artículo muestra cómo las prioridades de desarrollo cambiantes han influido en los tipos de impactos que se están documentando. Se utilizó un meta-análisis para examinar 98 estudios de acuerdo con: (i) la envergadura de los efectos reportados en relación con los objetivos de desarrollo de equidad social, crecimiento económico y sostenibilidad ambiental; (ii) el alcance de los efectos, que van desde impactos a mediano plazo a más largo plazo; y (iii) la precisión de medición (identificación, descripción o cuantificación) de los impactos.

Se han evaluado impactos para menos de la mitad de los 118 millones de hectáreas con forrajes mejorados que se encuentran documentados. Aunque Brasil representa el 86% de la superficie sembrada que se conoce, los informes de adopción de tecnología son, en general, irregulares, lo cual afecta a la precisión de las estimaciones globales. Más del 80% de los estudios relacionados con el impacto de forrajes tropicales reportaron efectos económicos, mientras que

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menos del 20% son estimaciones cuantitativas del impacto económico a largo plazo. Métodos y supuestos de valoración inconsistentes impidieron sumar, en forma válida, el impacto económico total. Se reportaron efectos sociales en menos del 60% de los estudios, y se enfatizaron los resultados a nivel de los hogares en cuanto a género y trabajo. La mayoría de los efectos reportados fueron no cuantitativos. Los efectos ambientales fueron reportados un poco más frecuentemente que los efectos sociales, con aumentos recientes en las estimaciones cuantitativas de la acumulación de carbono. Pocos estudios analizaron las ventajas y desventajas. Aproximadamente el 15% de los estudios fueron realizados por revisores independientes. Las prioridades de desarrollo más recientes − sostenibilidad ambiental, intensificación de sistemas, participación organizacional y capacidad de innovación − requieren de enfoques de mayor alcance para evaluar los impactos. Una mayor comercialización y coordinación con organizaciones de desarrollo y ambientales pueden generar una mayor demanda de forrajes mejorados.

Introduction

Increasing consumer demands for animal products are radically changing crop and livestock systems throughout the world (Delgado et al. 1999; FAO 2009). Despite reduced meat consumption per capita in some countries of Europe and the Americas (Kanerva 2011; Larsen 2012), the higher incomes of growing populations, especially in China and India, are stimulating greater global demand for and trade of livestock products (Delgado et al. 1999; Fu et al. 2012). In order to produce sufficient feed for more animals, an intensification process that improves the productivity of crop and livestock systems needs to continue – but at a more urgent pace (McDermott et al. 2010).

Two general strategies can intensify crop and livestock systems, namely the use of: (i) feed grain concentrates; and (ii) grass and legume forages (Herrero et al. 2010; Bouwman et al. 2011), while improving animal breeds and health status can improve feed efficiency. A dramatic and steady increase in the use of feed grains has already occurred (Delgado 2005; Thornton 2010). Now, one-third of all arable land is dedicated to crop production for use as animal feed (Goodland and Anhang 2009), although there is increasing demand for feed grains for use as food and biofuel (Dixon et al. 2010; Taheripour et al. 2010). Monocrop practices can cause environmental damage (Clay 2004), such as water and air pollution from high levels of chemical fertilizer and pesticide use (Steinfeld et al. 2006). Furthermore, the geographic isolation of grain-producing areas from livestock areas requires significant energy inputs for transportation and nutrient supplies (Pimentel and Pimentel 2007). Consequently, total net greenhouse gas (GHG) emissions associated with grain feedlot systems are estimated to be 15% higher than emissions from intensive forage grazing systems (Pelletier et al. 2010). In total, the production of livestock accounts for at least 51% of global anthropogenic GHG emissions (Goodland and Anhang 2009).

Often grown on non-arable lands, grass and legume forages can generate both positive and negative changes to economic, social and environmental landscapes. In striving to estimate global impacts of improved forages, a meta-analysis approach was used to review impact-related studies from the past 2 decades, associated with forage research, development, training and extension (RDTE) activities throughout the tropics, including Africa, Asia, Australia and the Americas. In addition to geography, the term global is interpreted as being comprehensive. Therefore, serving as a general framework for systematic analysis is a "triple bottom-line" concept (Elkington 1997) of social, economic and environmental changes caused by technological innovations, which has been employed by Embrapa (Avila 2001; Avila et al. 2008). In 2 ways, this paper is an extension of a review on adoption of tropical legumes conducted by Shelton et al. (2005), with: (i) the inclusion of sown grass pastures; and (ii) estimates of global impacts after adoption.

Methods

RDTE innovations of improved forages within a livestock supply chain

In order to substantiate causal relations between improved forages and a potentially wide range of different impacts, a generalized forage-livestock supply chain was developed. The supply chain with 4 links: input, production, transformation and marketing (Figure 1), can represent: (i) small-scale farmers who manage a diversity of crop and animal husbandry activities for home consumption and local markets; and (ii) large-scale operations specializing in meat and/or dairy production for national and international commodity markets. Forage innovations can change both products and processes of the supply chain. Products are improved forage *germplasm*, whereas processes are affected by *innovations* of farmers working with scientists and development workers. Improved forages are rarely a stand-alone off-the-shelf technology. In most cases, the technology input requires training and extension efforts to match forages with production systems, and develop or co-develop best practices of cultivation, harvest and optimal use as a feed for a particular type of animal (Horne et al. 2000; Peters et al. 2003). Stakeholders and beneficiaries of improved-forage RDTE include a diversity of participants along the supply chain, including suppliers of seeds or planting material, farmers and producer organizations, and marketers, traders and general consumers, who are affected positively by services or by negative externalities.

An array of effects on social, economic and environmental landscapes

A common distinction, *outcomes* versus *impacts*, although not clear-cut, is often used to clarify the types of effects and the times at which they occur. Adapted definitions from OECD-DAC (2002) and CGIAR (Walker et al. 2008) illustrate the conceptual difference: *Outcomes* (or *intermediate* or *Stage I impacts*) are the short- and medium-term effects resulting from an innovation. They represent changes in behavior, goods and services, either on- or off-farm, which occur between the completion of a project or program and the achievement of impacts. Technology-focused studies typically assess outcomes at a geographically specific scale after adoption has occurred and there is evidence of effects, such as costs and benefits. *Impacts* (or *Stage II impacts*) are a longer-term concept. They are the positive and negative, macro-level effects on identifiable areas or population groups caused by an innovation, directly or indirectly, intended or unintended. These effects can be socio-cultural, institutional, economic, environmental, etc. Impact studies are conducted to assess 'bigger picture' impacts generated by large-scale adoption, which lead to notable changes in social, economic and environmental landscapes.

The *breadth of effects* describes the different outcomes and impacts of RDTE innovations on different landscapes. With respect to a *social landscape*, improved forages affect individuals, households, communities and nations. Intermediate outcomes include increases or decreases in labor use of family members. Other possible social effects include enhanced farmer participation in producer or community organizations. Fostered farmer participation in, and capacity building of, organizations along a supply chain can lead to significant institutional change, with greater influence in policy decisions that can ultimately result in improved well-being and equity. An *economic landscape* also changes in many ways as a result of forage RDTE innovations. At the farm level, savings in input use or factor efficiencies generate different outcomes, such as reduced requirements for labor, rainfall/water or fertilizer. Also, cultivation of improved forages can lead to greater productivity, typically measured in yield of biomass, energy or protein per unit area. Nevertheless, forages are an intermediate product and are typically used for other purposes such as animal feed. Improved forages can enhance efficiencies of product transformation that result in higher farm gross and net revenues (profits). At an international scale, economic impacts of improved forages can include changes to the performance of a livestock subsector with respect to its enhanced competitiveness and comparative advantage. Such analyses often include examination of government policy interventions (e.g. subsidies, taxes and tariffs on inputs, outputs, imports and exports) on sector performance.

Effects of improved forages on the *environmental landscape* are often both positive and negative, and can lead to tradeoffs with social and economic objectives. On-farm performance outcomes result from better abilities to withstand pests, diseases, flooding and drought. Improved forages can also cover soils faster and more completely, thereby reducing erosion and weed infestations. Deep root structures can access water during dry seasons and store carbon in soils. Leguminous forages, in particular, fix nitrogen in soils, thereby improving soil health and fertility. Such improvements in on-farm performance can generate potentially significant benefits by preventing losses of biomass production and improving overall farm resilience to weather shocks. At farm and landscape levels, negative impacts of improved forages include invasiveness of some species and loss of local biodiversity (Chudleigh and Bramwell 1996; Steinfeld et al. 2006; Stevens and Falk 2009). Other impacts can arise from a cumulative effect of better farm productivity at larger scales, including changes to downstream water flows, water quality and sedimentation. Whether off-farm environmental effects are beneficial or detrimental depends on specific site contexts and management practices, thereby posing challenges to accurate measurement of impacts.

A meta-analysis approach was used to examine diverse effects from improved forage germplasm and associated knowledge-sharing innovations. Although the task of identifying studies for inclusion could be considered simple, identification requires a clear operational definition of the phenomenon being examined (Rudel 2008). The process of reviewing the studies enabled the comprehensive specification of effects on landscapes (Figure 1), which, in turn, served as the analytical framework for case selection. Via web-based literature searches, reviews of references within papers, and communications

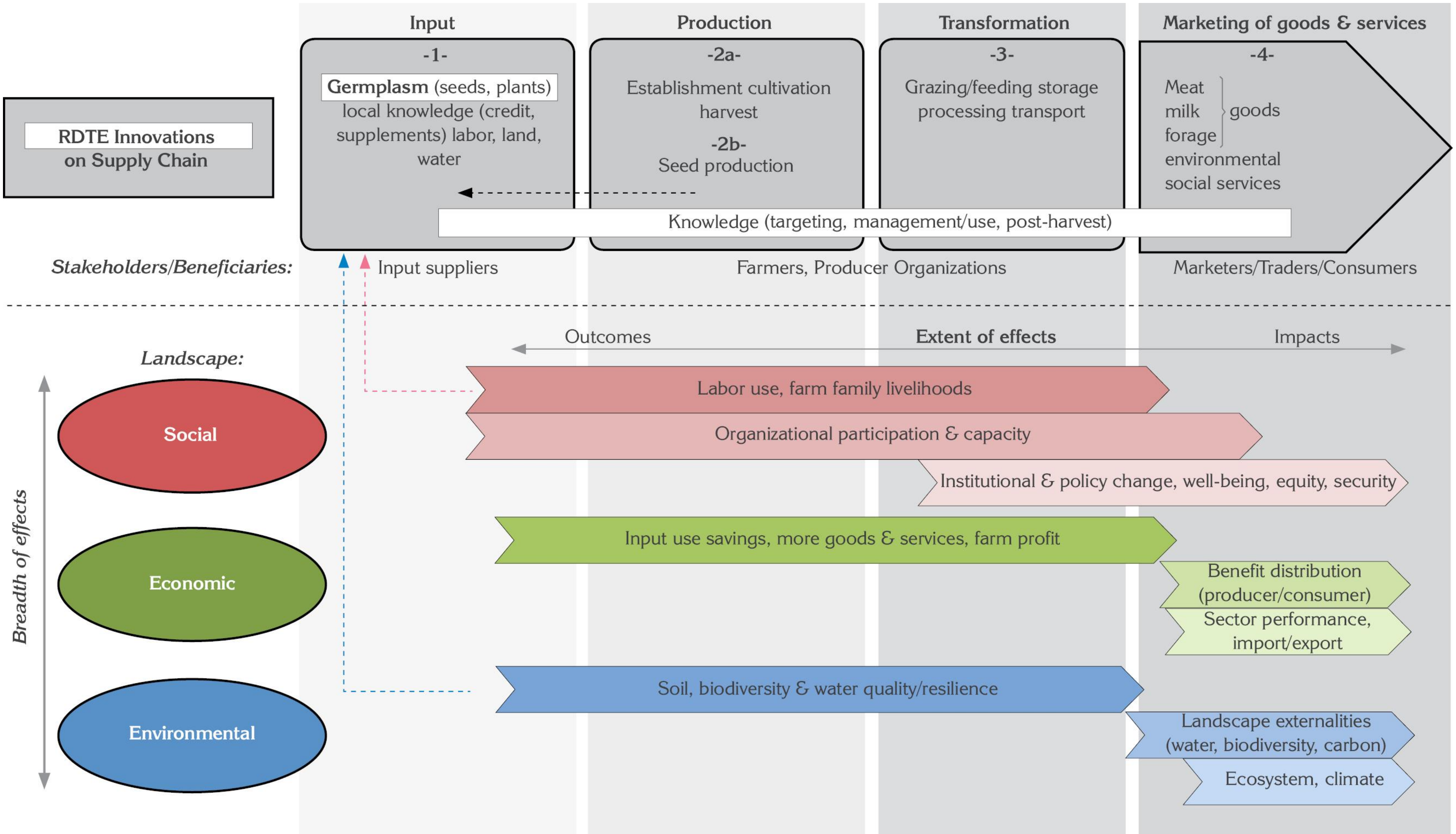

Figure 1. An array of effects on landscapes associated with RDTE innovations along a generic supply chain of improved forages.

with forage experts, a pool of over 170 studies was collected, and 98 were selected for use within the sample. Many disqualified studies were characterizations of existing forage-livestock systems or were studies of farm trials or adoption − without any description or quantification of impact. Although the search was conducted in 4 languages, most studies were written in English, with 4 in Spanish, 1 in Portuguese and none in French.

Table 1. Reported effects (% of total) per type and extent of effect and measurement precision.

Type of effect	Outcomes			Impacts		
	mentioned	*described*	*quantified*	*mentioned*	*described*	*quantified*
Social	2	11	11	1	1	2
Economic	1	3	18	0	0	21
Environmental	7	5	8	1	4	7

Many impacts remain undocumented within the literature due to financial, technical and other restrictions, which often prevent a comprehensive assessment of forage innovations. In order to minimize publication bias (Rothstein et al. 2005) that would reduce estimates of global impacts, the dataset was expanded to include "non-impact" studies, such as project reports and other documents, which also describe impacts. For countries where only information on technology adoption or productivity increases was available, authors were contacted in an effort to obtain grey literature of impacts. Although the sample represents a diversity of countries from tropical Africa, Asia, Australia and Latin America, a paucity of the smaller, less-populous countries became evident.

Keywords pertaining to the types of effects, along with synonyms, were used to identify their presence or absence. Reported effects within a study sometimes represented more than one location or type of forage. Therefore, reported effects were larger than the number of studies. Review of the units of analysis and associated text permitted the determination of: (i) outcomes versus impacts; and (ii) the measurement precision used within the analysis. There were 3 categories of measurement precision: (i) simple mention or identification; (ii) narrative or qualitative description; and (iii) quantitative analysis. All economic estimates were adjusted according to inflation and are reported in 2005 US$.

Results and Discussion

Approximately 118 Mha planted with improved forages have been documented, with Brazil accounting for 86% of the known planted area (IBGE 2007; Landers 2007; CIAT 2013). Nevertheless in all countries, the irregular reporting of technology adoption and incomplete analysis of associated impacts (<50% of adopted area) distort the accuracy of global adoption and impact estimates.

Nearly 80% of the impact-related studies were published between 1999 and 2013. Within the sample, more than 200 different types of effects were reported. Nevertheless, approximately 2/3 of the effects were intermediate, not longer-term, larger-scale impacts. Although economic effects were most frequently reported, fewer than 20% of all reported effects were quantified economic impacts. Environmental and social impacts were even less frequently quantified, with 7 and 2%, respectively, of the total types of effects reported (Table 1). More than 34% of reported effects were mentions or brief descriptions of change. Although such results were not quantitative, the information provided aids in better understanding the global impacts of improved forages.

Earlier studies tended to report outcomes rather than impacts. The progression of extending analysis to longer-term impacts could be a consequence of increasing scientific capacity, availability of new assessment methods and policy priorities to understand larger-scale effects. In the face of multiple confounding factors, which hinder the substantiation of cause-and-effect arguments, studies are increasingly using mixed quantitative and qualitative methods, such as detailed narratives or diagrams of causal impact pathways, which typically acknowledge a broader array of effects (e.g. Cramb 2000; Pathak et al. 2004; Connell et al. 2010; Ayele et al. 2012). Nevertheless, fewer than 15% of studies were conducted independently of personnel affiliated with the program or project. Limited collaboration with evaluation experts and organizations may have prevented the use of new assessment methods and approaches.

Analyses of economic impacts employed inconsistent estimation methods and assumptions, thereby preventing a valid summation of total economic benefit of the studies. Review of economic impacts reported within the sample revealed 9 critical methodological shortcomings, many of which have been highlighted in other meta-analyses of economic benefits (Raitzer 2003; Raitzer and Lindner 2005; McClintock and Griffith 2010):

1. Estimates were based on the results employing different estimation methods, which include economic surplus models, cost-benefit accounting or unsubstantiated expert opinion.

2. Estimates of economic impacts represented different periods of time. Benefits were reported as annual estimates or the net present value (NPV) that represented different multi-year periods. Moreover, different rates (5 and 10%) were used to discount the future value of benefits, thereby substantially affecting the magnitude of NPV estimates.

3. Economic impacts were reported in terms of gross economic benefit or net of costs.

4. Costs were inconsistently defined across the studies. Reported costs included research and development (R&D), training and education (T&E) and adoption. R&D and T&E costs largely pertain to public sector organizations that finance such activities (though private companies produce and market seeds). Estimation of these costs often requires the use of numerous assumptions regarding staff time and other investments attributable to an improved forage. Meanwhile, farmers face a variety of costs related to adoption of technology. Such private costs include those pertaining to: (i) working capital associated with planting improved forages and purchasing more animals; (ii) capital investments such as infrastructure (e.g. corrals, barns, fencing); and (iii) opportunity costs of land and labor. Opportunity costs of land could be significant, if land previously produced crops or generated positive environmental externalities (e.g. biodiversity, carbon storage, water flow regulation). Labor costs of innovation, such as those related to advancing, acquiring, adapting and/or sharing knowledge were not included. While some studies discussed and analyzed a portion of these costs, no study addressed all potential costs.

5. Descriptions and types of data on technology adoption were inconsistent. Studies exhibited wide variation with respect to geographic scope, intensity of use per farm and duration of use. More than 50% of studies reporting economic impacts did not use empirical data on which to base estimates of adoption of technology, but instead depended solely on expert opinions (Table 2).

6. Transparency in the documentation of analytical methods was not consistent across the studies.

7. In the face of inherent uncertainty of costs, adoption and discount rates, sensitivity analyses of changes in parameter estimates were rarely performed.

8. Despite many economic estimates representing largely ex-ante, or a combination of ex-post and ex-ante, time horizons, scenario analyses were not included to examine the effects of assumptions employed to represent future conditions (e.g. yield improvement, input and output prices, climate change). In addition, economic analyses of substitute inputs, such as feed grain concentrates, were not conducted.

9. Economic analyses emphasized production performance with little acknowledgement or discussion of the economic values derived from decreased risk of crop, food and income failures. Furthermore, benefits associated with enhanced environmental conditions/resilience and improved social well-being/security remain largely unrecognized.

Despite the biases and limitations inherent to the sample, large-scale economic impacts from grasses were evident in Latin America (Table 2). In contrast, impacts from grasses and legumes were more evenly reported from Africa, South-east Asia and Australia. Consequently, the traditional biological distinction between grasses and legumes was replaced with a producer/market contrast of smallholder local market versus largeholder national/international market. The economic benefits from new spittlebug-resistant *Brachiaria* varieties in Latin America were the largest reported (Rivas and Holmann 2004; Costa et al. 2009). Benefits resulting from *Stylosanthes* varieties resistant to anthracnose disease were less substantial, perhaps due to less rigorous adoption and economic impact analysis. Other large-scale economic impacts from grasses were realized in Australia (Chudleigh and Bramwell 1996). Economic benefits from some forage species were estimated in different years. Economic benefits of *Stylosanthes* and *Leucaena* reported in Australia point to expanding use and economic impact (Rains 2005; Shelton and Dalzell 2007). For *Stylosanthes* in Brazil, the estimated value of nitrogen in soils exceeded the value as a feed (Costa et al. 2009). Despite substantial investment and reported adoption in South-east Asia (Phaikaew et al. 2004; Guodao and Chakraborty 2005; Stür et al. 2007) and South Asia (Ramesh et al. 2005), only one empirical analysis of economic impact has been conducted in Indonesia (Martin 2010).

Table 2. Summary information: economic impacts of improved forages.

Country/ region	NPV[1] (MUS$ 2005)	Annual (MUS$ 2005)	Forage(s)	Area ($x10^3$ ha)	Reference	Type of adoption data
Smallholder / local market						
W. Africa	19 (96)[2]		*Stylosanthes guianensis, S. hamata*	19 (52)	Elbasha et al. 1999	Statistics & survey
W. Africa	46[2]		*Stylosanthes* spp., *Centrosema pascuorum, Aeschynomene histrix*	32	Tarawali et al. 2005	Stats & survey
W. Africa	491[2]		*Vigna unguiculata*	1400	Kristjanson et al. 2002	Stats, survey & modeling
Indonesia	1010		*Pennisetum, Gliricidia, Leucaena, Sesbania*	n.r.	Martin 2010	1/3 value of future cattle sales
Kenya		7.9	*Calliandra calothyrsus*	~82	Place et al. 2009	Survey
Uganda, N. Tanzania, Rwanda		2.2	*Calliandra calothyrsus*	~103	Place et al. 2009	Survey
India		?	*Stylosanthes* spp.	>250	Ramesh et al. 2005	Experts
Thailand		0.75	*Stylosanthes*	>300	Phaikaew et al. 2004	Experts
China		22	*Stylosanthes*	>200	Guodao and Chakraborty 2005	Experts
Largeholder/ national, international markets						
Australia	1387	37[3]	*Cenchrus ciliaris*	6915	Chudleigh and Bramwell 1996	Stats, experts & extrapolation
Australia	244	7[3]	*Stylosanthes* spp.	1154	Chudleigh and Bramwell 1996	Stats, exp. & extrap.
Australia	659	17[3]	All improved pastures	7772	Chudleigh and Bramwell 1996	Stats, exp. & extrap.
C. America, Mexico	1790	243[4]	*Brachiaria* spp.	3287	Holmann et al. 2004	Seed sales
Colombia, C. America, Mexico	4413	497	*Brachiaria* spp.	4429	Rivas and Holmann 2004	Seed sales
Mexico		41[4]	Improved forages & technology	n.r.	Espinosa García and Wiggins 2003	Experts
Australia		~0.9	*Clitoria ternatea*	100	Conway 2005	Experts
Australia		2	*Centrosema pascuorum*	5	Cameron 2005	Experts
Australia		22.4	*Stylosanthes scabra, S. hamata*	1500	Rains 2005	Experts
Australia		15	*Stylosanthes scabra, S. hamata*	1000	Noble et al. 2000	Stats, expert
Australia		15	*Leucaena leucocephala*	100	Mullen et al. 2005	Expert
Australia		69	*Leucaena leucocephala*	150	Shelton and Dalzell 2007	% cattle offtake
Brazil	6269	1826[5]	*Brachiaria brizantha* cv. Marandu	23621	Costa et al. 2009	Seed sales
Brazil		13.5[5]	Seed production	n.r.	Costa et al. 2009	Seed sales
Brazil	5749	772[5]	*Panicum maximum* cv. Tanzania	4746	Costa et al. 2009	Seed sales
Brazil	4499	1640[5]	*Panicum maximum* cv. Mombasa	10074	Costa et al. 2009	Seed sales
Brazil	7	1.7	*Stylosanthes capitata* + *S. macrocephala* (cv. Estilosantes Campo Grande)	200	Costa et al. 2009	Seed sales
Brazil		33	*Pueraria phaseoloides*	480	Valentim and Andrade 2005a	Expert
Brazil		4	*Arachis pintoi*	65	Valentim and Andrade 2005b	Expert
USA		7	*Arachis glabrata*	8	Williams et al. 2005	Expert
USA		2.4[6]	*Aeschynomene americana*	65	Sollenberger and Kalmbacher 2005	Expert
USA		0.5[6]	*Desmodium heterocarpon*	14	Sollenberger and Kalmbacher 2005	Expert

[1]NPV = Net present value; [2]Net costs of RDTE and adoption (establishment and additional cattle); [3]Break-even cost to prevent negative impact from forage plants, being annual cost to reduce NPV of benefits to zero; [4]50% adoption rate assumption; [5]Estimate of final year of seed sale data (2006); [6]Estimates from Shelton et al. (2005).

Inquiry into environmental benefits of improved forages increased in sophistication from their on-farm productivity changes to include quantitative inquiry into nutrient cycling (Rao et al. 1996), direct seeding of crop-pasture rotations (Embrapa 2004), trade-offs between the use of forage legumes as fodder or green manure (Quintero et al. 2009a), conservation agriculture (Landers 2007; Kassam et al. 2010; Silici 2010) and the co-benefits associated with integrated management of striga weeds, insect pests and soil health (Khan et al. 2011). Analyses also expanded to examine off-farm impacts associated with environmental benefits of reduced erosion and downstream sedimentation and pollution (Quintero et al. 2006, 2009b; White et al. 2007) and carbon and biodiversity benefits from silvopastoral systems (Pagiola et al. 2007). Each of these analyses examined the effects of comprehensive farm management, which typically contains a component of improved forages. In addition, reporting of carbon storage and associated climate change mitigation continues to expand from analyses of deep-rooting *Brachiaria* grasses in Colombia (Fisher et al. 1994) to Brazil (Pinto et al. 1996; Tarre et al. 2001; Silva et al. 2004; Fisher et al. 2007; Marchão et al. 2009; Tonucci et al. 2011), *Leucaena* in Australia (Shelton and Dalzell 2007) and grasslands in Latin America (Mannetje et al. 2008) and worldwide (FAO 2010; Peters et al. 2012).

Attributing some off-farm environmental impacts to improved forages can be tenuous. For example, the adoption of improved forages cannot be considered a sufficient condition to avoiding deforestation. Other factors affecting the conservation of forests, such as local and national policies and their enforcement, are also needed for forest protection. Nevertheless, the contribution of improved forages to intensification and land/forest saving can be considered a necessary condition. Serving as a logical narrative to substantiate a causal technology-forest link is that intensification enables similar quantities of livestock products to be produced on smaller land areas (White et al. 2001; Kaimowitz and Angelsen 2008; Ewers et al. 2009; Connell et al. 2010; Cohn et al. 2011). Despite the challenges of attributing “saved” areas to improved forages, the magnitude, importance and value of ecosystem services from these original land uses can be substantial. Even without including emissions from land use change, estimates of a plausible mitigation potential of livestock and pasture management options in mixed and rangeland-based production systems of the tropics could contribute approximately 4% of global agricultural GHG mitigation, with a corresponding economic value of approximately US$1.3 billion per year at a price of $20 per ton CO_2-equivalent (Thornton and Herrero 2010).

The most commonly reported social impacts were at the family level, with savings in family labor, especially that of women and children (e.g. Connell et al. 2010; Ahmed 2012; Maxwell et al. 2012), and family nutrition and food security (Kassa et al. 2000). At the organizational level, social benefits included increased farmer and stakeholder participation and capacities along links of the supply chain (Khanh et al. 2011; Shiferaw et al. 2011; Ayele et al. 2012; Stür et al. 2013). Measurement of larger social impacts remains difficult, since many factors are likely to affect the functioning and status of political processes, national security, equity and well-being. Although estimates of economic benefits were disaggregated according to wealth/poverty by Rivas and Holmann (2004) and showed substantial purchasing power benefits accruing to the less-wealthy consumers of animal products, notions of development and associated social benefit are often considered to contain aspects of increased local capacity to achieve impact – not merely the results of technological change. In order to address measurement and valuation challenges that come with broader definitions of social benefit, quantitative analytical methods are being combined with or complemented by qualitative methods. Such analyses are part of a new breed of impact analyses that increasingly recognize processes of social change along the entire forage-livestock supply chain, from inputs and cultivation to feeding and marketing (Connell et al. 2010; Shiferaw et al. 2011; Ayele et al. 2012; Stür et al. 2013).

Conclusion

Improved grass and legume forages have generated substantial impacts across uncountable social, economic and environmental landscapes. Past claims that the adoption of improved forages, especially legumes, is relatively poor across all tropical farming systems (Squires et al. 1992; Thomas and Sumberg 1995; Pengelly et al. 2003) may, however, continue to echo in some regions. Despite a broadening of inquiry to include outcomes identifying and describing a larger diversity of impacts, the limited sample of studies was probably biased with a tendency to report only larger, relatively homogeneous impacts, which are easier to measure. Consequently, impacts highlighted above are conservative and represent a fraction of the global impacts.

Impact evaluation continues to evolve in an attempt to improve aid and development processes (Stern et al. 2012). Results from the systematic meta-analysis of impact-related documents reveal how efforts changed to better understand cause-and-effect relationships between RDTE activities and impacts. Such an evolution corresponds to 3 general prescriptive approaches associated with the theory of evaluation that focus on: (i) analytical methods and experimental design; (ii) human and social values used in conducting evaluations; and (iii) users of the results of the evaluation (Alkin 2012). With regards to methods, the sample of impact-related documents shows increasing efforts to expand forage RDTE to address the performance of entire livestock supply chains. Furthermore, more analyses are recognizing and attempting to evaluate environmental and social benefits. This combination of expanded inquiry, in terms of extent and breadth of impact, is improving our knowledge of how forages affect change processes. Regarding human and social values, assessments of forage RDTE increasingly include stakeholder narratives. Such documentation efforts not only provide valuable contextual perspectives, but they also help to improve quantitative impact analyses by substantiating causal arguments of change. As for the use of results, many evaluations of impact are expanding the influence of forages by affecting policy decisions on research, development and conservation investment − ranging from site-specific to global contexts. Although the direct representation of many impact studies may be limited, generalizable accounts of lessons learned can help inform such decisions. Furthermore, additional inquiry into informational needs of diverse investors, from farmers to international organizations, can improve communication and targeting of improved forages, and thereby help achieve widespread beneficial impacts on social, economic and environmental landscapes.

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Molecular genetic variability, population structure and mating system in tropical forages

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Abstract

Microsatellite (SSR) markers were developed for the following tropical forage species, using accessions available from the plant genetic resources (PGR) collections held by EMBRAPA (Brazilian Agricultural Research Corporation): *Brachiaria brizantha*, *B. humidicola*, *Panicum maximum*, *Paspalum* spp., *Stylosanthes capitata*, *S. guianensis*, *S. macrocephala*, *Calopogonium mucunoides* and *Centrosema* spp. The markers were used to analyze population structure and genetic diversity, evolution and origin of the genetic variability in the center of origin, mating systems and genetic resources in EMBRAPA's germplasm bank. The results shed light on the amount of genetic variation within and between populations, revealed the need in some cases for further plant collection to adequately represent the species in PGR collections, allowed us to assemble core collections (subsets of the total collections) that should contain most of the available diversity and (in the case of the legumes) showed the need to avoid unwanted outcrossing when regenerating conserved material. The data will allow plant breeders to better select accessions for hybrid production, discriminate between genotypes and use marker-assisted selection in breeding programs. Our results will also underpin the construction of genetic maps, mapping of genes of agronomic interest and numerous other studies on genetic variability, population structure, gene flow and reproductive systems for the tropical forage species studied in this work.

Resumen

Se desarrollaron marcadores microsatélite (SSR) para las siguientes especies forrajeras tropicales, usando accesiones de germoplasma disponibles en las colecciones de recursos genéticos mantenidas por EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária): *Brachiaria brizantha*, *B. humidicola*, *Panicum maximum*, *Paspalum* spp., *Stylosanthes*

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capitata, *S. guianensis*, *S. macrocephala*, *Calopogonium mucunoides* y *Centrosema* spp. Los marcadores se usaron para analizar la estructura de las poblaciones y su diversidad genética; la evolución y el origen de la variabilidad genética en los respectivos centros de origen; los sistemas reproductivos; y los recursos genéticos en el banco de germoplasma de EMBRAPA. Los resultados arrojaron luz sobre la magnitud de la variación genética dentro de y entre poblaciones; revelaron la necesidad, para algunas especies, de incrementar las colecciones de germoplasma y así obtener una adecuada representación de las especies en las colecciones de recursos genéticos; permitieron establecer colecciones núcleo (subconjuntos de colecciones grandes), representativas de la diversidad genética disponible en las respectivas colecciones completas; y mostró, para las leguminosas, el riesgo de cruzamientos no deseados al multiplicar semilla de las accesiones conservadas. La información obtenida facilitará a los fitomejoradores la selección de accesiones para la generación de híbridos, la distinción entre genotipos, y el uso de la selección asistida por marcadores en programas de fitomejoramiento. Los resultados también ayudarán para la construcción de mapas genéticos, el mapeo de genes de interés agronómico y otros estudios tales como la variabilidad genética, la estructura de poblaciones, el flujo de genes y el sistema reproductivo, para las especies de forrajes tropicales estudiadas en este trabajo.

Introduction

Grasses from the genera *Brachiaria*, *Panicum* and *Paspalum* and legumes from the genera *Stylosanthes*, *Calopogonium* and *Centrosema* are among the most widely sown tropical forage species. The genus *Brachiaria* comprises about 100 species, notably palisade grass (*B. brizantha* syn. *Urochloa brizantha*) and Koronivia grass (*B. humidicola* syn. *Urochloa humidicola*). *B. humidicola* is particularly recognized for its tolerance of poorly draining soils, seasonal flooding and infertile acid soils (Miles et al. 1996). Guinea grass (*Panicum maximum*) has been widely introduced and exploited in most tropical and subtropical countries because of its high yield and nutritional content and its wide adaptability to diverse ecological niches. The genus *Paspalum* includes around 350 species, most of which are native to tropical/subtropical America (Zuloaga and Morrone 2005); approximately 220 species are found in Brazil (Valls 1987; Rua 2006). Several species are of economic importance for forage, turf and ornamental purposes in different parts of the world. Bahia grasses (*P. notatum*, Notata group) are particularly important and are widely used for forage, mainly in the southern USA. *P. atratum* (Plicatula group) is of growing interest for use as forage in areas subjected to periodic flooding.

All tropical forage grasses studied are polyploid and reproduce through aposporous apomixis with pseudogamy (Miles et al. 1996; Savidan 2000) but sexual genotypes have been identified in the germplasm collections (Nakajima et al. 1979; Jungmann et al. 2009a).

Stylosanthes is globally the most extensively sown tropical forage legume genus and Brazil (with 45% of its 48 species) is its major center of diversity. Three species (*S. capitata, S. guianensis* and *S. macrocephala*) have been sown for forage in Brazil because of their ability to grow on acid soils and hold leaves during the dry season when the grasses usually dry. *Calopogonium mucunoides* is one of the most widely used legumes in Brazil. It has high nitrogen-fixing capability and moderate drought tolerance, and is cultivated in soils with low pH and low fertility. The genus *Centrosema* comprises about 34 species, including some that are adapted to poorly drained and seasonally flooded areas, acid and low-fertility soils (Keller-Grein et al. 2000). *C. molle* (often called *C. pubescens*) is used throughout the tropics as a cut-and-carry forage, protein bank and cover crop.

Most of Brazil's 172 million hectares of pastures have been sown to the aforementioned grasses, and tropical legumes are being introduced to improve both forage quality and soil fertility. The Brazilian Agricultural Research Corporation (EMBRAPA) has released several cultivars of *B. brizantha*, and maintains germplasm collections of all genera and species mentioned above as genetic resources for its plant breeding programs. Despite the importance of sown tropical forages for the Brazilian cattle industry and the extensive literature on genetic diversity of the species mentioned above, little information is available on the molecular genetic diversity to be found in the germplasm collections and on the mating systems of some of these species, hindering advances through plant breeding programs.

In this paper we describe the results of a joint effort between EMBRAPA and the University of Campinas to generate basic molecular genetic information for these species. We have used simple sequence repeats (SSR) to evaluate the genetic diversity and population structure of the Brazilian germplasm collections of all species cited above and to estimate mating systems for some of them.

Methods

Material and methods can be found in the references listed in Table 1.

Results

The numbers of SSR (Simple Sequence Repeat) markers developed and the analyses performed for each grass and legume species are shown in Table 1, together with the relevant references.

In *B. brizantha*, the genetic similarities among 172 accessions and 6 cultivars of this species were estimated using 20 SSR markers. Similarity index values ranged from 0.40 to 1.00. A Bayesian analysis performed using the STRUCTURE software revealed the presence of 3 clusters with different allelic pools. This analysis is valuable for the performance of crosses to explore heterosis; however, the mode of reproduction of the accessions and ploidy barriers constrain effective exploration. A grouping analysis using the neighbor-joining method was consistent with the STRUCTURE analysis, and a combination approach suggested that this germplasm collection exhibits limited genetic variability despite the presence of 3 distinct allelic pools. There was no correlation between the genetic and geographic distances of the accessions.

The evaluation of the *B. humidicola* germplasm (60 accessions) with 27 SSR markers revealed a highly structured collection in 4 major clusters. The sole sexual accession did not group with any of the clusters. Genetic dissimilarities did not correlate with either geographic distances or genetic distances inferred from morphological descriptors. Additionally, the genetic structure identified in this collection did not correspond with differences in ploidy level. Alleles exclusive to either sexual or apomictic accessions were identified and the association of these loci with apospory is being studied.

The germplasm of *P. maximum* comprised 396 accessions that were evaluated with 30 SSR markers for genetic diversity. Four genetic clusters were identified in the collection using STRUCTURE analysis, and these results were confirmed using AMOVA (analysis of molecular variance). The largest genetic variation was found within clusters (65.38%). This study revealed that the collection of accessions from the *P. maximum* region of origin was a rich source of genetic variability. The geographical distances and genetic similarities among accessions did not indicate a significant association between genetic and geographical variation, supporting the natural interspecific crossing between *P. maximum*, *P. infestum* and *P. trichocladum* as the origin of the high genetic variability and the existence of an agamic complex formed by these 3 species.

In *Paspalum*, new specific SSR markers were developed for *P. notatum* and *P. atratum*, and used to evaluate a germplasm collection of 214 accessions of 35 different species. Based on distance-based methods and a Bayesian approach, the accessions were divided into 3 main species groups, 2 of which corresponded with the previously described Plicatula and Notata *Paspalum* groups. In more rigorous analyses of *P. notatum* accessions, the genetic variation evaluated using 30 SSR loci revealed 7 distinct genetic groups and a correspondence of these groups with the 3 botanical varieties of the species (*P. notatum* var. *notatum*, *P. notatum* var. *saurae* and *P. notatum* var. *latiflorum*).

For *S. capitata*, 192 accessions were analyzed using 15 SSR markers and the STRUCTURE analysis grouped the accessions into 4 distinct genetic clusters with a Nei's GST value of 11%. The average genetic distance was 0.50. The low genetic diversity between groups (11%) was expected because most of the accessions were collected in only 2 Brazilian States and might not represent the diversity in natural populations of the species. In *S. macrocephala*, 134 accessions were analyzed using 13 SSR markers. The STRUCTURE analysis grouped these accessions into 5 distinct clusters with a Nei's GST of 27%. The average genetic distance between accessions was 0.54. Accessions of *S. macrocephala* collected in the State of Bahia were distributed in all 5 clusters and 1 cluster was formed mostly by accessions collected in this State. As the Bahia State cluster showed the highest genetic diversity, we hypothesized that this State might be the center of origin of the species and suggested that more collections should be done to confirm our hypothesis. *S. guianensis* is the most diverse species of *Stylosanthes* and its taxonomic classification is controversial. We analyzed 150 accessions of *S. guianensis* using 20 SSR markers and the STRUCTURE analysis grouped these accessions into 9 groups that in general correlated with the taxonomic classification (Costa 2006). The genetic diversity among the groups obtained with STRUCTURE as shown by Nei's GST was 46%, while the average genetic distance was 0.66, which is in agreement with the high phenotypic diversity observed in this species. For all *Stylosanthes* species, some differences were observed between the clusters obtained with STRUCTURE and the one obtained based on genetic distances and NJ clustering. This is probably due to the different methods and assumptions of both approaches.

Table 1. New polymorphic SSR markers developed and analyses performed in each species.

Group	Species	Polymorphic SSR markers developed	Analyses performed	References
Grasses	*Brachiaria brizantha*	28	SSR transferability / genetic diversity	Jungmann et al. 2009a; Vigna et al. 2011a
	B. humidicola	65	SSR transferability / genetic diversity	Jungmann et al. 2009b, 2010; Vigna et al. 2011b
	Paspalum spp.	17	SSR transferability / genetic diversity	Cidade et al. 2013
	Paspalum notatum	11	SSR transferability	Cidade et al. 2009
	P. atratum	19	SSR transferability	Cidade et al. 2010
	Panicum maximum	86	SSR transferability / genetic diversity	Sousa et al. 2011a; 2011b
Legumes	*Calopogonium mucunoides*	23	Genetic diversity / core collection / mating system	Sousa et al. 2010; 2012
	Centrosema molle	26	Mating system / SSR transferability	Sousa et al. 2009; 2011c
	Stylosanthes capitata	18	Genetic diversity / core collection / mating system	Santos et al. 2009a; Santos-Garcia et al. 2011, 2012b
	S. guianensis	20	Genetic diversity / mating system	Santos et al. 2009b; Santos-Garcia et al. 2011, 2012a
	S. macrocephala	13	Genetic diversity / core collection	Santos et al. 2009c; Santos-Garcia et al. 2012b

In *C. mucunoides*, 195 accessions were analyzed using 17 SSRs and according to the STRUCTURE analysis, these accessions were grouped into 6 genetic clusters that correlated with their origin. The average genetic distance was 0.42 and the NJ clustering based on the pairwise distances was in agreement with the STRUCTURE analysis.

A total of 26 SSRs from *C. molle* were used to assess the marker transferability to 11 other *Centrosema* species. Data obtained with the transferable markers were used to study the genetic relationships among the 12 *Centrosema* species. Nineteen of the 26 SSRs amplified in at least one *Centrosema* species and were able to show polymorphism among accessions of these species. The 12 species were grouped in 3 distinct clusters based on the STRUCTURE analysis that correlated with the NJ clustering. Based on the genetic data, collection trips can be planned to improve the genetic diversity of the collections and crosses can be planned to explore genetic diversity and, potentially, heterosis between the genetic groups.

Stylosanthes, Calopogonium and some *Centrosema* species are often considered to be predominantly self-pollinating but outcrossing was previously observed by phenotypic analysis. By using SSR data and progeny analysis to estimate the outcrossing rate in *S. capitata*, *S. guianensis*, *Centrosema molle* and *Calopogonium mucunoides,* we have shown that all species present a mixed mating system with predominance of autogamy, with outcrossing rates between 16% in *C. mucunoides* and 31% in *S. capitata*. Seed multiplication in the germplasm collections of these species has ignored the existence of outcrossing and we consider that this might have caused contamination of accessions.

Finally, for *S. capitata, S. macrocephala* and *C. mucunoides* we have assembled core collections to represent 100% of the genetic diversity estimated with molecular markers. In *S. capitata*, only 13 accessions could represent the same genetic diversity present in the 192 accessions studied, whereas the genetic diversity of the 134 *S. macrocephala* accessions could be represented by as low as 23 accessions. In *C. mucunoides*, 15 accessions could represent the genetic diversity observed in the 195 accessions of the collection. In breeding programs, priority can be given to the evaluation of the core collections and the whole collection can be used as a backup resource, reducing costs and time for analysis.

Discussion and Conclusion

Molecular techniques have been used to study genetic diversity in tropical forage species (notably *Stylosanthes* and its major pathogen, *Colletotrichum gloeosporioides*) for about 20 years, but the use of SSR markers is relatively new, particularly in apomictic grasses. This research was the first systematic attempt to use microsatellite analysis on a wide range of species that are significant in Brazil; it represents a significant collaboration between EMBRAPA and the University of Campinas. Valuable information on the genetic diversity and the mating systems of the studied species was obtained. This information together with the developed SSR markers can be used directly in plant breeding programs and the study of natural populations. Additionally this study can be used as a base for future research that can further improve the use of the available genetic resources and accelerate the genetic improvement of these species. This should include the development of mapping populations and QTL mapping of agronomic traits such as disease resistance and biomass production.

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Brachiaria hybrids: potential, forage use and seed yield

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Abstract

A brachiaria breeding program initiated in 1988 at CIAT (Centro Internacional de Agricultura Tropical, Cali, Colombia) combined desirable attributes found in accessions of *Brachiaria brizantha* and *B. decumbens*. Three apomictic hybrids have been released (cvv. Mulato, Mulato II and Cayman). Mulato showed agronomic potential but seed yields were low. Trials in Central America demonstrated the superiority of Mulato II, a vigorous grass with deep and branched roots, giving it excellent drought tolerance in the Brazilian Cerrado and Mexico, plus outstanding nutritional value. Following trials in Mexico and Thailand, evaluating 155 new hybrids for 7 years, cv. Cayman was released due to strong waterlogging tolerance. Research on production, quality and seed yields of brachiaria hybrids in Asia, the Americas and Africa from 2003 to 2013 is summarized in this paper.

Resumen

Un programa de mejoramiento de brachiaria iniciado en 1988 en el CIAT (Centro Internacional de Agricultura Tropical, Cali, Colombia) combinó los atributos deseables encontrados en accesiones de *Brachiaria brizantha* y *B. decumbens* y condujo a la liberación de 3 híbridos apomícticos (cvs. Mulato, Mulato II y Cayman). Mulato mostró potencial agronómico pero la producción de semilla fue baja. Ensayos conducidos en Centroamérica demostraron la superioridad de Mulato II, un pasto de crecimiento vigoroso y con raíces profundas y ramificadas que proporcionan excelente resistencia a la sequía en el Cerrado brasileño y México. Mulato II también tiene un valor nutritivo sobresaliente. Después de ensayos en México y Tailandia, evaluando 155 nuevos híbridos durante 7 años, cv. Cayman fue liberado debido a su alta tolerancia de inundación. En este trabajo se resume la investigación conducida en Asia, las Américas y África durante 2003−20013, sobre la producción, calidad y rendimiento de semilla de híbridos de brachiaria.

Introduction

The registration of Mulato hybrid brachiaria (*Brachiaria ruziziensis* x *B. brizantha*) by Grupo Papalotla in 2004 (Miles et al. 2004) and the granting of Plant Variety Rights (PVR) in 2002, marked a significant breakthrough for tropical perennial forage cultivars. Until 2001, *Brachiaria* spp. cultivars used commercially were derived without genetic modification directly from natural germplasm collected in Africa or selected fromgermplasm collections in Australia and tropical America. The development in Belgium of a tetraploidized sexual ruzi grass (*B. ruziziensis*) (Swenne et al. 1981) and further studies by Ndikumana (1985) and Valle et al. (1994), led to Embrapa (Empresa Brasileira de Pesquisa Agropecuária) providing tetraploid, sexual ruzi grass to CIAT in 1988. The breeding program at CIAT combined desirable attributes found in accessions of *B. brizantha* and *B. decumbens.*

In 2001, Grupo Papalotla, a Mexican seed company, was granted the exclusive rights worldwide, to produce, research and commercialize the first generation of brachiaria hybrids developed by CIAT from 2001 to 2010. Mulato showed considerable agronomic potential but seed yields were low (Hare et al. 2007a). Fortunately, trials in tropical America found Mulato II had excellent drought tolerance in the Brazilian Cerrado and Mexico and its seed yields in Thailand were between 60 and

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100% higher than the highest seed yields of Mulato (Hare et al. 2007b; 2007c). From 2003 to 2008, further detailed studies were conducted in Mexico and Thailand on 155 new hybrid brachiaria lines resulting in 4 lines, BRO2/1718, BRO2/1752, BRO2/1794 and BRO2/0465 being granted PVR. BRO2/1752 produced similar dry matter (DM) yields to Mulato II, and in trials in Mexico demonstrated good waterlogging tolerance.

This paper summarizes research on production, quality and seed yields of brachiaria hybrids in Asia, the Americas and Africa from 2003 to 2013.

Regional evaluations and research

Asia

Field studies at Ubon Ratchathani University, Thailand (15° N), between 2003 and 2007, showed that Toledo palisade grass (*Brachiaria brizantha*), Mulato and Mulato II produced more total DM and leaf DM, particularly during the dry season, than other brachiaria grasses (Hare et al. 2009), Mulato II also producing significantly more leaf than Toledo palisade grass and Mulato. It is the production of green leaves that makes Mulato II a particularly attractive forage for livestock (Mutimura and Everson 2012).

From 2005 to 2008, 15 hybrid brachiaria lines were evaluated at Ubon Ratchathani University for DM production, quality (in terms of leaf percentage) and seed production (Table 1). Only 3 lines, BRO2/0465 and MXO2/1263 in the wet season in Trial 1 and cv. Cayman in the wet season in Trial 2, produced more DM than Mulato II. BRO2/0768 was the only line that produced a higher percentage leaf DM than Mulato II, in the wet season of Trial 1 and in the dry season of Trial 2 (Table 1). BRO2/1794 produced significantly more seed than all other hybrids in both trials. Mulato II, Cayman, BRO2/0465, BRO2/0768 and BRO2/1718 produced similar seed yields, averaging 150 kg/ha.

Table 1. Average wet season (May−October) and dry season (November−April) dry matter yields, leaf percentage and pure seed yields of hybrid brachiaria lines from 2005 to 2008 in Ubon Ratchathani, Thailand.

Hybrid line	Dry matter yields				Leaf percentage				Pure seed yields	
	Trial 1		Trial 2		Trial 1		Trial 2		Trial 1	Trial 2
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry		
	(kg/ha)				(%)				(kg/ha)	
Mulato II	8674	2886	10566	2801	74	91	72	83	129	141
Cayman	9252	2837	12597	3059	62	84	55	79	136	108
BRO2/0465	11477	3050	11594	2861	68	86	66	78	166	144
BRO2/1794	9420	2655	11149	2901	58	81	55	77	277	244
BRO2/1718	8569	2517	10394	3318	69	87	67	76	172	197
BRO2/1747	8728	2950	10539	2752	61	83	56	77	88	107
BRO2/1245	8889	3348	10399	2773	68	79	64	79	76	37
BRO2/0771	9032	3447	10866	2955	74	82	71	82	52	76
BRO2/0799	9282	2646	10734	2492	66	86	63	83	114	90
BRO2/1372	7602	2966	8722	3212	70	83	66	77	36	22
BRO2/1728	9515	2966	10558	2834	61	83	56	80	99	79
BRO2/0768	8542	3219	10653	3078	80	90	74	88	142	123
BRO2/1485	8901	3287	11790	2984	71	80	65	80	39	55
BRO2/1452	7451	2343	7667	2126	67	79	67	80	85	113
MXO2/1423	8809	3090	11076	2624	62	78	56	75	61	24
MXO2/1263	10853	3178	10859	2670	66	83	66	80	84	83
LSD ($P<0.05$)	2060	751	1349	569	4.9	4.8	3.8	4.4	69	64

From 2008 to 2011, a further 28 hybrid brachiaria BRO6 lines were evaluated alongside Mulato II, *B. brizantha* cvv. Marandu and Toledo, and the promising BRO2 lines, 1794, 0465, 1718 and 1372. None of the BRO6 lines produced significantly more DM than Mulato II. Mulato II produced significantly higher percentage of leaf DM than all other lines in both wet and dry seasons, except for BRO6/1922 in the dry season. Mulato II and BRO2/1718 produced significantly more seed than all the BRO6 lines. In this trial, BRO2/1794 produced less seed than Mulato II, BRO2/1718 and BRO2/0465.

Field trials conducted by the Thailand Department of Livestock Development (DLD) from 2004 to 2006 in northern Thailand (18° N; 319 m asl), showed that Mulato and Mulato II produced significantly higher DM yields than ruzi grass, but ruzi grass produced 3−4 times the seed yields of Mulato and Mulato II (Phunphiphat et al. 2007). In the dry season, Mulato and Mulato II produced 60% more DM than ruzi grass. In seed production trials conducted by DLD from 2007 to 2008 in northern Thailand at the same location, Mulato II produced significantly more seed (500 kg/ha) than Cayman (290 kg/ha), BRO2/1718 (250 kg/ha), BRO2/1794 (390 kg/ha), BRO2/0465 (145 kg/ha), Mulato (220 kg/ha), 10 MX02 lines (180 kg/ha), 8 BRO4 lines (157 kg/ha) and 20 BRO5 lines (40 kg/ha).

Further trials conducted in Thailand by DLD showed that leaf DM digestibility of Mulato II and 3 hybrid brachiaria lines averaged 75% and stem DM digestibility averaged 60%.

Research during 2005−2007 at the Tropical Pasture Research Center, Chinese Academy of Tropical Agricultural Sciences (CATAS) in Hainan, China (19° N), on acid soils (pH 4.9−5.5), with annual rainfall of 1600 mm, found that Mulato and *B. brizantha* cv. Reyan 6 produced significantly more DM than Mulato II, *B. decumbens* and *B. humidicola*, even though Mulato II had a higher leaf:stem ratio. In Guangxi, China (25° N), Mulato II was found to be very susceptible to winter cold, with only 5% plant survival in May, following 2 days below 0 °C in December. This was similar to results from Florida, USA, where Mulato II pastures are considerably weakened by winter temperatures below 0 °C and recover slowly in spring (Vendramini et al. 2012).

The Americas

Seed production trials were conducted at Warnes, Bolivia (19° S; 423 m asl), from 2007 to 2010 on hybrid brachiaria lines BRO5, MXO2 and BRO4. Over 4 years, only 1 new line, MXO2/2552, produced more seed than Mulato II, due to a high seed yield in 2010. In 2009, seed yields were low overall, due to dry conditions during anthesis and seed set. Further trials from 2008 to 2010 at the same location, found that none of the BRO6 lines produced significantly more seed than Mulato II.

In Mexico, evaluations on the hybrid brachiaria collections commenced in 2005 at Santa Elena, Oaxaca (16° N; 4−8 m asl; 800−1200 mm annual rainfall; 6−8 months dry season) on very sandy soils, with low organic matter, N and P and a pH of 5.6. To date, studies have been conducted on 15 BRO2 lines, 38 BRO5 lines, 28 BRO6 lines and 74 BRO9 lines.

Within the BRO2 lines, 4 lines (1718, 0465, 1752 and 1794) were selected from the Mexico and Thailand studies and granted PVR. Further trials with BRO2/1752 showed that it displayed superior waterlogging tolerance compared with Mulato II and 38 BRO6 lines. It was subsequently released as cultivar Cayman.

The 3 released cultivars (Mulato II, Cayman and BRO2/0465) produced 30 tonnes, 21 tonnes and 18 tonnes DM/ha respectively, 120 days after cutting. After 30 days subsequent regrowth, Cayman and BRO2/0465 produced significantly more DM (700−740 kg DM/ha) than Mulato II (100 kg DM/ha).

In the USA, forage production and nutritive value of Mulato II and Cayman under grazing were studied at the University of Florida, Beef Research Unit (29° N; 45 m asl) from 2010 to 2011 (João Vendramini pers. comm.). Under intensive grazing every 2 weeks, both hybrids had significantly higher percentage of leaf (70−80%), crude protein (19−21% in leaves, 10−12% in stems) and in vitro organic matter digestibility (73−75% leaf, 59−61% stem) than under grazing every 4 and 6 weeks, similar to the high quality achieved under cutting in Thailand.

Africa

Improved hybrid brachiaria grasses were evaluated in Rwanda (2° S) at a lower rainfall site (1400 mm/year) and a higher rainfall site (1800 mm/year) that had high soil Al levels (47 meq/100g of soil), against local signal grass (*B. decumbens*) and a naturalized buffel grass (*Cenchrus ciliaris*). At the lower rainfall site, BRO2/1485 and the local signal grass produced more DM than buffel grass and the brachiaria hybrids BRO2/0465 and BRO2/1452. When asked to rank the grasses, farmers selected Mulato II as the preferred cultivar at both sites, because of its year-round production of green forage without any input of fertilizer, high DM production, palatability, drought tolerance, quick regrowth, persistence, perenniality, and being easy to cut and carry (Mutimura and Everson 2012).

Commercial pasture development

In Vietnam, Mulato II is used as cut-and-carry forage by dairy farmers because of its high protein levels, palatability and digestibility (Raf Somers pers. comm.). In Thailand, Laos and Malaysia, expansion of planting of Mulato II has been slow, due to the strong competition from far cheaper ruzi grass seed produced in Thailand.

In the Pacific region, since 2007 over 10,000 hectares of Mulato II pastures have been established in Vanuatu, where it is primarily used for beef cattle grazing. In

Africa, Mulato II pastures have been established on nearly 1000 smallholder farms in Kenya, Tanzania and Ethiopia and on larger farms in Congo and Uganda. In Rwanda, 50 hectares of Mulato II have been planted to provide vegetative planting material for famers. In Burundi, Mulato II is currently being evaluated on research stations.

In the Americas, nearly 200,000 hectares of Mulato II pastures have been established since 2005 for both dairy and beef cattle grazing.

Commercial seed production

Mulato II and Cayman seed produced in Thailand and Laos is harvested by hand. In Thailand, the seed is ground-swept and in Laos it is harvested by knocking the seed from seedheads. The seed is hand-cleaned by the farmers and then acid-scarified in Thailand to reach 98% purity, 90% viability and 80% germination.

In tropical America, seed is produced only in Mexico (Mulato II and Cayman) and Brazil (Mulato II), with all seed being ground-swept using machinery (Pizarro et al. 2010). The seeds are cleaned and acid-scarified at a central location in each country.

Conclusion

In many trials in Asia, Africa and the Americas, the hybrid brachiaria cultivars have consistently produced significantly more dry-season forage than other tropical grasses and other brachiaria grasses. Because of its excellent drought tolerance, year-round production of green forage, leafiness, high digestibility and higher crude protein levels than most other tropical grasses, cv. Mulato II is a valuable grass for improved beef and dairy production. Cultivar Cayman also exhibits many of the qualities of Mulato II, and its outstanding waterlogging tolerance adds a new dimension to the hybrid brachiaria collection. Research is now continuing to evaluate the new BRO9 collection to select lines with high seed yields and high forage quality, as well as persistence and tolerance to grazing, or outstanding drought tolerance, or upright form with high DM yields for cut-and-carry forage systems, or good shade tolerance.

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Animal production from new *Panicum maximum* genotypes in the Amazon biome, Brazil

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Keywords: Guineagrass, forage breeding, liveweight gain, carrying capacity, forage allowance.

Introduction

The *Panicum maximum* breeding program coordinated by the Brazilian Agricultural Research Corporation (Embrapa) has been evaluating and selecting genotypes under different soil and climatic conditions, with the objective to release new cultivars adapted to the diverse regions of Brazil. For the Amazon biome, small-plot experiments carried out in Acre between 2003 and 2005 allowed the selection of some promising genotypes (Valentim and Andrade 2005; Valentim et al. 2006; Andrade and Valentim 2009), with higher potential for forage growth than cultivars on the market. The objective of this work was to compare 2 new *P. maximum* genotypes with cv. Tanzânia in relation to carrying capacity and animal performance, when managed under rotational stocking in the Amazon biome.

Methods

This grazing experiment was carried out between October 2010 and September 2012 in Rio Branco, State of Acre, Brazil. The local climate is Am according to Köppen-Geiger classification and the soil is classified as Haplic Plinthosol. Three *Panicum maximum* genotypes (accession PM32, hybrid PM46 and cv. Tanzânia) were tested in a randomized block design with 2 replications. Each experimental unit was 1.0 ha divided into 3 paddocks of 0.33 ha, managed under a 42-day rotation with a rest period of 28 days. Three Aberdeen Angus x Nelore heifers were used as tester animals. Additional animals (regulators) were placed in or removed from each paddock according to pasture height targets (PM32 and Tanzânia, pre-grazing 70−75 cm and post-grazing 30−35 cm; PM46, pre-grazing 50−55 cm and post-grazing 25−30 cm). Pastures were fertilized with 240 kg/ha of reactive rock phosphate, 100 kg/ha of simple superphosphate and 50 kg/ha of potassium chloride at establishment, and 300 kg/ha of urea was applied annually.

Animals were weighed every 28 days after a fasting period of 16 h. Carrying capacity in AU (450 kg) per ha was calculated according to the mean weight and number of animals/days in each experimental unit. Animal production (kg/ha of live weight) was calculated based on animal daily gain and the number of animals/day in each experimental unit. Green forage allowance was calculated for each grazing cycle, dividing the green dry matter (kg/ha) by stocking rate (kg/ha of live weight), as proposed by Sollenberger et al. (2005). Data obtained for the various stocking cycles were grouped into rainy (October−April) or dry (May−September) season and were analyzed according to a randomized complete block design with repeated measures in time, using a mixed model with the fixed effects of genotype, season and their interactions, and the random effect of block. Significant interactions ($P<0.10$) were conveniently broken down. Least squares means were compared using Fisher's protected LSD ($P<0.10$).

Results

There was a significant ($P<0.10$) genotype x season interaction for carrying capacity, with hybrid PM46 showing consistently a lower carrying capacity than the other genotypes during the rainy seasons (Table 1). The Plinthosol of the experimental area became waterlogged during the February−March period in both years and this decreased growth of hybrid PM46, which is highly intolerant of soil waterlogging (Andrade and Valentim 2009). To avoid stand loss of hybrid PM46, animals were removed for about 60 days each year and this lowered its carrying capacity during the rainy seasons.

The grazing management adopted, with adjustment of stocking rate based on predetermined pasture height goals, ensured a similar green forage allowance ($P>0.10$)

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for the genotypes throughout the experimental period (Table 1). Despite this, hybrid PM46 supported greater (P<0.10) daily liveweight (LW) gains, demonstrating its superior forage quality to the other genotypes. This is related to the plant architecture of this grass, with smaller plant size than traditional *P. maximum* cultivars like Tanzânia, maintaining a pasture structure more favorable to high forage intake, with higher percentages of green leaves and lower percentages of dead material (Farinatti et al. 2012).

The highest animal production (P<0.10) was obtained during the second rainy season, but it did not vary among genotypes (P>0.10), with an annual average of 847 kg/ha/yr of LW gain (Table 1). This rather high production per unit area is a consequence of the level of nitrogen fertilizer used (135 kg/ha/yr of N) and the climatic conditions of the region. Thus, the higher daily gains of the heifers on hybrid PM46 compensated for the lower carrying capacity of this pasture during the rainy seasons.

Table 1. Carrying capacity, forage allowance, animal performance and production of heifers (Aberdeen Angus x Nelore) on *Panicum maximum* genotypes under grazing during 2 years.

Genotype	2010 − 2011		2011 − 2012		Annual mean
	Rainy	Dry	Rainy	Dry	
		Carrying capacity (AU/ha)[2]			
PM46	2.69 Ba[1]	2.23 Ab	2.79 Ba	2.28 Ab	2.51
PM32	3.42 Ab	2.50 Ac	3.83 Aa	2.63 Ac	3.14
Tanzânia	3.30 Aa	2.30 Ac	3.45 Aa	2.54 Ab	2.93
Mean	3.14	2.34	3.36	2.48	2.86
		Green forage allowance (kg DM/kg LW)			
PM46	1.75	1.53	1.52	1.83	1.66 A
PM32	1.50	1.37	1.33	1.59	1.49 A
Tanzânia	1.60	1.69	1.43	1.50	1.56 A
Mean	1.61 a	1.53 a	1.42 a	1.64 a	1.55
		Animal performance (g/hd/d)			
PM46	718	646	682	639	672 A
PM32	520	533	559	510	532 B
Tanzânia	470	488	568	499	509 B
Mean	569 a	556 a	603 a	550 a	571
		Area production (kg LW/ha)			
PM46	455	400	485	384	862 A
PM32	434	390	558	389	886 A
Tanzânia	360	334	523	371	794 A
Mean	416 b	375 b	522 a	381 b	847

[1]Means followed by different letters, upper-case in columns and lower-case in rows, differ at P<0.10.
[2]AU = 450 kg live weight.

Conclusion

The *Panicum maximum* hybrid PM46 has greater potential for beef cattle production under grazing in the Amazon biome than accession PM32 and cv. Tanzânia, but it should be released for areas with well-drained soils.

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BRS Mandobi: a new forage peanut cultivar propagated by seeds for the tropics

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Keywords: *Arachis pintoi*, forage breeding, forage legume, mixed pastures.

Introduction

Forage peanut (*Arachis pintoi*) is a forage legume of relatively recent use in beef and dairy cattle feeding. Although it is native exclusively to Brazil, the first cultivar, Amarillo, was released in Australia in 1987. Forage peanut has a great number of favorable attributes for establishing grass-legume mixtures, which guarantee the persistence and high yields of high quality forage in cultivated pastures. Therefore, studies with this species have intensified in many tropical regions of the world (Assis and Valentim 2009). In the state of Acre, western Brazilian Amazon, 137,000 ha are cultivated with forage peanut, cv. Belmonte, benefiting thousands of producers, with an annual economic impact of USD 46M (Embrapa 2012). Social and environmental impacts resulting from the use of this legume are also highly positive. However, despite the success achieved in mixed pastures with forage peanut, expansion of the area sown has been relatively slow because vegetative propagation using stolons is labor-intensive and requires large quantities of limited vegetative material. In Brazil, Embrapa has coordinated the development of new forage peanut cultivars and their evaluation in different biomes. The objective of this work is to present a new cultivar of forage peanut propagated by seeds, developed by Embrapa for use in tropical regions, in partnership with Unipasto (Association for Promotion of Forage Breeding Research).

Methods

The Brazilian Forage Peanut Evaluation Network started in 1999 and included experiments in the states of Acre, Bahia and Distrito Federal. Development of the new cultivar was based on mass selection over 5 years, followed by evaluation of environmental adaptation in pure stands and in mixed pastures in the western Brazilian Amazon. During the evaluation process, selection was performed to improve speed of establishment, dry matter yield, nutritive value and seed production. Evaluations also occurred under rotational grazing in mixed pastures with *Cynodon nlemfuensis, Brachiaria brizantha* and *B. humidicola*, in intensive systems. The new cultivar was registered with the Ministry of Agriculture, Livestock and Food Supply of Brazil in 2008 and its protection process was finalized in 2011, after conducting tests for distinctness, homogeneity and stability. While developing the cultivar, the semi-mechanized seed production system of forage peanut was also established (Assis et al. 2011).

Results and Discussion

The new forage peanut cultivar, named BRS Mandobi, presents long and wide leaflets, with high intensity of bristles on the abaxial face of basal leaflets. The basal leaflet apex is acute, whereas the predominant form of the apical leaflet apex is obtuse and its form is obovate. Flowers are yellow and the hypanthium is long. Mandobi presents large stipules in its free portion, not welded to the petiole. Its fruits are also large, compared with cvv. Amarillo and Alqueire-1 (Assis et al. 2011).

Mandobi is adapted to tropical and equatorial regions over a rainfall range of 1,200 to 3,500 mm/year. It has high vigor, good leaf:stem ratio and a high degree of tolerance to soil waterlogging. The new cultivar shows good establishment, high dry matter yield and high seed yield (Table 1). It is estimated that in one kilogram there are 6,500 seeds. In the environmental conditions of Acre, a mixed pasture with marandugrass (*B. brizantha* cv. Marandu), *Pueraria phaseoloides* and Mandobi reached an annual carrying capacity of 2.5 AU/ha without irrigation or nitrogen fertilization. Mandobi has persisted for more than 10 years in mixed pastures with

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Cynodon nlemfuensis, Brachiaria brizantha and *B. humidicola* under intensive rotational grazing. Its use in regions with prolonged drought (above 4 months) is the subject of further research.

Five diseases caused by fungi have been observed in Mandobi, without serious consequences (Gonçalves 2011): forage peanut rust (*Puccinia arachidis*), anthracnose (*Glomerella cingulata*; anamorph: *Colletotrichum gloeosporioides*), *Mycosphaerella* leaf spot (*Mycosphaerella berkeleyi*), stem rot and *Athelia* leaf blight (*Athelia rolfsii*) and *Rhizoctonia* leaf blight (*Thanathephorus cucumeris*).

Table 1. Main traits of *Arachis pintoi* cv. BRS Mandobi, the new forage peanut cultivar developed by Embrapa for use in tropical regions.

Trait	Value	Observation	Reference
Ground cover	90%	16 weeks after planting, spacing of 0.5 x 0.5 m	Assis et al. 2008
Forage yield	9−15 t/ha	10 months after planting	Balzon et al. 2005
Pure seed yield	3000 kg/ha	18−21 months after planting	Valentim et al. 2009
Annual carrying capacity	2.5 AU/ha	mixed Marandugrass-Mandobi pastures	Andrade et al. 2012
Crude protein	22.8%	average of rainy and dry seasons	Santos 2012
Neutral detergent fiber	53.8%	average of rainy and dry seasons	Santos 2012
Acid detergent fiber	27.6%	average of rainy and dry seasons	Santos 2012
Dry matter digestibility	65.8%	annual average	Oliveira et al. 2011

In Brazil, Amarillo is the only cultivar whose seeds are available in the market, imported from Bolivia and Peru. Amarillo seeds are sold at a very high price, which prevents wide adoption of forage peanut in mixed pastures (Embrapa 2012). In January 2013, the price was around USD 45/kg. Moreover, the cost of local production of Mandobi seeds, based on the production system developed by Embrapa, was around USD 8/kg in 2011 (Assis et al. 2011), showing that national production of seeds using appropriate technologies can significantly reduce the price of seed, enabling its wider adoption by producers.

Conclusion

The development of cv. Mandobi, combined with an efficient seed production and distribution system, should increase the supply of forage peanut seeds at reduced cost. This presents a new option for producers in the tropics wishing to introduce a persistent, high-yielding forage legume of high quality into their production systems by planting seeds.

Mandobi is also an excellent option for recovery of degraded pastures in the Amazon, especially in regions with impermeable soils, where marandugrass is dying.

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Selecting new *Brachiaria humidicola* hybrids for western Brazilian Amazon

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Keywords: Agronomic evaluation, forage breeding, genotypic values, mixed models, nutritional evaluation.

Introduction

Brachiaria humidicola is a perennial and stoloniferous forage grass, with excellent adaptation to infertile soils and waterlogging. Producers in northern Brazil have become interested in this species, especially after the degradation of large areas of lowly permeable soils sown with *B. brizantha* cv. Marandu, which is highly intolerant of soil waterlogging (Andrade and Valentim 2006). However, since only 3 cultivars of *B. humidicola* are registered in Brazil (Mapa 2013), there is a need to develop new cultivars adapted to the range of environmental conditions experienced. Recently, Embrapa Beef Cattle obtained intraspecific hybrids of *B. humidicola* that need to be evaluated agronomically, aiming at subsequent grazing trials and future releases. The objective of this study was to evaluate and select apomictic and sexual hybrids of *B. humidicola* under the environmental conditions of the state of Acre, in western Brazilian Amazon.

Methods

The research was conducted at the Experimental Station of Embrapa Acre in western Brazilian Amazon. Fourteen sexual and apomictic hybrids of *B. humidicola* and cv. Tully were evaluated, in a randomized block design with 6 replications in plots of 3 m^2. These hybrids were previously evaluated and selected from a small plot experiment with 50 hybrids at Campo Grande, MS, Brazil (Figueiredo et al. 2012).

The hybrids were planted by stolon pieces in December 2008 and, after establishment, plots were standardized with cuts in April and July 2009. The experimental period was November 2009−June 2011, with 8 harvests, 2 in the dry season and 6 in the rainy season. The agronomic traits evaluated were: total dry matter (DM) yield and leaf DM yield, obtained after determining the percentage of leaves in the samples. Additionally, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin concentrations were obtained from July 2009 and July 2010 harvests (dry season) and January, March and November 2010 harvests (rainy season).

The data were processed using a mixed model methodology, in that variances were estimated by restricted maximum likelihood and genotypic values were predicted by the best linear unbiased prediction methods. Initially, one analysis per harvest was run to verify the existence or not of heterogeneity of residual variances. Afterwards, analysis of the data including all harvests was conducted simultaneously. Phenotypic data were multiplied by h_i/h_g, as proposed by Resende et al. (2008), where h_i is the square root of heritability in harvest *i* and h_g is the square root of the mean of the heritabilities in all harvests. The significance of variances was verified by analysis of deviance (Resende et al. 2008). Genotypes were ranked on a selection index, in which the predicted genotypic values were standardized and traits received different weightings: total DM yield was weighted by 0.4; leaf DM yield by 0.4; NDF by 0.1; and ADF by 0.1. The other traits were not included in the selection index owing to lack of genetic variability.

Results and Discussion

There was genetic variability for total DM yield, leaf DM yield, NDF and ADF (Table 1). The lack of genetic variability for CP and lignin concentrations prevented selection of hybrids based on these traits. The ranking of genotypes was influenced by harvest, since the variance

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of genotype x harvest interaction was significant for all traits. Estimates of heritability in the genotype mean were moderate to high for total DM yield, leaf DM yield and NDF, but low to moderate for ADF. The remaining estimates were not significantly different from zero. The genotypic correlations between harvests were low to medium for total DM yield, leaf DM yield, NDF and ADF, indicating no high coincidence of the best genotypes in all harvests, which reinforces the importance of including the effect of genotype x harvest in the model (Table 1). According to individual repeatabilities, 5, 4, 7, 15, 21 and 14 harvests would be required to achieve a determination coefficient of 80% in the plot evaluation of permanent phenotypic value for total DM yield, leaf DM yield, and CP, NDF, ADF and lignin concentrations, respectively. The experiment conducted has high accuracy for total DM yield, leaf DM yield and NDF; moderate for ADF; and low accuracy for CP and lignin (Table 1).

Table 1. Estimates of general mean (m), genotypic variance (V_g), variance of the genotype x harvest interaction (V_{gh}), mean genotype heritability (h^2_m), genotypic correlations between harvests (r_{gh}), individual repeatability (r) and accuracy (Acc) for agronomic and nutritional traits of *Brachiaria humidicola* genotypes.

Trait[1]	m	V_g	V_{gh}	h^2_m	r_{gh}	r	Acc (%)
TDM (kg/ha/harvest)	3038.8	119550.4**	353175.5**	62.7**	0.2529	0.4394	79.15
LDM (kg/ha/harvest)	1576.4	63624.6**	78528.1**	76.6**	0.4476	0.5019	87.55
CP (%)	5.52	0.0034 ns	0.1114**	7.4 ns	0.0298	0.3712	27.33
NDF (%)	70.24	0.2980**	0.5300**	58.1**	0.3599	0.2085	76.22
ADF (%)	36.65	0.0754**	0.2303**	37.1**	0.2468	0.1624	60.89
LIG (%)	4.01	0.0026 ns	0.2706**	2.8 ns	0.0097	0.2209	16.68

[1]TDM: total dry matter yield; LDM: leaf dry matter yield; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; LIG: lignin. ** Significant by the χ^2 test, at P<0.01.

Classification of hybrids based on the selection index (Table 2) showed 9 genotypes superior to cv. Tully. Among these, there are 4 apomictic and 5 sexual hybrids. Sexual hybrids may be recombined in the recurrent selection program, while apomictic hybrids can advance to evaluation trials with animals, being candidates for release as new cultivars for western Brazilian Amazon.

Table 2. Ranking of *Brachiaria humidicola* hybrids and cv. Tully, based on the selection index obtained from predicted genotypic values by the method of best linear unbiased prediction.

Rank	Genotype	Rank	Genotype	Rank	Genotype
1	216 (S)[1]	6	297 (S)	11	138 (S)
2	289 (S)	7	350 (S)	12	111 (A)
3	193 (A)	8	146 (A)	13	29 (A)
4	242 (A)	9	185 (S)	14	3 (A)
5	88 (A)	10	Tully (A)	15	64 (S)

[1](S): sexual hybrid; (A): apomictic hybrid.

Conclusion

The variability and genetic superiority among the hybrids allows selection of apomictic genotypes for further evaluation with animals, with the ultimate release of new cultivars adapted to the environmental conditions of western Brazilian Amazon. The superior sexual hybrids identified will allow recombination of these genotypes, enabling new cycles of evaluation and selection in the breeding program of *B. humidicola*. While the experimental precision for selection of hybrids is high for agronomic traits, precision of the nutritional traits is much lower and must be improved in order to minimize environmental influences.

Acknowledgments

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Development, isolation and characterization of a new non-virulent mimosine-degrading *Klebsiella pneumoniae* strain from the rumen liquor of German steers by using IBT-Göttinger bioreactor

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Keywords: Leucaena toxicity, rumen microflora, DHP.

Introduction

The tropical legume *Leucaena leucocephala* (leucaena) has many uses, including: a potential source of firewood and timber; for soil erosion control (Dijkman 1950); to provide shade; to enhance soil fertility; and as a nutritious forage for animal feed (Ruskin 1977). It is widely used as forage for cattle in tropical agriculture (Shelton 1998). In Myanmar, leucaena is used as a protein source in urea-molasses multi-nutrient blocks for ruminants (Ni Ni Maw et al. 2004).

However, the use of leucaena as ruminant feed is not without problems, because it contains mimosine, a toxic anti-nutritional factor limiting its use as animal feed. Jones (1981) reported the absence of toxicity when leucaena was fed to goats and cattle in Hawaii and Indonesia. According to the low dihydroxypyridine (DHP) in urine of those animals, it was assumed that they could degrade mimosine and DHP. Hawaiian goats, but not Australian goats, could degrade 3,4-DHP ruminally (Jones and Megarrity1983). Inoculation of susceptible animals with rumen liquor containing mimosine-degrading bacteria protected against DHP toxicity in ruminants (Jones and Lowry 1984).

For maintaining mimosine-degrading bacteria, the donor animals should be fed on leucaena continuously and it is expensive to maintain their veterinary care. Hence, we tried to develop mimosine-degrading ruminal bacteria using a fermenter, intending to produce a source of inoculum for the routine control of leucaena toxicosis in ruminants.

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Materials and Methods

The donor steer was from the Institute of Animal Physiology and Nutrition, Faculty of Agriculture, Georg-August University of Göttingen, Germany, and had never consumed leucaena leaves. Rumen liquor was obtained via a rumen cannula and immediately placed in a conical flask, flushed with carbon dioxide, stoppered, maintained under carbon dioxide, and frozen at 4 °C in a refrigerator for 2 weeks to kill the protozoa. Before subsequent fermentation, rumen liquor was thawed. Fermentation was commenced with 520 mL of rumen liquor.

The IBT-Goettinger Bioreactor was a modified form of that used by Böhnel and Zeggu (1993). The medium 98-5 for rumen microbes (Allison et al. 1990), without mimosine, was supplied continuously. Infusion of mimosine started at 25 mg/d, increasing by 25 mg of mimosine every second day for 16 days, when 200 mg/d was infused. Daily samples were taken and mimosine degradability of the sample was assessed by colorimetric assay. Purification of the mimosine-degrading bacterial strain was done from the samples showing mimosine degradability. After that, tests for biochemical properties and molecular identification were conducted. For proof that bacterial strains were non-pathogenic, a mouse bioassay was carried out to determine the lethal dose.

Results and Discussion

Although the rumen liquor of the donor steer was unable to degrade mimosine initially, infusing gradually increasing amounts of mimosine for 16 days (maximum of 200 mg mimosine/d) enabled the liquor to degrade mimosine (Figure 1). It is possible that some ruminal bacteria became adapted to degrading mimosine, as found by Ruskin (1977). According to molecular identification, genotypic characteristics of this isolate were almost identical (94% similarity) to those of reference *Klebsiella pneumoniae* from GenBank entries held by the National Centre for Biotechnology Information (NCBI). Although this isolate is similar to pathogenic *K. pneumoniae*, it has some different biochemical properties (Biolog test). In this experiment, gradually increased amounts of mimosine infused into rumen liquor induced adaptation in the bacteria first, so they could degrade mimosine. Mice inoculated with the recommended dose of *K. pneumoniae* (3.3 x 10^8 CFU/mL) showed no clinical signs of klebsiellosis, as reported by Thakker et al. (2006).

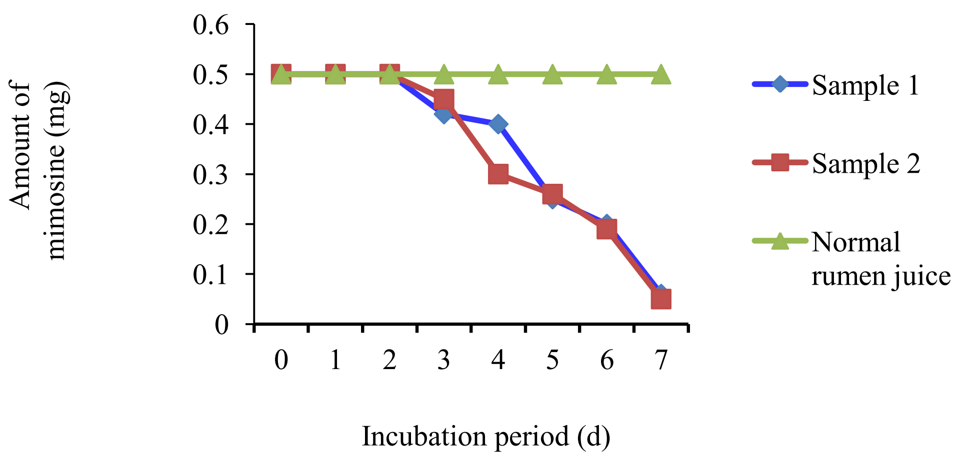

Figure 1. Effects of treatment with mimosine on the ability of rumen liquor to degrade mimosine. Sample 1 was taken on day 15 of treatment with mimosine, when dosage was 200 g/d; sample 2 was taken on day 16 of treatment.

Conclusion

This study has shown that treatment of rumen liquor with mimosine increases the ability of the bacteria to degrade mimosine. *Klebsiella pneumoniae* isolated from this experiment was effective at degrading mimosine. Studies are needed to compare the effectiveness of this bacterium in protecting animals fed leucaena, in comparison with *Synergistes jonesii*. An experiment to measure these parameters will be conducted in Myanmar.

Acknowledgments

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Development of leucaena mimosine-degrading bacteria in the rumen of sheep in Myanmar

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Keywords: *Leucaena leucocephala*, anti-nutritive factor, toxicity symptoms, urinary concentration.

Introduction

Myanmar has an agricultural base, and about 70% of people reside in rural areas. They depend for survival on agriculture and small-scale crop production, with ruminant livestock consuming fibrous agricultural residues. For optimal ruminant production, concentrates are needed as supplements to these residues. As concentrates are expensive, researchers are testing alternative protein sources like legumes, including foliage from leguminous trees such as leucaena (*Leucaena leucocephala*). Leucaena is the most widely used leguminous tree as a ruminant feed because it is rich in protein (~ 22%) and contains easily digestible fiber (23% neutral detergent fiber, 16.6% acid detergent fiber; Ni Ni Maw 2004). Khin Htay Myint (2005) noted that 25% of leucaena in the ration tended to increase nitrogen retention without decreasing dry matter and organic matter digestibilities.

However, leucaena leaves contain a toxic non-protein amino acid, called mimosine. Research workers have endeavored to reduce mimosine toxicity in animals fed leucaena in Myanmar (Aung Aung 2007; Wink Phyo Thu 2010) and one avenue of research was the development of mimosine-degrading bacteria in the rumen of sheep fed leucaena. In this paper we describe an experiment tracing the development of mimosine-degrading bacteria in the rumen of sheep.

Materials and Methods

The experiment was carried out using 9 male sheep aged 6−8 months and weighing 11−18 kg. The animals were divided into 3 groups and fed the following 3 diets: rice straw + mixed concentrate (G1); rice straw + mixed concentrate + 50% leucaena leaves (G2); and rice straw + mixed concentrate + gradually increased amount of leucaena leaves from 10% to 50% level (4 days for each level) (G3). Body temperatures of sheep were measured daily, when the animals were checked for clinical signs and symptoms of mimosine toxicity. Mimosine concentration in urine was determined at the end of the experiment.

Results

Results of clinical examinations and urinary mimosine concentrations of sheep after feeding 50% leucaena leaf are shown in Table 1. Animals from all groups had the same average body temperature (37.5 ºC). Clinical signs appeared 4 days after feeding 50% leucaena in the total diet for animals in G2. The most prominent signs were hair loss, dullness and decrease in feed intake. However, no clinical signs of leucaena mimosine toxicosis were observed in animals from G3 throughout the experimental period, although urinary mimosine levels in this group were high.

Discussion

The loss of hair by animals from the group fed 50% leucaena after 4 days from the start of feeding is similar to the reports of Hegarty et al. (1964). Toxic symptoms in cattle were reported by other research workers (Jones and Hegarty 1984). The failure of toxicity to alter body temperature of the sheep agrees with the experiment of Jones et al. (1978), where rectal temperature of all cattle showing toxic symptoms was normal. However, in the experiment of Hegarty et al. (1964), the body temperature of sheep was 105 °F (40.6 °C) after 4 days of infusion of mimosine and higher than that of sheep from this experiment. The absence of toxic symptoms when the animals were fed on gradually increased amount of leucaena leaves was unexpected, as mimosine levels in urine of this group were high. Molecular identification of bacterial strains from rumen liquor will be performed to check the possibility that mimosine-degrading strains

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were present. Subsequently, rumen liquor from the sheep which developed the ability to degrade mimosine will be transferred to other ruminants to encourage more extensive use of leucaena in Myanmar.

Table 1. Clinical examination of sheep with and without leucaena in the diet.

Group[1]	Clinical signs			
	Body temperature (°C)	Loss of weight	Hair loss	Urinary mimosine (mg/L)
1	37.5	Nil	Nil	0
2	37.5	Slightly	Present	0.006
3	37.5	Nil	Nil	0.16

[1]Group 1: rice straw + mixed concentrate; Group 2: rice straw + mixed concentrate + 50% leucaena; Group 3: rice straw + mixed concentrate + gradually increased amount of leucaena from 10% to 50% level (4 days for each level).

Conclusion

This study has shown that gradual introduction of leucaena leaf into the diet of sheep can prevent the appearance of symptoms of mimosine toxicity in the sheep. This suggests that rumen microflora have adapted to the changes in dietary composition through the slow introduction of leucaena. This has far-reaching implications for how leucaena can be used in the diets of animals, especially in cut-and-carry systems.

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Morphophysiological adaptations of *Brachiaria humidicola* cultivars under grazing

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Keywords: Phyllochron, lamina length, leaf lifespan, number of green leaves, continuous stocking.

Introduction

Pastures of *Brachiaria humidicola* are broadly used in Brazil, especially in soils with low fertility and poor drainage (Valle et al. 2010). Currently, 3 cultivars are commercially available: Tully, Llanero and BRS Tupi (available since 2012). Despite the extensive use of this species, research has focused primarily on aspects related to herbage and animal production, while only a few studies aimed at understanding morphological adaptations of the plants when subjected to grazing and contrasting environmental conditions. Therefore, the aim of this work was to evaluate some morphological traits of 2 *B. humidicola* cultivars when continuously stocked during 2 seasons of the year.

Methods

The experiment was carried out at the EMBRAPA National Beef Cattle Research Center, in Campo Grande, MS, Brazil (20°27' S, 54°37' W; 530 m asl), from January to December 2012. The climate, according to the Köppen-Geiger classification, is rainy (tropical savanna), subtype Aw. The soil of the experimental area is classified as an Oxisol (red clayed latosol), with poor drainage and subject to temporary flooding. Pastures of 2 *Brachiaria humidicola* cultivars (BRS Tupi and Tully) were established in early 2004 and, since 2010, were fertilized annually with 30 kg P_2O_5/ha, 30 kg K_2O/ha and 50 kg N/ha. The experimental area comprised 9 ha, divided into 6 paddocks of 1.5 ha. Nelore steers were used with an average live weight of 220 kg. Treatments consisted of the 2 cultivars assigned in a complete randomized block design with 3 replications. The area was continuously stocked with stocking rate adjusted in order to maintain a relatively constant sward height of 20 cm throughout the year. Sward height was monitored weekly at 30 points per experimental unit using a sward stick (Barthram 1985). Morphogenetic and structural traits were assessed on 15 marked tillers per experimental unit. The following variables were measured: phyllochron, leaf lamina length and leaf lifespan. At the end of each cycle of data collection (on average 4 weeks), new tillers were marked. Data were grouped by season of the year (dry and rainy seasons) and analyzed by a mathematical model, where cultivars and seasons were considered fixed effects and the interactions between them and blocks as random effects. All statistical analyses were performed by the method of least squares using the General Linear Model of SAS (SAS Institute 1996). Means were compared using a significance level of 5%.

Results and Discussion

Canopy heights remained relatively constant throughout the year and within the targets, for both cultivars. During the dry season, average sward heights were 18.2 ± 2.4 cm and 17.6 ± 2.8 cm for Tully and BRS Tupi, respectively; during the rainy season, the values were 20.1 ± 1.9 cm for Tully and 21.9 ± 1.4 cm for BRS Tupi. The number of green leaves per tiller (NGL) (5.2 leaves/tiller) was not affected by season of the year, but Tully had more green leaves than BRS Tupi (5.6 vs 4.7 leaves/tiller). The NGL seems to be a genotypic constant (Lemaire and Chapman 1996) and may vary between species and even among cultivars. This characteristic is related to leaf lifespan (LLS) expressed by the number of intervals of leaf appearance. Thus, the greater NGL for Tully can be attributed to the higher LLS, mainly during

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the dry season (Table 1). Moreover, LLS was constant for BRS Tupi between seasons, apparently an adaptation to produce the same amount of leaves in a given time. According to Robson (1969), there are several strategies for producing the same quantity of leaves, including variations in rates of appearance and elongation of leaves and duration of the elongation period. The phyllochron showed the same pattern as LLS, i.e., phyllochron values for BRS Tupi were constant between seasons, while Tully displayed higher values for phyllochron during the dry period (Table 1). The values for phyllochron for BRS Tupi and Tully were similar during the rainy season but Tully had lower values than BRS Tupi during the dry period.

Table 1. Leaf lifespan, phyllochron and final lamina leaf length for 2 *Brachiaria humidicola* cultivars continuously grazed in 2 seasons of the year.

Season of the year	Leaf lifespan (days)		Phyllochron (days/leaf)		Leaf lamina length (cm)	
	Cultivars					
	BRS Tupi	Tully	BRS Tupi	Tully	BRS Tupi	Tully
Dry	123.7Ab[1] (± 19.30)[2]	220.5 Aa (± 19.42)	24.2Ab (± 2.88)	35.2 Aa (± 2.89)	8.60 Ba (± 0.52)	7.3 Aa (± 0.52)
Rainy	109.6 Aa (± 16.85)	113.8 Ba (± 19.42)	24.1 Aa (± 2.51)	20.4 Ba (± 2.89)	12.9 Aa (± 0.45)	8.6 Ab (± 0.45)

[1]Means within columns followed by the same upper-case letters or in rows followed by the same lower-case letters do not differ (P>0.05). [2]Values in parenthesis correspond to standard error of the mean.

Another interesting result concerned the final leaf lamina length (FLL). For Tully, FLL reduced only slightly from wet to dry season, while for BRS Tupi the reduction was around 35%. This pattern indicates that the cultivars present different strategies to adapt to changes in the environment. BRS Tupi seems much more effective at using available resources to expand leaves rapidly when growing under non-limiting conditions, while Tully seems to have a more conservative strategy, being able to tolerate a more limiting environment. In this way, it seems that BRS Tupi could adapt better to fertile or flooded soils with no extreme dry period, while Tully could tolerate infertile soils and a more prolonged drought period.

Conclusion

Different cultivars of *Brachiaria humidicola* present different patterns of adaptation to the environment. Since BRS Tupi is more effective at using available resources to grow faster in non-limiting conditions and Tully is more tolerant of poor soil fertility and prolonged drought periods, farmers can choose the appropriate cultivar for their particular circumstances.

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Reciprocal recurrent selection in the breeding of *Brachiaria decumbens*

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Keywords: Apomixis, cultivar, intraspecific hybridization, signalgrass.

Introduction

Pastures of *Brachiaria decumbens* cv. Basilisk radically changed the scenario of livestock production in central Brazil in the early 1970s and in fact, promoted the development of this vast region. However, despite the reasonable biomass yields and nutritional value when grown on these tropical acid soils, its susceptibility to grassland spittlebugs has limited its use. The breeding of *B. decumbens* in Brazil has been restricted to interspecific crosses using cv. Basilisk as a pollen donor due to the lack of compatible sexual ecotypes within this species. Recently, the successful chromosome duplication of a sexually reproducing diploid accession produced 3 successful events (Simioni and Valle 2009), enabling intraspecific crosses. This paper reports the onset of the research to obtain superior apomictic hybrids in *B. decumbens* using reciprocal recurrent selection (RRS), a cyclic breeding strategy.

Methods

Four hundred and fifty-seven intraspecific hybrids, obtained from crossings between the 3 sexual plants artificially tetraploidized by colchicine, and the apomictic tetraploid cv. Basilisk, represented the base population of this work. The reproductive mode of these hybrids was determined by microscopic analysis of cleared ovaries (Young et al. 1979). A chi-squared test was performed on this population to verify whether the genetic segregation for apomictic to sexual plants fitted the expected model for a tetrasomic monogenic inheritance. The 80 F_1 sexual plants identified were crossed with cv. Basilisk in order to produce full-sib progeny (1^{st} year of a RRS). For this, each sexual plant was vegetatively propagated and transplanted into a uniform pasture of cv. Basilisk. The sexual plants were spaced 5 m from each other to avoid crossing between them and to enhance crosses with the apomictic genitor. Besides natural crossing, manual crossings were also performed using pollen of the apomictic genitor onto flowers of the sexual plants. Inflorescences were bagged and the seeds harvested 20 days after pollination.

Seeds were harvested on each sexual plant and germinated in the greenhouse. The seedlings were planted in the field in December 2012, and will be evaluated for agronomic traits, nutritive value and resistance to spittlebugs during the 2013 growing season (3 cuts every 35 days in the rainy season; 1 cut in the middle and 1 at the end of the dry season) as the 2^{nd} year of RRS. The progeny will be selected using a selection index, which will take into account information for all traits evaluated. The recombination of selected sexual progeny will be done in the third year, in a crossing block comprising only the sexual plants that gave rise to these selected progeny. Seeds will be harvested and a new improved sexual population produced to start the next cycle of selection. At the end of the second year, superior hybrids with high performance will be identified. If these hybrids are apomictic, they can enter the hybrid evaluation program as candidates for a new cultivar (Figure 1).

Results and Discussion

Of the 457 hybrids in the base population, 153 were subjected to characterization of their mode of reproduction. Of these, 80 were sexual and 73 apomictic, a proportion of apomictic to sexual plants not significantly different from the 1:1 ratio (P>0.05) (Table 1). This result corroborates the proposed hypothesis of a single dominant gene controlling apomixis (Valle and Savidan 1996).

Crossings between the 80 sexual tetraploid plants and the apomictic cv. Basilisk were performed in a crossing block scheme to obtain full-sib progeny. The percentage

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of filled seed ranged from 0.0% to 20.7% across sexual plants, with an average of 3.96%, showing that the percentage of filled seeds produced by artificial crossing was very low in intraspecific *B. decumbens* crosses. These progeny are under evaluation in the field and will be selected for further recombination. In each selection cycle, superior apomictic hybrids will be identified to proceed to cultivar development. *B. decumbens* had never been bred before and only a single cultivar is available commercially.

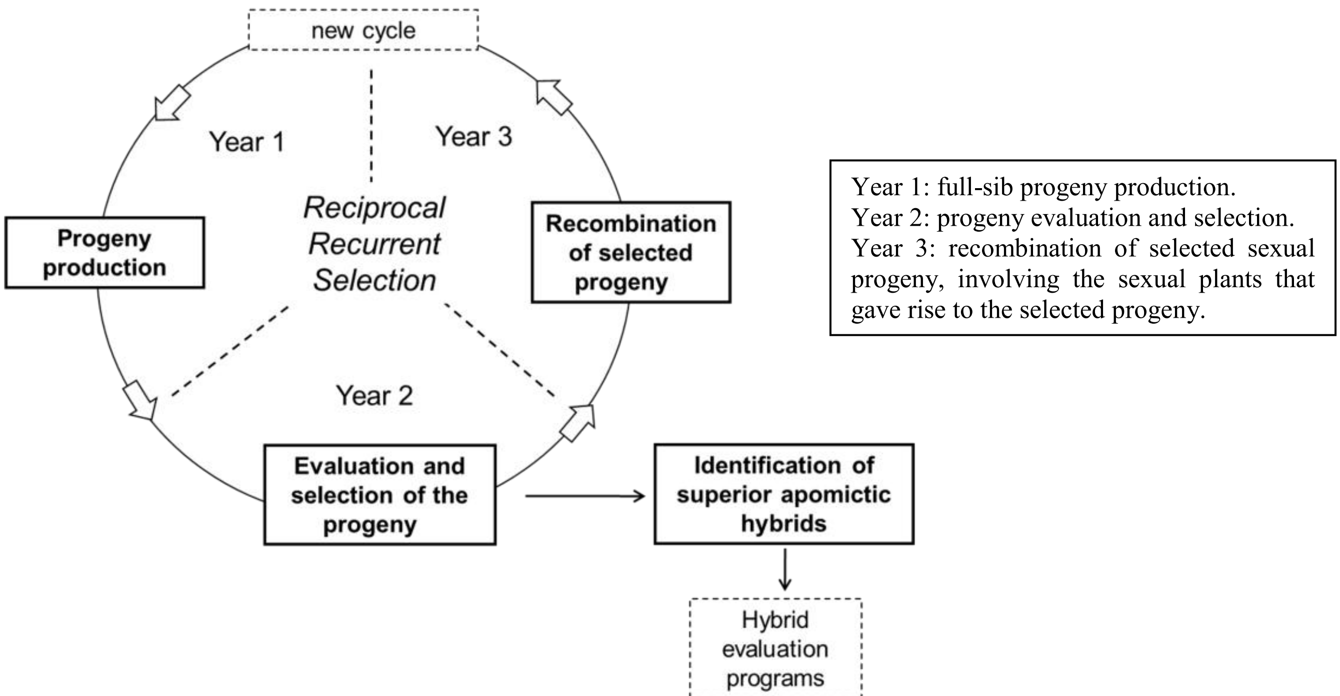

Figure 1. Reciprocal recurrent selection scheme in *Brachiaria decumbens* and relation with the hybrid evaluation programs underway at Embrapa Beef Cattle.

Table 1. Number of hybrids evaluated for mode of reproduction.

	Apomictic	Sexual	Total
Number observed	73.0	80.0	153.0
Number expected	76.5	76.5	153.0
χ^2 test: 0.32 (P > 0.05)			

Conclusion

The inheritance of apomixis in intraspecific crosses of *Brachiaria decumbens* has a monogenic control with the apospory allele dominant to sexuality, which is in agreement with other species of *Brachiaria*. The prospect of generating novel variability and selecting new cultivars is now a reality and should promote grassland diversification in the tropics where cv. Basilisk is already extensively and successfully used.

Acknowledgments

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Effects of two intake levels of *Leucaena leucocephala* on rumen function of sheep

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Keywords: Browse trees, voluntary intake, digestibility, protein supplements, rumen ammonia, rate of passage.

Introduction

Ruminant production systems based on grass pastures often produce only poor animal performance as expressed by growth or reproductive rates. The nutrient imbalance affecting rumen function is due to low energy and protein intake.

The incorporation of leguminous forages such as *Leucaena leucocephala*, in the diet of ruminants, can stimulate rumen function by providing protein-rich forage (Barros-Rodríguez et al. 2012). This increases the availability of compounds such as ammonia, amino acids and peptides as well as branched short-chain fatty acids, which are produced as a result of degradation of proteins. These substances promote fiber breakdown by acting as ruminal growth activators for rumen bacteria, especially cellulolytic bacteria (Hoover and Stokes 1991). This study aimed to evaluate the effects of 2 intake levels of *L. leucocephala* (leucaena) on rumen function of sheep fed *Pennisetum purpureum*.

Materials and Methods

The experiment was conducted over 60 days at the Faculty of Veterinary Medicine, University of Yucatán, Mérida, using 18 Pelibuey sheep, cannulated in the rumen and with an average weight of 33.3 ± 6.13 kg. A completely randomized design was usedwith 3 replicates of the following 3 treatments: T1− 100% grass, T2− 20% leucaena + 80% grass, T3− 40% leucaena + 60% grass.

Dry matter (DM) degradation was determined using the technique described by Ørskov et al. (1980), incubating at 4, 8, 12, 24, 36, 48, 72, 48 and 96 hours. Rumen constants of DM were analyzed by Graph Pad Prism (GraphPad 2003) that fits the data of the exponential equation $Y = a + b (1 - e^{-ct})$ (Ørskov and McDonald 1979). Concentration of NH_3 and pH were determined on rumen liquid samples, collected 6 hours after feeding. The samples were filtered through cotton gauze and 10 mL of rumen liquor from each sample was stored in 0.5% HCl (1:1) for later analysis of NH_3. The pH was measured immediately after collection with a portable potentiometer (Hanna pHep family). Apparent digestibility of DM and voluntary intake were estimated directly in metabolic cages. Data were analyzed by ANOVA and means were compared by the Tukey test using SAS statistical software (SAS 2002).

Results and Discussion

Degradation rate of forage was higher on Treatments T2 and T3 than on the pure grass diet (Table 1).

Voluntary intake and apparent digestibility of DM of the diets containing legume were higher than those of the pure grass diet. Ammonia concentration in rumen liquor was also higher on the diets containing leucaena and rumen pH level declined as proportion of legume in the diet increased.

For all parameters evaluated, there were no differences between T2 and T3 (P>0.05). The lack of a major response in T3 can probably be attributed to a crude protein vs digestible energy imbalance caused by higher leucaena intake in T3 (data not shown), leading to low efficiency of synthesis of microbial protein in the rumen (Calsamiglia et al. 2010). These results are consistent with those reported by Barros-Rodríguez et al. (2012).

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Table 1. In situ degradation of DM, apparent digestibility of DM, voluntary intake, ruminal pH and ammonia concentration.

	Treatments			Significance level
	T1	T2	T3	
DM degradation (%)				
Pennisetum purpureum				
t0	17.68	17.68	17.68	
a[1]	19.02±1.52[a2]	19.27±1.27[a]	17.88±1.36[a]	>0.05
b	47.49±1.47[a]	46.59±1.16[a]	47.58±1.24[a]	>0.05
c	0.038±0.003[b]	0.050±0.003[a]	0.052±0.003[a]	>0.05
a+b	66.51[a]	65.86[a]	65.46[a]	>0.05
r^2	0.95	0.97	0.97	
Leucaena leucocephala				
t0	34.27	34.27	34.27	
a	32.53±1.42[a]	31.99±1.54[a]	32.33±1.63[a]	>0.05
b	52.81±1.70[a]	49.17±1.41[a]	49.02±1.49[a]	>0.05
c	0.029±0.003[b]	0.061±0.004[a]	0.059±0.004[a]	>0.05
a+b	85.34[a]	81.16[a]	81.35[a]	>0.05
r^2	0.96	0.97	0.97	
Apparent DM digestibility (%)	52.52±2.17[b]	56.5±2.28[a]	57.6±2[a]	0.002
VI (g/kg $LW^{0.75}$)	67.58±0.053[b]	86.94±6.09[a]	83.72±2.31[a]	0.0001
pH	6.86±0.03[a]	6.62±0.02[b]	6.56±0.03[c]	0.0001
NH_3 (mg/L)	25.33±3.78[b]	36.06±6.31[a]	33.38 ±4.56[a]	0.005

[1]a: soluble fraction (in percentage); b: insoluble but potentially degradable fraction (in percentage); c: degradation rate (in percentage per hour); a+b: potential degradation; VI: voluntary intake; LW: live weight; t0: time zero.
[2]Means within rows with different superscripts differ significantly (P< 0.05).

Crude protein concentration of grass was apparently a limiting factor for ruminal digestion, restricting microbial function and limiting energy intake, as indicated by the low rumen ammonia levels on the pure grass diet. The additional protein provided by the leucaena would have increased availability of ammonia for rumen microflora, stimulating microbial growth and increasing rate of breakdown of the forage, which increased rate of passage of the diet and consequently voluntary intake. Kakengi et al. (2001) obtained similar trends with leucaena supplementation in the diet of cattle. The reduction in pH with increasing level of leucaena in the diet indicates increased production of volatile fatty acids from the increased intake and breakdown of the forage. These findings support the results reported by Osakwe and Steingass (2006) that, as leucaena proportion in the diet increases, rumen function improves.

Conclusions

Providing supplements of leucaena in the diet of sheep can improve rumen function by increasing the rate of degradation of forages and stimulating voluntary intake. This increases availability of nutrients for metabolic processes resulting in improved growth rates, wool growth and reproductive performance. Our results indicate that providing leucaena at more than 20% of the diet would not produce additional benefits. It would seem preferable to supplement twice as many sheep with this lower level. Further studies may be warranted to determine how effective lower levels of supplementation might be.

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Effects of irrigation and season on performance of grazed pastures of *Brachiaria brizantha* cv. MG5

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Keywords: Pasture production, liveweight gains, tropical grass.

Introduction

In Brazil, livestock production is based primarily on systems with continuously grazed natural or cultivated pastures (FAO 2009). Intensifying animal breeding, handling and knowledge strategies is necessary to obtain improvements in production indices for economically feasible and sustainable grazing systems (Cedeño et al. 2003). Beef cattle breeding on irrigated pastures, like all forms of intensive grazing, requires the use of forage species with high potential for production and quality; *Brachiaria brizantha* cv. MG5 is considered a good option. This study aimed to evaluate the effects of irrigation on the performance of *B. brizantha* cv. MG5 grazed rotationally by beef cattle at different times of the year.

Methods

An area of 4 ha of *B. brizantha* cv. MG5 on the Experimental Farm at FESP, Minas Gerais, Brazil, was divided into 32 paddocks. An area of 2 ha was irrigated by a conventional sprinkler system and the remaining 2 ha was not irrigated. Ten steers grazed the irrigated area and 10 grazed the non-irrigated area. Animals were shifted between paddocks every 2 days, providing 30 days rest for each paddock and a 32-day grazing cycle. At the end of each cycle the animals were weighed. Animal weight data were analyzed over 2 seasons (November−February, summer, 4 cycles; and April−July, winter, 4 cycles) in 2011/2012. For quantitative and qualitative forage evaluation, samples from each paddock were collected before animals entered and immediately after removal by using a 1.0 m^2 board launched randomly, and cutting the forage 20 cm from the soil. Harvests were done between June and August 2011 (winter) and November 2011 and January 2012 (summer). Green samples were weighed to determine production of green mass (PGM) and subsamples were taken for drying to assess the content of total dry matter (TDM). The data were submitted to statistical analysis using the GENES program (Cruz 2006).

Results

Production of green and dry mass as well as voluntary intake was higher in summer than in winter and irrigation increased production of both green and dry mass (Table 1).

Table 1. Effects of season and irrigation on dry matter content (DM), production of green mass (PGM), apparent intake of green mass (IGM), production of dry mass (PDM) and apparent intake of dry mass (IDM) of *Brachiaria brizantha* cv. MG5.

Factor	DM	PGM	IGM	PDM	IDM
	(%)	(kg/ha)			
Winter	32.95a[1]	1917b	552b	591b	171b
Summer	33.66a	2606a	764a	806a	238a
Irrigated	33.62a	2371a	664a	740a	208a
Non-irrigated	32.99a	2153b	652a	657b	201a

[1]Means within columns and factors followed by the same letter do not differ according to the Scott and Knott test (1974).

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Mean daily weight gains by steers were increased by irrigation in winter (P<0.05) but not in summer (Table 2). Weight gains in winter were lower than summer gains on both irrigated and non-irrigated pastures, but the differences were significant only on the non-irrigated area (P<0.05).

Table 2. Effects of season and irrigation on mean liveweight gains (LWG) of steers (kg/d).

	Summer		Winter	
	Irrigated	Non-irrigated	Irrigated	Non-irrigated
LWG	0.68a[1]	0.68a	0.50a	0.16b

[1]Means followed by the same letter do not differ according to the Scott and Knott test (1974).

Conclusion

This study has shown that there is no merit in irrigating *B. brizantha* cv. MG5 pastures during the summer months but that weight gains are improved by irrigating in winter. The economics of such a strategy will depend on the particular situation.

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Structural characteristics of Puerto Rico stargrass (*Cynodon nlemfuensis*) pastures under different frequencies and severities of defoliation

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Keywords: Leaf area index, tiller population density, pasture height, leaf blade:stem ratio, forage bulk density.

Introduction

An understanding of the effects of frequency and severity of defoliation on the sward structure of pastures is essential for identifying pasture management strategies that are more efficient in forage usage in tropical environments (Da Silva and Nascimento Jr 2007; Euclides et al. 2010). Some pasture species have not yet been investigated in this way, e.g. cultivars of *Cynodon.* The potential of this grass in tropical environments suggests that it should be given priority (Pedreira 2010). This study was designed to evaluate the effects of 2 frequencies (90 and 95% light interception) and severities (20 and 30 cm residue) of defoliation on the structural characteristics of Puerto Rico stargrass pasture (*Cynodon nlemfuensis*) during 3 seasons (fall, winter and spring).

Materials and Methods

The experiment was carried out at Pesagro-Rio, Seropédica, RJ, Brazil (22°45' S, 43°41' W; 33 m asl). The climate is type AW (Köppen), with a dry season from April to September and wet season from October to March. The experimental area consisted of 16 plots of 300 m² each (experimental unit) of Puerto Rico stargrass (*C. nlemfuensis*) arranged under a randomized complete block design in a 2 x 2 factorial arrangement with 4 replications. The treatments were a combination of 2 intervals (90 and 95% light interception − LI) and 2 severities (20 and 30 cm residue) of defoliation. The trial started on March 26, 2012 and lasted until December 27, 2012, including fall (March 26−June 20), winter (June 21−September 22) and spring (September 23−December 21) seasons. Grazing of pastures commenced when the canopy reached 90 or 95% LI and continued until the residual height was 20 or 30 cm, using crossbred Holstein x Gir animals (average weight 450 kg).

A day before the start of grazing, the height (HEI) of pastures was evaluated (40 measurements per plot), and 4 samples per paddock were collected to estimate herbage mass, through cuts made close to the ground, using a circular frame of 0.20 m^2. The samples were weighed, before sorting into leaf blade, stem and dead components, which were weighed and dried in a fan-forced oven at 55 ºC for 72 hours to obtain their dry mass. Based on these data and the heights of pastures, forage bulk density (FBD) was calculated by dividing the herbage mass by the average height of the grass, and leaf blade:stem ratio (LB:S) was determined. Tiller population density (TPD) and leaf area index (LAI) were estimated by cutting a forage sample, contained within a 0.3 x 0.3 m frame, a day before the entry of animals to paddocks, and measurements made of leaf blades with a leaf area meter (LI-COR LI-3100).

Data were analyzed by PROC MIXED of SAS®, version 9.0, and variance analysis was based on the following sources of variation: LI, residue height, season and their interactions, which were considered as fixed effects, and the effect of blocks as a random effect. The treatment means were estimated and compared by the "LSMEANS" and by the PDIF (P<0.05), respectively.

Results and Discussion

Tiller population density differed with severity of defoliation and with season (P<0.05) (Table 1). Highest TPD occurred at the heavier defoliation level (20 cm) in winter and lowest at 30 cm defoliation in the fall (P<0.05)

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with remaining values intermediate. LAI reached its highest levels at 20 cm defoliation in the fall and lowest levels at 20 cm defoliation in spring (P<0.05), with remaining values intermediate.

Table 1. Tiller population density (TPD) and leaf area index (LAI) of Puerto Rico stargrass (*Cynodon nlemfuensis*) pastures under 2 severities of defoliation during the fall, winter and spring.

Season	Severity of defoliation		s.e.
	20 cm	30 cm	
	TPD (no. of tillers/m^2)		
Fall	1189 bc[1]	992 c	74
Winter	1583 a	1287 b	74
Spring	1194 b	1183 b	74
	LAI		
Fall	4.0 a	3.4 b	0.2
Winter	3.3 b	3.4 b	0.2
Spring	2.7 c	3.7 ab	0.1

[1]Means followed by the same lower-case letters within parameters do not differ (P>0.05).

There were significant interactions between season, frequency and severity of defoliation for HEI, LB:S and FBD (Table 2). There was a trend for swards grazed when they reached 95% LI to have greater HEI at this time than those grazed at 90% LI. The lowest heights at the commencement of grazing were in pastures grazed at 90% LI to either 20 or 30 cm residue. LB:S varied between 0.61 and 0.37, both values being for pastures grazed at 95% LI to 30 cm. There was a tendency for LB:S to be highest in the fall but results were inconsistent. There was no consistent pattern for FBD with the highest recording being for pasture grazed at 90% LI to 20 cm in winter and the lowest in pasture grazed at 95% LI to 30 cm in the fall (P<0.05). There was a trend for pastures to be shortest and densest in winter. These findings suggest that pastures of Puerto Rico stargrass can be grazed at 90% LI and to 20 cm without jeopardizing LB:S, a good indicator of forage quality, or FBD, which has a significant impact on the ability of animals to select a high quality diet.

Table 2. Effects of 2 frequencies and 2 severities of defoliation on height (HEI), leaf blade:stem ratio (LB:S) and forage bulk density (FBD) of Puerto Rico stargrass (*Cynodon nlemfuensis*) pastures prior to grazing during fall, winter and spring.

Season	Light interception (%)				s.e.
	90		95		
	Residue (cm)		Residue (cm)		
	20	30	20	30	
	HEI (cm)				
Fall	47 cd[1]	47 cde	50 ab	48 bc	1
Winter	30 g	36 f	47 cd	47 cd	1
Spring	46 de	46 de	51 a	44 e	1
	LB:S				
Fall	0.54 b	0.49 d	0.54 b	0.61 a	0.01
Winter	0.47 de	0.49 d	0.43 ef	0.60 a	0.02
Spring	0.48 de	0.51 cd	0.46 de	0.37 f	0.02
	FBD (kg/ha/cm)				
Fall	122 de	155 bc	129 de	116 e	7
Winter	215 a	176 bc	181 b	126 de	11
Spring	129 de	133 d	129 de	156 c	3

[1]Means followed by the same lower-case letters within parameters do not differ (P>0.05).

Conclusions

It seems that pastures of Puerto Rico stargrass can be grazed at frequent intervals and to a residue of 20 cm without any significant detriment. Data on dry matter yields and crude protein concentrations with this management strategy would provide greater confidence in adopting such a regime.

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Growth of Bali bulls on rations containing *Sesbania grandiflora* in central Lombok, Indonesia

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Keywords: Bali cattle, growth rate, agroforestry, fodder trees.

Introduction

The demand for meat in Indonesia is currently growing by up to 8% per year, with beef cattle fattening identified as a major livestock industry (Purwantara et al. 2012). Bali cattle (*Bos javanicus*) account for almost 27% of total beef cattle in Indonesia; they are the predominant breed in the eastern islands and are highly favored by smallholder farmers for their high fertility, low calf mortality and generally higher price at markets (Purwantara et al. 2012). Lombok in west Nusa Tenggara is one of the biggest suppliers of Bali cattle in Indonesia.

A major constraint to improving the overall productivity of Bali cattle is their slow growth rate, due to lack of readily available, inexpensive, high-quality protein sources. Fodder tree legumes, such as sesbania (*Sesbania grandiflora*), offer a fast-growing, low-cost source of protein (Evans and Rotar 1987). Farmers in Lombok have established a unique and productive integrated farming system by planting sesbania trees along the bunds of rice paddies, providing forage and timber without significantly compromising rice yield (Dahlanuddin and Shelton 2005).

As only the central part of Lombok is intensively planted with sesbania, a collaborative project funded by the Australian Centre for International Agricultural Research (ACIAR) is underway aiming to: (a) characterize the existing cattle fattening systems; and (b) assess the impact of differing levels of sesbania feeding on the growth rate of Bali bulls from weaning to maturity (about 30 months old).

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Materials and Methods

Objective 1 − Pre-trial

Three typical cattle fattening groups were selected in central Lombok in the hamlets of Montong Oboq, Bun Prie and Repok Nyerot. Commencing March 2012, animal weights, feed regimes and sale prices were monitored regularly to understand the fattening profiles of the 3 groups.

Objective 2 – Feeding trial

Within each of these groups, a semi-controlled feeding trial was begun in July 2012, using 20 male Bali calves with an average age of 7.6 ± 0.4 months and mean live weight (LW) of 90 ± 5.8 kg. Bulls were randomly allocated to the 3 villages in August 2012. Farmers were requested to feed sesbania to these bulls at rates up to 20% (fresh weight) of total diet in Montong Oboq, 40% in Bun Prie and 60% in Repok Nyerot. A rice bran supplement of 0.5 kg fresh weight/100 kg LW was supplied for farmers at Bun Prie and Repok Nyerot, where higher levels of sesbania were being fed. The actual amounts and proportions of different feeds offered were recorded on 6 consecutive days in March 2013. Live weight was measured monthly.

Results and Discussion

The pre-trial profiles of the 3 groups are presented in Table 1. Farmers in Repok Nyerot achieved the highest daily gains and sale weights, but the monthly profit margin was slightly lower than for those in Montong Oboq. The higher gains were thought to be due to higher levels of sesbania feeding. This aspect was tested in the subsequent feeding trial. Montong Oboq had the longest fattening period (12.5 ± 1.3 months), as they started with the lightest bulls (119 ± 15 kg).

Table 1. Pre-trial profiles of groups fattening Bali cattle at 3 sites in central Lombok (March–July 2012).

Categories	Montong Oboq	Bun Prie	Repok Nyerot
No of farmers	30	19	30
No of cattle/household/period	1	1−2	2−3
No of cattle monitored	8	30	23
Initial live weight (kg)	119 ± 15	195 ± 5	188 ± 13
Sale weight (kg)	268 ± 3	237 ± 6	312 ± 11
Fattening period (months)	12.5 ± 1.3	4.8 ± 0.4	8.3 ± 0.9
Average daily gain (kg/hd/d)	0.44 ± 0.13	0.32 ± 0.03	0.58 ± 0.05
Animal value at start (Rp $x10^4$/kg)	3.3 ± 1.4	3.0 ± 0.1	3.3 ± 0.3
Animal value at sale (Rp $x10^4$/kg)	2.3 ± 0.2	3.0 ± 0.1	3.0 ± 0.1
Margin (Rp/head/month)	49.9 ± 4.3	29.9 ± 3.1	47.1 ± 5.3

In the feeding trial, farmers could not achieve the recommended levels of sesbania feeding (Table 2).

Table 2. Growth rates of Bali bulls fed sesbania foliage at 3 sites in central Lombok (August 2012–March 2013).

Variables	Montong Oboq	Bun Prie	Repok Nyerot
Land ownership (ha)	0.39 ± 0.15	0.23 ± 0.01	0.53 ± 0.08
No of farmers selected for trial	6	6	8
No of sesbania trees/farmer	199 ± 37	321 ± 79	326 ± 31
No of bulls allocated to village	6	6	8
Starting weight of bulls (kg)	87 ± 3	93 ± 2	89 ± 1
Requested level of sesbania in diet (% fresh)	20	40	60
Actual proportion of sesbania in diet (% fresh)	29 ± 6	49 ± 2	45 ± 4
Amount of rice bran offered (kg DM/hd/d)	0	0.9 ± 0.1	1.2 ± 0.1
Average daily gain (kg/hd/d)	0.35 ± 0.02	0.34 ± 0.02	0.50 ± 0.04

Despite similar proportions of sesbania in the diet, daily gains were higher at Repok Nyerot (0.50 kg/hd/d) than at Bun Prie (0.34 kg/hd/d); gains at Montong Oboq were 0.35 kg/hd/d, where sesbania feeding was least and rice bran was not fed. Differences in growth rates may have been related to variation in feeding practices by individual farmers, i.e., total dry matter offered/day and differing quality of the grass offered.

These data offer a basic understanding of sesbania feeding systems in Indonesia and their productivity. Growth rates were comparable with previously recorded data, namely 0.38 kg/d for bull calves of similar age fed 30% sesbania; however, they were much higher than 0.2 kg/d achieved in traditional fattening systems comprising diets of predominantly local grass species (Dahlanuddin et al. 2013).

Conclusion

Although some difficulties occurred with this on-farm research, the study suggests that the inclusion of sesbania in the fattening diet can boost animal growth rates. The trial will continue to monitor the growth path on-farm until the bulls reach maturity.

Acknowledgments

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Selection of psyllid-resistant forage varieties from an inter-specific breeding program of *Leucaena leucocephala* with *L. pallida*

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Keywords: Psyllid damage, leafiness, better branching, forage legumes.

Introduction

Leucaena (*Leucaena leucocephala*) pastures for beef cattle production are productive and sustainable; however, susceptibility to the psyllid insect (*Heteropsylla cubana*) has limited expansion of current commercial cultivars into more humid areas (>800 mm/yr) (Shelton and Dalzell 2007). Psyllids can also cause intermittent damage in lower rainfall regions during humid periods.

The psyllid, which arrived in Australia in 1986, is a leaf-sucking insect specific to the *Leucaena* genus, feeding on the growing tips of susceptible cultivars (Bray 1994). Psyllid damage can reduce production by as much as 50−70% in humid regions and 20−50% in subhumid environments (Bray 1994; Mullen and Shelton 2003). Work on psyllid resistance in the *Leucaena* genus through the 1990s showed that several *Leucaena* species, including the tetraploid *L. pallida,* had good levels of resistance (Mullen et al. 2003).

A breeding program to develop psyllid-resistant varieties began in 2002 at The University of Queensland (UQ) based on the F1 inter-specific hybrids between *L. leucocephala* and *L. pallida* (known as 'KX2'), developed at the University of Hawaii (Brewbaker 2008). Between 2002 and 2005, UQ initiated a program of recurrent selection in an attempt to produce stable outcrossed KX2-derived lines, but inbreeding depression for yield and poor forage quality led to a change in the breeding strategy, and a backcrossing program was implemented between 2005 and 2008. Two cycles of backcrossing to elite *L. leuco-cephala* ssp. *glabrata* material were completed, followed by 2 cycles of progeny testing and selection for self-compatibility to achieve stability and uniformity (2009−2012). Forty elite psyllid-resistant lines were then evaluated to identify the most suitable lines for release to industry. This paper describes the results of these trials.

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Methods

Six replicates of 40 elite lines were compared, with 4 commercial *L. leucocephala* cultivars (Peru, Cunningham, Tarramba and Wondergraze) as controls. The randomized block design experiment was conducted at the Redlands Research Station (27°53' S, 153°25' E), 30 km east of Brisbane, Australia, from October 2011 to March 2013. The station receives an annual rainfall of 1322 mm and was an ideal location to conduct psyllid-resistance trials owing to the high challenge from the insect over significant periods of each year. Each plot contained 12 plants of each line, spaced 0.5 m apart. Buffer and border rows of the highly susceptible cv. Peru were included to ensure even psyllid pressure across the site. Psyllids were controlled with dimethoate until plants were well established.

Traits assessed were:

- Psyllid damage rating of growing tips (PDR) (1 = no damage, 9 = dead) (Wheeler 1988)
- Yield index based on basal diameter^2 × height (Stewart et al. 1992), adjusted to a 1−5 scale
- Floral development rating (FDR) (1 = vegetative, 5 = mature seed pods)
- Leafiness and branching ratings (1 = low, 5 = high)
- In vitro dry matter digestibility (IVDMD) of the first fully expanded leaves from 10 plants per plot.

Minitab was used to create 'box and whisker' plots for each trait. The elite lines were then compared with the controls with respect to the median, lower and upper quartiles, range and skewness of the data.

Results and Discussion

All elite lines tested were superior to the commercial cultivar controls in psyllid damage rating (PDR), but had higher variability (Figure 1). The median PDR was 2.8 for the elite lines compared with ~7.8 for controls. This difference was evidence that the breeding program was successful, as a PDR <3 indicates minor damage under psyllid attack, while a PDR of >8 indicates severe damage and large production losses. The digestibility and associated spread for the elite lines (median of 69%) were similar to the value for controls (median of 68.3%). High forage quality is one of the most important characteristics of leucaena, and a lower digestibility in the elite lines was expected due to the lower IVDMD of *L. pallida.* The results indicate that selection for psyllid resistance will be possible without seriously compromising digestibility.

Yield index for the elite lines (median of 2.8) was similar to that of the controls (median of 2.6). However, the yield index was measured following a period of low psyllid challenge, so that yield differences due to psyllid damage were minimized. The range for this trait was large, reflecting the genetic diversity of the breeding lines. Elite lines were similar to the controls in leafiness, branching and floral development ratings, with the observed range in these traits again higher for the elite lines than for the controls. Regarding floral development, it is important to select lines that seed adequately to meet seed-production demands, but not excessively so as to pose a weed risk. When all parameters were considered, together with minimum criteria established for each trait (including seed availability), 8 of the 40 breeding lines were selected to be carried forward in the commercialization process.

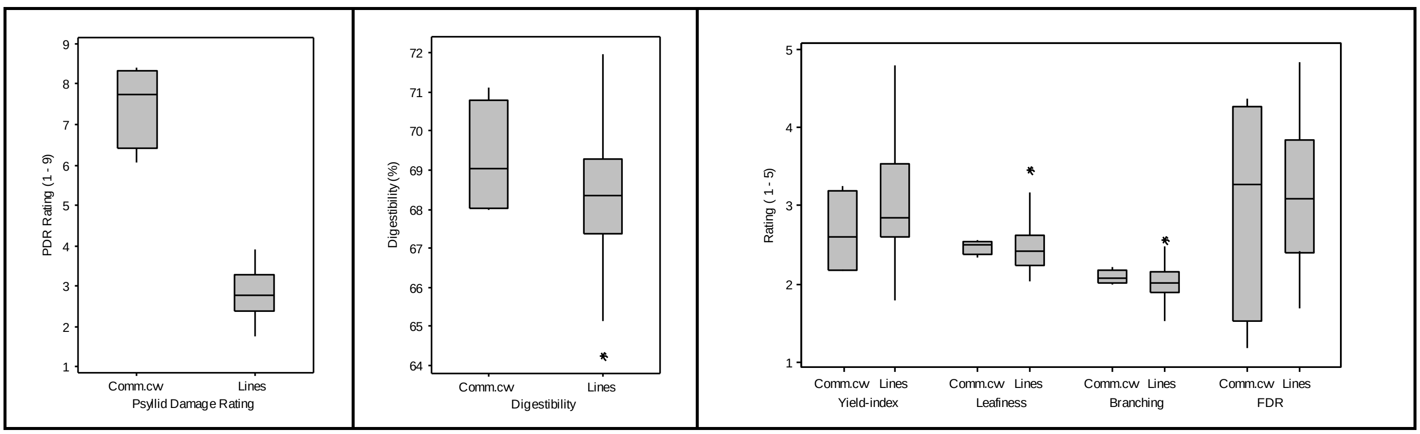

Figure 1. Box plot of psyllid damage ratings, digestibility and agronomic traits for leucaena breeding lines compared with commercial cultivars. FDR = floral development rating.

Conclusion

The breeding program has successfully developed elite lines with superior psyllid-resistance compared with the commercial cultivars. This was achieved without significant reduction in yield, forage quality or floral development characteristics. Plant Breeder's Rights will be sought over the period 2013−2015 for eventual release of at least one of these lines to industry.

Acknowledgments

The statistical assistance of Mr Allan Lisle, and the laboratory assistance of Mr Peter Isherwood for DMD analysis were greatly appreciated. This project was funded jointly by The University of Queensland and Meat and Livestock Australia.

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Survey of pasture species and management, manure management, milk production and reproduction on pasture-based dairy farms in Florida and Georgia

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Keywords: Grazing, forages, sustainability, management system.

Introduction

Traditionally, most dairy farms in the south-eastern United States confine cows to barns or on pasture lots year-round and feed stored forages and concentrated feeds (Fontaneli et al. 2005). Often, much of the feed is purchased; however, the cost of purchased feed and fuel has risen rapidly in the last 5 years (NASS 2009). In addition, a significant amount of capital is tied up in buildings, machinery and manure management systems on the farms. For these reasons, many dairy farmers have shown an interest in or started transitioning to pasture-based dairy systems (Ricks and Hardee 2012). The management practices and production results of pasture-based dairy farms in the south-east appear to vary widely (Macoon et al. 2011), but have not been described. The objective of this study was to document pasture and crop management, manure management and milk production on pasture-based dairy farms in Florida and Georgia.

Methods

A survey was designed and consisted of 62 questions covering 7 areas, including farm business structure, young stock management, milking herd management, pasture and crop management, feeding management, manure and nutrient management, and environment and sustainability. The survey focused on the year from summer 2011 to spring 2012. Dairy farmers in Florida and Georgia were invited by telephone calls, emails and announcements in newsletters to participate. Data were collected by personal interviews from September 2012 to March 2013, and analyzed using Microsoft Excel.

Results

The survey was conducted on 23 dairy farms, involving approximately 29,000 cows and 17,000 heifers, about 15% of all dairy cows in Florida and Georgia. Rotational stocking was employed by 13 (57%) of the respondents. During the warm season, all 23 farms grew warm-season perennial grasses, and during the cool season, 18 farms grew cool-season annual grasses. The total area of warm-season perennial grassland was 5,012 ha, with mixed-species pastures occupying 2,630 ha (52%) and non-mixed pastures occupying the remaining 2,382 ha. Of the non-mixed grass pastures, areas were: 878 ha (37%) of bermudagrass (*Cynodon* spp.), which included Tifton 85, common bermudagrass, Florakirk bermudagrass and coastal bermudagrass; 1,114 ha (47%) of stargrass (*Cynodon nlemfuensis*); 100 ha (4%) of limpograss (*Hemarthria altissima*); and 289 ha (12%) of bahiagrass (*Paspalum notatum*), including cvv. Pensacola, Tifton 9 and Argentine. The total area of cool-season annual grasses was 1,475 ha, with mixed cool-season annual grasses on 878 ha (59%) and non-mixed cool-season annual grasses on 678 ha (41%). Of the non-mixed grasses, oats (*Avena sativa*) was the most common (482 ha, 71%), followed by triticale (x*Triticosecale* spp.) on 144 ha (21%) and annual ryegrass (*Lolium multiflorum*) on 52 ha (8%). The most popular mixture of cool-season grasses was annual ryegrass and oats, established on 374 ha (43%). Warm-season annual grasses were established on 2,358 ha, with corn (*Zea mays*) on 938 ha (40%), sorghum (*Sorghum bicolor*) on 850 ha (36%), crabgrass (*Digitaria sanguinalis*) on 400 ha (17%) and pearl millet (*Pennisetum glaucum*) on 168 ha (7%).

Thirteen farms (57%) treated fall army worm (*Spodoptera frugiperda*) with pesticide, while 16 farms (70%) controlled weeds with herbicides and 11 farms also used a machine to cut weeds. No manure or com-

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mercial fertilizer was used on grass paddocks on 10 farms (43%), while 3 farms (13%) used only commercial fertilizer, 1 farm (4%) used liquid manure only, 1 farm (4%) used solid and liquid manure, 3 farms (13%) used liquid manure and commercial fertilizer, and 5 farms (22%) used solid and liquid manure plus commercial fertilizer. Ten farms (43%) applied no manure or fertilizer to cropland, 9 farms (39%) used all their liquid and solid manure plus commercial fertilizer, 3 farms (13%) used only liquid and solid manure, and only 1 farm (4%) used liquid manure and commercial fertilizer on cropland.

Average milk production was 27 ± 7 kg/cow/d during the winter and 20 ± 7 kg/cow/d during the summer. The rolling herd mean yield was 7,794 ± 1,773 kg/cow/yr. Average somatic cell count was 246,292 ± 69,614 cells/mL during the winter and 365,292 ± 78,587 cells/mL during the summer. Six farms (26%) utilized a year-round breeding strategy, while the remaining farms practised various seasonal breeding strategies. Three farms (20%) employed 100% seasonal breeding. The most calvings were reported in October (11 farms, 48%), while 14 farms (61%) reported the fewest calvings in August. Non-breeding periods were reported by 18 farms (78%). Summer breeding was avoided owing to low conception rates; summer calving was avoided because of calving problems at this time (9 farms, 50%), and cows were not bred during October−November (11 farms, 61%) to avoid calving during the summer.

Conclusion

Grass varieties, fertilizer practices, milk production and reproduction all varied widely among pasture-based dairy farms in the south-eastern United States. Survey results will help direct subsequent research and extension programs to gather more knowledge, help promote sustainable agriculture and meet farmers' needs from university Extension staff.

Acknowledgments

We thank the farmers who participated in the survey and the Extension agents who helped to contact them. This study was financially supported by USDA-SARE grant LS11-243 "Improving the Welfare of South Eastern Dairy Families through the Adoption of Sustainable Production Practices".

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Morphogenic responses of two *Brachiaria* genotypes to clipping frequency

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Keywords: Marandu palisadegrass, Mulato II brachiariagrass, defoliation, leaf appearance, pasture.

Introduction

Tropical grasslands represent an important resource for the Brazilian cattle industry, which is heavily dependent on grazed pastures. Total pasture area in the country totals 196 Mha (23% of the country's land area) (FAO 2013). The genus *Brachiaria* represents around 85% of cultivated pastures in Brazil (Moreira et al. 2009), 40% of which are established with *B. brizantha* cv. Marandu (Barbosa 2006). Mulato II is a new hybrid brachiariagrass cultivar, which has been developed to improve agronomic characteristics, broaden the range of adaptation, and ensure high forage production and nutritive value. It has also been viewed as a means of reducing the dependence on the Marandu palisadegrass monoculture (Argel et al. 2007).

The use of new cultivars should be based on adequate understanding of physiological processes and growth potential under a range of management practices. Morphogenic characteristics allow for accessing herbage accumulation potential through the measurement of tissue synthesis and senescence in forage plants. Management practices such as defoliation frequency can modify assimilate partitioning in the forage plant, affecting morphogenic characteristics related to growth rate and forage nutritive value. The objective of this research was to describe and explain morphogenic differences between Marandu palisadegrass and Mulato II brachiariagrass as affected by harvest frequency.

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Methods

The experiment was conducted in the Department of Animal Science ESALQ/USP, Piracicaba, SP, Brazil (22°42' S, 47°30' W; 580 m asl). Average annual rainfall is about 1,300 mm and temperature ranges between 10 °C and 35 °C. The soil at the experimental area is a Kandiudalfic Eutrudox. Plots were established with *Brachiaria brizantha* cv. Marandu and *Brachiaria* cv. Mulato II in October 2010. The evaluation occurred during the summer season (December 22, 2011−March 20, 2012). Two experiments were conducted simultaneously in adjacent areas, one irrigated and another non-irrigated. The design of each experiment was a randomized complete block in a 2 x 2 factorial arrangement, with the 2 grasses and 2 harvest frequencies (28 and 42 days) and 4 replications, totaling 16 experimental units of 20 m² (4 m x 5 m). Harvests were made 10 cm from the soil surface.

Ten tillers were evaluated for the following characteristics: (a) leaf blade length and (b) leaf type, classified as expanding, expanded, senescing and dead. Leaves were classified as: expanding, when their ligules were not exposed; expanded, when the ligule was visible and/or growth ceased; senescing, when part of the leaf blade showed signs of senescence (yellowing and necrosis); and dead, when more than 50% of the leaf blade was senesced. Degree of leaf senescence was estimated visually. The stem length was measured from the soil level to the ligule of the youngest fully expanded leaf. Means were calculated using "LSMEANS" statement, and comparisons made with "PDIFF" based on a Student t-test ($P<0.05$). Data for the experiment were analyzed using the GLM Procedure of SAS.

Results and Discussion

Marandu palisadegrass had higher leaf appearance rate than Mulato II (Figure 1A). This was due to the higher number of leaves per tiller in Marandu (Figure 1B) and the similar length of leaves in the 2 grasses (8.8 cm, P=0.7789). Leaf elongation rate increased from 0.64 to 1.24 cm/tiller/d with lower harvest frequency (Figure 1C). This characteristic is related to an increase in leaf area resulting in an exponential increase of shoot mass in swards clipped every 42 days relative to those clipped every 28 days. The 42-d clipping frequency resulted in a 3-fold stem elongation rate compared with the 28-d frequency (Figure 1D). The stem component is often related to decreased nutritive value and intake by grazing ruminants.

Despite the higher stem elongation rate, which is often associated with light competition among tillers, senescence rate was not affected by clipping frequency (0.0055 cm/tiller/d, P=0.0723). Light competition among tillers was not sufficient to cause accelerated death of mature leaves in the base of the sward.

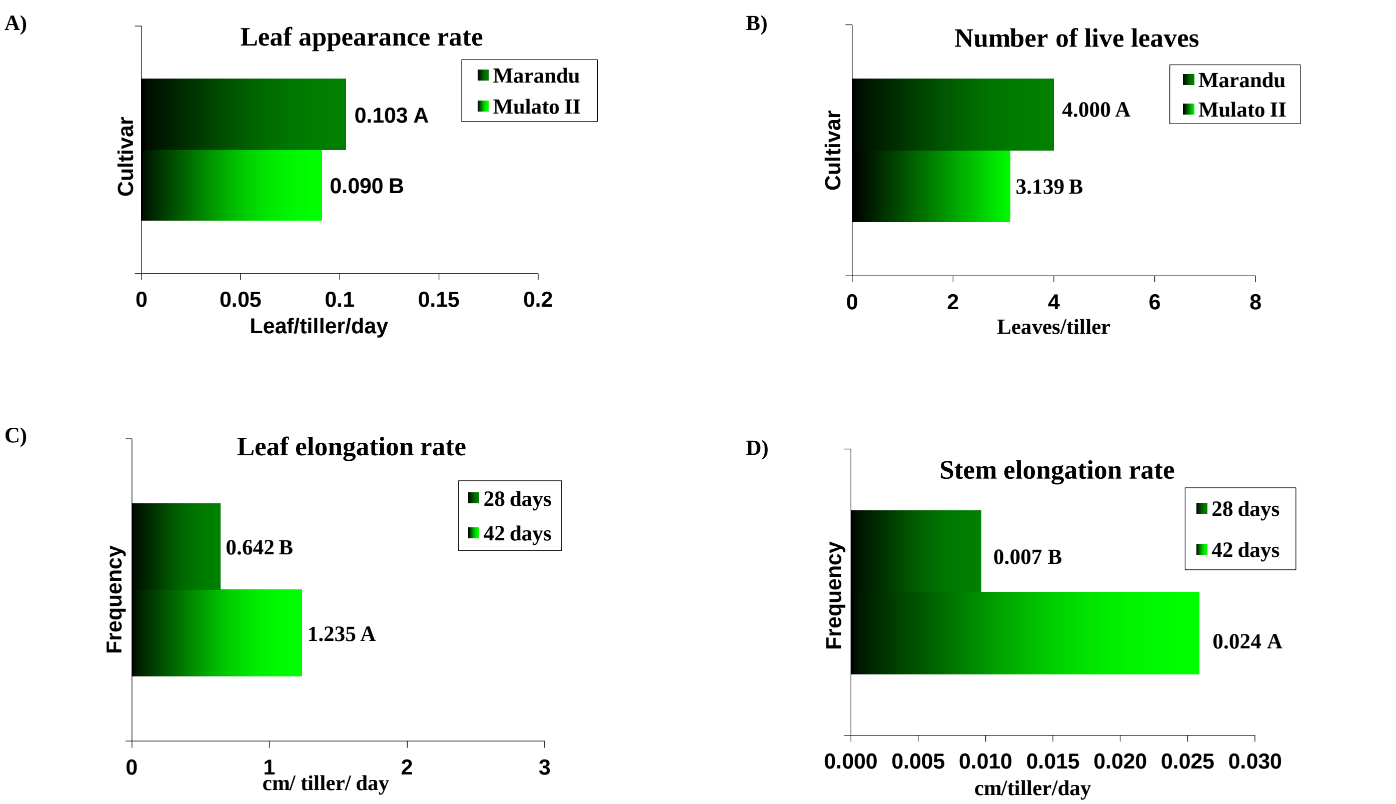

Figure 1. Morphogenic characteristics of 2 *Brachiaria* genotypes in response to clipping frequency. Within parameters, means with different letters are different (P<0.05).

Conclusion

The higher morphogenic rates and growth potential of Marandu palisadegrass than Mulato II may help explain the different growth responses in these genotypes. Harvesting every 28 days would reduce stem proportion in the herbage mass harvested, resulting in better quality forage for animals. However, reduced dry matter production could be expected.

Acknowledgments

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Sward structural characteristics of perennial peanut genotypes as affected by harvest frequency

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Keywords: Light interception, *Arachis,* leaf angle, leaf area index, forage legumes, tropical legumes.

Introduction

Despite the high potential of tropical forage species, herbage production, nutritive value and animal production in Brazilian livestock production systems are lower than what should be obtained from both a biological and operational point of view (Pedreira and Mello 2000). Even with these limitations, the livestock industry is often able to sustain high levels of productivity (animal product per hectare) by using good animal genetics and supplementation. Reducing production costs, however, will likely depend on the identification and incorporation of a high-quality forage resource, in terms of both improving diet quality of grazing animals and sustaining pasture soil productivity.

The search for economically viable and sustainable forage production alternatives has been the subject of a great deal of research in many parts of the world. Among the alternatives explored, the diversification of pastures by the introduction of forage legumes in traditional production systems has been suggested, mainly to improve soil chemical characteristics (increased nitrogen levels) and improve forage quality (Perez 2004; Valentim and Andrade 2004). Promising legume germplasm is available in the tropics, but before these materials are incorporated into commercial systems, they need to be evaluated for adaptation, production and persistence in specific micro-environments. In addition, interactions involving grazing management strategies and genotypes should be described and explained, so that their agronomic potential can be explored. The aim of this study was to characterize sward structure of 4 perennial peanut genotypes, subjected to 2 harvest management strategies, in southeastern Brazil.

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Methods

The experiment was conducted at the Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ) in Piracicaba, SP, Brazil (22°42′ S, 47°30′ W; 580 m asl). Treatments consisted of all possible combinations between 4 perennial peanut (*Arachis pintoi*) genotypes: Alqueire-1, Amarillo, Belmonte and Mandobi and 2 harvest frequencies: 28 and 42 days. The soil was a Typic Hapludoll. The experimental design was completely randomized with 3 replications in a 5 x 2 factorial, totaling 30 experimental units of 18 m^2 separated by 1-m aisles. Light interception, leaf area index and foliage angle were measured using a model LAI-2000 canopy analyzer (Li-Cor, Lincoln, Nebraska, USA). Eight readings were taken per experimental unit immediately before each harvest using a ratio of 4 measurements at ground level and a comparative measure above the canopy. A 180-degree protection was used in the field of view of the sensor (lens) as recommended by the manufacturer for use in plots (Welles and Norman 1991). The experimental units were measured before and after the harvests; the residual height was 10 cm.

Results and Discussion

There were significant effects for frequency of harvest (P=0.0105) and genotype (P=0.0037) in pre-harvest measurements for leaf area index (LAI) (Table 1). Plots harvested every 42 d showed higher LAI than those harvested every 28 d, due to increase in the amount of leaves that are related to the exponential increase in shoot mass. Overall, Belmonte had greatest LAI as a result of better adaptation to local edaphoclimatic conditions and genetic differences among the genotypes. Light interception (LI) was modified only by harvest frequency (P=0.0242) with longer intervals increasing LI for all genotypes. This is related to increase in the LAI, leaves being the most important plant part for capturing light and performing photosynthesis. There was an interaction effect of harvest frequency x genotype (P=0.0393). Amarillo increased foliage angle when harvested with longer clipping intervals as a result of adaptation in the architecture, changing the orientation of the leaves to optimize LI with a greater LAI. When harvested every 28 d, Alqueire-1 and Mandobi had greater foliage angle than Belmonte. On the other hand, when harvested every 42 d, there was no difference among genotypes (Table 1). It is possible that modification in sward structure happens mainly during the initial 28 days of regrowth.

Table 1. Leaf area index (LAI), light interception (LI) and leaf angle of perennial peanut genotypes submitted to 2 cutting frequencies.

	Genotype				
Cutting frequency (days)	Alqueire-1	Amarillo	Belmonte	Mandobi	Mean
			LAI		
28	3.0	3.6	3.6	3.0	3.3 b[1]
	(0.24)	(0.25)	(0.25)	(0.25)	(0.12)
42	3.4	3.4	5.1	3.7	3.9 a
	(0.39)	(0.41)	(0.39)	(0.41)	(0.20)
Mean	3.2 B[1]	3.5 B	4.3 A	3.4 B	
	(0.23)[2]	(0.23)	(0.23)	(0.23)	
			LI (%)		
28	89	91	92	90	90 b
	(1.37)	(1.43)	(1.43)	(1.43)	(0.71)
42	94	93	95	92	94 a
	(2.24)	(2.38)	(2.24)	(2.38)	(1.15)
Mean	91	92	93	91	
	(1.31)	(1.39)	(1.33)	(1.39)	
			Leaf angle (degree)		
28	41 Aa	36 Bb	39 ABa	41 Aa	39
	(1.42)	(1.47)	(1.47)	(1.47)	(0.73)
42	41 Aa	46 Aa	43 Aa	40 Aa	42
	(2.30)	(2.44)	(2.30)	(2.44)	(1.19)
Mean	41	41	41	41	
	(1.35)	(1.42)	(1.37)	(1.43)	

[1]Capital letters compare genotypes and lower-case letters compare frequencies by Student's t-test (P<0.05).
[2]Numbers in parentheses are the standard error of the mean.

Conclusion

The findings from this study help to explain how different genotypes react structurally to different cutting frequencies. How this is reflected in production of dry matter is the subject of a different study.

Acknowledgments

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Effects of season and year of evaluation in the selection of *Brachiaria humidicola* hybrids

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Keywords: Genotype by environment interaction, environmental effects, nutritive value, tropical grasses.

Introduction

Brachiaria humidicola is well adapted to infertile and acid soils with poor drainage or temporary flooding (Keller-Grein et al. 1996). It is widely used in Brazil in wetlands and areas of marginal land characterized by waterlogged soils. During the evaluation stages of the breeding program of this species, genotypes are generally tested using consecutive cuts within different seasons for at least 2 years. The 'Cerrado' region, where most animal production takes place in Brazil, has 2 well defined seasons: spring-summer with warm weather and rain; and autumn-winter with cooler, dry weather. Thus, owing to environmental variation, especially related to climate, it is important to investigate the main effects of environmental factors (years and seasons), as well as interactions between genotypes and environmental factors, in order to have greater confidence in the selection of superior hybrids of *B. humidicola* on the basis of agronomic and nutritional traits.

Methods

Fifty hybrids resulting from crossing *B. humidicola* cv. BRS Tupi with a sexual accession, both hexaploid (2n = 6x = 36), were evaluated with the 2 parents as controls. They were established by seedlings in a complete randomized block design with 8 replications, using plots of 2.0 m². Each experimental unit was subjected to 7 cuts during the rainy seasons and 2 cuts during the dry seasons in 2008 and 2009. The production of total green matter was weighed and a sample of approximately 300 g of green material was taken for separation and determination of the dry weight of leaves, stems and dead material. With this information, we calculated total dry matter (TDM), leaf dry matter (LDM), leaf percentage (%L) and leaf:stem ratio (LSR). After 7 days a visual assessment of regrowth was performed. This was a combined score ranging from 0 (poor) to 6 (excellent) of plant density (percentage of tillers shooting) and speed of regrowth. Leaf samples were analyzed for: crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (Lig) concentrations and in vitro organic matter digestibility (IVOMD) using near-infrared reflectance spectroscopy (NIRS) (Marten et al. 1985). The traits were analyzed statistically for the 9 cuts together using the following model: $y = Xf + Pb + Zg + Nk + Qa + Ti + Wt + Om + e$, where y is the data vector, f is the effect of combining cut-season-year (assumed fixed) added to the overall mean, b is the effect of blocks, g is the genotypic effect, k is the effect of the blocks x hybrids interaction, a is the effect of the hybrids x years interaction, i is the effect of the seasons x hybrids interaction, t is the effect of the hybrids x times x years interaction, m is the effect of the blocks x cuts interaction within season and year, and e is the error term. X is the incidence matrix of fixed effects and P, Z, N, Q, T, W and O are the incidence matrices of random effects b, g, k, a, i, t and m, respectively. The analyses were performed using the statistical package Statistical Analysis System (SAS Institute 2002).

Results and Discussion

The year effect was significant ($P<0.001$) for LDM, %L, regrowth, CP and NDF (Table 1), showing that overall mean performance of hybrids varied between the 2 years. This is due to climatic fluctuations between years causing one year to be more favorable than the other. There was a significant effect of seasons for most characters, except for NDF and ADF, since the rainy season promotes more growth than the dry season. Within agro-

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nomic characters, the years x seasons interaction was significant only for percentage of leaf blades (%L) ($P<0.001$). Regarding nutritive value, NDF, IVOMD and lignin showed a significant years x seasons interaction, which is understandable since fiber deposition is highly correlated with climate and plant age and strongly affects digestibility.

Table 1. P-values of the F statistic for fixed effects and Wald Z statistic for random effects for agronomic and nutritive value traits of *Brachiaria humidicola* hybrids evaluated over 9 cuts.

Effects	LDM	TDM	%L	LSR	Regrowth	CP	NDF	ADF	IVOMD	Lig
Fixed effects										
Year	0.001	0.103	0.001	0.055	0.001	0.001	0.004	0.091	0.422	0.098
Season	0.001	0.001	0.001	0.001	–	0.001	0.888	0.775	0.001	0.046
Y x S	0.135	0.159	0.001	0.582	–	0.140	0.016	0.205	0.001	0.001
Cut (Y – S)	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002
Random effects										
Hybrid	0.001	0.001	0.001	0.467	0.001	0.001	0.014	0.038	0.001	0.093
H x Y	(.)	.	.	.	0.154	0.001	0.370	0.115	.	.
H x S	.	.	.	0.075	.	.	0.032	.	0.075	0.372
H x Y x S	.	.	.	0.032	0.001	.	.	.	.	.
H x C (Y – S)	0.001	0.001	0.001	.	0.001	0.040	0.190	0.131	0.187	0.387

(.) Nulls value for Wald Z statistic.

There was significant variation between cuts in both years and seasons for all characters. It is important to verify the existence of genetic variability among hybrids for response to environmental factors. While significant genetic divergence was observed for most characters, no significance was observed for the effects of interactions with years and/or seasons for the characters studied (Table 1), implying that hybrids behaved consistently in the seasons and/or years of evaluation. The hybrids x cuts interaction within seasons and years, however, was significant for almost all agronomic traits ($P<0.001$), except for LSR (.) as opposed to that observed for the characters of nutritive value, with only CP influenced by this effect.

Conclusion

We conclude that the interactions of hybrids with years and/or seasons were not important, but there was marked variation explained by the interaction of cuts within seasons and years. This suggests that breeders should not correct for the effects of years and seasons in models used in the statistical analysis, once the use of a parsimonious model involving the general effect of the cuts can properly select hybrids for agronomic and nutritive value traits.

Acknowledgments

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PROGARDES™: a legume for tropical/subtropical semi-arid clay soils

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Keywords: *Desmanthus*, species blend, persistence.

Introduction

The range of available sown pasture legumes for the vast heavy clay soil regions of northern Australia has long been regarded as being deficient (Burt 1993; Jones and Clem 1997; Pengelly and Conway 2000). Indeed immense areas of northern Australia's semi-arid clay soil regions have no sown pasture legume with proven adaptation and persistence through the long annual dry seasons (Gardiner and Swan 2008).

The genus *Desmanthus* is a Mimosaceae legume containing some 24 species which are native to the Americas and range from being herbaceous to suffruticose in habit (Luckow 1993). *Desmanthus* is one of the very few legumes consistently observed to persist under heavy grazing on clay soils in their native environments (Pengelly and Conway 2000). Numerous accessions of *Desmanthus* were collected and introduced into Australia by various institutions, notably CSIRO and QDPI, over the past 50 years (Reid 1983; Pengelly and Liu 2001), as potential legumes for clay soils. After years of multi-site field evaluation of *Desmanthus* and other species, in 1991 QDPI released 3 *Desmanthus* cultivars, cvv. Marc (*D. virgatus)*, Bayamo (*D. leptophyllus*) and Uman (*D. pubescens*), which were marketed as a blend named "Jaribu" (Cook et al. 1993). Currently, only cv. Marc is available commercially with a focus on southern subtropical Queensland markets. However, Pengelly and Conway (2000) state that, owing to Marc's low dry matter production, its contribution to animal diets and soil N is limited.

Development of Progardes™

In the 1990s Chris Gardiner at James Cook University, Townsville, started to survey legumes at a number of old abandoned trial sites across remote northern and central western Queensland's semi-arid clay soil regions (<500 mm AAR). He found that various *Desmanthus* accessions were the only surviving and thriving legumes of all species sown in those environments. These particular accessions had persisted for a decade or more (now more than 2 decades) and had survived grazing, drought, flooding, fire and frosts and had therefore withstood the full gambit of environmental stresses (Gardiner and Swan 2008). The selection and breeding of plants from these survivors and others from other similar old sites and their subsequent evaluation in new trials and seed multiplication have led to the development of new lines of *Desmanthus* for northern Australia and similar environments**.**

The best of these varieties have been released by Agrimix Pty Ltd, JCU's commercialization partner, as a blend named Progardes™ (www.progardes.com.au). Progardes being, **Pro** for protein, **gar** for Gardiner and **des** for *Desmanthus* The blend comprises new selections of the species *D. bicornutus, D. leptophyllus* and *D. virgatus* giving it a broad range of early to late maturity types, plus a range of habits (herbaceous to suffruticose) and edaphic and climatic tolerances. In recent times Progardes™ has been sown in trials and demonstration areas totaling some 2000 ha in a wide range of environments including: open Downs, cleared Gidgee/Boree and Brigalow land types across Queensland and via a number of sowing techniques including aerial, blade ploughing, stick raking, broadcasting onto cultivated seedbeds in a buffel grass renovation and broadcasting onto unprepared native grass pasture, all with considerable success. By the end of the 2012/13 season, Agrimix had some 12,000 ha commercially sown.

As with most crops and pastures, the key to successful establishment is one of timing, and with Progardes™ in northern Australia planting at the end of the dry season/start of the wet season gives the establishing plants a good opportunity to come away with the sown or native

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grasses. Annual rainfall on our rangelands is highly variable (McKeon 2006), so the common practice is to plant a mixture of hard and soft (scarified) seed, which gives some insurance against false seasonal starts. Scarification typically enhances germination from about 10% to 70−80%. Experience is showing that 2 or 3 wet seasons may be required for the Progardes™ to become well established in the harsher drier environments such as on the Mitchell Grass Downs, but only one season in the more favorable Brigalow region of Queensland.

Grazing and feeding trials with both sheep and cattle have been undertaken. Steers grazing a buffel grass-Progardes™ mixed pasture in central Queensland gained an additional 40 kg live weight over a 90-day study period compared with steers on an adjacent buffel grass-only pasture during a cool dry winter (Gardiner and Parker 2012). In a feeding study with sheep, adding Progardes™ to a Flinders grass diet resulted in a positive N balance and significantly improved intake and weight gains over that of sheep fed only Flinders grass (Ngo 2012). Concentrations of crude protein in leaf, stem and pods with seeds of Progardes™ have been recorded as being 20.2, 11.9 and 17%, respectively, while energy (ME) levels of the whole plant were 10.3 MJ/kg DM.

Commercial seed crops are being grown successfully on the Atherton Tablelands and the Burdekin Irrigation areas; some hay has also been effectively made from Progardes™. It is expected that even small amounts of Progardes™ will improve animal production in the targeted dry environments owing to what Bell (2009) terms the marginal value of feed that such nutritious plants offer. Kretschmer and Pitman (2001) stated that as little as 10% of a legume in poor quality grass pasture would make significant impacts on animal intake and therefore production. In a 250 ha trial in central Queensland's Brigalow region, sowing Progardes™ into renovated buffel grass resulted in a legume density of 7 plants/m^2, with a frequency of 75% at the end of the 1st growing season. This indicates potential compatibility with buffel, where few legumes, as stated by Robertson et al. (1997), have been successful. Reid (1983) noted, when collecting *Desmanthus* in Mexico, that in certain regions on clay soils it often occurred with the introduced buffel grass. Its compatibility with buffel suggests too that it has potential to alleviate buffel run-down, a widespread problem which reduces animal production.

Conclusions

Progardes™ appears to be an appropriate legume for improving livestock production in terms of increased liveweight gains, reproductive rates, turn-off and wool production, particularly in the semi-arid clay soil regions of northern Australia, where few, if any, other herbaceous sown pasture legumes are currently adapted. Widespread plantings by commercial graziers will confirm whether this potential can be realized and what contribution the legume can make to sustainability of pastures in these regions.

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Potential of Australian bermudagrasses (*Cynodon* spp.) for pasture in subtropical Australia

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Keywords: IVDMD, forage quality, seed set.

Introduction

In Australia, little work has been carried out on the improvement of tropical or subtropical pastures in recent years. There seems to be an increasing demand for pastures that can withstand heavy grazing, while producing high yields of high quality forage in the humid subtropical regions of Australia. Most graziers, who live in these areas, have small acreages, which they graze intensively. *Cynodon* spp. represent a potential source of grasses with these attributes. This study will evaluate the pasture potential of a large number of bermudagrass (*Cynodon* spp.) ecotypes collected from across Australia.

Materials and Methods

The studies were conducted on a highly fertile vertisol at the University of Queensland, Gatton Research Station (27.54° S, 152.34° E), Queensland, Australia. Included were 850 *Cynodon* ecotypes collected from 5 climatic zones across Australia and 70 *Cynodon* turf cultivars assembled from Australia and USA. The 920 genotypes were split into 2 groups based on collection time. Experiment 1 included 440 entries and was planted on August 20, 2008. Experiment 2 included 480 entries and was planted on April 20, 2009. For each grass a 2 m x 2 m plot was established from a single plug. Each experiment was arranged as an augmented Latin square (ALS) design and included a single replication of each ecotype with 5 repeated check entries (Legend, Wintergreen, CT-2, 81-1, 40-1), replicated 5 times and Latinized throughout the field. Experiment 1 was harvested on March 25, 2010 and experiment 2 on April 29, 2010. Using a standard lawn mower with cutting height set to 5 cm, a middle strip 45 cm wide and 1 m long was cut from each quadrant after the front and rear of the plot had been trimmed to remove 'edge' effects. The grass was collected, weighed fresh and sub-sampled to determine moisture content, for calculating dry matter (DM) yield per unit area. In vitro dry matter digestibility (IVDMD) (Minson and McLeod 1972) was determined for the top 20 and bottom 5 ecotypes on the basis of yield from each experiment. Seed set of the grasses was determined from X-ray images taken of flower heads using a Faxitron-X-Ray machine.

Results

Average DM yields for experiments 1 and 2 were 8.9 and 7.7 t/ha, respectively, with a large range (1–23 t/ha) for each experiment (Figure 1). Average values for IVDMD of the top 20 ecotypes in experiments 1 and 2 were 47% and 48%, respectively, and for the bottom 5 ecotypes were 51% and 47%, respectively. Most ecotypes (520 of the 920) were completely sterile, and there was a large range in seed set per head (1−150 seeds/head) for the remaining ecotypes (see Plate 1). On average the highest-yielding ecotypes were collected from warm environments (Table 1).

Discussion

These preliminary yield data suggest there is great potential within the assembled Australian *Cynodon* germplasm to identify forages with agronomic potential

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for the humid subtropics. The top 20 ecotypes on the basis of yield from both experimental areas produced 14−23 t/ha, similar to biomass production of other tropical forage grasses grown in Queensland; for example, blue couch (*Digitaria didactyla*) 7−11 t/ha, pangola grass (*D. eriantha*) 10−20 t/ha, creeping bluegrass (*Bothriochloa insculpta*) 10−15 t/ha and bahiagrass (*Paspalum notatum*) 8−20 t/ha (Cook et al. 2005).

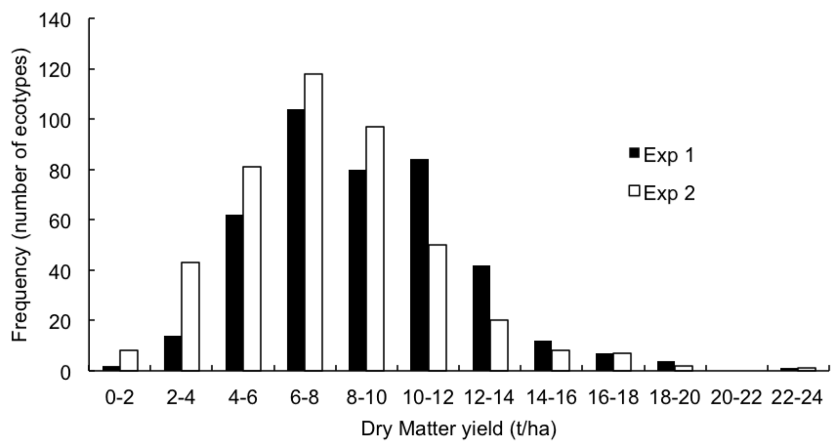

Figure 1. Frequency distribution: Dry matter production of *Cynodon* ecotypes evaluated in 2 field experiments at Gatton, Queensland.

Table 1. Average dry matter production of *Cynodon* ecotypes grouped by climatic zone of collection site.

Climatic zone	Experiment 1 DM (t/ha)	Experiment 2 DM (t/ha)
Tropical	9.09	7.81
Subtropical	8.61	7.90
Semi-arid	9.05	7.91
Mediterranean	−	7.63
Temperate	7.88	7.38
Mean	8.91	7.73

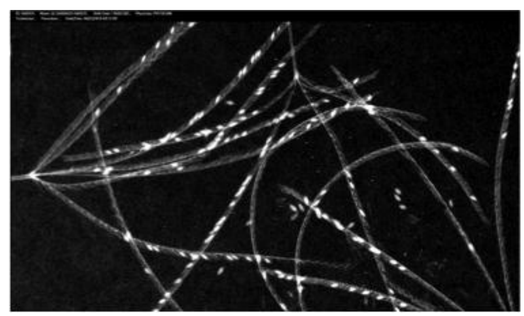

Plate 1. X-ray image of *Cynodon* inflorescences showing seed set.

In addition, the biomass production observed in this study compares well with commercial production for *Cynodon* forage cultivars in USA with USDA releases Tifton 68 and Coastal Bermuda producing 12 and 14 t/ha, respectively (Burton 2003). The IVDMD values of about 48% compare favorably with those of forages like pangola grass (45−70%), bahiagrass (48−70%) and commercial *Cynodon* forages from the USA (40−60%) (Hill et al. 1993; Cook et al. 2005). It should be noted that the biomass and digestibility levels reported here for Australian *Cynodon* ecotypes represent underestimates, since the swards were uncut for 12−19 months and would have reached a 'closed' canopy stage well before the harvest date.

The development of seeded types should also be possible, since some high-yielding grasses also had high seed set. The *Cynodon* ecotypes of this study were tested in a subtropical environment and not surprisingly, on average, the highest-yielding grasses were collected from tropical, subtropical or semi-arid climatic zones with the lowest-yielding ecotypes from the temperate zone.

Conclusion

There is potential to select among Australian *Cynodon* ecotypes, particularly among germplasm from warmer zones, material with high yields, digestibility and seed set, for cultivar development in humid subtropical regions.

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Effect of cutting interval on yield and quality of three brachiaria hybrids in Thailand

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Keywords: Mulato II, forage production, nutritive value, harvest frequency.

Introduction

In field trials in Thailand, *Brachiaria* hybrid cv. Mulato II produced significantly more green leaf, particularly during the dry season, than other brachiaria grasses (Hare et al. 2009). Cultivar Cayman produced more dry matter (DM) than Mulato II in 1 of 3 wet seasons, and line BRO2/1794 produced similar DM yields to Mulato II (Pizarro et al. 2013). The objective of this field study was to determine the effects of varying cutting intervals on growth and forage quality of hybrid brachiaria grasses in Thailand.

Materials and Methods

This study was conducted on the Ubon Ratchathani University farm in the eastern part of NE Thailand, on 3-year-old plots used for seed production studies in 2009 and 2010 (Bouathong et al. 2011). The trial was a randomized complete block design, with 2 cultivars (Mulato II and Cayman) and the line BRO2/1794, 4 cutting intervals (30, 45, 60, 90 days) and 4 replications. Plots were cut to 5 cm above ground level on May 24, 2011, and 200 kg/ha NPK (15:15:15) was applied. Thereafter, the same amount of fertilizer was applied every 45 days until November 18, 2011, when the study ended. Traits evaluated included dry matter (DM) yields and concentrations of crude protein (CP), acid detergent fiber (ADF) and neutral detergent fiber (NDF) of both leaves and stems.

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Results

Increasing cutting interval significantly increased both stem and total DM yields and reduced the percentage of leaf, but had no effect on leaf DM production (Table 1). Mulato II produced significantly less stem but more leaf DM than Cayman and BRO2/1794. BRO2/1794 produced more stem and lower percentage of leaf than Mulato II and Cayman.

Mulato II produced significantly more leaf DM than Cayman at 30, 45 and 90-day cutting intervals and more than BRO2/1794 at all 4 cutting intervals, and had a greater percentage of leaf than Cayman and BRO2/1794. Cayman had a greater percentage of leaf than BRO2/1794 at all 4 cutting intervals (Table 1).

Increasing cutting interval significantly reduced CP concentrations and increased ADF and NDF concentrations in stems and leaves (Table 2). Cayman and BRO2/1794 had higher stem CP levels than Mulato II at 30 and 45-day cutting intervals and both had lower levels than Mulato II at the 60-day cutting interval. BRO2/1794 had lower leaf CP levels than both Cayman and Mulato II at most cutting intervals. Overall, Mulato II had higher leaf ADF and stem and leaf NDF levels than both Cayman and BRO2/1794 at most cutting intervals (Table 2).

Discussion and Conclusion

The significantly higher leaf DM production and percentage of green leaf and significantly lower stem DM production of Mulato II than Cayman and BRO2/1794 at all cutting intervals support the conclusion of Argel et al. (2006) that high production of green leaves makes Mulato II an extremely attractive forage for livestock. Mulato II would seem to offer advantages over the other 2 hybrids for sowing in Thailand. Based on the data in this study, an optimum cutting interval for the genotypes tested would involve a compromise between quantity and quality. Cutting at 30-day intervals would produce CP levels 3−4 percentage points higher than cutting at 45- and 60-day intervals, but DM production would be 20% lower than cutting at the longer intervals. Extending cutting intervals to 90 days would greatly increase DM production but CP concentrations in leaf would be down to maintenance levels for non-lactating and non-reproducing animals. The appropriate cutting interval will depend on the usage to which the forage is put and what combination of yield and quality is desired.

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Table 1. Effects of cutting interval on stem and leaf dry matter production and percentage of leaf of 3 hybrid brachiaria genotypes.

Cultivar/Line	Cutting interval (days)			
	30	45	60	90
	Total dry matter (kg/ha)			
Mulato II	11238	13240	12932	18500
Cayman	10840	12824	13944	21197
BRO2/1794	10246	12944	14108	19786
LSD (P<0.05)	1862			
	Stem dry matter (kg/ha)			
Mulato II	2371	3840	4372	8552
Cayman	2954	5048	6227	13441
BRO2/1794	3298	6058	8335	14345
LSD (P<0.05)	1144			
	Leaf dry matter (kg/ha)			
Mulato II	8867	9400	8560	9948
Cayman	7886	7766	7717	7756
BRO2/1794	6948	6886	5773	5441
LSD (P<0.05)	944			
	Leaf %			
Mulato II	78.9	71.0	66.2	53.9
Cayman	72.7	60.7	55.5	36.5
BRO2/1794	67.8	53.2	40.9	27.6
LSD (P<0.05)	2.6			

Table 2. Effects of cutting interval on mean crude protein (CP), acid detergent fiber (ADF) and neutral detergent fiber (NDF) concentrations in stem and leaf of 3 hybrid brachiaria genotypes.

Cultivar	Cutting interval (days)			
	30	45	60	90
		Stem CP (%)		
Mulato II	8.8	4.7	5.4	2.8
Cayman	9.4	5.5	4.8	2.5
BRO2/1794	9.2	5.4	4.7	2.7
LSD (P<0.05)		0.38		
		Leaf CP (%)		
Mulato II	12.6	9.4	9.5	7.1
Cayman	13.2	9.9	8.9	7.2
BRO2/1794	12.2	9.2	8.8	6.7
LSD (P<0.05)		0.37		
		Stem ADF (%)		
Mulato II	34.6	35.1	39.1	44.2
Cayman	34.9	37.3	39.4	43.6
BRO2/1794	33.4	34.4	41.2	44.2
LSD (P<0.05)		0.42		
		Leaf ADF (%)		
Mulato II	27.8	28.7	31.3	31.6
Cayman	26.2	27.4	29.3	30.1
BRO2/1794	26.6	27.3	28.8	29.2
LSD (P<0.05)		0.33		
		Stem NDF (%)		
Mulato II	62.2	68.2	69.0	74.3
Cayman	61.4	65.2	68.3	70.5
BRO2/1794	61.2	65.6	70.9	71.7
LSD (P<0.05)		0.55		
		Leaf NDF (%)		
Mulato II	55.5	59.0	60.8	63.3
Cayman	51.9	55.5	57.4	58.0
BRO2/1794	53.0	57.4	57.0	58.4
LSD (P<0.05)		0.39		

Effect of cutting interval on yield and quality of two *Panicum maximum* cultivars in Thailand

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Keywords: Guinea grass, forage production, nutritive value, Mombasa, Tanzania, harvest frequency.

Introduction

Tanzania guinea grass [*Panicum maximum* cv. Tanzania (cv. Purple in Thailand)] has been grown in Thailand for over 20 years and has proven to be a high quality forage (Phaikaew et al. 2007). Mombasa guinea grass (*Panicum maximum* cv. Mombasa) was introduced to Thailand in 2007 and commercial seed production commenced in 2008, because of a demand for seed in Central and South America (Hare et al. 2013). It is a larger and taller cultivar than Tanzania. A series of studies have been undertaken at Ubon Ratchathani University, Thailand, to study the agronomic differences between these cultivars. The effects of cutting were examined in the first of these studies.

Materials and Methods

This study was conducted for two 180-day periods on the Ubon Ratchathani University farm from July 9, 2010 to January 5, 2011, and from May 23, 2011 to November 18, 2011 on plots planted in May 2010. The trial was a randomized complete block design, with 2 cultivars (Mombasa and Tanzania), 4 cutting intervals (30, 45, 60 and 90 days) and 4 replications. At the beginning of each 180-day period, the plots were cut 5 cm above ground level and 200 kg/ha NPK (15:15:15) was applied. The same amount of fertilizer was applied thereafter every 45 days. Traits evaluated included dry matter (DM) yields and concentrations of crude protein (CP), acid detergent fiber (ADF) and neutral detergent fiber (NDF) of both leaves and stems.

Results

Increasing cutting interval significantly increased stem and total DM yields and significantly reduced the percentage of leaf, but had no effect on leaf DM production in both years (Table 1). Mombasa produced 17−21% more total DM and 18−24% more leaf DM than Tanzania, but similar amounts of stem DM and percentage of leaf.

Increasing cutting interval significantly reduced CP concentrations and increased ADF and NDF concentrations in stems and leaves (Table 2). Mombasa had lower stem and leaf CP levels than Tanzania at all cutting intervals and higher stem fiber levels than Tanzania but similar leaf fiber levels.

Discussion and Conclusion

The higher total (17−21%) and leaf (18−24%) DM production from Mombasa than from Tanzania supports earlier findings under grazing in Central and South America, where Mombasa produced 28−40% more DM than Tanzania (Cook et al. 2005). It is the greater production of green leaf that has increased the demand for Mombasa rather than for Tanzania. Even though the quality of Tanzania was superior to Mombasa in terms of CP and stem fiber levels, Mombasa's greater DM production appeals to farmers. An optimum cutting interval based on the data in this study, would involve a compromise between quantity and quality. Cutting at 30-day intervals will produce the highest quality forage in terms of CP concentrations, but DM production is lower than from longer cutting intervals. The appropriate cutting interval will depend on what combination of yield and quality is desired for the particular application.

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Table 1. Effects of cutting interval on stem and leaf dry matter production and percentage of leaf of Mombasa and Tanzania guinea grasses.

Cultivar	Cutting interval (days)							
	Year 1				Year 2			
	30	45	60	90	30	45	60	90
	Total dry matter (kg/ha)							
Mombasa	9848	10865	12435	12002	8176	9823	9596	10177
Tanzania	7558	8011	9570	12075	6876	8082	6519	10662
LSD (P<0.05)	2434				1719			
	Stem dry matter (kg/ha)							
Mombasa	2352	3354	4343	5199	1546	2421	2475	3783
Tanzania	1646	2410	3413	5673	1327	1866	1692	4081
LSD (P<0.05)	1216				739			
	Leaf dry matter (kg/ha)							
Mombasa	7496	7511	8092	6803	6630	7402	7121	6394
Tanzania	5912	5601	6157	6402	5549	6216	4827	6581
LSD (P<0.05)	1340				1046			
	Leaf (%)							
Mombasa	76.4	69.3	65.2	56.9	81.2	75.6	74.4	62.9
Tanzania	78.4	70.7	64.5	53.0	80.7	76.8	73.9	62.1
LSD (P<0.05)	4.9				2.9			

Table 2. Effects of cutting interval on mean crude protein (CP), acid detergent fiber (ADF) and neutral detergent fiber (NDF) concentrations in stem and leaf of Mombasa and Tanzania guinea grasses.

Cultivar	Cutting interval (days)							
	Year 1				Year 2			
	30	45	60	90	30	45	60	90
	Stem CP (%)							
Mombasa	5.1	3.5	4.4	2.9	5.6	2.3	2.4	2.2
Tanzania	5.9	5.1	4.9	4.2	5.8	3.0	3.2	2.3
LSD (P<0.05)	2.5				0.5			
	Leaf CP (%)							
Mombasa	9.3	7.1	7.8	5.6	10.4	5.9	5.3	4.3
Tanzania	10.9	9.9	8.9	7.2	10.6	6.6	6.4	5.1
LSD (P<0.05)	2.4				0.7			
	Stem ADF (%)							
Mombasa	42.1	45.1	47.6	48.4	40.3	41.5	45.0	50.8
Tanzania	41.7	42.2	45.3	48.5	41.4	42.3	44.7	53.6
LSD (P<0.05)	2.1				0.4			
	Leaf ADF (%)							
Mombasa	37.2	36.5	38.3	38.8	35.6	37.2	37.5	40.1
Tanzania	36.2	36.9	38.1	37.5	34.6	37.2	38.2	39.7
LSD (P<0.05)	1.9				0.4			
	Stem NDF (%)							
Mombasa	70.1	74.2	74.3	76.6	69.3	73.3	74.3	77.4
Tanzania	70.3	69.9	72.8	75.5	68.9	70.9	72.4	75.6
LSD (P<0.05)	2.6				0.2			
	Leaf NDF (%)							
Mombasa	64.7	65.3	65.8	66.2	62.3	64.3	66.4	68.0
Tanzania	64.3	65.8	65.6	65.6	62.3	65.5	66.3	66.6
LSD (P<0.05)	1.6				0.3			

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Tropical grass growth functions modeling by using a non-linear mixed model

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Keywords: *Pennisetum* hybrid, data analysis, pasture yield, plant components.

Introduction

Non-linear growth curves are used for modeling plant physiological variables. Agronomists often use non-linear curves to fit data of plant growth because, unlike the coefficients of polynomials, their parameters can have biological meaning. The change in the parameters of curves fitted to data from measurements repeated over time can show how they are affected by environmental changes. The linear model approach improperly estimates the error terms when there are repeated measurements in experiments. We therefore used a maximum likelihoods approach coupled with Bayesian statistics, using a tropical cut-and-carry grass as an example.

Methods

An experiment was conducted at the experimental site "Papaloapan" of the National Institute for Agriculture, Forestry and Livestock (INIFAP), located in Isla, Veracruz, Mexico (18°06' N, 95°31' W; 65 m asl). The climate is hot and subhumid, with rainy summers and an average annual rainfall of 1,000 mm, and average temperature of 25.7 °C (Enríquez-Quiroz and Romero-Mora 1999).

The experiment was established in July 2011 and the assessments were made from August 24, 2011 to January 23, 2012. A forage stand of the *Pennisetum* hybrid cv. Maralfalfa was harvested at different stages (different subplots) of growth [30, 60, 75, 90, 105, 120, 135, 150, 165 and 180 days after planting (DAP)] and morphological components of the grass (leaf, stem, dead material) and sward height were measured. Plot size was 5 x16 m.

We used non-linear models by NLMIXED procedures in SAS, and a Bayesian approach with WINBUGS software for estimating the growth curve. We used a multiple non-linear growth function to model, and assumed that the response variable followed a multivariate normal distribution. A comparison of biologically relevant coefficients and the estimated standard error, and measures of adjustment were obtained. The results showed a clear advantage of non-linear mixed models over linear mixed models. The proposed model for the response variable (y) is a hierarchical normal distribution with mean $\mu(t)$ and variance σ^2, where

$$\mu(t) = \frac{a}{1+be^{-ct}} + \varepsilon \qquad (1)$$

the elements of the mean $\mu(t)$: *a, b, c* are parameters, *t* is the time variable, and $\varepsilon \sim n(0, \sigma_e^2)$.

Results

The estimated growth curve parameters are presented in Table 1, the growth dynamics of total plant mass, morphological components (leaf, stem) and sward height are shown in Figure 1. Overall, total plant mass, and leaf and stem increased slowly during days 0−60, after which rate of growth accelerated from day 61 to day 150 and then decreased almost to zero as growth was counteracted by increase in dead material.

Conclusion

The non-linear mixed logistic model gives a good fit for the growth of the tropical forage grass *Pennisetum* hybrid cv. Maralfalfa, for each of the growth components. This curve suggests that the best time to harvest this grass is at 150 days [corresponding to the inflection point of the derivative of equation (1)] if the aim is to maximize total dry matter production.

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Table 1. Estimated parameters of growth curves under the model, for cumulative herbage mass (kg DM/ha), cumulative leaf mass (kg DM/ha), cumulative stem mass (kg DM/ha) and sward height (cm) of *Pennisetum* hybrid cv. Maralfalfa.

	Total herbage mass		Leaf mass		Stem mass		Sward height	
Parameter	Estimate	Standard Error	Estimate	Standard Error	Estimate	Standard Error	Estimate	Standard Error
a	37.2552	2.8047	7.4043	0.7344	25.9946	1.7894	2.4227	0.06972
b	336.01	383.7	33.9985	28.0675	852.21	1182.94	12.7706	4.7777
c	5.2302	1.1168	3.7793	1.0138	6.029	1.3138	4.369	0.636
R^2	0.95		0.94		0.95		0.99	
AIC[1]	195.0		111.0		175.1		-3.6	
MSE[1]	30.9621		1.8837		15.9621		0.0414	

[1]AIC = Aiakaike Information Criterion, MSE = Mean Square Error.

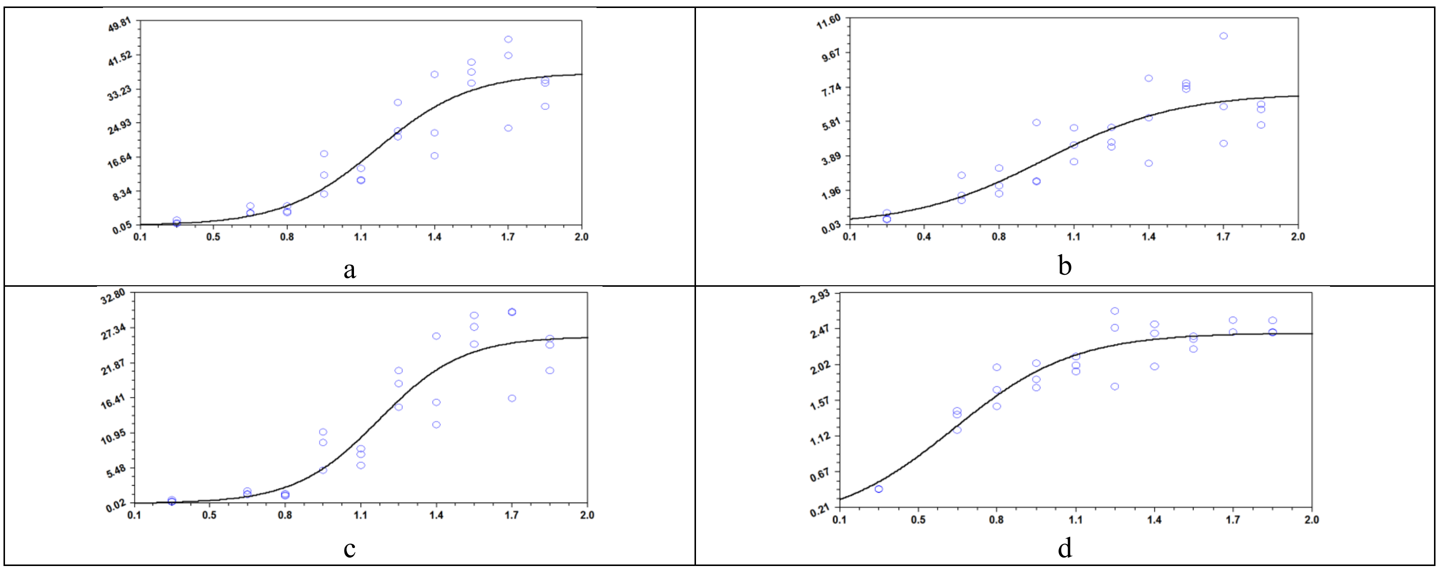

Figure 1. Growth curves for the variables, a) total cumulative herbage mass (t DM/ha); b) cumulative leaf mass (t DM/ha); c) cumulative stem mass (t DM/ha); and d) sward height (m) of *Pennisetum* hybrid cv. Maralfalfa. The horizontal axis is time in days x 100.

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Potential of *Panicum maximum* as a source of energy

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Keywords: DM yield, cellulose, lignin, combustion power.

Introduction

The use of plant biomass as a source of energy presents many advantages, mainly that it is a renewable, clean source of energy. Many tropical grasses have excellent potential as energy crops. The main one in Brazil is *Pennisetum purpureum* (elephant grass) owing to its very high yields. However, it is vegetatively propagated, thus more difficult to establish than seed-propagated species. The use of *Panicum maximum* (guinea grass) is a possible alternative for use as a source of energy, due to its high yields as well as seed propagation. The objective of this research was to evaluate the potential of different *P. maximum* genotypes for use as energy crops, in comparison with elephant grass.

Methods

Fourteen *P. maximum* genotypes (guinea grass), including 3 commercial varieties (cvv. Milênio, Mombaça and Tobiatã), and *P. purpureum* cv. Napier (elephant grass) were evaluated at Embrapa Beef Cattle in Campo Grande, MS, Brazil, in a randomized block design with 3 replications. The soil was a dark red latosol fertilized with 50 kg/ha P_2O_5 (superphosphate) and 50 kg/ha K_2O (potassium chloride) at planting**.** Plots consisted of 4 rows, 3 m long, spaced 0.5 m apart. Genotypes were seeded in November 2007 and cut at 20 cm from the soil in March 2008. Every 120 days thereafter, plots were harvested at 20 cm from the soil, the cut material was weighed and a sample taken, which was weighed and separated into plant parts: leaf, stem and dead matter. After drying at 65 °C for 3 days, leaf and stem samples were ground through a 1 mm screen and evaluated for quality through NIRS (Near-Infrared Reflectance Spectroscopy). A ground sample of each was sent to Embrapa Forestry, in Curitiba, PR, Brazil, for evaluation of combustion power in a digital adiabatic calorimeter, according to NBR 8633 (Associação Brasileira de Normas Técnicas 1984). Data were analyzed by SAS.

Results and Discussion

The elephant grass (cv. Napier) produced 30.6 t/ha/yr dry matter (DM) (Table 1), less than the levels obtained by Urquiaga et al. (2006) in Brazil, who obtained at least 30 t/ha of stems with a minimal application of fertilizers and N from biological N fixation. *Panicum maximum* yields were lower and varied from 13.8 to 21.4 t/ha/yr. The yields recorded for guinea grass are much lower than the 49.1 t/ha in 9 months growth on a dark red latosol fertilized with 60 kg N/ha, 60 kg K_2O/ha and 60 kg P_2O_5/ha reported by Fernandes et al. (2009). Leaf percentage in the guinea grass genotypes (range 51.7−71.5%) was higher than the 48.3% for Napier. Culm yield of Napier was very high (18.1 t/ha/yr), compared with a maximum of 10.5 t/ha for *P. maximum.*

In general, elephant grass presented the highest neutral detergent fiber (NDF) and lowest lignin concentrations in leaves (Table 1), and the highest crude protein (CP) and lowest cellulose in stems (Table 2). For second-generation ethanol production, cultivars with the highest cellulose concentrations and lowest lignin concentrations are desirable. Second-generation ethanol is produced from plant biomass, which is mainly composed of cellulose, a polymer formed by chains of glucose. The breakdown of cellulose into simple glucose molecules allows microorganisms to ferment this simple sugar and subsequently ethanol is produced. On this basis, the guinea grass genotypes are preferable, because of their higher cellulose concentrations in leaves (mean of 29.5%, Napier 28.8%) and stems (mean of 35.6%, Napier 31%). These cellulose concentrations agree with those obtained

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by Morais et al. (2009), but are lower than the 41.2% for elephant grass culms fertilized with 100 kg N/ha (as urea), split 1/3 at planting and 2/3 after 50 days (Quesada et al. 2004).

Table 1. Yield and quality of 14 *Panicum maximum* genotypes and *Pennisetum purpureum* cv. Napier.

Genotype	DM (t/ha)	Leaf %	NDF	Cellulose	Lignin[1]	Cellulose yield (t/ha)	Lignin yield (t/ha)	Combustion power (%)
			% in leaves					
PM40	13.8	63.8	74.7	28.8	3.2	4.5	0.6	16.9
PM271	18.9	58.6	74.7	29.1	3.5	6.0	0.9	16.7
PM41	17.1	71.5	76.2	30.3	3.6	5.7	0.9	17.1
PM322	17.9	51.7	73.8	29.2	3.5	6.0	0.9	16.8
PM186	16.0	70.4	76.0	30.3	3.6	5.1	0.9	17.1
PM4	19.1	63.8	74.2	30.2	3.6	6.6	0.9	16.9
PM30	21.4	64.2	74.1	30.0	3.5	7.2	1.2	17.0
PM190	15.7	57.5	74.3	29.1	3.5	5.1	0.9	17.1
PM145	15.4	60.7	75.0	28.9	3.3	4.8	0.6	16.9
PM23	13.5	54.2	74.5	29.5	3.4	4.5	0.6	17.2
PM10	21.4	60.7	75.4	28.7	3.6	7.2	1.2	17.3
Milênio	20.3	57.4	74.6	28.9	3.6	6.6	1.2	17.0
Mombaça	19.4	64.1	74.4	29.3	3.5	6.6	0.9	16.7
Tobiatã	17.7	69.8	76.4	30.4	3.6	5.7	0.9	17.1
Napier	30.6	48.3	79.2	28.8	3.0	9.3	1.5	17.7
MSD[2]	3.9	6.0	1.1	0.7	0.2	4.5	0.3	0.5

[1]Extracted with sulphuric acid.
[2]Mean difference for significance by Waller-Duncan mean comparison.

Table 2. Quality characteristics of 14 *Panicum maximum* genotypes and *Pennisetum purpureum* cv. Napier.

Quality characteristic	*P. maximum* genotypes	*P. purpureum* cv. Napier
	(%)	
Leaf CP	10.9−12.4	11.9
Leaf digestibility	51.6−58.2	57.1
Stem CP	4.7−6.2	6.8
Stem digestibility	41.1–48.0	46.3
Stem NDF	77.9−79.9	78.0
Stem cellulose	35.0−36.3	31.0
Stem lignin	5.6−6.2	5.6

On the other hand, *P. maximum* genotypes presented slightly higher lignin concentrations in leaves (mean of 3.5%, Napier 3.0%) and stems (mean of 5.9%, Napier 5.6%). However, DM yield is an important consideration and the high yield obtained with Napier meant that total cellulose accumulated by Napier (9.3 t/ha/yr) (Table 1) was much higher than for *P. maximum* (4.5−7.2 t/ha/yr). The total quantities of lignin accumulated were also higher for Napier (1.5 t/ha/yr) than for guinea grass (0.6−1.2 t/ha/yr). Biomass may also be used for direct burning to produce charcoal. Elephant grass presented a higher combustion power (17.7%) than the guinea grass genotypes (16.7−17.3%); however, the difference was small (Table 1).

Conclusion

Our data indicate that guinea grass genotypes may be used as alternative sources of biomass for energy production as ethanol or charcoal. Six genotypes were most promising, including cv. Mombaça, and produced about two-thirds of the DM yield of Napier. The savings in planting from seed as opposed to cuttings would need to be weighed up against the reduced total production relative to elephant grass.

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Performance of Nelore cattle on *Panicum maximum* pastures in the Brazilian Cerrado

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Keywords: Tropical grass, average daily gain, herbage allowance, liveweight gain, stocking rate.

Introduction

With the increased demand for meat products, associated with environmental concerns due to global climatic and land use changes, the need to efficiently use pasture and forage resources will increase (Boval and Dixon 2012). Efforts to breed and release new forage cultivars must incorporate these concerns, ensuring that introduced material is of high quality, especially under grazing (Euclides et al. 2008). In this study, we aimed to evaluate the average daily weight gain and stocking rate of Nelore beef cattle grazing new genotypes of *Panicum maximum* during the rainy season in the Brazilian Cerrado.

Methods

The study was conducted at the Embrapa Cerrados Research Center (15°35' S, 47°42' W; 1007 m asl) from November 10, 2011 to April 14, 2012. The average annual rainfall for the region is 1,230 mm, concentrated in the period from October to March. The soil of the experimental area is classified as an Oxisol. The *Panicum maximum* genotypes PM32 and PM45 were evaluated using cv. Massai as the control treatment. The experiment followed a completely randomized block design with 4 replications of 1.3 ha each, totaling about 16 ha. Nelore steers (*Bos indicus*) with an average age of 20 months and initial weight of 280 kg were used to assess weight gains. Each paddock contained 3 tester animals and regulator animals varying in number to maintain a forage allowance of 9% (kg DM/100 kg BW/d). The paddocks were divided in half and grazed on a rotation of 28 days grazing and 28 days rest. All paddocks received 100 kg N/ha/yr applied equally in November and January. Tester animals were weighed every 28 days, after a 16-h fast. To estimate herbage mass, 12 samples were collected pre-grazing using squares of 1 m² in each half of the paddock. The rate of dry matter accumulation was estimated by the difference between herbage mass after and before grazing. The results were analyzed using PROC GLM (SAS Institute 1996) and comparison of means was performed by the Tukey test ($P<0.05$).

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Results and Discussion

Average daily gain (ADG) ranged from 0.83 to 1.00 kg/hd/d and was affected by genotype ($P<0.05$), with highest values observed for PM32 and lowest for cv. Massai (Table 1). PM45 and Massai supported higher stocking rates than PM32, owing to the higher rate of herbage accumulation (44, 52 and 63 kg DM/ha/d, respectively, for genotypes PM32, PM45 and Massai). Gain per hectare reflected the stocking rate, but there was no significant difference between genotypes ($P>0.05$). Pre-grazing herbage mass was not affected by genotype ($P>0.05$), while leaf bulk density was highest for PM45 and lowest for PM32 ($P<0.05$). The high leaf bulk density of PM45 was not reflected in higher ADGs.

In a study with Marandu grass pastures (*Brachiaria brizantha* cv. Marandu) managed rotationally with an offering of 10%, ADG was 0.63 kg/hd/d and gain per hectare was 612 kg live weight/ha in 140 days (Herling et al. 2011). In pastures of cv. Massai managed rotationally, the maximum ADG during the rainy season ranged from 0.60 to 0.70 kg/hd/d (Euclides et al. 2008). In our study, the observed ADGs were higher than values presented in the literature for tropical grasses, which is surprising considering that the forage allowance at the end of the trial period, including the rate of herbage accumulation, was only about 6%, i.e., below the target of 9% initially desired. The ADG for genotype PM32 was similar to that obtained by Sollenberger and Jones (1989) evaluating dwarf elephant grass (cv. Mott) with an average herbage allowance of 4.9% during the summer (0.97 kg/hd/d).

Table 1. Average daily gain, productivity, stocking rate, leaf bulk density and herbage mass for grazed *Panicum maximum* genotypes during the rainy season in Planaltina, DF, Brazil.

Genotype	ADG (kg LW/hd/d)	Productivity (kg LW/ha)	Stocking rate (AU/ha)[2]	Leaf bulk density (kg/ha/cm)	Pre-grazing herbage mass (kg DM/ha)
PM32	1.00 a[1]	423.9 ns	3.88 b	44.5 b	4602 ns
PM45	0.90 ab	501.0 ns	5.03 a	65.1 a	4336 ns
cv. Massai	0.83 b	458.7 ns	4.88 ab	49.3 ab	5618 ns
CV%	9.38	20.17	14.36	16.8	18.94

[1]Means followed by the same letter within columns do not differ by the Tukey test (P>0.05). [2]Animal Unit = 450 kg live weight (LW).

Conclusion

While the new accessions PM32 and PM45 supported higher ADGs than cv. Massai, this was not reflected in higher productivity in the case of PM32. Further studies are needed to determine how these new genotypes can be used as alternatives to Massai for increasing the productivity of cattle in the region.

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Quality seed production of range grasses − A major constraint in revitalizing tropical pastures

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Keywords: Seed setting, seed germination, hormonal solutions.

Introduction

Only 4% of India's geographical area of 326.82 Mha is under pastures. Socioeconomic and ecological consequences of land degradation are affecting 85 Mha of rangelands/grasslands. To provide sufficient milk for the ever-growing population, current milk production of 128 Mt must increase to 160 Mt by 2020. To make this possible, an additional 825 Mt of green fodder is required. Increasing the area producing green fodder is difficult because of severe competition from food crops. Revitalizing the denuded grasslands is the most plausible means for improving the availability of green fodder. This needs mission mode programs with participation of the people.

One impediment to increased green fodder production is limited availability of good quality seed. In India only 25−30% of the required seed is available for sowing cultivated fodder and 20% and 15% for range grasses and legumes, respectively (Anon. 2011). Forage seed production encounters several problems, namely: poor seed setting; extreme climatic conditions; seed shattering and non-synchronization in maturity; and the presence of empty seeds. Empty seeds with partially developed embryos and poor nutrient reserves fail to germinate. The standard germination percentage of most tropical grasses is around 20−30 percent. Several studies have been conducted to enhance germination by treating with GA_3 or KNO_3 or by other means of dormancy removal. However, the major reason for the low germination percentage is formation of 'false' seeds (dry floral parts without fully developed seeds). Wobus and Weber (1999) emphasized the role of hormones in combination with other seed metabolites, including sugar, in seed maturation. Understanding reproductive biology and harvest scheduling are also important for enhancing formation of pure, germinating seeds. Hence, the present study was conducted to understand seed setting and the effects of external hormonal application on seed germination.

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Methods

Seed-setting studies

To study seed setting, the annual range grass, *Pennisetum pedicellatum,* commonly known as *Dinanath* grass in India, was chosen. The seed harvested in bulk was examined for the presence of caryopses in the spikelets by manual defluffing. Separately, 10 individual inflorescences of *P. pedicellatum* var. Bundel Dinanath-1 and Bundel Dinanath-2 were taken and caryopses from each spikelet were separated manually by slightly pressing at the bottom. The presence or absence of caryopses in spikelets was noted for further calculation. The seed setting percentage was calculated by counting the total number of spikelets per panicle and spikelets carrying a true caryopsis.

Hormonal studies on seed germination

The effects of hormones on seed germination were studied by spraying 25, 50 and 100 ppm IAA (indoleacetic acid) on Guinea grass (*Panicum maximum* var. BG-2) inflorescences in the field. The IAA solutions were prepared and 0.05% tween-80 was added to enhance adsorption of the solutions. Ten inflorescences were selected and the spray was applied twice at 4-day intervals. The individual treated inflorescences were collected along with controls at maturity and the seed was bulked for further germination studies, with 3 replications, using the standard procedure in sand. Germination was recorded till no further increase in the number of seedlings was observed.

Seed-ripening studies

The effects of hormonal solutions on seed ripening were studied by dipping the cut panicles of *Panicum maximum* in hormonal solutions and water. Five guinea grass panicles were collected at the anthesis stage from the field. The panicles were dipped in 100 ppm and 200 ppm solutions of IAA, kinetin and water. A control without water was also studied. Matured seeds from each treatment were collected separately. The experiment was conducted under ambient room temperature with 3 replications.

Results and Discussion

The 2 released *Dinanath* grass varieties BD-1 and BD-2 were distinctly marked for single floret and 3 florets per sessile spikelet, respectively. The percentage of well-developed seeds per panicle was found to be 96% and 92% in BD-1 and BD-2, respectively, whereas in normal bulk-harvested seed lots, only 20−60% of spikelets contain filled seeds. The naked seeds, i.e., caryopses, obtained showed 93% germination. Thus, formation of pure germinating seed (caryopses) in *Dinanath* was found to be >90%. Observations revealed that a lot of caryopses were dropped during harvesting in bulk harvests due to the species' floral structure. Thus, harvesting at physiological maturity is crucial to optimize recovery of spikelets with caryopses. In this context, the development of physiological and harvesting maturity indices for bulk harvesting of *Dinanath* grass should be given high priority in research programs.

External application of IAA at 25, 50 and 100 ppm at the panicle emergence stage in *Panicum maximum* (var. BG-2) substantially increased germination, with the highest germination (45%) being obtained at 100 ppm IAA (Table 1). Rate of germination in IAA treatments was also higher than in the control. These results might be due to an increased number of pure germinating seeds or an increase in germination per se. Barazesh and McSteen (2008) showed that hormones play an important role in inflorescence development in grasses. A positive response of guinea grass to auxin (IAA) paves the way for further exploration of phytohormone-induced seed development in range grasses.

Table 1. Effects of indoleacetic acid (IAA) treatment of Guinea grass panicles on seed germination.

Treatments	Germination (%)		
	Day 4	Day 6	Day 8
Control	15.3	23.3	26.0
IAA 25 ppm	19.3	33.3	34.7
IAA 50 ppm	20.0	34.0	38.7
IAA 100 ppm	23.3	40.7	44.7

The cut panicles of *Panicum maximum* showed varying degrees of liveliness after treatment with different solutions. Cut panicles without water dried early, followed by those dipped in kinetin and water, with panicles dipped in IAA solution remaining viable for longest. In *P. maximum* under field conditions, spikelets shattered within a week after anthesis. If liveliness can be maintained in cut panicles, the shattering loss can be minimized and more mature seed can be collected than with bulk harvest.

Conclusions

Seed setting as such is not a problem in the annual range grass *Pennisetum pedicellatum* but shattering of caryopses during bulk harvest leads to a low germination percentage in harvested seed. Physiological and harvesting maturity indices must be developed to retain caryopses in the harvested spikelets. External application of phytohormone IAA at 100 ppm during anthesis could be one strategy for increasing subsequent seed germination. Additionally, in order to decrease harvesting costs in grasses with non-synchronous maturity, increased liveliness following IAA treatment could be a mechanism for optimizing yield of high quality seed by allowing more time for seed maturity after harvest.

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Agronomic evaluation of 324 intraspecific hybrids of *Brachiaria decumbens* in Brazil

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Keywords: Apomixis, hybrids, signalgrass, forage breeding.

Introduction

Brachiaria decumbens cv. Basilisk is certainly the most planted pasture grass in the tropics owing to its adaptation to poor and acid soils, typical of the tropics, plus good animal performance. This cultivar was responsible for a radical change of scenario in central Brazil in the early 1970s and is the only cultivar available commercially. Efforts to breed this species have been recent, as the successful somatic chromosome duplication of sexually reproducing diploid plants of *B. decumbens* was accomplished only in 2009 (Simioni and Valle 2009). Subsequently, intraspecific crosses with natural apomictic tetraploid accessions were carried out. Breeding is needed since cv. Basilisk is susceptible to grassland spittlebugs, which limit its widespread use. A recurrent selection scheme has been devised to improve the species and the first progeny are now under evaluation. This paper reports the preliminary results of the agronomic evaluation of 324 intraspecific hybrids of *B. decumbens*.

Methods

Four hundred and fifty-seven intraspecific hybrids, obtained from crossing 3 sexual plants artificially tetraploidized by colchicine with the apomictic tetraploid cv. Basilisk, comprise the Embrapa Beef Cattle base population of *B. decumbens*. Of these, 324 hybrids were selected (mass selection) and vegetatively propagated to produce 10 cuttings each. These were transplanted to a field trial at Embrapa Beef Cattle, Campo Grande, MS, in an 18 x 18 lattice design, with 2 replications and 5 plants per plot at a spacing of 1.0 x 0.5 m. The commercial cv. Basilisk was used as a control. This paper reports the results of 2 cuts during the dry season of 2012 (July 6, mid-season, and October 2, end of dry season).

Agronomic evaluations were made for total dry matter (TDM), leaf dry matter (LDM), leaf percentage (%L), leaf:stem ratio (LSR) and regrowth rating (REG). Regrowth rating was a combined score from 0 (poor) to 6 (excellent) of plant density (percentage of tillers shooting) and speed of regrowth. Data were analyzed using the restricted maximum likelihood/best linear unbiased prediction procedure (REML/BLUP), implemented in the software SELEGEN REML/BLUP (Resende 2002).

Results and Discussion

The accuracy observed in the analysis of the 2 cuts varied from 17% to 74% for %L and TDM, respectively (Table 1), considered by Resende and Duarte (2007) as of medium to very high precision for the traits evaluated. This means that the results are trustworthy with the exception of %L (17%). The latter could mean that there is little variation in leaf content among the hybrids during the dry season, when plant growth is limited.

There were significant ($P<0.01$) differences between hybrids for TDM and LDM by the likelihood ratio test (Table 1), indicating genetic variability among the hybrids, thus allowing for gains through selection. For %L, LSR and REG, no significant difference was observed ($P>0.05$). The interaction genotype x cut was significant for the majority of parameters except for LSR and REG, thus complicating the ranking of hybrids by TDM, %L and LDM in the same season.

The seasonality of production of fodder plants is a reflection of the reduction in the availability of light, temperature and rainfall, that usually occurs in the winter in the central region of Brazil. Thus like most tropical forage species, *B. decumbens* also fails to develop well

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during this period of unfavorable environmental conditions. Valle et al. (2000), when evaluating interspecific hybrids of *Brachiaria*, obtained average production of TDM and LDM in the rainy season 4 times those in the dry season.

For most parameters evaluated, cv. Basilisk presented the highest overall means, except for LSR, highlighting the exceptional performance of this cultivar. Even when a selection pressure of 2.5% was applied (best 8) there were no hybrids better than cv. Basilisk, except for LSR and REG. These are preliminary results and this evaluation will continue for at least 7 cuts. As some cuts will occur during the rainy season, differences between hybrids might become more evident.

Table 1. Genetic variance ($\hat{\sigma}_g^2$), genotype x cut interaction variance ($\hat{\sigma}_{gc}^2$), heritability estimates based on progeny means (h^2_m), accuracy (Acgen) and BLUP mean values of *Brachiaria decumbens* hybrids for agronomic traits, evaluated in 2 cuts at Embrapa Beef Cattle, Campo Grande.

	TDM[1]	%L	LDM	LSR	REG
$\hat{\sigma}_g^2$	95197.01**	0.85	22136.57**	0.22	0.02
$\hat{\sigma}_{gc}^2$	17832.64**	16.55**	11967.91**	0.06	0.12
h^2_m	0.55	0.03	0.47	0.23	0.14
Acgen	74.2	17.3	68.6	48.0	37.4
Overall mean	1485.95	50.90	754.39	2.34	2.20
Basilisk mean	2117.69	50.87	1018.78	2.12	2.24
$Mean_{10\%}$	1924.85	51.19	938.84	2.83	2.30
$Mean_{5\%}$	1990.52	51.24	968.94	3.06	2.34
$Mean_{2.5\%}$	2050.75	51.31	1003.11	3.33	2.38

[1]TDM: Total dry matter; %L: Leaf dry matter percentage; LDM: Leaf dry matter; LSR: Leaf:stem ratio; REG: Regrowth (0 = poor to 6 = excellent); Overall mean: BLUP mean value of the 324 hybrids; $Mean_{10\%}$: BLUP mean value of the best hybrids considering a selection intensity of 10%; $Mean_{5\%}$: BLUP mean value of the best hybrids considering a selection intensity of 5%; $Mean_{2.5\%}$: BLUP mean value of the best hybrids considering a selection intensity of 2.5%.

Conclusion

No hybrids of *B. decumbens* performed better than cv. Basilisk during this dry season study, but there was genetic variability among the hybrids. The presence of hybrid x cut interactions indicates that evaluation in the rainy season is necessary to predict the genotypic value of hybrids with greater reliability for selection. Nutritive value determination as well as spittlebug resistance will also be included in the future to rank these hybrids for selection.

Acknowledgments

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Vegetative propagation of *Stylosanthes scabra*

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Keywords: Root development, indolebutyric acid, auxins, hormones, gibberellins, cytokinins.

Introduction

One way to multiply genetically desirable plants in a more uniform manner is to use vegetative propagation, such as using plant cuttings. This technique multiplies a single plant into several, in an easy low-cost system (Fachinello et al. 2005), so it is useful in plant breeding. The technique may be enhanced through the use of hormonal regulators, with indolebutyric acid (IBA) being most frequently used, because of its stability in hydro-alcoholic solution, low sensitivity to biological degradation and good establishment of adventitious roots (Fachinello et al. 1995). In spite of its large-scale use for fruit production, the technique is not commonly used for forage species, such as *Stylosanthes.* We evaluated different concentrations of IBA for root establishment and initial development of *Stylosanthes scabra* cuttings.

Materials and Methods

The experiment was conducted at the Federal Rural University of Pernambuco, Recife, PE, Brazil. Treatments were 4 IBA concentrations (1, 2, 3 and 4 g/L) applied to *S. scabra* plant cuttings (20 cm long, 2.5 mm diameter), selected from a collection obtained in Bom Jardim, PE. The experiment was fully randomized with 3 replicates, and the experimental unit was a set of 15 x 500 mL plastic recipients, each with a single cutting, with a 1:3 sand:vermiculite mixture. The cuttings had flat cuts at the top, and inclined ones at the bottom, and were inserted into the respective IBA solutions to 2.5 cm from the bottom for 10 seconds, and transferred to the experimental units. At the end of the experiment (60 days), percentage of cuttings with roots, root length and root and shoot dry masses were recorded and subjected to regression analysis using ASSISTAT.

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Results and Discussion

All parameters measured responded to IBA concentration according to an exponential relationship (Figure 1). Highest root length was determined at 421 cm at 1.90 g/L IBA concentration (Figure 1A), which supports the finding of Schuster et al. (2011) that root length in *Arachis pintoi* cuttings peaked at IBA concentrations about 2 g/L. Percentage of rooted cuttings was highest at IBA concentrations of 1 and 2 g/L (Figure 1B), while highest shoot dry mass (1.67 g) occurred at 2.28 g/L IBA (Figure 1C), and highest root dry mass (246 mg) at 1.71 g/L IBA (Figure 1D). Pasqual et al. (2001) also reported negative effects of higher IBA concentrations on fruit plant cuttings, possibly due to hormonal imbalances among auxins, gibberellins and cytokinins as reported by Cunha et al. (2012).

Conclusion

The 2 g/L IBA concentration seems the most promising for use in stimulating root development on cuttings of *Stylosanthes scabra* in breeding programs. Use of this technique should enhance the success rate in striking cuttings and accelerate the selection process without risks of cross-pollination contaminating selection lines.

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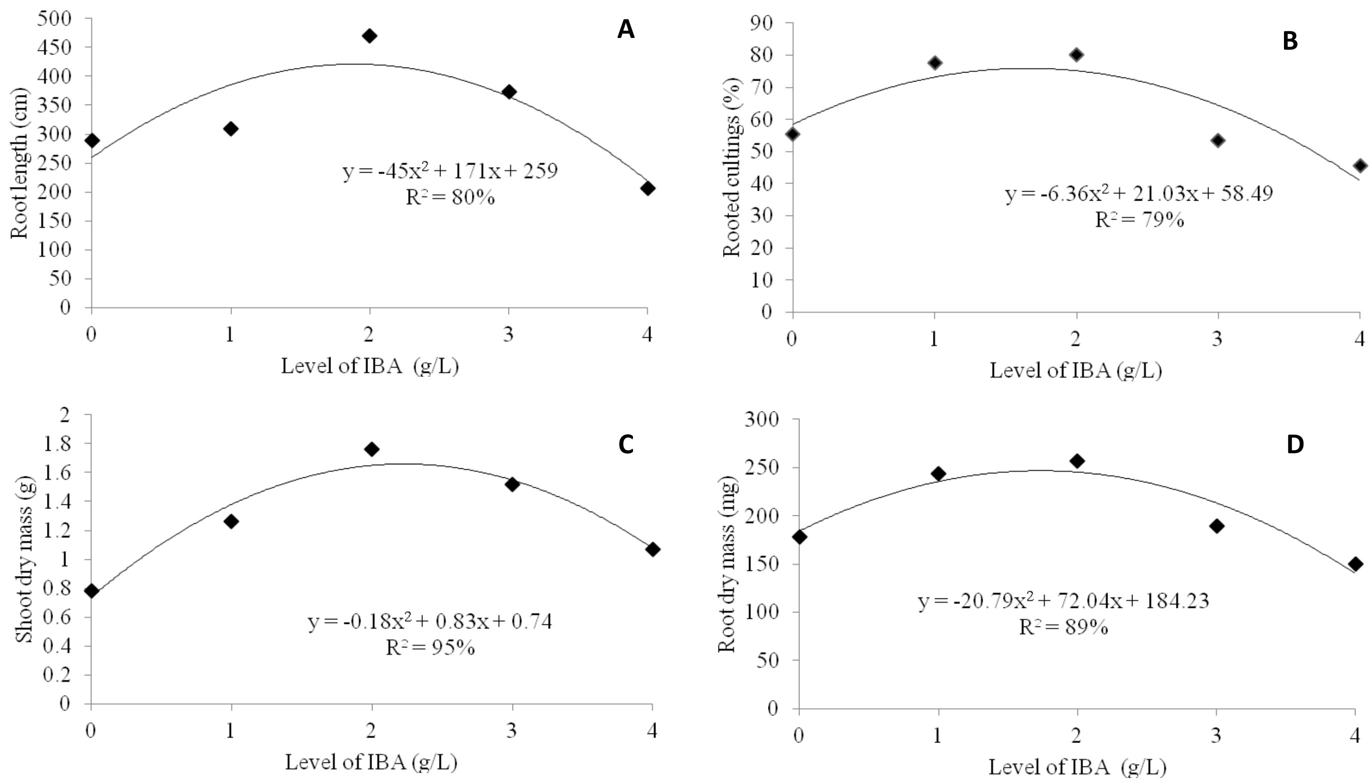

Figure 1. Effects of concentration of indolebutyric acid (IBA) on: (A) root length; (B) percentage of rooted cuttings; (C) shoot dry mass; and (D) root dry mass of *Stylosanthes scabra* cuttings.

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Agronomic and nutritional evaluation of intraspecific crosses in *Brachiaria decumbens*

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Keywords: Hybrids, apomixis, selection, forage breeding, signalgrass.

Introduction

Brachiaria decumbens cv. Basilisk is the single most important forage grass used for pastures in the tropics. This cultivar has exceptional adaptation to acid soils, vigorous growth, ease of establishment, and good forage value throughout the year, but these favorable characteristics are counteracted by its susceptibility to insect pests − spittlebugs. Breeding to produce improved cultivars within this species was impossible until 2009 due to the lack of compatible sexual ecotypes. With the success of somatic chromosome duplication of sexually reproducing diploid plants of *B. decumbens* (Simioni and Valle 2009), intraspecific crosses with natural apomictic tetraploid accessions were finally possible. This abstract reports the results of the agronomic and nutritional evaluation of 50 pre-selected intraspecific hybrids of *B. decumbens*.

Methods

Four hundred and fifty-seven intraspecific hybrids, obtained from crosses between 3 sexual plants artificially tetraploidized by colchicine and the apomictic tetraploid cv. Basilisk, comprise the Embrapa Beef Cattle base population of *B. decumbens*. Of these, 50 hybrids were selected (mass selection), vegetatively propagated and transplanted to a field trial at Embrapa Beef Cattle, Campo Grande, MS, Brazil, in a randomized complete block design, with 4 replications and 5 plants per plot with spacing of 1.0 x 0.5 m. The commercial cv. Basilisk was used as a control. The evaluation began in July 2011 and continued for 6 cuts: 2 cuts during the dry season (July 20 and September 28, 2011) and 4 cuts during the rainy season (November 04 and December 09, 2011, and January 07 and February 29, 2012).

Agronomic evaluations were made for total dry matter (TDM), leaf dry matter (LDM), leaf dry matter percentage (L), leaf:stem ratio (LSR) and regrowth (REG). The regrowth score was a combined score from 0 (poor) to 6 (excellent) based on density scores (percentage of tillers showing regrowth) and speed of regrowth. A leaf sample from each plot, previously dried and ground, was used for the analysis of crude protein (CP), in vitro dry matter digestibility (IVDMD), neutral detergent fiber (NDF) and lignin (LIG) by near-infrared reflectance spectroscopy (NIRS) (Marten et al. 1989).

Data were analyzed using the restricted maximum likelihood/best linear unbiased prediction procedure (REML/BLUP), implemented in the software SELEGEN REML/BLUP (Resende 2002), with the following univariate model: $y = Xm + Zg + Wp + e$, where y is the data vector; m is the fixed effect (combination cut-block); g is the genetic effect (random); p is the permanent environmental effect (random); and e is the random error. X, Z and W are the incident matrices for m, g and p, respectively.

Results and Discussion

The joint analysis (not shown) detected highly significant differences ($P<0.01$) for hybrids for all traits evaluated. Significant differences were detected in the hybrids x harvests interaction for TDM, LDM, L, LSR, REG, CP and IVDMD, but not for NDF and LIG. Thus, genetic variation was present among the hybrids for all traits

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evaluated, and performance of the hybrids differed significantly across harvests for all except 2 of these traits. The selection accuracy estimates in the joint analysis ranged from 62 to 93% for the evaluated traits, which are considered moderate to high values.

The TDM BLUP overall mean of the hybrids (902 kg/ha) was higher than for cv. Basilisk (829 kg/ha) and the mean of the best performing 10 hybrids was 33% superior to cv. Basilisk (Table 1). The hybrid with highest performance (1196 kg/ha) yielded 367 kg/ha more than cv. Basilisk, which demonstrates the excellent prospects for this breeding program. For the traits related to leaf, the component of the forage with highest nutritional value, overall mean values for LDM, L and LSR of the hybrids were 21.3, 13.7 and 50% superior to cv. Basilisk, respectively. The hybrid with the highest LDM (770 kg/ha) had almost 60% more LDM than the cultivar and the hybrid with highest LSR (4.0) had more than twice as much leaf as the control. Traits related to the leaf component in the forage are very important in breeding programs, because leaves are preferentially consumed by cattle and have higher nutritive value than stems. The overall mean REG of the hybrids did not differ from the cv. Basilisk mean (3.3); however, the mean REG of the best 10 hybrids and the best hybrid were 15 and 39% superior to the cultivar, respectively. For nutritive value traits, overall means for CP and IVDMD of the hybrids were higher than for the cultivar, while NDF and LIG were lower than for the cultivar. This shows that it is feasible to identify and select hybrids with better nutritive value. The highest CP recorded amongst the hybrids (13.2%) was 1.5 percentage units higher than for cv. Basilisk (Table 1). Some of the best 10 hybrids for nutritive value traits were coincident with the best 10 hybrids for agronomic traits (not shown), indicating that it is possible to select hybrids with high performance for both agronomic and quality traits. Further evaluation for other traits like resistance to spittlebugs and seed production needs to be considered before superior hybrids can be identified to continue to the next phase of the breeding program.

Table 1. BLUP mean values of the *Brachiaria decumbens* hybrids and cv. Basilisk for agronomic and nutritive-value traits, evaluated in 6 harvests at Embrapa Beef Cattle.

	TDM[1] (kg/ha)	LDM (kg/ha)	L (%)	LSR	REG
$Mean_1$	1196	700	64.0	4.0	4.6
$Mean_{10}$	1103	673	62.9	3.3	3.8
Overall mean	902	536	58.2	2.4	3.3
Basilisk mean	829	442	51.2	1.6	3.3
	CP (%)	IVDMD (%)	NDF (%)	LIG (%)	
$Mean_1$	13.2	74.9	59.6	2.4	
$Mean_{10}$	12.9	74.4	60.6	2.5	
Overall mean	12.4	73.8	61.7	2.6	
Basilisk mean	11.7	73.6	63.3	2.8	

[1]TDM: Total dry matter; LDM: Leaf dry matter; L: Leaf dry matter percentage; LSR: Leaf:stem ratio; REG: Regrowth (0, poor to 6, excellent); CP: leaf crude protein percentage; IVDMD: leaf in vitro dry matter digestibility; NDF: leaf neutral detergent fiber percentage; LIG: leaf lignin percentage. $Mean_1$: BLUP mean value of the best hybrid; $Mean_{10}$: BLUP mean value of the best 10 hybrids; Overall mean: BLUP mean value of all 50 hybrids.

Conclusion

There is genetic variability for a range of agronomic and nutritive value traits in *Brachiaria decumbens* hybrids, making it possible to select superior hybrids with higher performance than cv. Basilisk. This selection process is underway and results will be reported elsewhere.

Acknowledgments

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Dry matter intake by beef steers on Piatã palisadegrass (*Brachiaria brizantha* cv. BRS Piatã) pasture

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Keywords: Structural characteristics, sward height, nutritive value, stocking rate.

Introduction

Beef production in Brazil is primarily based on tropical pastures and 85% of these pastures are *Brachiaria brizantha* cv. Marandu. Such a monoculture could prove disastrous, if some pest or disease emerged. With the goal of further diversifying pastures and contributing to the sustainability of the pasture production system, *B. brizantha* cv. BRS Piatã was released by the Brazilian Agricultural Research Corporation (Embrapa). Little is known about the characteristics of this cultivar in relation to grazing management and plant-animal interactions. Dry matter (DM) intake by grazing animals is influenced by the structural characteristics of tropical grasses (Stobbs 1973; 1975), and the presence of stem and dead material at the grazing horizon limits bite depth (Carvalho et al. 2008). Under such conditions, it is common to observe a reduction in bite rate and increases in time per bite and daily grazing time (Difante et al. 2009), resulting in inefficient harvesting and lower herbage intake. The aim of this work was to estimate the DM intake by beef steers grazing Piatã palisadegrass managed at 3 grazing intensities.

Methods

Place and time

The experiment was conducted at Embrapa Beef Cattle, Campo Grande, MS, Brazil, during the summer and autumn of 2010.

Treatments and experimental design

The Piatã palisadegrass pasture was grazed at 3 intensities of continuous stocking, represented by 15, 30 and 45 cm sward height. The experimental design was a randomized block design with 3 treatments and 2 replicates. Six paddocks measuring 0.7 ha were used and received 80, 40 and 40 kg/ha of N, P_20_5 and K_20, respectively. Three steers (testers) were kept in each paddock, and additional steers (grazers) were added to or removed from all paddocks as necessary to maintain the sward height imposed in each treatment.

Experimental evaluations

Sward height was measured weekly at 60 random points per paddock. For determination of herbage mass and morphological components of the pasture (leaf blade, stem and dead material), 15 samples per paddock were cut at ground level using a 1 m x 1 m frame. All samples were divided, with 1 subsample weighed fresh and oven-dried at 65ºC to determine dry herbage mass, and the other separated into leaf (leaf blades), stem (stems and leaf sheaths) and dead material before drying to determine percentages of the components. Two hand-plucked samples were taken from each paddock. They were oven-dried, ground to pass through a 1-mm screen, and analyzed to obtain estimations of crude protein (CP), neutral detergent fiber (NDF) and acid detergent lignin (ADL) concentrations and in vitro organic matter digestibility (IVOMD) via near-infrared reflectance spectroscopy (NIRS) (Marten et al. 1985). Dry matter intake (DMI) was estimated using the n-alkanes as external markers (Dove and Mayes 2006). The test animals (3 per paddock) were dosed twice-a-day for 12 days (Penning 2004) using 200 g of dotriacontane (C_{32}) in each application. The determination of n-alkanes of the forage and faeces samples, within the range of C-chains between 27 and 35, followed the methodology proposed by Dove and Mayes (2006) and DMI was estimated according to the equation proposed by Dove and Mayes (1991).

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Statistical analyses

The data were analyzed using the Mixed Procedure in SAS (1996). The model included the random effect of blocks, the fixed effects of sward height, season and interactions between them. If appropriate, the means were compared with a Tukey test at a 5% significance level.

Results and Discussion

Average sward heights were maintained relatively stable and within pre-determined limits of variation during the entire experimental period, and averaged 14.2 ± 1.1, 30.0 ±1.3 and 42.6 ± 1.3 cm, respectively, for 15, 30 and 45 cm treatments. To maintain these target sward conditions, an increase in the stocking rate (SR) was needed as the grazing height declined. During summer the means were 7.3, 6.9 and 5.9 steers/ha; and in autumn 3.6, 3.4 and 3.4 steers/ha for swards managed at 15, 30 and 45 cm, respectively. The higher (P=0.0007) SR in summer was a consequence of the greater herbage accumulation rate (P=0.0161) at that time of year (83.4 and 49.3 kg/ha/d for summer and autumn, respectively), which would have resulted from more favorable climatic conditions and the application of N fertilizer during summer.

No sward height by season (P>0.05) interaction was detected for any of the variables studied. Herbage mass (HM) increased as sward height increased, but there was no effect of season on HM (P=0.1203) (Table 1). Swards grazed at 15 cm presented higher leaf percentage and leaf:stem ratio than those grazed at 30 and 45 cm, so steers on the shorter pastures would have been able to more readily select a diet high in leaf. No sward height effect was found for CP (P=0.4757), IVOMD (P=0.8790) or NDF (P=0.8973) concentrations in plucked samples, and the averages were, respectively, 12.8%, 63.8% and 73.7%. Apparent dry matter intake for steers grazing swards at 15 cm was higher than for those grazing pastures at either 30 or 45 cm (Table 1). As there were no quality differences in plucked samples, it seems probable that steers grazing swards at 30 or 45 cm with lower leaf percentage and consequently lower leaf:stem ratio had difficulty selecting high levels of leaf (Table 1). These findings were in agreement with several other studies (Carvalho et al. 2008; Difante et al. 2009), in which sward structure was found to be more important than nutritive value of leaf in determining herbage intake.

Table 1. Means, standard errors of the difference (s.e.) and probability levels (P) for herbage mass, leaf percentage, leaf:stem ratio and apparent dry matter intake (DMI) by steers in Piatã palisadegrass pastures subjected to 3 grazing intensities. Means followed by the same letter in the same row do not differ (P>0.05).

	Sward height (cm)				
	15	30	45	s.e.	P
Forage mass (kg DM/ha)	1.757 c	2.999 b	4.411 a	249	0.0003
Leaf %	23.1 a	16.6 b	16.8.b	1.2	0.0181
Leaf:stem ratio	1.24 a	0.83 b	0.65 b	0.1	0.0083
DMI (% live weight)	2.94 a	1.80 b	1.70 b	0.2	0.0003

Conclusion

Sward height seems to be critical in management of Piatã palisadegrass pastures. It is important to keep pastures at a height where dry matter availability is adequate and sufficient leafy material is accessible for grazing animals to satisfy appetite. A grazing height of 15 cm appears adequate to satisfy these requirements.

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Status of animal feed resources in Rwanda

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Keywords: Animal feed, zero grazing, seasonality.

Introduction

Animal feed resources remain a major constraint for livestock development in tropical Africa. In Rwanda, grazing lands are shrinking sharply because crop cultivation is progressively encroaching on grazing areas with increasing human pressure (Mutimura and Everson 2011). Therefore, over 60% of households cultivating less than 0.7 ha, and owning livestock, practize zero-grazing, where farmers cut-and-carry forage and crop residues to feed animals that are kept exclusively under sheds (MINAGRI 2009). In general, the main feed for dairy cattle under a zero-grazing system is Napier grass (*Pennisetum purpureum*). For more than a decade, efforts to improve the feed resource base and feeding management have been based on the introduction, characterization and evaluation of exotic forage species, including grasses and legumes. The main aim of this study was to identify and document the status of improved forages as animal feed resources and for use in environmental protection in Rwanda.

Materials and Methods

Rwanda is located in central Africa, immediately south of the equator (1°4' to 2°51' S, 28°63' to 30°54' E). It has a surface area of 26,338 km^2, and is landlocked, being 1,200 km from the Indian Ocean and 2,000 km from the Atlantic Ocean.

A feed inventory survey was conducted in 19 of the 30 districts in the country including 4 districts in the Southern Province, 4 in the Eastern Province, 4 in the Western Province, 4 in the Northern Province and 3 in the Kigali peri-urban area. The structured questionnaire was administered in 8 households per sector (local administration division under the district) by a team of 5 scientists cum extension staff; three sectors per district were sampled. The key information collected included data on: feed resources (feed resource calendar depicting: types, amounts, level of use, sources; means of acquisition; costs); and stakeholders in the feed value chain.

Data analysis was of descriptive statistics (frequencies) computed using the SPSS 16.0 for Windows.

Results and Discussion

Thirty feed types were indicated as feed resources produced on farm. These included grasses, legumes, crop residues, brewers' and home wastes, and non-conventional feeds. The major feed used during the rainy season was Napier grass (*Pennisetum purpureum*), which accounted for 20% of the feeds. It was followed by roadside grass (10.5%) and maize stover (8%). The least used feed resources were groundnut haulms (1.1%) and home wastes (0.1%). The high variability of feed resources indicates the shortage of feedstuffs in the country (Mutimura and Everson 2011). Although crop residues were key feed resources, livestock owners used them opportunistically. Few households fed conserved feeds because they could not produce enough to conserve. This observation agrees with findings in central and southern plateau areas of Rwanda, where conserved feeds (silage and hay) had the lowest ranking within the common feed resource inventory in smallholder dairy households (Kamanzi and Mapiye 2012). However, in peri-urban areas on dairy farms (small or large), silage is used to feed dairy cows (Nyiransengimana and Mbarubukeye 2005). Hay from grasses, especially *Brachiaria* grasses, was used up to 3.7% by farmers. The most common ones were the hybrids Mulato II and Mulato, which have been disseminated since 2008 (Mutimura and Everson 2012).

During the rainy and wet seasons, the feeds most sourced off-farm were roadside grass (17%), banana peels (8.4%) and sweet potato vines (8.1%). Farmers also purchased forages from neighboring farmers or concentrates from the markets. The most purchased feeds were maize bran (11%), commercial concentrate (9.6%) and rice bran (8.9%). Interestingly, multi-purpose trees (MPTs) were harvested free of charge from neighboring farms and comprised up to 2.6% of feed resources.

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MPTs and grasses are planted on contour bands for erosion control and soil amendment, rather than MPTs being seen by farmers as feed resources of commercial importance. In contrast, farmers in neighboring countries consider that MPTs, especially *Calliandra calothyrsus,* are commercially viable and valuable home-grown feed resources (Kabirizi 2003).

Conclusion

Despite efforts to improve forage productivity and quality in Rwanda, farmers still experience feed shortages in both wet and dry seasons, mainly because of limited land availability. While feed from neighboring farms and feed markets helps to eke out the feed resource base, these are not long-term solutions. More innovative solutions that integrate home-grown forages, crop residues and off-farm feed resources into a complete package of interventions for sustainable household land use are needed. This should be given priority in research in Rwanda.

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Establishment of *Leucaena leucocephala* cv. Tarramba in eastern Indonesia

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Keywords: Leucaena establishment, transplanting, soil type, leaf tissue analysis.

Introduction

The adaptability and productive value of *Leucaena leucocephala* cv. Tarramba (Tarramba) in eastern Indonesia has been observed since 2001 (Nulik et al. 2004), with many farmers and other stakeholders currently requesting seed for planting. Tarramba has greater forage and timber production, and psyllid tolerance than other commercial cultivars and locally grown leucaena. The variability of soils and climate in eastern Indonesia means that establishment techniques specific to the region are required to achieve maximum adoption and utilization of Tarramba.

Materials and Methods

A number of sites differing in soil type in west Timor (8 sites) and east Sumba (4 sites) were selected to study the establishment of Tarramba (Table 1). Management varied among sites for factors such as exclusion of animals during establishment and competition from weeds and trees. Scarified seed (imported from Australia) was washed and immersed in clean water until the seed became imbibed (4−12 hours). The seed was germinated in petri-dishes (radicle length between 0.5 and 1.5 cm) and sown into polybags (December 2011) or directly seeded into the field between the rows, when planting maize.

Seedlings in polybags were well watered and pressed to prevent soil loss and wilting during transplanting to the field in January and February 2012. Plant height and basal stem diameter were measured to determine yield via a growth index (GI) (GI = height x basal diameter^2) (Stewart et al. 1992). Fifty plants from each site were identified for measurements. Mean GI data presented here are from a single measurement in October 2012. Young fully expanded leaves were collected from leucaena trees established at the Kambatatana sites in east Sumba to identify nutrient deficiencies, as the soils were highly eroded, poor karst limestone soils.

Results and Discussion

As anticipated, Tarramba performed differently according to method of planting and the environmental conditions and management during establishment. Soil type (particularly water holding capacity and fertility) and competition from weeds and shading affected the capacity of the plants to grow to their potential (Table 1). Plant growth was greatest in vertisols and alluvial sediments, such as at Batulesa in Kupang District and at Wanga in east Sumba, followed by Ponain on a vertisol, then Tesbatan II and Oebola Dalam on alfisols, with poorest growth during establishment on poor karst limestone soils in Kambatatana and at Naunu, where competition from weeds and trees was high. Tissue analysis of leaf samples (youngest fully expanded leaves) indicated that there were deficiencies of P, K and S in samples taken from one or both Kambatatana sites (Ruaysoongnern et al. 1989; Mullen et al. 2003) (Table 2). Nevertheless, the nutritional value of Tarramba was superior to that of the native grasses present at Kambatatana, particularly in crude protein concentration (19% vs <3%).

Best seedling growth occurred at sites that involved transplanted seedlings from polybags, which allowed plants to establish for 2 months with daily watering

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without competition from trees or weeds, e.g. at the Batulesa and Wanga sites (GI>4000), with Ponain the next best site (GI = 1500). Transplant sites that were not well weeded did poorly (GI of Tesbatan 1 was 198). Establishment by direct seeding was best where competition from trees was minimized, e.g. GI at Oebola Dalam was 1180. Direct seeding of imbibed seeds into maize rows was best with good weed management, although competition from actively growing maize was initially high. This planting method provided excellent growth conditions (sufficient soil moisture content and low competition) following harvest of the maize.

Table 1. Site description, management practices and Tarramba growth index (± standard deviation).

Region	Site	Soil type	Animals excluded (y/n)	Planting method	Weed competition[1]	Competition from trees[1]	Survival rate (%)	Growth index
East Sumba	Wanga	Vertisol	y	Transplanted	1	1	85	4137 ±28
	Laindeha	Vertisol Inceptisol	n	Transplanted	3	2	75	NA
	Kambatatana (2 sites)	Vertisol (site 1) Limestone (site 2)	y	Transplanted	3	2	100	974 ±164[2] 272 ±15[3]
West Timor	Oebola Dalam	Alfisol	y	(a) Direct seeded into maize[4] (b) Transplanted	1	2	90	1181 ±92
	Naunu	Mollisol Inceptisol	y	(a) Direct seeded into maize[4] (b) Transplanted	1	4	90	137 ±43
	Ponain	Vertisol	y	Transplanted	2	2	80	1501 ±36
	Tesbatan (I)	Vertisol	y	Transplanted	5	5	95	198 ±20
	Tesbatan (II)	Alfisol	y	Transplanted	1	1	10	1259 ±59
	Oeli	Alfisol	y	Transplanted	1	1	95	840 ±92
	Dusun 1	Alfisol	y	Transplanted	1	1	80	482 ±37
	Batulesa	Vertisol	n	Transplanted	1	1	75	5430 ±63

[1]Where 1 is low and 5 is high.
[2]Planted on alluvial flats below a limestone hill with better vertisols.
[3]Planted on a highly eroded limestone ridge.
[4]No measurements made, visual assessment only.

Table 2. Nutrient concentrations in young fully expanded leaves of leucaena plants harvested November 24, 2012.

Item	Al	B	Cu	Fe	Mn	Zn	Ca	K	Mg	Na	P	S
	(mg/kg)						(%)					
Kambatatana (ridge)	48	99	10	103	53	21	1.90	0.94[1]	0.42	0.04	0.12[1]	0.19[1]
Kambatatana (gully)	205	129	7	181	35	41	2.09	0.94[1]	0.37	0.05	0.11[1]	0.44
Adequate		>22	>4.40	>44	n/a	>13	>0.44	>1.65	>0.22	n/a	>0.27	>0.26
Deficient		<18	<3.60	<36	n/a	<11	<0.36	<1.35	<0.18	n/a	<0.22	<0.22

[1]Levels indicate deficiency (Ruaysoongnern et al. 1989; Mullen et al. 2003).

Conclusions

This study has shown that *Leucaena leucocephala* cv. Tarramba can be established successfully in easternIndonesia, when seed is prepared appropriately for germination, seedlings are allowed to establish without grazing, and competition from weeds and trees is minimal. Transplanting from polybags will give better initial growth than direct seeding, even into well weeded maize. Despite reduced plant growth in karst limestone soils owing to nutrient deficiencies, the forage produced is of better quality than existing native grasses even in the dry season. This cultivar shows great potential for improving livestock performance in eastern Indonesia regardless of soil type.

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Performance of young Nelore bulls grazing marandu grass pasture at different heights

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Keywords: Pasture management, pasture yields, liveweight gains, plant composition, grazing intensity, *Brachiaria brizantha*.

Introduction

Brazil is one of the largest beef cattle producers in the world with approximately 200 M head. The Industry relies predominantly on warm-season grass pastures, with approximately 90% of animals finished on pastures.

One of the main factors for the intensification of animal production systems based on pasture is appropriate management. Adjustment of stocking rate to maintain optimum forage allowance is essential. Studies on forage allowance have resulted in a better understanding of the response of forage crops and animals to changes in grazing intensity.

The purpose of this study was to evaluate management strategies for beef cattle systems grazed at different heights (15, 25 and 35 cm) in *Brachiaria brizantha* cv. Marandu in terms of pasture production and animal performance.

Material and Methods

The experiment was conducted at Animal Science Department, São Paulo State University, Jaboticabal, SP, Brazil (21º15'22" S, 48º18'58" W; 595 m asl). The trial was set up in an area of *Brachiaria brizantha* cv. Marandu pastures established in 2001 on a red latosol.

Fertilizer was applied at the rate of 90 kg N/ha in the rainy season. According to the Köppen classification, the climate of Jaboticabal is characterized as Awa, or subtropical with dry winters and rainy summers. The experimental period was from January to April 2012 during the rainy season. Experimental paddocks (6.0 ha) were managed under continuous stocking, with variable stocking rates to give 3 grazing heights (15, 25 and 35 cm) using young Nelore bulls.

Forage quantitative and structural components were measured monthly using samples collected from the sites at medium height and separated into leaf blades, stems and leaf sheaths, and dead matter. All forage included within the perimeter of the rising plate (0.25 m^2) was collected at soil level. Individual animal performance was measured by weighing animals at the start and end of the experiment, after a 12-hour period of complete fasting.

Data were analyzed by a complete randomized design with 3 grazing heights and 2 replications (paddocks) with 6 animals per paddock, and harvest date in repeated measures over time. Data were analyzed using the GLM procedure of SAS.

Results and Discussion

Total herbage mass and leaf and stem proportions decreased, and dead material increased along the 4-month experimental period (Table 1). Herbage mass increased in response to grazing height, while structural characteristics did not respond uniformly to grazing height.

With the decline in both rainfall and temperatures in March−April in Brazil, tropical grasses begin to senesce, resulting in a higher proportion of stem and dead material, with the effect most obvious in swards managed at greater heights. In general, crude protein and digestibility values decreased, while cell wall increased over the experimental period.

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Table 1. Total herbage mass and mass of the components in *Brachiaria brizantha* cv. Marandu pastures managed at 3 forage heights under a continuous stocking system during the 4-month rainy season.

Variable	Height (cm)	27/01/2012	25/02/2012	23/03/2012	21/04/2012
Forage mass (kg/ha)	15	6542Ca[1]	4934Cb	3524Cc	2114Cd
	25	8297Ba	8646Ba	7208Bb	5770Bc
	35	13512Aa	11614Ab	10058Ac	8502Ad
Leaf mass (%)	15	38.7Ba	33.9Ab	24.9Ac	16.0Ad
	25	44.5Aa	31.1Ab	25.0Ac	18.9Ad
	35	37.4Ba	34.3Ab	26.0Ac	17.7Ad
Stem mass (%)	15	28.5Ab	31.9Aa	27.6Bb	23.2Cc
	25	28.2Ab	33.3Aa	30.6Ab	27.9Bc
	35	26.3Ab	32.3Aa	31.4Aa	30.6Aa
Dead mass (%)	15	32.7Bd	34.1Ab	47.4Bc	60.7Aa
	25	27.2Cd	35.5Ab	44.3Ac	53.0Ba
	35	36.2Ac	33.3Ad	42.4Ab	51.6Ba

[1]Means followed by the same lower-case letters in rows and upper-case letters in columns for each analyzed factor are not significantly different according to Tukey's test at 10% probability.

Animal ADG increased in response to pasture height (Table 2), mainly related to higher herbage allowance in this pasture. According to Poppi and MacLennan (2007), average daily weight gain from tropical pastures during the wet season ranges from 0.5 to 0.7 kg/d depending on herbage allowance. Our observations at the greater herbage heights conform with this suggestion.

Table 2. Initial and final weights and performance of young Nelore bulls grazing *Brachiaria brizantha* cv. Marandu pastures at 3 different heights during the rainy season.

Height (cm)	Variable			
	IW (kg)	FW (kg)	ADG (kg/d)	AG (kg/ha/d)
15	243.0a[1]	279.2c	0.3b	2.5c
25	242.6a	314.9b	0.6a	3.6[a]
35	245.9a	330.0a	0.7a	3.2b

[1]Means followed by the same lower-case letter within columns for each analyzed factor are not significantly different according to Tukey's test at 10% probability.

Conclusion

While increasing herbage grazing height increased animal weight gains, there was a plateauing effect between 25 and 35 cm. The increased grazing pressure to maintain pastures at the lower height more than compensated for the slightly higher daily gains at the higher grazing height. Grazing intensity should be manipulated to obtain a compromise between gains per individual and gains per hectare.

Acknowledgments

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Seasonal expression of apospory in bahiagrass

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Keywords: Reproduction, apomixis, meiosis, embryo sac, parthenogenesis, polar nuclei.

Introduction

Flowering plants can reproduce sexually (outcrossing and/or selfing) and/or asexually. Sexual reproduction implies the successful completion of meiosis and double fertilization for the formation of both the embryo and the endosperm. In contrast, gametophytic apomixis is an asexual mode of reproduction through seeds, that involves parthenogenetic embryo development from a cytologically unreduced egg cell (2n). Apospory is the process by which unreduced gametophytes are formed after a series of mitotic divisions of somatic cells (2n) in the ovary. This occurs independently from the sexual meiotic process; and therefore, both sexual and apomictic pathways may coexist simultaneously.

Apospory is inherited in bahiagrass (*Paspalum notatum* Flüggé) as a single dominant Mendelian factor with distorted segregation (Martínez et al. 2001), and its degree of expression was reported to vary throughout the flowering season in *P. cromyorrhizon*, a close relative of bahiagrass (Quarin1986). Bahiagrass is a perennial warm-season grass widely used for forage and utility turf in the south-eastern US due to its persistence in sandy, infertile soils. Diploid races reproduce sexually and are highly self-incompatible (Acuña et al. 2007), while polyploids are classified as pseudogamous apomicts (pollination is required) (Quarin 1999). Sexual tetraploid genotypes have been experimentally created (Quesenberry and Smith 2003; Quesenberry et al. 2010) and successfully used in crosses (Acuña et al. 2009). Cyto-embryological analysis has been used to determine the mode of reproduction in bahiagrass (Martínez et al. 2001; Acuña et al. 2007). At anthesis, sexual plants produce spikelets having only a single *Polygonum* type meiotic embryo sac (SES), characterized by bearing the egg apparatus close to the micropyla, a large binucleated central cell and a group of antipodal cells at the chalazal end (Figure 1a). Highly apomictic plants produce ovules having single or multiple aposporous embryo sacs (AES), which present the egg apparatus and a central cell with 2 polar nuclei, and no antipodal cells (Figure 1b). Some tetraploid bahiagrass races are also able to produce ovules that have the sexual meiotic megasporocyte together with one or more aposporous sacs (AES+SES), and these plants are classified as facultative apomictic. The objective of this study was to characterize the reproductive mode of 5 wild dwarf bahiagrasses, a highly apomictic hybrid (Acuña et al. 2009) and cv. Argentine at different times during the flowering season and under different nitrogen (N) fertilizer rates.

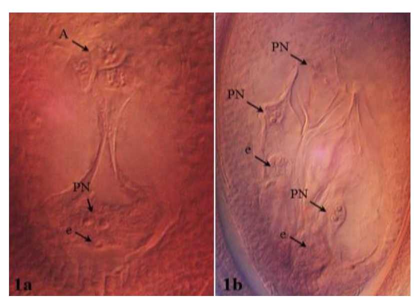

Figure 1. Types of embryo sacs: (a) ovule bearing an SES; (b) ovule having at least 3 AES. A = antipodals, e = egg cell, and PN = polar nuclei. Magnification: x 312.5.

Methods

The study was conducted on a bahiagrass sward planted in the fall of 2011 at the Agronomy Forage Research Unit, near Gainesville, FL, USA. The experimental design was a randomized complete block design in a strip split-split-plot arrangement with 3 replications. Main

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plots consisted of 8 bahiagrass genotypes: 5 wild dwarf bahiagrasses (3 Fpen 7; 3 Fpen 9; Fldw 5-1; Fldw 6-4; and Fldw 6-5), 2 highly apomictic hybrids (Hyb 3; and Hyb 14), and cv. Argentine. Subplots were the dates of plant defoliation at 10 cm (May and June) and sub-subplots were different N fertilizer rates (0, 60 and 120 kg N/ha) applied randomly using ammonium nitrate immediately after mowing the plots. Inflorescences were collected at anthesis at 3 different times during the flowering season: (a) spring: before mowing the plots (only the facultative apomicts were flowering); (b) summer; and (c) fall. In the summer and fall inflorescences were collected only from plots mowed in May. Additionally, only the genotypes 3 Fpen 9, Fldw 6-5, Hyb 3 and Argentine were analyzed in the summer and fall. For each embryo sac observation, 2 different inflorescences at anthesis were fixed in FAA (18 Ethanol 70%:1 Formaldehyde 37%:1 glacial acetic acid). Pistils were dissected out of the spikelets and cleared following the methodology described by Young et al. (1979). At least 20 ovules per sample were observed using a differential interference contrast microscope.

Results and Discussion

The 5 dwarf genotypes were classified as facultative apomicts, since all of them produced florets having AES, SES or both AES+SES in the same ovule in the inflorescences collected in the spring. The proportion of AES varied (29−55%) among the dwarf plants. All genotypes showed a high number of ovules bearing AES+SES. Ovules bearing SES were produced with a lower frequency, ranging from 12 to 20%. Overall, the production of aborted or immature embryo sacs (AbES) was low, except for the genotype Fldw 6-5 (20%). Nitrogen fertilizer did not affect apospory in any of the genotypes; therefore results from the 3 N rates were averaged within season (summer and fall) for each genotype. Genotypes Hyb 3 and Argentine bahiagrass reproduced by apomixis as reported by Acuña et al. 2009. These genotypes were classified as highly apomictic because more than 90% of the embryo sacs produced in either the summer or fall were AES, while the remainder were AbES (Figure 2).

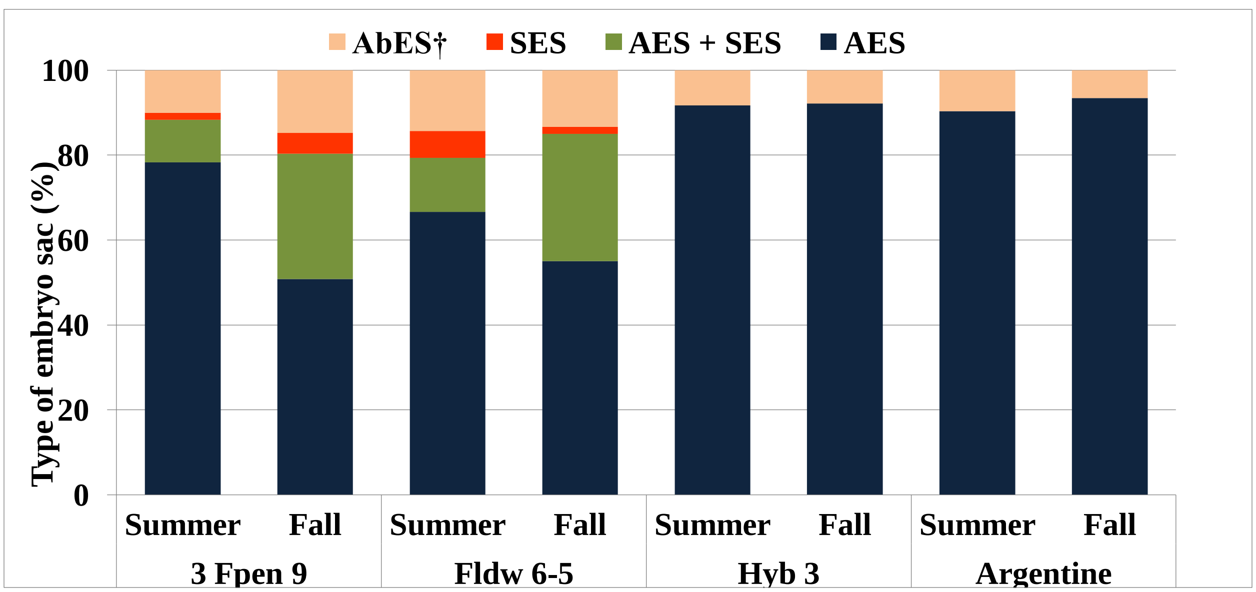

Figure 2. Types of embryo sac observed in 2 facultative apomictic (3Fpen9 and Fldw6-5) and two obligate apomictic (Hybrid 3 and Argentine) bahiagrass genotypes at two different stages of the flowering season.
†AbES: aborted or immature embryo sacs; SES: meiotic embryo sacs; AES: aposporic embryo sacs; AES+SES: AES and SES embryo sacs in the same ovule.

The two facultative apomictic dwarf plants performed similarly in their modes of reproduction in the summer and fall (Figure 2). Apospory was expressed in greater proportion in the summer (60−80% of the embryo sacs were AES); however, in the fall the production of AES+SES increased, reaching similar values as in the spring. Therefore, the potential for apomictic reproduction increases as the plants reach peak flowering (summer). Hence, the environmental conditions that affect flowering may also be involved with the expression of apospory, as Quarin (1986) proposed for *P. cromyorrhizon*.

Conclusion

The 5 wild dwarf genotypes behaved as facultative apomictic and the expression of apospory varied throughout the flowering season. The potential for apomixis (AES) increased as the plants reached their peak flowering (summer); however, the potential for sexual reproduction (AES+SES and SES) increased in the spring and fall. Apospory was consistently expressed throughout the flowering season in the highly apomictic genotypes Hyb 3 and Argentine. These results have implications for seed harvesting.

In general, the dwarf genotypes produced more AbES than the highly apomictic plants, so a lower seed set may be expected from them. Considering that each embryo sac had the same opportunity to develop a viable seed, time of seed harvest can be used to manipulate objectives in a breeding program. When new genetic variability is desired, seed should be harvested in the spring or fall. In contrast, when harvest occurs during peak flowering, progeny will be highly uniform. As N fertilizer had no impact on the expression of apospory in these genotypes, application of fertilizer to improve seed production can proceed without concern about likely impact on reproductive pathway.

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Morphological divergence among progeny of *Macroptilium lathyroides* accessions from the semi-arid region of Pernambuco, Brazil

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Keywords: Tropical legumes, population, selection, genetic diversity, heritability.

Introduction

Macroptilium is a legume genus with approximately 20 species, usually annual or biennial, herbaceous and distributed mainly in the Americas. It is widely used as a forage resource in grasslands and usually fixes atmospheric N.

Martins et al. (2001) indicated that half-sib family selection with progeny testing is the most common plant breeding method used in Brazil. In the scientific literature, however, there are few studies dedicated to *Macroptilium* spp.

This study evaluated morphological divergence among *Macroptilium* spp. progeny from accessions collected in 4 counties located in the semi-arid region of Pernambuco State, NE Brazil.

Methods

The experiment was carried out at the Animal Science Department of the Federal Rural University of Pernambuco (UFRPE), NE Brazil. Accessions of *Macroptilium lathyroides* were collected in Bom Jardim, Caetés, Sertânia and Tupanatinga counties, in the semi-arid region of Pernambuco State. Seeds originating from 15 progeny of these accessions were planted and morphological characteristics of the seedlings assessed from October 2012 to January 2013.

Seeds were planted in 20-L pots filled with soil (Ultisol) collected from the 0−20 cm soil layer in Arcoverde, PE. The soil presented the following chemical characteristics: pH (H_2O) = 6.6; Mehlich-I P = 57 mg/dm^3; K^+ = 0.41cmol$_c$/dm^3; Na^+ = 0.25 cmol$_c$/dm^3; Ca^{2+} + Mg^{2+} = 3.65 cmol$_c$/dm^3; Ca^{2+} = 2.9 cmol$_c$/dm^3; Al^{3+} = 0.0 cmol$_c$/dm^3; H^++Al^{3+} = 2.28 cmol$_c$/dm^3; organic C = 6.11 g/kg; soil organic matter = 10.53 g/kg. No lime was added. Before planting, seeds were scarified with sulphuric acid for 10 min.

A complete randomized design was used with 3 replications. Treatments were different accessions of *Macroptilium lathyroides*. The following response variables were assessed: average plant height (from ground level to the tallest leaf); leaf and leaflet dimensions (length and width; longitudinal and latitudinal measurements using a pachymeter); leaf number per plant; and general aspect of the plant (desirability) using a grading scale ranging from 1 (low) to 5 (high). These evaluations were performed every 45 days. Variance analyses were performed using the software GENES (Cruz 2001). The Scott-Knott test was used ($P<0.05$) for comparing means.

Results and Discussion

Only plant height, leaf number and desirability showed any variation between accessions ($P<0.05$). Four accessions (#2 from Bom Jardim; #12 from Tupanatinga; #14 and #15 from Caetés) showed highest values. In an evaluation of the legume genera *Calopogonium*, *Centrosema*, *Macrotyloma* and *Macroptilium,* Veasey et al. (1999) observed genetic variability for morphological characteristics within the *Macroptilium* genus. Garcia et al. (2003) assessed the genetic variability of *Macroptilium erythroloma* in Rio Grande do Sul, Brazil, and used a protein polymorphism test to reveal greater similarity among individuals from the same population than between populations, indicating populations with genetic activity.

Progeny presented heritability estimates ranging from 6.47 to 59.6% for leaflet width and plant height, respectively (Table 1). These heritability values are of low to medium magnitude, indicating that these plant responses may be highly affected by the environment.

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Table 1. Morphological traits and their estimated heritability of 15 *Macroptilium lathyroides* accessions collected in the semi-arid region of Pernambuco State, Brazil.

Accessions	PH[1]	LL	LW	LLL	LLW	LN	Des
			cm			Nº	
1(BJ)[2]	64.98b[3]	5.67a	5.02a	3.16a	2.04a	9.85b	2.62b
2 (BJ)	105.83a	6.09a	5.83a	3.47a	2.30a	19.88a	3.94a
3 (BJ)	66.55b	4.88a	4.18a	2.78a	1.92a	6.85b	2.25b
4 (BJ)	79.97a	5.68a	4.94a	3.34a	2.15a	12.36b	2.94b
5 (BJ)	85.44a	4.92a	4.15a	3.00a	2.24a	10.19b	2.94b
6 (BJ)	81.92a	4.87a	4.48a	2.86a	1.76a	10.66b	3.00b
7 (BJ)	76.61b	4.26a	4.33a	2.62a	1.92a	10.07b	2.92b
8 (BJ)	66.01b	6.11a	5.34a	3.42a	2.39a	9.59b	2.95b
9 (BJ)	84.5a	5.79a	4.59a	3.11a	2.11a	8.94b	2.83b
10 (BJ)	65.58b	5.15a	4.87a	2.83a	2.12a	10.94b	2.69b
11 (BJ)	65.92b	5.66a	5.15a	2.87a	2.00a	11.91b	3.03b
12 (T)	97.33a	5.86a	4.69a	3.58a	1.92a	20.58a	3.66a
13(C)	66.33b	3.18a	3.05a	2.02a	1.47a	12.41b	2.66b
14(C)	95.00a	5.63a	5.26a	3.22a	2.11a	16.66a	3.50a
15 (S)	97.25a	4.87a	5.01a	2.96a	2.33a	17.14a	3.55a
Mean	78.90	5.22	4.75	3.01	2.06	12.20	3.01
CV(%)	18.23	16.36	15.87	15.36	17.36	35.47	17.28
h^2	59.60	52.26	45.80	39.74	6.47	55.35	48.67

[1]PH = plant height; LL = leaf length; LW = leaf width; LLL = leaflet length; LLW = leaflet width; LN = leaf number; Des = desirability (grading scale ranging from 1 to 5, where 1 is less desirable and 5 more desirable).
[2]BJ = Bom Jardim county; T = Tupanatinga county; C = Caetés county; S = Sertânia county.
[3]Means followed by the same letter within columns do not differ by Scott-Knott test (P>0.05).

Conclusion

The variation in plant height, leaf number and desirability among these accessions of *Macroptilium lathyroides* indicates that there is merit in collecting further germplasm of this species and determining if these characteristics can be reflected in increased dry matter production and forage quality.

Acknowledgments

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BRS Paiaguás: A new *Brachiaria* (*Urochloa*) cultivar for tropical pastures in Brazil

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Keywords: Grass cultivar, animal performance, palisade grass, tropical pastures, pasture diversification.

Introduction

Approximately half the world's beef is produced in the tropics and subtropics, almost exclusively on pasture. While the world production of beef and veal increased 91% over the past 40 years, the increase in the tropics was 200%. Brazil has today the largest commercial cattle herd in the world (about 190 M head) and is the world's largest exporter of beef. The area of cultivated pasture increased from 30 to 100 Mha between 1970 and 1995 (IBGE 2006). This area has now stabilized or decreased despite the increase in beef production, reflecting the gain in productivity per ha. Part of the cultivated pasture expansion till 1995 resulted from the replacement of native pasture, and part from the opening of the Brazilian Cerrados, but the main contributing factor to the increase in livestock production was the use of more productive cultivars and intensification in the management of cultivated pastures.

The demand for productive and high quality forages continues to be high. Very few cultivars are commercially available, and the majority of these display apomictic reproduction, resulting in no novel genetic variation. New cultivars are urgently needed to increase pasture diversification as insurance against the extensive monocultures formed in central Brazil. The cultivars released, mainly by Embrapa, were developed mostly by selection from the natural variability in germplasm collections, reflecting the success of this methodology, and account for over 70% of the forage seed available commercially. This paper presents data on a new cultivar of *Brachiaria brizantha*, selected for soils of medium fertility with a well defined dry season.

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Methods

Developing a new cultivar involves 2 years of agronomic evaluation in plots under a cutting regimen and morphological characterization of germplasm collections (about 100 accessions) to select the best 20−25 for regional trials (another 2 years) still in plots under cutting, to evaluate the genotype x environment interactions and finally, selection of the 2−4 most promising ones for use in grazing trials over another 2 years (Figure 1). Parallel trials are carried out to evaluate resistance to biotic (pests and diseases) and abiotic (tolerance of drought, flooding, shade, toxic Al in soils; response to fertilizers) stresses, and seed-production technology to gather the necessary information to properly recommend a new cultivar. BRS Paiaguás has been under evaluation at Embrapa Beef Cattle, Campo Grande, MS, Brazil for over 18 years and was derived from an accession introduced from Nairobi, Kenya, in a collection gathered by CIAT in 1984.

Results and Discussion

Cultivar BRS Paiaguás was selected for its productivity, vigor and seed production and, although it has no resistance to spittlebugs (the most important insect pest in pastures in Brazil), it has high potential production during the dry season, with high leaf percentage and good nutritional value. In regional trials it showed high dry matter production, though lower than *B. brizantha* cv. Xaraés, with limited spittlebug damage in the plots. This cultivar is adapted to soils of medium fertility and behaves similarly to *B. brizantha* cv. Marandu in response to fertilizers. In grazing trials, when compared with *B. brizantha* cv. BRS Piatã, released as an alternative for dry season grazing, it showed even higher potential, since it has more forage growth and better nutritional value, resulting in higher animal gains per head and per unit area (Table 1).

Figure 1. BRS Paiaguás in plots and in a pasture in Campo Grande, MS, Brazil (2013).

It is important to note that in the 2011 and 2012 dry seasons, BRS Piatã pastures had to be destocked for 2 months (August−September) owing to lack of available forage, while BRS Paiaguás remained stocked and maintained animal gains. Average daily gains (ADG) and carrying capacity were higher (P<0.05) on BRS Paiaguás during the dry season. Overall ADGs for the 3 years of evaluation for BRS Paiaguás and BRS Piatã were 0.52 and 0.44 kg/hd/d (P=0.0039), respectively. Maintaining pastures at 15 cm height resulted in higher carrying capacity but lower ADGs and lower gain per unit area than keeping pastures at 30 cm height. BRS Piatã pastures had more encroachment of weeds at 15 cm than BRS Paiaguás.

Table 1. Average daily gain and carrying capacity over 3 years of 2 *Brachiaria brizantha* cultivars.

Cultivar	Rainy season	Dry season
	ADG (kg/hd/d)	
BRS Paiaguás	0.66 aA[1]	0.31 aA
BRS Piatã (control)	0.59 aA	0.13 bB
	Carrying capacity (AU/ha)	
BRS Paiaguás	3.60 aA	3.50 aA
BRS Piatã (control)	3.56 aA	1.8 bA

[1]Means for different parameters followed by different upper-case letters within columns and different lower-case letters within rows are different (P<0.05).

Conclusion

BRS Paiaguás deserves the status of cultivar, based on the production of total dry matter and leaf blades and vigor, especially during the dry season, when it accumulates forage of high nutritional value, resulting in good weight gains per animal and per ha. The differences in animal performance and gain per unit area observed in the comparison trial with BRS Piatã indicate that neither cultivar should be grazed below 30 cm.

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Herbage accumulation, nutritive value and persistence of Mulato II in Florida

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Introduction

Grasses in the *Brachiaria* genus are the most widely grown forages in tropical America, occupying over 80 Mha (Boddey et al. 2004). Mulato II is apomictic and a vigorous, semi-erect cultivar resulting from 3 generations of crosses including original crosses between ruzigrass (*B. ruziziensis*) and signalgrass (*B. decumbens* cv. Basilisk, apomictic tetraploid). According to Peters et al. (2003), Mulato produced 25% more herbage mass than palisadegrass (*Brachiaria brizantha*) and koroniviagrass (*B. humidicola*) under similar management practices. Although Mulato II shows promise as forage in tropical regions, herbage accumulation and persistence in subtropical areas is unknown. This publication summarizes results of the research with Mulato II conducted in Florida in the last 5 years.

Methods

South Florida

This experiment was conducted on Mulato II in Ona, FL (27°26' N, 82°55' W) between August and November in 2007 and 2008. Treatments were the factorial combinations of 3 stubble heights (2.5, 7.5 and 12.5 cm) and 2 harvest frequencies (2 and 4 weeks) in a randomized complete block design with 4 replicates**.** Plot size was 3 x 2 m with 1-m alley between plots. Samples were analyzed for in vitro digestible organic matter (IVDOM) and crude protein (CP) concentrations. Mulato II ground cover was determined as a measurement of persistence at the end of the experimental period using a 1-m^2 quadrat divided into 100 squares. The proportion of squares with Mulato II plants was reported.

Central Florida

The study was conducted in Gainesville, FL (29°44' N, 82°16' W) from June 2008 to June 2010. Treatments were Mulato II treated as an annual (planted in 2008 and 2009), Mulato II treated as a perennial (planted in 2008 only), Tifton 85 (*Cynodon* hybrid) (planted in 2008), and Tifleaf 3 pearl millet (*Pennisetum glaucum*) and Hayday sorghum-sudangrass (*Sorghum bicolor*) (both planted in 2008 and 2009), arranged in a randomized complete block design with 4 replicates. The annual treatment for Mulato II was included to compare the use of this grass with the annual species pearl millet and sorghum-sudangrass, while the perennial Mulato II treatment was included to compare persistence and productivity over time with Tifton 85 bermudagrass. Plots were 5 x 5 m with a 1-m alley between plots. Seeded grass was planted on June 2008 and 2009. Tifton 85 was planted vegetatively using 100 plugs per plot. In general, perennials were harvested every 5−6 weeks throughout the summer, with slightly longer intervals during cool autumn weather. An area of 2.88 m^2 was harvested with a sickle-bar mower from the center of the plot to a 10-cm stubble height. Herbage accumulation, IVDOM and CP were determined. A 2 m x 0.5 m-frame, divided into 100 squares, was placed at 3 locations in each plot to determine the persistence of the plants at the end of the experimental period in 2009 and 2010. At each location where the frame was placed, cover was estimated visually in 25 of these squares. Cover was the average of the 75 estimates per plot.

North Florida

The study was conducted in Marianna, FL (30°52' N, 85°11' W). Treatments were 3 forage species, Tifleaf 3

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pearl millet, Hayday sorghum-sudangrass and Mulato II arranged in a completely randomized design with 3 replicates. Pastures (0.6-ha experimental units) were established in June 2008 and June 2009 in a prepared seedbed. Pastures were stocked continuously using a variable stocking rate. Two heifers (Angus crossbred) were assigned as testers to each experimental unit. Additional heifers of comparable age and weight to the testers were introduced or removed to maintain similar forage stubble height (≈ 30 cm) across experimental units. Herbage mass, nutritive value, stocking rate and average daily gain per head and per ha were evaluated.

Results

In south Florida, there was a quadratic decrease in herbage accumulation from 2.0 to 1.6 t/ha with decreasing stubble height. Conversely, herbage CP increased linearly with decreasing stubble height (from 14 to 17%), while IVDOM was unaffected (66 vs 67%). Mulato II ground cover decreased linearly from 87 to 74% as stubble height decreased from 12.5 to 2.5 cm.

In central Florida, Hayday and Tifleaf 3 established more rapidly than Mulato II; however, Mulato II had greater herbage accumulation later in the fall. The perennial treatments (Mulato II and Tifton 85) had greater herbage accumulation overall than the annual treatments and Tifton 85 had greater ground cover than Mulato II in 2009 (73 vs 36%) and 2010 (73 vs 12%). Mulato II had greater IVDOM than Tifton 85 (67 vs 63%), Hayday (64%), and Tifleaf 3 (62%).

In north Florida, in year 1, there were no differences in herbage allowance (0.9 kg DM/kg body weight), average daily gain (0.5 kg/d) and gain/ha (168 kg) among treatments. However, in year 2, Mulato II had greater herbage allowance (2.0 vs 0.7 kg DM/kg BW) and ADG (0.78 vs 0.41 kg/d) than Tifleaf 3 and Hayday but similar gain/ha (302 kg).

Conclusions

In central and north Florida, Mulato II may behave as an annual or biennial forage and its greater herbage accumulation and nutritive value make it a suitable alternative to Tifton 85 and warm-season annual forages. In contrast, in south Florida, Mulato II behaves as a perennial forage; however, forage persistence is reduced if it is cut to short stubble heights. This management strategy should be avoided.

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