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Table of Contents

Research Papers

Short Communications

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Research paper

Selection of effective strains of *Bradyrhizobium* for Caatinga stylo (*Stylosanthes seabrana*)

Selección de cepas efectivas de Bradyrhizobium *para* Stylosanthes seabrana

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Abstract

Caatinga stylo (*Stylosanthes seabrana*) is recommended as a forage legume for permanent and long-term ley pastures on clay soils of southern (cv. Primar CPI92838B) and central (cv. Unica CPI110361) Queensland. The release of the 2 cultivars was contingent on the availability of an effective and persistent strain of *Bradyrhizobium*, because suitable effective nitrogen-fixing strains do not occur naturally in the soils of the target regions. Effective strains of *Bradyrhizobium* (strains CB3480 and CB3481), suitable as inocula for Caatinga stylo, were selected from nodule material collected in Bahia, Brazil.

This paper documents soil-pot and field experiments that led to the selection of these persistent and effective strains of *Bradyrhizobium* and the eventual release of CPI92838B and CPI110361, respectively, as cvv. Primar and Unica.

Keywords: Inoculation, nitrogen fixation, nodulation, soil temperature, survival.

Resumen

Stylosanthes seabrana (Caatinga stylo) es una leguminosa forrajera recomendada para pasturas permanentes o, en rotación con cultivos, pasturas de larga duración en suelos arcillosos tanto en la zona sur (cv. Primar CPI92838B) como en la zona centro (cv. Unica CPI110361) de Queensland, Australia. La liberación de estos cultivares estuvo sujeta a la disponibilidad de una cepa efectiva y persistente de *Bradyrhizobium*, debido a que este tipo de cepas fijadoras de nitrógeno no ocurre en forma natural en los suelos de la región. A partir de material nodular recolectado en Bahia, Brasil, fueron seleccionadas como efectivas las cepas *Bradyrhizobium* CB3480 y CB3481, y adecuadas como inóculos para esta especie.

Este trabajo documenta la investigación realizada en materas y a nivel de campo que llevaron a la selección de estas cepas persistentes y efectivas de *Bradyrhizobium* y a la eventual liberación de *S. seabrana* CPI92838B y CPI110361, respectivamente, como cvs. Primar y Unica.

Palabras clave: Fijación de nitrógeno, inoculación, nodulación, supervivencia, temperatura del suelo.

Introduction

The selection and release of adapted accessions of Caatinga stylo [*Stylosanthes seabrana*; by the taxonomic database GRIN (www.ars-grin.gov), now considered to be *S. scabra*] for planting on clay soils and suitable effective and persistent strains of *Bradyrhizobium* arose from a series of experiments over several years. During field evaluation of accessions of *Stylosanthes*, at a range of sites in Queensland during the early 1990s (Edye 1994; Edye et al. 1998), it was observed that the accessions corresponding to Caatinga stylo were poorly and ineffectively nodulated and frequently were not nodulated at all. In addition, most accessions grew well for 1 or 2 years, then began to show classical signs of nitrogen deficiency [R.A. Date and (the Late) L.A. Edye unpublished data].

Strains of *Bradyrhizobium* isolated from some of the few nodules formed on plants from each of these sites were shown to be ineffective in nitrogen fixation, when tested in either aseptic tube culture or soil-pot assessments

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(R.A. Date unpublished data). Similar trials with strains of bradyrhizobia, isolated from other species of *Stylosanthes* but from the same geographical area (i.e. states of Bahia and Minas Gerais, Brazil) and already held in CSIRO's germplasm collection, similarly failed to form effective N-fixing associations with the exception of strains CB2126 and CB3053 (Date 2010). These failures led to the collection of new nodule material from Brazil in 1992 and 1994. The isolation and authentication of new strains from these nodules were completed in 1995 and preliminary effectiveness was demonstrated on a range of accessions of *S. seabrana* the following year (Date 2010).

In addition to the absence of suitable effective strains of bradyrhizobia, soil-pot experiments determined that plants, grown in soil from Edye's evaluation sites (Edye 1994; Edye et al. 1998), did not nodulate while soil nitrogen was available but subsequently nodulated effectively and grew satisfactorily, provided suitable *Bradyrhizobium* had been applied at the time of sowing. This observation indicated that, under field conditions, applied inoculum strains would need to persist for 1 or more growing seasons before plants would nodulate. Nitrogen inhibition of nodulation has been documented for a number of legumes (Streeter 1988; Carroll and Mathews 1990) and is demonstrated in the Caatinga stylo soil-pot experiments, where regrowth of uninoculated controls showed classical nitrogen-deficiency symptoms, whereas inoculated treatments did not (see plant dry weight differences between uninoculated and strain treatments in the ST series experiments below).

Strains CB3480 and CB3481 were isolated and selected from the nodule samples collected in Brazil in 1992. This success led to further collections of *S. seabrana* germplasm and nodule material in 1994. Morphological classification of the new germplasm (Date et al. 2010) and isolation and selection of additional strains of *Bradyrhizobium* have provided additional plant and bradyrhizobial lines for further selection work.

On these clay soils producers prefer to sow the small-seeded legumes on the surface (rolled or with shallow scarifying) prior to the onset of seasonal rainfall. Soil-surface temperatures under these conditions frequently exceed 50–60 °C for 4–6 h/d and are lethal to rhizobia on surface-sown inoculated seed where rhizobia may not survive more than 2–3 days (McInnes and Date 2005). An alternative method of introducing the inoculum to the system is required.

The work reported in this paper was completed over several years, as new germplasm of both *S. seabrana* and bradyrhizobia became available, with the aim of providing effectively nodulating cultivars of Caatinga stylo for the clay soil regions of southern and central Queensland.

Material and Methods

Bradyrhizobium

New strains of bradyrhizobia obtained from the 1992 and 1994 nodule collections in Brazil were authenticated as *Bradyrhizobium* by reinoculation of seedling plants of *S. seabrana* growing in aseptic tube culture (Norris and Date 1976), i.e. completing Koch's postulate. The dry weights of the same plants also served as an initial assessment of the nitrogen-fixation effectiveness of the isolates. From a bacteriological aspect, the new isolates (strains) were not typical of bradyrhizobia isolated from other species of *Stylosanthes*. They grew only on acidified nutrient agar and were very slow-growing (10–12 vs. 6–8 days) and then only to pin-head size (± 20% that of other bradyrhizobia; R.A. Date unpublished data).

Soil-pot technique

Air-dried soil from some of Edye's field sites (Table 1) was passed through a 5 mm sieve, then potted to within 15 mm of the top of 15 cm pots lined with plastic bags. The amount of dry soil varied between 1,800 and 1,950 g/pot depending on soil type. Pots were then watered to 95% field capacity and maintained at this level by being mounted on circular tables (10 pots per table) on an automatic watering machine (Andrew and Cowper 1973), which circulated the tables with pots on an endless chain belt around a glasshouse twice each 24 hours. There were 2 replicates of each strain x soil treatment. No fertilizer/nutrients were added to the potted soils.

Surface-sterilized and pre-germinated seed of *S. seabrana* CPI110361, later released as cv. Unica, was sown at 10 seeds per pot and later thinned to 6 plants, which were inoculated with 1 mL suspension of a peat culture of the test strains of bradyrhizobia. Plant tops were harvested at 6–8 weeks, dried at 60 °C for 48 hours and weighed. In instances where there were few differences between uninoculated controls and strain treatments, plants were allowed to regrow for a further 6–8 weeks and again plants tops were harvested and weighed. Dry weights of whole tops and leaf were used as indices of the effectiveness of the strain treatments. After harvest plant roots were washed out of soil and observations made on level of nodulation.

Table 1. Sites used by Edye (1995) for cultivar evaluation and soil used in soil-pot* and field† experiments (Northcote 1979; Edye 1995, 1997; Isbell 1996; Pengelly and Staples 1996; Edye et al. 1998).

Site	Lat (S)/Long (E) (decimal deg.)	Soil type (Isbell 1996) and Northcote (1979) classification
Banoona†	26.906/149.136	Vertisol
Brian Pastures*	25.650/151.750	Dark brown clay complex Ug5.24
Cardigan	20.228/146.644	Red Duplex
Gunalda	25.993/152.542	Red Podzolic Dr3.21
Hillgrove*	19.637/145.793	Euchrozem or Black Earth
Holyrood*†	26.817/148.750	Red Duplex Dy2.33
Lansdown†	19.648/146.823	Solodized Solonetz
Mt Garnet	17.717/145.150	Red Earth Duplex Dr2.51
Narayen brigalow*†	25.665/150.871	Self-mulching Brown Vertisol Ug5.32
Narayen granite*†	25.683/150.867	Mottled Yellow-red Podzolic Dy3.41
Roma Research Station†	26.578/148.765	Fertile Black Earth Ug5.22
Rostock	26.918/149.423	Self-mulching Grey Vertisol
Southedge	16.983/145.350	Red Earth Gn2.12

Experimental series

Four soil-pot experiments were completed:

- ST09 tested 8 strains of bradyrhizobia: 7 new strains from the nodule material collected in 1992 and strain CB3053 from the early screening work (Date 2010) plus an uninoculated control using soil from the Narayen granite, Narayen brigalow, Brian Pastures and Holyrood sites (Table 1).
- ST25 tested 23 new strains from nodule material collected in 1992, an uninoculated control and control with N added using soil from Narayen granite and Narayen brigalow sites (Table 1).
- ST50 tested 47 new strains from nodule material collected in 1994, 1 of the best strains (CB3480) from ST25, an uninoculated control and a control with N added using soil from the Narayen granite, Narayen brigalow, Hillgrove and Holyrood field sites (Table 1).
- ST130 tested a further 94 strains from nodule material collected in 1994, 12 existing strains, 1 'diagnostic strain' (Date and Norris 1979), 3 of the best strains from ST50 and 8 strains isolated from other species of *Stylosanthes*, plus uninoculated controls and controls with N added (1 of each on alternate tables of automatic watering machine) using soil from Narayen granite and Holyrood field sites (Table 1).

Data analysis and classification methods

For the soil-pot experiments data sets of plant top dry weights, leaf dry weights and nitrogen content were used as ratio attributes for each strain. These data were analyzed using simple ANOVA [strain x replication (x harvest time where appropriate)] and the strain means used as attributes in PATN (Belbin 1989) analysis. The means were range-standardized (TRND module) to provide the input data for ASO (with the Gower Metric option) to obtain symmetric matrices, which were classified by the hierarchical routine FUSE (UPGMA option). The routine GDEF was used to determine group composition and DEND (Dendrogram) to display group structure.

Field experiments

Two series of field trials were established: 1 in January 1995 to assess the strains of bradyrhizobia currently available; and another in January 1996 to assess new strains developed from glasshouse soil-pot assessment of bradyrhizobia arising from new material collected in Brazil in 1994. In each year experiments were established at the CSIRO field stations at Lansdown (solodic soil) and Narayen (granite soil) and in a red earth soil at Holyrood, near Roma (see Table 1). Field plots were comprised of a series of 5 m rows, each row representing a single strain of *Bradyrhizobium*. There were 3 replications of each strain treatment, randomized within replications. Each strain was prepared as a peat-based inoculum with more than 100 million cells per gram. Seed of cv. Unica was inoculated at the commercial rate of 250 g peat per 25 kg seed using a 5% solution of methyl cellulose, allowed to air dry and then sown the same day. Supplementary irrigation was used to ensure establishment of the Caatinga stylo. Plots were maintained weed-free in the establishment year only.

Harvests each year were made by taking 5 randomly selected 10 cm diameter core samples (to a depth of 15 cm) per row, recording the dry weight of plant tops from this area and washing out root samples to collect nodules for strain identification. Fluorescently labelled specific antibody typing (Somasegaran and Hoben 1985) was used to identify strains forming nodules.

Inoculum delivery

Experiments assessing alternative methods of delivery of the inoculum strains were established at 3 sites in southern Queensland. Treatments (Table 2), comparing surface vs. deep placement (8–10 cm) of the inoculum either on the seed of a preceding wheat crop or on inert plastic prills, were established at Roma Research Station, Holyrood and Banoona (see Table 1). *Bradyrhizobium* strain CB3546 was used to inoculate wheat seed (Treatments 1 and 2), plastic prills and Caatinga stylo seed in Treatments 3 and 4, respectively, and CB3481 to inoculate Caatinga stylo in Treatment 2 (see Table 2). The use of these 2 strains was to assess which method of inoculum introduction contributed most to the nodule population. Strain CB3546 is an antibiotic (rifamycin and streptomycin) -resistant variant of CB3481 and was equally effective in nitrogen fixation in sand-jar assay (6.2, 4.3, 5.5, 5.9 and 0.2 g dry weight/jar, respectively, for CB3481 mother culture, CB3481 commercial peat, CB3546 mother culture, CB3546 peat culture used in experiment and uninoculated control). It became obvious that strain CB3546 was serologically distinct from CB3481 and this method of distinguishing between the 2 strains proved simpler than attempting antibiotic-resistance assay of the strains forming the nodules.

In an adjacent area at the Banoona site, 2 commercial 10-ha areas (1 of cv. Primar and 1 of cv. Unica Caatinga stylo) were established by Queensland Department of Agriculture, Forestry and Fisheries (QDAFF) using commercially prepared peat inoculant of CB3481, 2 months later in 1996 than the alternative delivery experiment.

Results

More than 700 isolates, obtained from the nodules collected in 1992 and 1994, nodulated Caatinga stylo in the aseptic tube-culture authentication tests. In a sand-jar glasshouse nitrogen-fixation effectiveness assessment, based on plant dry weight as an index of nitrogen-fixation effectiveness (Date 2010), 154 of these were selected for further evaluation in the soil-pot experiments (Table 3).

ST09

Most strain treatments were better than uninoculated controls in the Narayen granite, Narayen brigalow and Holyrood soils and there were significant differences among strains within these 3 soils (Table 4). There were no significant differences between strain treatments and the uninoculated control plants growing in the Brian Pastures soil at the corresponding harvest time (Table 4), nor were there any differences after 4 successive regrowth periods. When soil was washed from plant roots after the fourth regrowth period, none of the plants was nodulated, suggesting that soil nitrogen remained at levels high enough for good plant growth and too high for plants to nodulate.

Table 2. Alternative delivery treatments for introducing *Bradyrhizobium* strains CB3546 and CB3481 for surface-sown Caatinga stylo (*Stylosanthes seabrana*) cv. Unica.

Treatment	Pretreatment June 1996	Inoculation December 1996
T1	Wheat inoculated with CB3546, sown 8–10 cm depth	Uninoculated Unica, surface-sown[1]
T2	Wheat inoculated with CB3546, sown 8–10 cm depth	Uninoculated Unica, surface-sown[1] 1996 over plastic prills inoculated with CB3481 and drilled to a depth of 8–10 cm
T3	Uninoculated wheat, sown 8–10 cm depth	Uninoculated Unica, surface-sown[1] over plastic prills inoculated with CB3546 and drilled to a depth of 8–10 cm
T4	Uninoculated wheat, sown 8–10 cm depth	Unica inoculated with CB3546, surface-sown[1]

[1]Surface-sown = broadcasting seed on surface, then raking of the top 0.5 cm soil and rolling to simulate commercial conditions.

Table 3. List of strains of *Bradyrhizobium*, host species of isolation and country of origin.

Strain	Host species	Country
Strains already in collection		
CB2126[1]	*Stylosanthes hamata*	Jamaica
CB2152[2]	*Stylosanthes hamata*	USA
CB2229	*Stylosanthes guianensis*	Costa Rica
CB2248	*Stylosanthes guianensis*	Costa Rica
CB2464	*Stylosanthes guianensis*	Brazil MG
CB2534	*Stylosanthes guianensis*	Australia
CB2797	*Macroptilium atropurpureum*	Brazil MG
CB2843	*Stylosanthes guianensis*	Australia
CB2851	*Stylosanthes guianensis*	Australia
CB3053[3]	*Stylosanthes hamata*	Antigua
Strains isolated from Edye field trials		
CB3451	*Stylosanthes seabrana*	Australia
CB3452	*Stylosanthes seabrana*	Australia
CB3453	*Stylosanthes seabrana*	Australia
CB3454	*Stylosanthes seabrana*	Australia
CB3455	*Stylosanthes seabrana*	Australia
CB3456	*Stylosanthes seabrana*	Australia
CB3486	*Stylosanthes seabrana*	Australia
CB3487[2]	*Stylosanthes seabrana*	Australia
CB3602	*Stylosanthes macrocephala*	Australia
CB3608	*Stylosanthes seabrana*	Australia
Strains isolated from 1992 and 1994 collections[4]		
CB3480[1]	*Stylosanthes seabrana*	Brazil
CB3481[1]	*Stylosanthes seabrana*	Brazil
CB3483[2]	*Stylosanthes seabrana*	Brazil
CB3484	*Stylosanthes seabrana*	Brazil
CB3485[2]	*Stylosanthes seabrana*	Brazil
CB3488[2]	*Stylosanthes seabrana*	Brazil
CB3489[2]	*Stylosanthes seabrana*	Brazil
CB3490[2]	*Stylosanthes seabrana*	Brazil
CB3491	*Stylosanthes seabrana*	Brazil
CB3492[2]	*Stylosanthes seabrana*	Brazil
CB3493[2]	*Stylosanthes seabrana*	Brazil
CB3494[2]	*Stylosanthes seabrana*	Brazil
CB3495[2]	*Stylosanthes seabrana*	Brazil
CB3496[2]	*Stylosanthes seabrana*	Brazil
CB3497[2]	*Stylosanthes seabrana*	Brazil
CB3581	*Stylosanthes* sp.[5]	Brazil
CB3582	*Stylosanthes* sp.[5]	Brazil
CB3583	*Stylosanthes seabrana*	Brazil
CB3584	*Stylosanthes* sp.[5]	Brazil
CB3585	*Stylosanthes* sp.[5]	Brazil
CB3586	*Stylosanthes* sp.[5]	Brazil
CB3587	*Stylosanthes seabrana*	Brazil
CB3589	*Stylosanthes* sp.[5]	Brazil
CB3590	*Stylosanthes seabrana*	Brazil
CB3591	*Stylosanthes* sp.[5]	Brazil
CB3592	*Stylosanthes* sp.[5]	Brazil
CB3593	*Stylosanthes seabrana*	Brazil
CB3594	*Stylosanthes seabrana*	Brazil
CB3595	*Stylosanthes seabrana*	Brazil
CB3596	*Stylosanthes* sp.[5]	Brazil
CB3597	*Stylosanthes* sp.[5]	Brazil
CB3598	*Stylosanthes seabrana*	Brazil

Continued

Strain	Host species	Country
CB3599[3]	*Stylosanthes* sp.[5]	Brazil
CB3600[3]	*Stylosanthes seabrana*	Brazil
CB3601	*Stylosanthes* sp.[5]	Brazil
CB3603	*Stylosanthes seabrana*	Brazil
CB3604	*Stylosanthes seabrana*	Brazil
CB3605	*Stylosanthes seabrana*	Brazil
CB3606	*Stylosanthes seabrana*	Brazil
CB3607	*Stylosanthes seabrana*	Brazil
CB3609	*Stylosanthes seabrana*	Brazil
CB3610	*Stylosanthes seabrana*	Brazil
CB3611	*Stylosanthes seabrana*	Brazil
CB3612	*Stylosanthes seabrana*	Brazil
CB3613	*Stylosanthes seabrana*	Brazil
CB3614	*Stylosanthes seabrana*	Brazil
CB3615	*Stylosanthes seabrana*	Brazil
CB3616	*Stylosanthes seabrana*	Brazil
CB3617	*Stylosanthes seabrana*	Brazil
CB3618	*Stylosanthes seabrana*	Brazil
CB3619	*Stylosanthes seabrana*	Brazil
CB3620	*Stylosanthes seabrana*	Brazil
CB3621	*Stylosanthes seabrana*	Brazil
CB3622	*Stylosanthes seabrana*	Brazil
CB3623	*Stylosanthes seabrana*	Brazil
CB3624	*Stylosanthes seabrana*	Brazil
CB3625	*Stylosanthes seabrana*	Brazil
CB3626	*Stylosanthes seabrana*	Brazil
CB3627	*Stylosanthes seabrana*	Brazil
CB3628	*Stylosanthes seabrana*	Brazil
CB3629	*Stylosanthes seabrana*	Brazil
CB3630	*Stylosanthes seabrana*	Brazil
CB3631	*Stylosanthes seabrana*	Brazil
CB3632	*Stylosanthes seabrana*	Brazil
CB3633	*Stylosanthes seabrana*	Brazil
CB3634	*Stylosanthes seabrana*	Brazil
CB3635	*Stylosanthes seabrana*	Brazil
CB3636	*Stylosanthes seabrana*	Brazil
CB3637	*Stylosanthes seabrana*	Brazil
CB3638	*Stylosanthes seabrana*	Brazil
CB3639	*Stylosanthes seabrana*	Brazil
CB3639	*Stylosanthes seabrana*	Brazil
CB3640	*Stylosanthes seabrana*	Brazil
CB3641	*Stylosanthes seabrana*	Brazil
CB3642	*Stylosanthes seabrana*	Brazil
CB3643	*Stylosanthes seabrana*	Brazil
CB3644	*Stylosanthes seabrana*	Brazil
CB3645	*Stylosanthes seabrana*	Brazil
CB3646	*Stylosanthes seabrana*	Brazil
CB3648	*Stylosanthes seabrana*	Brazil
CB3649	*Stylosanthes seabrana*	Brazil
CB3650	*Stylosanthes seabrana*	Brazil
CB3651	*Stylosanthes seabrana*	Brazil
CB3652	*Stylosanthes seabrana*	Brazil
CB3653	*Stylosanthes seabrana*	Brazil
CB3654	*Stylosanthes seabrana*	Brazil
CB3655	*Stylosanthes seabrana*	Brazil

Continued

Strain	Host species	Country
CB3656	*Stylosanthes seabrana*	Brazil
CB3657	*Stylosanthes seabrana*	Brazil
CB3658	*Stylosanthes seabrana*	Brazil
CB3659	*Stylosanthes seabrana*	Brazil
CB3660	*Stylosanthes seabrana*	Brazil
CB3661	*Stylosanthes seabrana*	Brazil
CB3662	*Stylosanthes seabrana*	Brazil
CB3663	*Stylosanthes seabrana*	Brazil
CB3664	*Stylosanthes seabrana*	Brazil
CB3665	*Stylosanthes seabrana*	Brazil
CB3666	*Stylosanthes seabrana*	Brazil
CB3667	*Stylosanthes seabrana*	Brazil
CB3668	*Stylosanthes seabrana*	Brazil
CB3669	*Stylosanthes seabrana*	Brazil
CB3670	*Stylosanthes seabrana*	Brazil
CB3671	*Stylosanthes seabrana*	Brazil
CB3672	*Stylosanthes seabrana*	Brazil
CB3673	*Stylosanthes seabrana*	Brazil
CB3674	*Stylosanthes seabrana*	Brazil
CB3675	*Stylosanthes seabrana*	Brazil
CB3676	*Stylosanthes seabrana*	Brazil
CB3677	*Stylosanthes seabrana*	Brazil
CB3678	*Stylosanthes seabrana*	Brazil
CB3679	*Stylosanthes seabrana*	Brazil
CB3680	*Stylosanthes seabrana*	Brazil
CB3681	*Stylosanthes seabrana*	Brazil
CB3682	*Stylosanthes seabrana*	Brazil
CB3683	*Stylosanthes seabrana*	Brazil
CB3684	*Stylosanthes seabrana*	Brazil
CB3685	*Stylosanthes seabrana*	Brazil
CB3686	*Stylosanthes seabrana*	Brazil
CB3687	*Stylosanthes seabrana*	Brazil
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CB3698	*Stylosanthes seabrana*	Brazil
CB3699	*Stylosanthes seabrana*	Brazil
CB3700	*Stylosanthes seabrana*	Brazil
CB3701	*Stylosanthes seabrana*	Brazil
CB3702	*Stylosanthes seabrana*	Brazil
CB3703	*Stylosanthes seabrana*	Brazil
CB3704	*Stylosanthes macrocephala*	Brazil
CB3705	*Arachis* sp.	Brazil
CB3706	*Stylosanthes seabrana*	Brazil
CBX001	*Stylosanthes* sp.[5]	Brazil
CBX002	*Stylosanthes* sp.[5]	Brazil
CBX005	*Stylosanthes* sp.[5]	Brazil
CBX003	*Stylosanthes* sp.[5]	Brazil
CBX004	*Stylosanthes* sp.[5]	Brazil

Continued

[1]Strains used in 1995 and 1996 field trials.
[2]Strains used in 1996 field trials.
[3]Strains used in 1995 field trials.
[4]All strains isolated from nodule material collected in 1992 and 1994 from Bahia State, Brazil.
[5]Strains isolated from nodules formed on trap host *Macroptilium atropurpurem* inoculated with crushed nodules from *Stylosanthes* and authenticated by checking nodule-forming ability and effectiveness on *S. seabrana.*

Table 4. Plant top dry weights (g/pot) for Experiment ST09.

Strain	Soils (sites)			
	Brian Pastures	Narayen granite	Narayen brigalow	Holyrood
CB3053	8.1	11.0	5.6	11.1
CB3480	7.8	12.5	10.2	11.8
CB3581	8.2	10.3	4.7	9.4
CB3582	7.9	10.9	4.9	9.9
CB3584	8.0	9.4	3.5	7.9
CB3591	7.4	11.4	7.9	11.4
CB3592	8.0	11.5	8.5	11.0
CB3606	7.7	11.1	5.0	9.7
Uninoc control	7.6	9.6	3.7	7.6
LSD (5%)	0.6	0.6	0.6	0.6

ST25

At the 5-group level most strains formed effective nitrogen-fixing associations (Figure 1) but for some strains this was not obvious until after the regrowth harvest. Dry weight and leaf nitrogen data for a representative of each of the similarity groups are recorded in Table 5. Strains CB3480 and CB3586 were overall the most effective strains.

ST50

This group of strains showed some interaction responses between soils. Some differences were significant at the 1% level. Strains in Groups 1, 2, 3 and 4 were the most effective and had smaller dissimilarity values

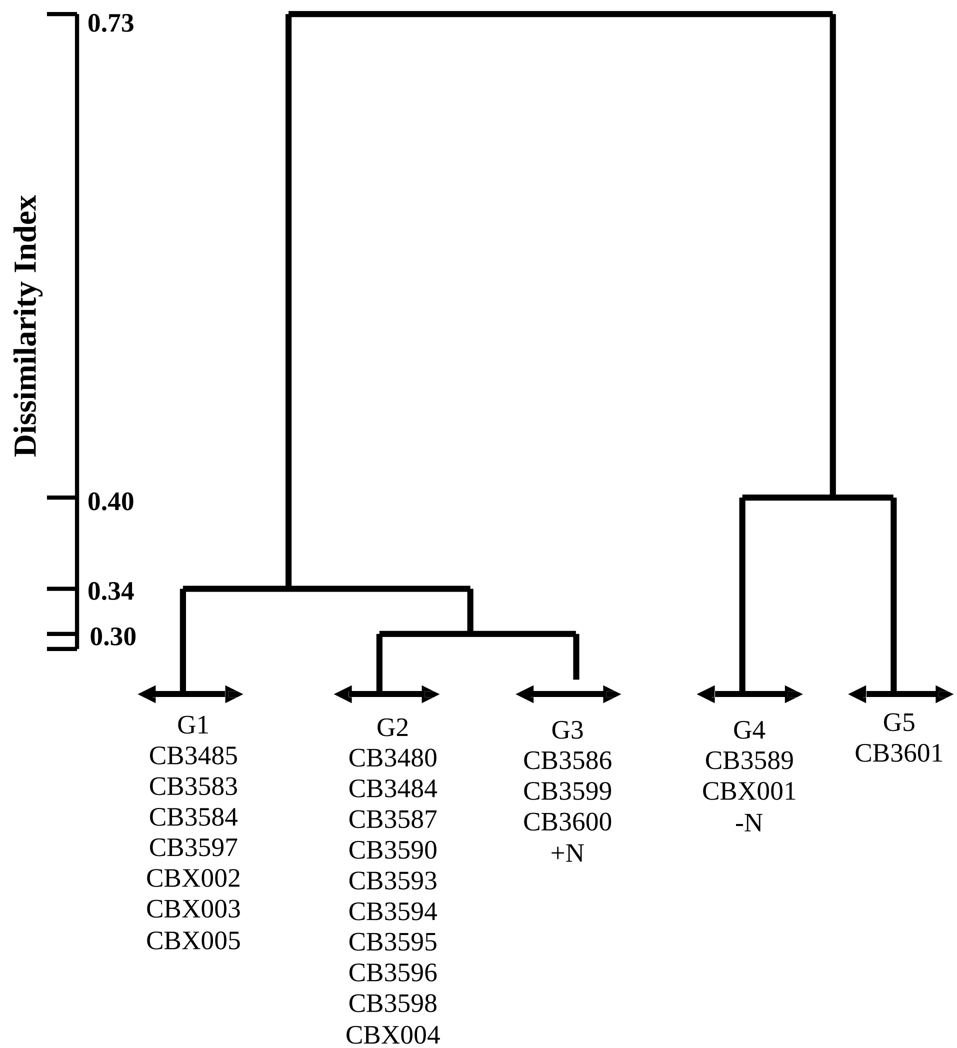

Figure 1. Similarity Groups G1–G5 of 23 strains of *Bradyrhizobium* effectiveness responses on Caatinga stylo (*Stylosanthes seabrana*) grown in Narayen granite and Narayen brigalow soils in soil-pot evaluations (ST25).

than the least effective strains in Group 5 (Figure 2 and Table 6). Strains CB3480, CB3489, CB3490, CB3491, CB3640, CB3673 and CB3684 were the best overall performers.

Group 5 strains had only 3–10 small (<0.5 mm) white nodules compared with many 0.5–1.5 mm nodules on plants in Groups 1, 2, 3 and 4. All nodules occurred at the root/lateral root/secondary root junctions.

ST130

As with Experiment ST50 there was a range of responses but most strains were effective (Figure 3 and Table 7). Dry weight data for the best-performing strains from the groups included those for strains CB3480, CB3481 and CB3489. There was a high level of dissimilarity between the effective strains in Groups 1, 2, 3 and 4 and those less-effective strains in Group 5.

Table 5. Top dry weight, leaf dry weight and leaf nitrogen content of Caatinga stylo grown in soil from Narayen granite (NG) and Narayen brigalow (NB) sites after inoculation with new strains of *Bradyrhizobium* collected in 1992 (ST25) with an unfertilized control and a N-fertilized control.

Group	Strain	Top dry weight (g)				Leaf dry weight (g)				Leaf nitrogen (mg)	
		NG		NB		NG		NB		NG	NB
		H1[1]	H2	H1	H2	H1	H2	H1	H2	H1	H1
G1	CB3583	3.2	3.1	17.3	3.4	2.6	1.5	8.2	1.6	53	131
G2	CB3480	5.0	2.4	19.1	4.0	2.1	1.2	10.0	1.8	54	229
G3	CB3586	4.3	2.7	20.3	3.6	2.3	1.3	10.8	1.7	57	238
G4	CB3589	2.1	3.1	14.5	2.8	2.3	1.5	7.7	1.3	33	95
G5	CB3601	2.2	2.1	16.4	1.6	1.8	1.1	8.2	0.7	21	93
Control	-N	2.2	2.5	12.1	1.4	2.4	1.3	6.5	0.7	28	79
Control	+N	3.5	2.6	20.8	3.4	2.4	1.3	11.1	1.6	41	206
	LSD (5%)	1.1	1.0	5.0	0.7	0.9	0.4	2.8	0.3	22	54

[1]H1 = Harvest 1; H2 = Harvest 2.

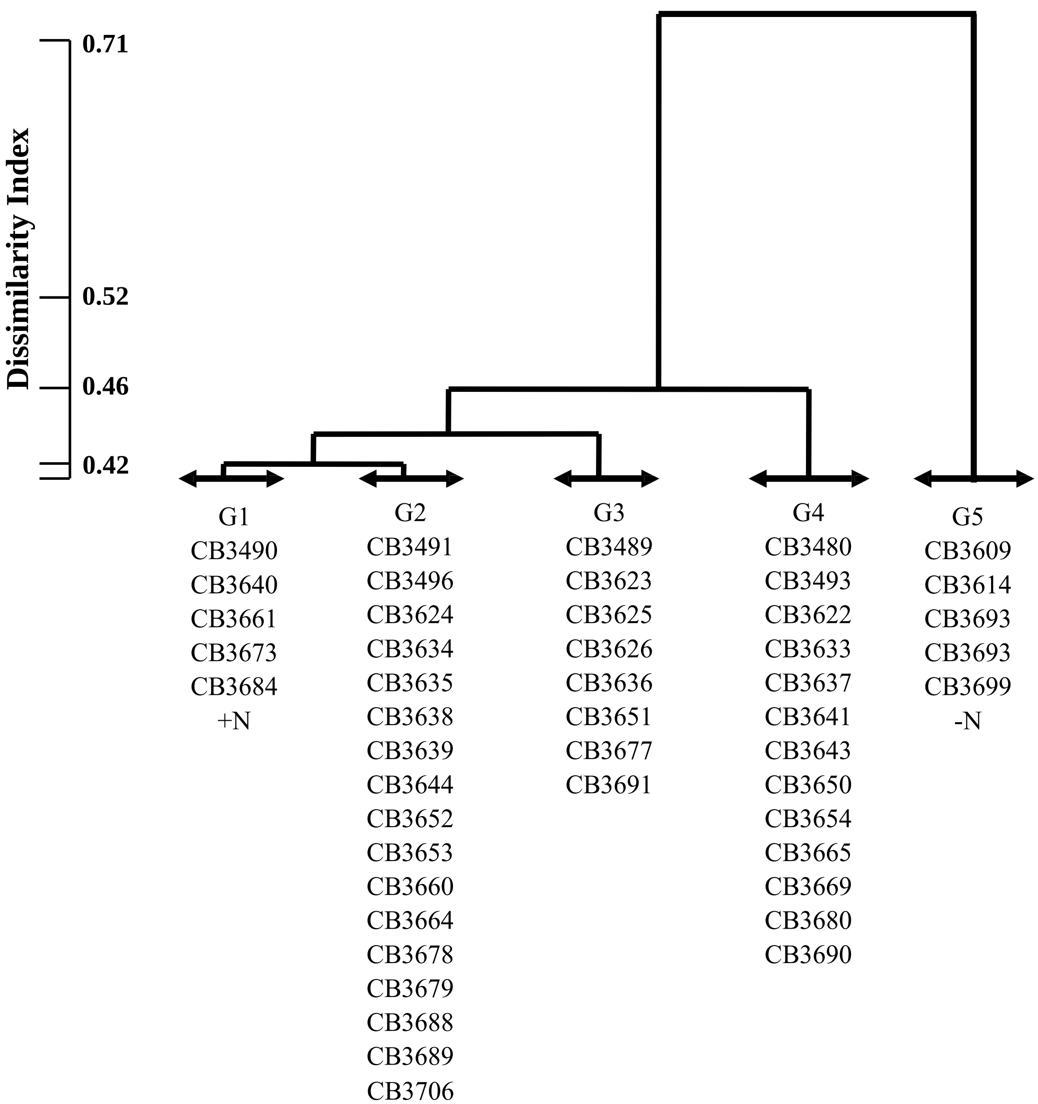

Figure 2. Similarity Groups G1–G5 of 47 new strains of *Bradyrhizobium* effectiveness responses on Caatinga stylo (*Stylosanthes seabrana*) grown in Narayen granite, Narayen brigalow, Holyrood and Hillgrove soils in soil-pot evaluations (ST50).

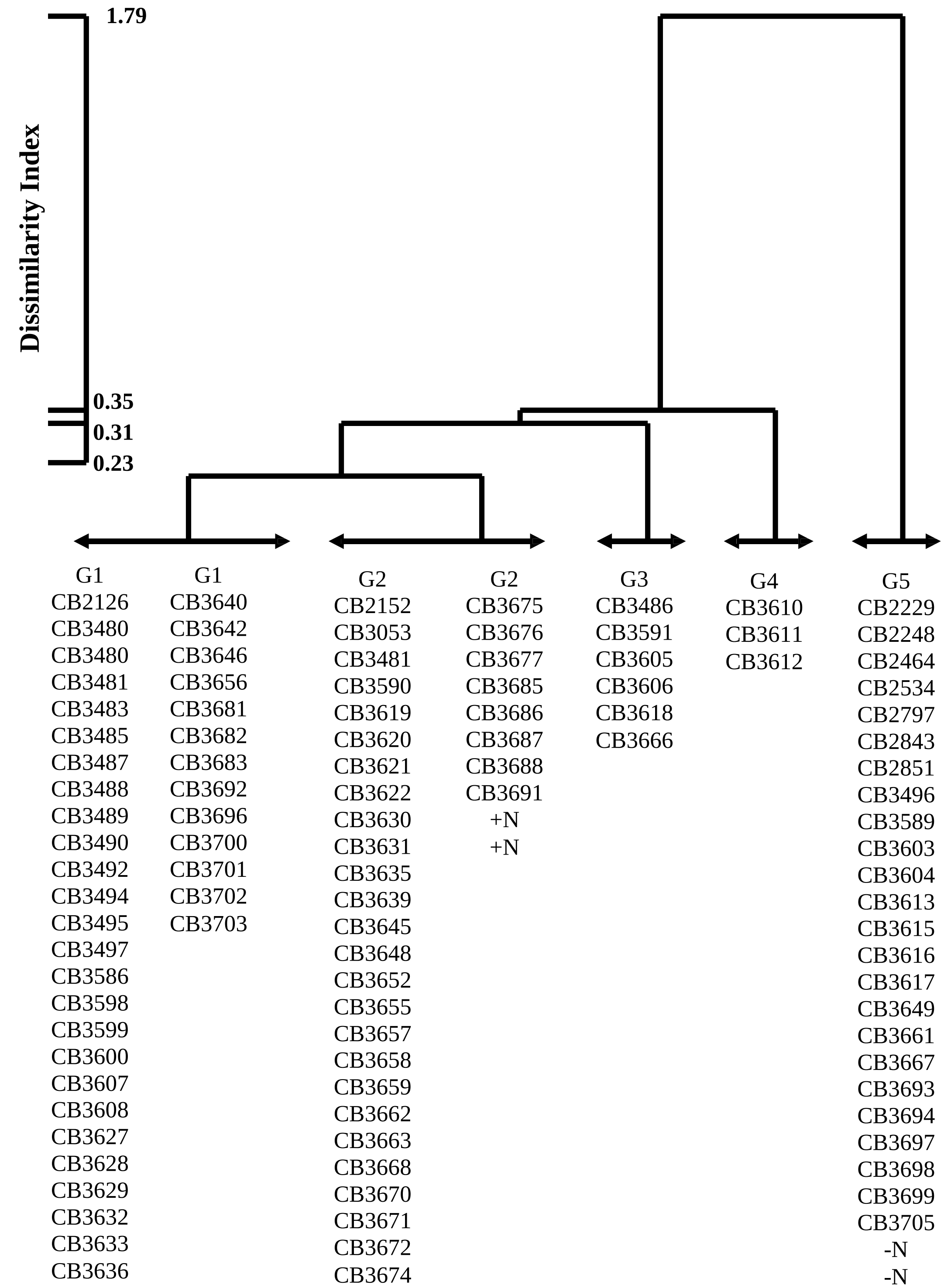

Figure 3. Similarity Groups G1-G5 of 94 new, 12 existing, 1 diagnostic and 3 of the best *Bradyrhizobium* strains from ST50 strains for effectiveness responses on Caatinga stylo (*Stylosanthes seabrana*) grown in Narayen granite, Narayen brigalow, Holyrood and Hillgrove soils in soil-pot evaluations (ST130).

Table 6. Plant-top dry weights for Caatinga stylo grown in soils from the Narayen granite (NG), Narayen brigalow (NB), Hillgrove and Holyrood sites inoculated with 47 new strains of *Bradyrhizobium* collected in 1994 (ST50) plus an unfertilized control and a N-fertilized control.

Group	Strain	Top dry weight (g/pot)							
		NG		NB		Hillgrove		Holyrood	
		H1[1]	H2	H1	H2	H1	H2	H1	H2
G1	CB3490	7.2	5.6	na[2]	6.9	7.7	6.5	5.8	6.2
G1	CB3640	7.6	5.5	na	7.4	8.3	7.5	6.3	6.6
G1	CB3661	3.6	3.2	na	3.6	7.9	4.8	4.4	5.8
G1	CB3673	6.2	5.3	na	6.2	7.9	5.4	5.5	5.5
G1	CB3684	6.3	5.4	na	8.3	7.9	6.5	5.7	5.7
G2	CB3491	7.4	5.1	na	6.8	8.0	7.3	6.8	7.6
G3	CB3489	8.1	6.2	na	7.5	7.1	6.1	6.3	6.8
G4	CB3480	7.7	5.3	na	8.7	8.6	7.1	6.5	6.8
G5	CB3699	2.2	0.7	na	3.3	7.8	5.1	4.4	4.0
Control	-N	2.1	0.8	na	2.9	9.2	6.8	3.9	4.1
Control	+N	4.7	9.0	na	9.3	7.9	9.5	5.7	7.6
	LSD (5%)	2.1	1.4		1.9	1.3	1.3	1.1	1.5
	LSD (1%)	2.8	1.9		2.6	1.7	1.7	1.5	2.0

[1]H1 = Harvest 1; H2 = Harvest 2.
[2]na = not available - many samples accidently destroyed. Of those recoverable top dry weight values ranged from 8.5 to 17.5 g/pot. Significantly there was no difference between samples for uninoculated and N-fertilized treatments.

Table 7. Plant top dry weights (g/pot) for best-performing strains of *Bradyrhizobium* on Caatinga stylo (*Stylosanthes seabrana*) grown in soils from Narayen granite and Holyrood sites inoculated with additional new strains of *Bradyrhizobium* collected in 1994 (ST130) plus an unfertilized control and a N-fertilized control.

Group	Strain	Whole tops				Leaves			
		Narayen granite		Holyrood		Narayen granite		Holyrood	
		H1[1]	H2	H1	H2	H1	H2	H1	H2
G1	CB3480	5.8	4.9	5.8	6.3	2.4	2.2	2.4	2.8
G1	CB3481	5.3	4.0	5.3	5.5	2.2	2.0	2.2	1.7
G1	CB3483	5.6	4.3	5.7	5.5	2.4	2.1	2.3	2.4
G1	CB3485	5.5	3.3	6.5	6.2	2.3	1.6	2.6	2.8
G1	CB3487	5.3	3.2	6.1	5.8	2.2	1.7	2.6	2.4
G1	CB3488	5.4	4.3	5.5	5.6	2.3	2.1	2.4	2.5
G1	CB3489	4.7	3.6	5.8	5.6	2.1	1.7	2.4	2.4
G1	CB3490	5.4	4.0	5.9	5.5	2.3	2.0	2.5	2.4
G1	CB3492	5.5	4.0	6.3	6.7	2.3	1.9	2.6	3.0
G1	CB3494	5.7	4.4	5.5	5.3	2.3	2.0	2.3	2.3
G1	CB3495	5.5	4.4	6.0	5.6	2.3	2.0	2.3	2.5
G1	CB3497	5.7	4.5	6.3	6.0	2.4	2.0	2.5	2.5
G2	CB3053	4.5	3.0	4.9	5.0	1.8	1.5	2.0	2.2
G2	CB3630	5.2	4.1	5.7	4.5	2.2	2.0	2.4	1.9
G3	CB3486	4.7	2.3	5.6	4.9	2.1	1.3	2.4	2.1
G3	CB3606	5.2	2.5	6.1	5.5	2.3	1.2	2.5	2.4
G4	CB3610	3.8	3.3	4.7	4.8	1.6	1.6	2.7	2.0
G5	CB3616	2.9	1.4	3.6	2.6	1.4	0.8	1.8	1.2
G5	CB3693	1.9	0.7	3.3	2.8	0.9	0.4	1.6	1.3
	-N	2.7	1.1	3.4	3.2	1.2	0.6	1.6	1.4
	+N	5.1	3.1	5.4	5.0	2.1	1.6	2.4	1.9
	LSD (5%)	0.6	0.9	0.7	1.2	0.3	0.4	0.4	0.6
	LSD (1%)	0.8	1.2	0.9	1.6	0.4	0.6	0.5	0.7

[1]H1 = Harvest 1; H2 = Harvest 2.

There were scattered small (<0.5 mm) nodules on the roots of plants in Group 5, whereas in Group 1 both tap and lateral roots were profusely populated with larger (1–1.5 mm) nodules. Similar but less numerous nodulation was recorded for plants in Groups 2, 3 and 4. Overall there were fewer nodules on plants grown in Narayen granite soil than in Holyrood soil.

Field experiments

In the trials sown in 1995 only strains CB3053, CB3480 and CB3481 had dry weight yields greater than the uninoculated controls. Strains CB2126, CB3599 and CB3600 failed to produce responses and were not harvested (Tables 8 and 9). Strains CB3053 and CB3481 formed the majority of nodules and only CB3481 maintained this high level in successive years. A significant level of 'contamination' of control plots by CB3481 was observed in later years (Tables 8 and 9).

As with the trials sown in 1995 only those plots in the trials sown in 1996 that demonstrated better growth responses than the uninoculated controls were harvested for plant dry weight yield and determination of the proportion of nodules formed by the inoculum strains (Tables 10 and 11). The proportional differences between inoculated and uninoculated treatments for plant dry weight yield increased in successive years and were greater at the Narayen granite and Lansdown sites than at Holyrood (Table 10). Strains CB3481, CB3494 and CB3495 formed a high proportion of the nodules at all sites and in all years (Table 11), although CB3494 failed to improve yield and form nodules in the year of sowing (1996). Strains CB3488 and CB3489 also formed a large proportion of the nodules at the Holyrood and Narayen granite sites but failed at Lansdown. There was significant 'contamination' of some control plots from adjacent inoculated rows in some replications (see ad hoc notes Table 12).

Table 8. Proportion of nodules formed by inoculum strains of *Bradyrhizobium* on Caatinga stylo (*Stylosanthes seabrana*) in trials sown in 1995. (See explanatory notes 1, 2 and 3).

Site	% nodules formed			
	CB3053[1]	CB3480	CB3481	Control[2]
Holyrood				
Apr-95	11	0	100	0
Apr-96	34	1	72	2 (2% CB3053)
Apr-97	0	0	75	0
Apr-98	-	-	-	-
Narayen granite				
Apr-95	25	1	44	0
Jan-96	39	8	96	1
May-97	78	0	98	0
Mar-98[3]	69		90	38
Lansdown				
May-95	31	0	47	0
Jan-96	81	25	86	0
May-96	-	34	81	0
May-97	-	-	59	31
Mar-98	11	-	67	13 (4% CB3053)

[1]Six strains CB2126, CB3053, CB3480, CB3481, CB3599 and CB3600 were assessed in the 1995 Strain Trial. Strains CB2126, CB3599 and CB3600 failed to respond and were not harvested.

[2]Values in control columns are for positive identification of CB3481. Values in brackets refer to the strain indicated.

[3]For Narayen Mar-98 and Lansdown May-97 and Mar-98, 2 of the 3 replicates of controls were adjacent to plots of CB3481.

Table 9. Relative yields (as % best treatment in each year) for inoculated Caatinga stylo (*Stylosanthes seabrana*) in strain trials sown in 1995. (See explanatory notes 1, 2 and 3).

Site	CB3053	CB3480	CB3481	Control[3]
Holyrood				
Apr-95	79	48	100	85
Apr-96	29	29	100	31
Apr-97	-	-	100	21
Apr-98	-	-	-	-
Narayen granite				
Apr-95	<------------------------------------Lost to wildlife[1]------------------------------------->			
Jan-96	54	61	100	25
May-97	30	8	100	6
Mar-98	22	-	100	32
Lansdown				
May-95	100	85	57[2]	49
Jan-96	23	19[2]	100	14
May-96	-	27	100	30
May-97	-	-	100	47
Mar-98	51	-	100	83

[1]Data for Narayen Apr-95 unreliable, due to grazing by wildlife prior to harvest.
[2]Data for CB3481 Lansdown May-95 and CB3480 in one replication may be unreliable due to accidental damage by wind drift herbicide.
[3]Yields of controls for Narayen Mar-98 and Lansdown May-97 and Mar-98 high due to contamination by effective N-fixing strains.

Table 10. Summary relative plant dry weights (% best for year) for inoculated Caatinga stylo (*Stylosanthes seabrana*) for strain trials sown in 1996.

Strain	Holyrood				Narayen granite			Lansdown		
	1996	1997	1998	1999	1996	1997	1998	1996	1997	1998
CB2152					35					
CB3480	100				49			59		
CB3481	88	76	97	88	30	34	44	74	63	47
CB3483		48	65	68		42	45		64	73
CB3485			45							
CB3486					100					
CB3488		54	75	53		42	90			
CB3489		74	81	87	75	49	79		58	
CB3490			67	100		34	68			100
CB3491									69	
CB3494		44	90	59		57	100			95
CB3495		100	99	91	87	100	86		100	90
CB3497		36	100							
Control	65	37	56	35	58	36	14	100	59	28

Table 11. Proportion (%) of nodules formed on Caatinga stylo (*Stylosanthes seabrana*) by inoculum strains for strain trials sown in 1996. (See explanatory notes 1 to 6).

Strain[1]	Holyrood				Narayen granite			Lansdown		
	1996	1997	1998	1999	1996	1997	1998	1996	1997	1998
CB2152	-	-	-	-	2	-	-	-	-	-
CB3480	15	-	-	-	0	-	-	0	-	-
CB3481	39	63	87	75	3	91	89	22	87	80
CB3483	-	0	0	0	-	0	0	-	3	5
CB3485	-	-	-	-	nd[6]	-	-	-	-	-
CB3486	-	-	-	-	-	nd	nd	-	-	-
CB3488	-	39	83	72		97	90			
CB3489	-	0	94	92	14	0	95	-	0	-
CB3490	-	-	85	91	-	80	94	-	-	83
CB3491	-	-	-	-	-	-	-	-	6	-
CB3494	-	96	93	100	-	96	97	-		100
CB3495	-	96	94	91	13	98	98		96	91
CB3497	-	51	19	-	-	-	-	-	-	-
Control	2[2]	2[3]	6[4] (CB3481) 34 (CB3495) 30 (CB3488)	6[5](CB3481) 42(CB3495) 0 (CB3488)	0	5	4	0	0	25

[1]Eighteen (18) strains were selected for assessment in 1996. Five (5) strains (CB2841, CB3487, CB3492, CB3493 and CB3496) failed to respond at any site and were not harvested.
[2]Holyrood 1996. Control tested against antiserum for only CB3481.
[3]Holyrood 1997. Control tested against only CB3481 (1.5%) and CB3495 (1.7%).
[4]Holyrood 1998. Control vs. CB3481 (6%), CB3495 (34%) and CB3488 (30%) – mostly in 2 of the 3 replicates where control plots were near or adjacent to indicated strain plots.
[5]Holyrood 1999. Control vs. CB3481 (6%), CB3495 (42%), CB3488 (0%) and CB3497 (0%).
[6]nd = not determined – antisera for serological identification not available.

Table 12. Ad hoc observational evidence for spread of strains CB3481 and CB3495 in strain trials sown in 1996.

Site	Year	Strain	Comment
Holyrood	1996	1.5% CB3481	Mostly in Rep 1, Control row not adjacent to an inoculated CB3481 row; no check for other strains
	1997	1.5% CB3481	Mostly in Rep 1, Control row not adjacent to an inoculated CB3481 row; no check for other strains
		1.7% CB3495	Mostly in Reps 1 and 2, Control row not adjacent to an inoculated CB3495 row
	1998	6% CB3481	Mostly in Rep 2, Control row 2 rows away from inoculated CB3481 row
		34% CB3495	Mostly in Reps 1 and 2, Control row adjacent to an inoculated CB3495 row in Rep 2
		30% CB3488	Mostly in Reps 2 and 3, Control row adjacent to an inoculated CB3488 row in both Reps
	1999	6.4% CB3481	Mostly in Rep 2, Control row not near an inoculated CB3481 row
		42% CB3495	Mostly in Rep 1, Control row immediately adjacent to an inoculated CB3495 row
Lansdown	1998	56% CB3495	Mostly in Reps 2 and 3, Control rows not adjacent to an inoculated CB3495 row but area under surface water due to cyclonic weather conditions in 1997

Inoculum delivery

For Caatinga stylo the introduction of the bradyrhizobia by inoculation of wheat seed sown in June (T1) was better than placing the bradyrhizobia 10 cm below the legume seed in the normal December-January sowing (T3) (Table 13). At the Holyrood site the combined total of nodules identifiable as CB3481 and CB3546 in T2 was similar to that for CB3546 alone in T1, but there was a trend for nodules from CB3481 to increase with time in T2 with a concomitant decline in those from CB3546. Nodule formation by strain CB3546 also declined in T1. There were similar but less marked declines at Roma Research Station and Banoona sites. Deep placement of the inoculum either on a preceding cereal crop (T1 and T2) or on inert plastic prills (beads) (T3) at the time of sowing the Caatinga stylo provided more nodules than when introduced as inoculum on the surface-sown legume seed (T4). The poor result in T4 confirms that inoculation of surface-sown seed for this legume is a risky practice (Table 13).

For farming systems for which these new cultivars have been selected, the preferred method of sowing the legume is as an associated crop with the cereal, often into dry soils and at high soil surface temperatures (>50 °C for 4–6 h/d; D.A. Eagles and R.A. Date unpublished data). Corresponding bare-soil temperatures at 2, 5 and 10 cm depths were, respectively, 40–50 °C for 6–8 h/d, 30–40 °C for 4–6 h/d, and frequently >30 °C for 8–10 h/d (D.A. Eagles and R.A. Date unpublished data). Temperature profiles at 0, 2, 5 and 10 cm depth for the Holyrood site are recorded in Figure 4 for the 1996-1997 season. Similar profiles were recorded for the 1997-1998 season and for the Narayen granite, Narayen brigalow and Roma Research Station sites in both seasons.

Discussion

This series of soil-pot assessments was aimed at selecting suitable strains of *Bradyrhizobium* for Caatinga stylo (*Stylosanthes seabrana*) in order to advance the release of the 2 new cultivars, Primar and Unica. Several strains, which showed particular promise, were selected and evaluated for their ability to survive in field situations and to form nodules in second and subsequent growing seasons, when soil nitrogen had been depleted. Twenty-four strains based on plant dry weight yield (used as an index of nitrogen-fixation effectiveness) were selected: 6 in trials sown in 1995 and 18 in 1996 at the Holyrood, Narayen granite and Lansdown sites.

These strains produced 2−7 fold increases in plant dry weight, especially in the 2nd, 3rd and 4th (for Holyrood) growing seasons, and accounted for the majority of nodules formed. Based on data from trials sown in 1995 (Tables 8 and 9), strain CB3481 was released to industry for the inoculation of the new cultivars, Primar and Unica, in 1997. As well as confirming the ability of CB3481 to satisfactorily nodulate *S. seabrana* over a 3-year period, the data from field trials sown in 1996 (Tables 10 and 11) have identified several additional strains that could serve as replacement strains for CB3481, if this became necessary. There was good evidence of the ability of these strains to spread to neighbouring areas as indicated by the proportions of strains CB3481, CB3488 and CB3495 found in uninoculated control plots (Tables 8, 10 and 12).

Only cv. Unica was used in these trials, due to limited availability of seed of cv. Primar seed at the time of experimentation; however, nitrogen-fixation effectiveness responses of the 2 cultivars in N-free glasshouse assessments (Date 2010) and in separate soil-pot experiments

Table 13. Percentage recovery of nodules containing inoculum strains CB3546 and CB3481 for alternative delivery trials at Holyrood, Roma Research Station and Banoona sites[1].

Site	Year	Treatment[2]				
		T1	T2/CB3546	T2/CB3481	T3	T4
Holyrood	1997	53	30	37	27	6
	1998	38	13	17	29	7
	1999	19	0	51	1	3
Roma Res. Stn	1997	5	7	0	5	3
	1998	4	7	17	5	4
Banoona	1997	-	-	-	-	-
	1998	1	3	27	0	0
	1999	2	2	16	6	1

[1]In random samples of nodules from adjacent QDAFF trials tested for strain CB3481 in May 1998, there were no nodules on Primar and 3% on Unica and in March 1999 values were 70 and 40%, respectively, for the 2 cultivars.
[2]Treatments T1, T2, T3 and T4 are described in Table 2.

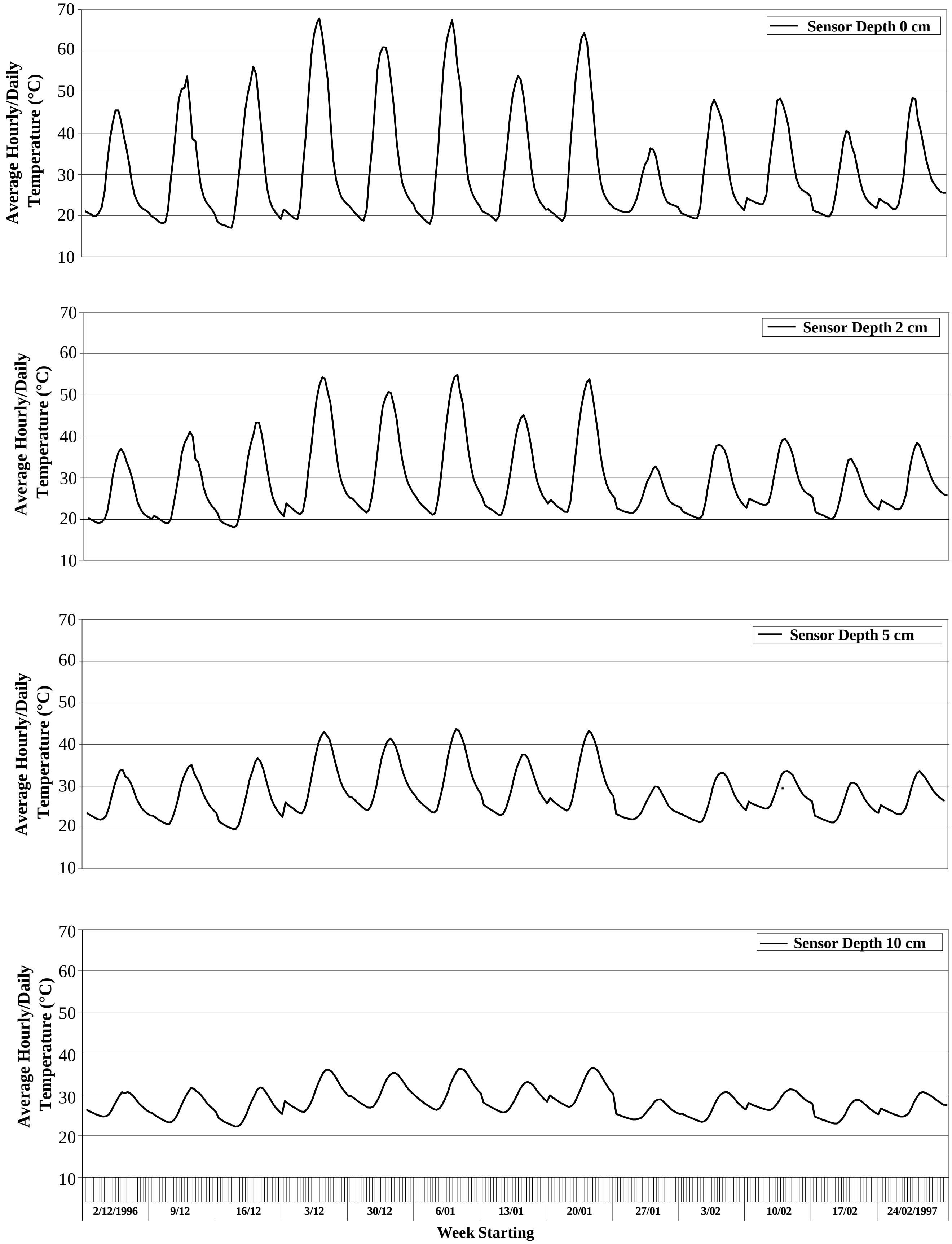

Figure 4. Holyrood site bare-soil temperature profiles 1996-1997 at 0, 2, 5 and 10 cm depth.

confirmed that both cultivars responded in the same way with the recommended strains of *Bradyrhizobium*. The good percentages of nodules identified as CB3481 on both cultivars in the commercial sowings (see Table 13) in the third growing season compared with the recoveries in the second growing season provide separate evidence of the inability of Caatinga stylo to nodulate, while soil nitrogen is available in the initial years, and that the recommended inoculum strain CB3481 persists into the second and third growing seasons.

The alternative delivery experiments suggest that deep placement of the inoculum, either by inoculation of a prior crop, e.g. wheat, or on an inert carrier, e.g. plastic prills, may confer an advantage over inoculation of surface-sown Caatinga stylo seed for producers in clay-soil cropping systems, where soil temperatures are detrimental to the inoculum *Bradyrhizobium*.

Commercially prepared inoculum (peat-based and freeze-dried forms) of strain CB3481 has been available since 1998. If new germplasm material is required for additional cultivar and strain selection and evaluation, the genetic resource collection of *S. seabrana* germplasm now resides with SARDI/Australian Pastures Genebank and the root-nodule bacteria (*Bradyrhizobium*) collection with the Centre for *Rhizobium* Studies (CRS), Murdoch University, Western Australia.

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Research paper

Botanical composition of Caatinga rangeland and diets selected by grazing sheep

Composición botánica de un pastizal del ecosistema Caatinga y de dietas seleccionadas por ovejas en pastoreo

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Abstract

Sheep dietary selection from species-diverse Caatinga rangeland of semi-arid northeastern Brazil has not been documented. This study examined the botanical composition of the available forage and diets of Dorper x Saint Ines ewes on thinned Caatinga over-seeded with *Cenchrus ciliaris* and *Urochloa mosambicensis*. Sixty-three species from 23 families, dominated by shrubs and short trees of low forage nutritive value, were identified in the vegetation. The botanical composition revealed, on average, high presence of 29.2% Malvaceae and 13.0% *C. ciliaris*. Using the microhistological technique, sheep showed, on average, 59.6% preference for dicotyledons throughout the year. However, selectivity indexes indicated, on average, greater selection for Poaceae during the rainy season (1.5) and for dicotyledons in the dry season (1.8) with a year-round aversion for Malvaceae (0.3). These findings suggest that Caatinga vegetation management should include Malvaceae thinning and greater incorporation of grasses and herbaceous legumes to improve rangeland carrying capacity.

Keywords: Brazil, continuous stocking, dietary selection, microhistological technique, principal components.

Resumen

El consumo selectivo por ovejas en pastoreo en la vegetación de Caatinga del semiárido noreste de Brasil ha sido muy poco documentado. En este estudio fue evaluada la composición botánica tanto del forraje potencial disponible como del consumido por ovejas Dorpers x Santa Ines en una Caatinga raleada y sobre-sembrada con las gramíneas *Cenchrus ciliaris* y *Urochloa mosambicensis*. En la vegetación se identificaron 63 especies de 23 familias, dominadas por arbustos y árboles pequeños de bajo valor forrajero. La composición botánica reveló, en promedio, alta presencia de especies de la familia Malvaceae (29.2%) y de *C. ciliaris* (13.0%). Utilizando la técnica microhistológica se encontró que a través del año las ovejas tuvieron, en promedio, una preferencia del 59.6% por las dicotiledóneas. Sin embargo, los índices de selectividad indicaron, en promedio, una mayor selección por las Poaceae durante la época de lluvias (1.5) y en la estación seca por las dicotiledóneas (1.8), y una aversión hacia las Malvaceae durante todo el año (0.3). Estos resultados indican que el manejo de la vegetación de la Caatinga debe incluir un control de las Malvaceae y una mayor incorporación de gramíneas y leguminosas herbáceas para mejorar la capacidad de carga de los pastizales en la zona.

Palabras clave: Brasil, componentes principales, pastoreo continuo, selección de dieta, técnica microhistológica.

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Introduction

Livestock production in semi-arid Brazil is based on Caatinga rangeland (Araújo Filho et al. 1998), which covers approximately 86% (83.5 Mha) of the region (3–16° S, 35–45° W) and 9.8% of Brazil's land area (IBGE 2012). Caatinga rainfall ranges from 200 to 800 mm/yr. The upper story of shrubs, small trees and prickly deciduous vegetation consists mostly of Leguminosae, Euphorbiaceae, Cactaceae and Bromeliaceae, while the herbaceous layer is comprised primarily of annual grasses and dicotyledons. The majority of this vegetation has low forage value and the upper story is partially inaccessible to sheep (Santos et al. 2010).

More efficient utilization of the natural vegetation for ruminant production requires greater knowledge of species preferred by grazing animals. To improve management of the Caatinga, studies of ecosystems should determine botanical composition, forage mass and nutritive value as well as soil characteristics (Heady 1975; Holechek et al. 2006; Albuquerque et al. 2008). Taken as a whole, this information will help land managers better understand soil-plant-animal-environment interrelationships and develop productive and sustainable management strategies.

Forage allowance and animal species have marked effects on native plant populations (Bhatta et al. 2001; Sankhyan et al. 2001). Albuquerque et al. (2008) observed that botanical composition of shrub/tree-pasture combinations under grazing tends to vary over time because palatable species suffer selective grazing pressure which can result in their decline, while those not consumed by animals tend to increase.

Due to differences in selectivity between browsers and grazers, intake of various forage species differs among animal species as does the concentration of dietary nutrients (Holechek et al. 2006). Dietary botanical composition can be estimated by collecting dietary samples via esophageal or rumen fistulae for examination using the point microscopic technique (Heady and Torrel 1959) or analyzing feces using the microhistological technique (Sparks and Malechek 1968). As the latter technique is less labor-intensive and is conducted with intact sheep grazing without interference and therefore not subject to possible inaccuracies in sampling, we chose to use this methodology.

Studies conducted in Northeast Brazil show that 70% of species from Caatinga rangeland contribute to the botanical composition of ruminant diets. Herbaceous species can contribute over 80% of diets in the rainy season, while woody perennials become more important in the dry season and can contribute up to 48.5% (Araújo Filho et al. 1998).

The objective of this study was to document the seasonal changes in botanical composition of available forage and the diets selected by sheep continuously grazing partially cleared Caatinga over-seeded with perennial grasses.

Materials and Methods

Rangeland study

The research was conducted from January 2011 to January 2012 in São Miguel (8°10'50'' S, 38°23'14'' W), which is located in Serra Talhada, Pernambuco, Brazil. The location is characterized by undulating relief, shallow, well-drained, medium to high fertility Luvisols, 439-m elevation, and a tropical semi-arid climate with a mean annual temperature of 25.7 °C. The vegetation is primarily composed of hyperxerophilic Caatinga, consisting of shrubland plus thorn and deciduous forest (CPRM 2005). Annual rainfall during the study, derived from a pluviometer, was 696 mm compared with a 10-year average of 674 mm (Figure 1).

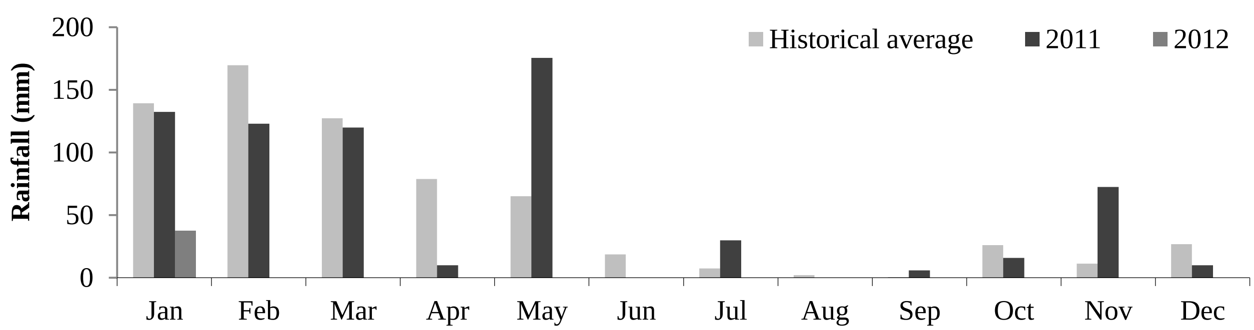

Figure 1. Rainfall during the experimental period and the medium-term (2001–2011) average. Source: São Miguel Farm, Serra Talhada, PE, Brazil.

The experimental area consisted of 38 ha of thinned Caatinga, with one-third of the area oversown with buffel grass (*Cenchrus ciliaris*) and sabi grass (*Urochloa mosambicensis*). During the study the paddock was continuously grazed by 70 crossbred sheep (Dorper x Santa Inês), free of parasites, with initial average body weight of 30.9 ± 4.4 kg and 10 months old. Water and salt were available at all times.

Soil analysis

Soil samples collected at depths from 0 to 20 cm and 20 to 40 cm were analyzed for particle size by the pipette method (EMBRAPA 1997) and for soil fertility at the Universidade Federal Rural of Pernambuco laboratories. The soil was sandy-loam, eutrophic, with: pH (H_2O) = 6.4; P (Mehlich-1) = 11.8 mg/dm^3; Na^+ = 0.17 cmol$_c$/dm^3; K^+ = 0.2 cmol$_c$/dm^3; Ca^{2+} = 3.5 cmol$_c$/dm^3; Mg^{2+} = 1.5 cmol$_c$/dm^3; Al^{3+} = 0.08 cmol$_c$/dm^3; $H^+ + Al^{3+}$ = 3.1 cmol$_c$/dm^3; organic carbon = 7.3 g/kg; and OM = 12.6 g/kg (Cavalcanti 1998).

Botanical composition of rangeland

We initiated sample collection when inflorescences appeared and sent them to the Dárdano Andrade Lima Herbarium (MOSS), Agronomic Institute of Pernambuco, Brazil for identification. Botanical composition was estimated by the dry-weight-rank method adapted from Jones and Hargreaves (1978) using 1-m^2 quadrats. Evaluation took place every 56 d from January 2011 to January 2012. In order to take into account vegetation heterogeneity, data points were located on nine 100-m transects, each with 20 sampling points at least 10 m apart.

Botanical composition of the diet

Commencing in March 2011, 2 fecal samples were collected from the rectums of 7 sheep at the same time as pasture assessments were conducted. Samples were stored in plastic bags at -15 °C. Dietary botanical composition was determined subsequently using microhistological techniques modified from Scott and Dahl (1980) by removing the abaxial and adaxial epidermis of the leaf blades (paradermal cuts). A set of microscopic slides was used as references by collecting fresh plant material from species considered abundant and possessing forage potential. The species chosen were divided into 3 distinct groups: Poaceae (*U. mosambicensis*, *Melinis repens*, *C. ciliaris* and *Brachiaria plantaginea*); Malvaceae (*Herissantia crispa*, *Sida galheirensis*, *Melochia tomentosa* and *Waltheria macropoda*); and other dicotyledons (*Cnidoscolus quercifolius*, *Aspidosperma pyrifolium*, *Croton sonderianus*, *Bauhinia cheilantha*, *Caesalpinia pyramidalis*, *Mimosa tenuiflora* and *Macroptilium martii*).

Epidermal structures were used for identification using photomicrographs from light microscopes equipped with cameras. These included shape and arrangement of epidermal cells, shape and presence of siliceous cells, types of glandular trichomes, types of stomata and shape of stomata subsidiary cells (Sparks and Malechek 1968). Fecal material was filtered with distilled water in ABNT No. 140 sieves with 0.105-mm pores. The residue was then subjected to the same procedure used for mounting reference slides. Five slides per cycle per animal were made where 20 field readings were photomicrographed by a light microscope set at 10x objective. The fragments obtained were recorded and then the relative frequency of each component was determined, according to a formula developed by Holechek and Gross (1982):

$$\% = \frac{\text{component frequency}}{\sum \text{of identified component frequencies}} \times 100$$

Selectivity index

Indexes were used to compare botanical composition of the pasture and diet. Pasture composition was used as the reference for dietary composition. If the index was <1, there was avoidance of the component, while if the index was >1, that component was selected at a greater level than its presence in the paddock (Heady 1975).

The selectivity indexes were calculated by Kulczynski formula (Hansen and Reid 1975; Alipayo et al. 1992; Bauer et al. 2008):

$$SI_{jk} = \frac{2\sum_{i=1}^{I} \min\ (P_{ij}, P_{ik})}{\sum_{i=1}^{I} (P_{ij} + P_{ik})} \times 100$$

where SI_{jk} is the Selectivity Index (%), P_{ij} and P_{ik} are percentages of the component i in the diet j and pasture k.

Statistical analysis

Data were subjected to descriptive statistics (mean and confidence interval at 5% probability) and multivariate analysis using Statistica, Version 7 (StatSoft Inc., Tulsa, OK, USA). Principal components analysis was used to simplify the data set, summarizing the information in a few components that retained maximum variation. Cluster analyses between the botanical composition of pasture and diet as a factor of similarity were undertaken by the Tocher Method, where the Euclidean average distance intragroup must be smaller than the average distance intergroup.

Results

Available forage

The floristic diversity of the vegetation included 23 families and 63 species from different strata: 10 trees, 29 shrubs and 24 herbs. There was a predominance of woody species, with a diverse, dispersed herbaceous layer of mostly annuals (Table 1).

Plant families with the largest numbers of species were: Leguminosae with its 3 subfamilies, Caesalpinoideae, Mimosoideae and Papilionoideae; Malvaceae, Poaceae, Euphorbiaceae and Cactaceae. Fifty-two percent of the families found in this study had only 1 species (Figure 2). Of the total number of genera, 51 (90%) were represented by only 1 species (Table 1).

Table 1. Plant species present in thinned Caatinga browsed by sheep at Serra Talhada, PE, Brazil. Species and family names are according to The Plant List taxonomic database (www.theplantlist.org).

Family	Scientific Name	Common Name
Herbaceous Stratum		
Amaranthaceae	*Froelichia humboldtiana* (Schult.) Seub.	Froelichia
Compositae	*Centratherum punctatum* Cass.	Perpétua-roxa
Boraginaceae	*Heliotropium tiaridioides* Cham.	Crista-de-galo
Cactaceae	*Melocactus bahiensis* (Britton & Rose) Luetzelb.	Coroa-de-frade
Cactaceae	*Tacinga inamoena* (K.Schum.) N.P.Taylor & Stuppy	Quipá
Cleomaceae	*Cleome spinosa* Jacq.	Mussambê
Cyperaceae	*Cyperus uncinulatus* Schrad. ex Nees	Barba-de bode
Convolvulaceae	*Ipomoea asarifolia* (Desr.) Roem. & Schult.	Salsa
Leg. Mimosoideae	*Mimosa sensitiva* L.	Malícia / Dormideira
Leg. Papilionoideae	*Macroptilium martii* (Benth.) Marechal & Baudet	Orelha-de-onça
Nyctaginaceae	*Boerhavia diffusa* L.	Pega-pinto
Poaceae	*Andropogon gayanus* Kunth	Capim-andropogon
Poaceae	*Aristida setifolia* Kunth	Capim-panasco
Poaceae	*Brachiaria plantaginea* (Link) Hitchc.	Capim-milhã
Poaceae	*Cenchrus ciliaris* L.	Capim-buffel
Poaceae	*Melinis repens* (Willd.) Zizka	Capim-favorito
Poaceae	*Urochloa mosambicensis* (Hack.) Dandy	Capim-corrente
Pontederiaceae	*Eichhornia paniculata* (Spreng.) Solms	Rainha-dos-lagos / Aguapé
Portulacaceae	*Portulaca halimoides* L.	Beldroega
Rhamnaceae	*Crumenaria decumbens* Mart.	-
Rubiaceae	*Spermacoce verticillata* L.	Vassourinha-de-botão
Rubiaceae	*Diodella teres* (Walter) Small	Engana-bobo
Rubiaceae	*Staelia virgata* (Link ex Roem. & Schult.) K.Schum.	Poaia
Plantaginaceae	*Angelonia cornigera* Hook.	-
Shrub Stratum		
Amaranthaceae	*Alternanthera brasiliana* (L.) Kuntze	Quebra-panela
Apocynaceae	*Allamanda blanchetii* A.DC.	Alamanda-roxa
Apocynaceae	*Calotropis procera* (Aiton) Dryand.	Algodão-de-seda
Boraginaceae	*Cordia leucocephala* Moric.	Moleque-duro
Bromeliaceae	*Bromelia balansae* Mez	Macambira-de-cachorro
Bromeliaceae	*Bromelia laciniosa* Mart. ex Schult. & Schult.f.	Macambira
Cactaceae	*Arrojadoa rhodantha* (Gürke) Britton & Rose	Rabo-de-raposa
Cactaceae	*Cereus jamacaru* DC.	Mandacaru
Cactaceae	*Pilosocereus gounellei* (F.A.C.Weber ex K.Schum.) Byles & G.D.Rowley	Xique-xique
Euphorbiaceae	*Cnidoscolus quercifolius* Pohl	Faveleira
Euphorbiaceae	*Cnidoscolus urens* (L.) Arthur	Cansanção
Euphorbiaceae	*Croton heliotropiifolius* Kunth	Quebra-faca
Euphorbiaceae	*Croton sonderianus* Müll.Arg.	Marmeleiro
Euphorbiaceae	*Jatropha mollissima* (Pohl) Baill.	Pinhão bravo
Euphorbiaceae	*Manihot carthaginensis* subsp. *glaziovii* (Müll.Arg.) Allem	Maniçoba

Continued

Family	Scientific Name	Common Name
Shrub Stratum		
Leg. Caesalpinioideae	*Bauhinia cheilantha* (Bong.) Steud.	Mororó
Leg. Mimosoideae	*Mimosa tenuiflora* (Willd.) Poir.	Jurema-preta
Leg. Papilionoideae	*Aeschynomene filosa* Benth.	Angiquinho
Leg. Papilionoideae	*Dioclea grandiflora* Benth.	Mucunã
Leg. Papilionoideae	*Indigofera suffruticosa* Mill.	Anileira
Malvaceae	*Herissantia crispa* (L.) Brizicky	Malva
Malvaceae	*Herissantia tiubae* (K.Schum.) Brizicky	Malva / Mela-bode
Malvaceae	*Sida galheirensis* Ulbr.	Relógio / Malva-branca
Malvaceae	*Wissadula periplocifolia* (L.) Thwaites	Veludo-branco
Malvaceae	*Melochia tomentosa* L.	Capa-bode
Malvaceae	*Waltheria macropoda* Turcz.	Malva-branca
Malvaceae	*Waltheria rotundifolia* Schrank	Malva-amarela
Passifloraceae	*Piriqueta guianensis* subsp. *elongata* (Urb. & Rolfe) Arbo	-
Verbenaceae	*Lantana camara* L.	Chumbinho
Arboreal Stratum		
Apocynaceae	*Aspidosperma pyrifolium* Mart.	Pereiro
Leg. Caesalpinioideae	*Caesalpinia pyramidalis* Tul.	Catingueira
Leg. Caesalpinioideae	*Chamaecrista hispidula* (Vahl) H.S.Irwin & Barneby	Visgo
Leg. Mimosoideae	*Anadenanthera colubrina* var. *cebil* (Griseb.) Altschul	Angico
Leg. Mimosoideae	*Prosopis juliflora* (Sw.) DC.	Algaroba
Leg. Papilionoideae	*Amburana cearensis* (Allemao) A.C.Sm.	Imburana-de-cheiro
Rhamnaceae	*Ziziphus joazeiro* Mart.	Juazeiro
Rubiaceae	*Mitracarpus longicalyx* E.B.Souza & M.F.Sales	-
Sapindaceae	*Talisia esculenta* (A. St.-Hil.) Radlk.	Pitombeira
Sapotaceae	*Sideroxylon obtusifolium* (Roem. & Schult.) T.D.Penn.	Quixabeira / Rompe-gibão

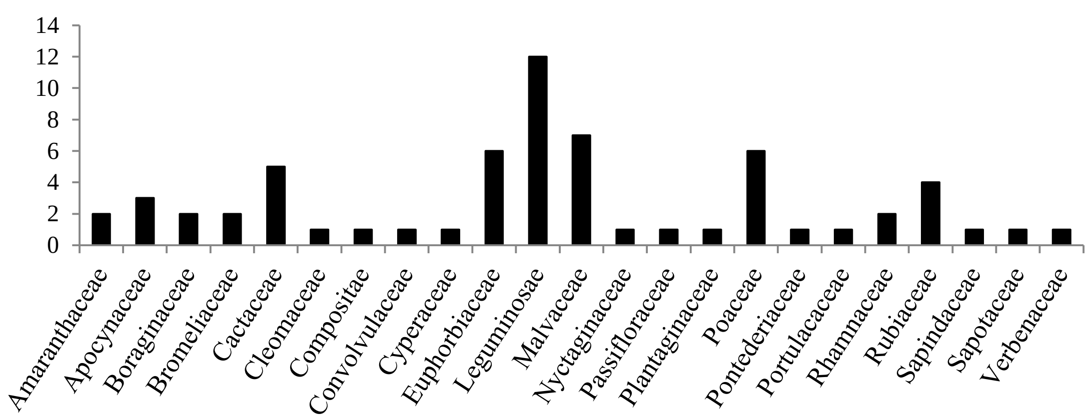

Figure 2. Number of species in different families in thinned Caatinga grazed by sheep at Serra Talhada, PE, Brazil.

Despite the floristic diversity (Table 1), botanical composition was dominated by "other Malvaceae" of low palatability to sheep (Santos et al. 2008) and the component "other species" (Table 2). During the dry season (Figure 1), prevalence of "other Malvaceae" increased, probably due to tolerance of the xerophytic environment (Prado 2003), which made it more competitive than less drought-tolerant species (Table 2).

Buffel grass (*C. ciliaris*) and the "other Poaceae" component were less prevalent but persisted throughout the year (Table 2), possibly because they are evergreen in the presence of minimal soil moisture (Hanselka et al. 2004). As the dry season progressed, the contribution of sabi grass (*U. mosambicensis*) and the "other species" component to available forage declined, the former due to drought dormancy (Skerman and Riveros 1990) and

Table 2. Botanical composition (% ± s.e.) of Caatinga with thinned over-story and an under-story grazed by sheep at Serra Talhada, PE, Brazil.

Component	Jan 11	Mar 11	Apr 11	Jun 11	Aug 11	Oct 11	Jan 12
Melochia tomentosa (Malv.)	3.3 ± 12.7	2.0 ± 10.8	3.1 ± 14.5	2.3 ± 12.1	0.8 ± 7.6	2.0 ± 10.4	1.2 ± 8.5
Cenchrus ciliaris (Poaceae)	10.7 ± 26.5	10.0 ± 26.1	8.7 ± 22.8	14.7 ± 30.5	17.5 ± 33.6	14.1 ± 28.7	15.0 ± 31.8
Urochloa mosambicensis (Poaceae)	2.9 ± 15.7	1.4 ± 9.7	2.6 ± 13.4	8.2 ± 24.7	9.6 ± 17.6	3.5 ± 15.0	4.0 ± 17.4
Caesalpinia pyramidalis (Leg.-Caesalp.)	1.5 ± 12.3	3.6 ± 14.3	0.2 ± 0.9	0.5 ± 3.9	2.7 ± 13.3	1.6 ± 10.0	11.4 ± 30.1
Diodella teres (Rubiaceae)	1.2 ± 5.7	8.8 ± 24.5	11.3 ± 27.3	11.0 ± 26.3	2.6 ± 14.7	4.8 ± 16.1	0.1 ± 0.7
Mimosa tenuiflora (Leg.-Mimos.)	1.0 ± 5.3	0.4 ± 4.2	0.6 ± 6.8	0.2 ± 2.3	1.1 ± 8.8	0.7 ± 5.8	2.7 ± 15.5
Croton sonderianus (Euphorb.)	14.2 ± 29.9	4.5 ± 19.3	3.2 ± 15.4	1.4 ± 7.7	3.9 ± 15.1	5.1 ± 20.0	8.6 ± 25.6
Macroptilium martii (Leg.-Papil.)	0.3 ± 1.3	5.0 ± 17.4	7.2 ± 23.1	3.4 ± 16.4	2.8 ± 16.5	0.7 ± 5.8	0.0 ± 0.0
Aspidosperma pyrifolium (Apocyn.)	3.1 ± 12.6	3.6 ± 15.4	1.0 ± 7.6	1.1 ± 8.5	1.7 ± 8.9	1.5 ± 10.6	5.5 ± 20.5
Ipomoea asarifolia (Convolv.)	0.4 ± 2.2	0.0 ± 0.0	0.8 ± 7.3	1.1 ± 7.8	1.2 ± 10.0	0.5 ± 4.6	1.5 ± 10.0
Cactaceae	1.3 ± 5.1	0.7 ± 7.2	1.5 ± 11.0	0.8 ± 9.1	1.2 ± 10.5	5.1 ± 20.0	1.2 ± 8.9
Other Malvaceae	21.6 ± 36.0	25.6 ± 36.0	18.3 ± 30.9	27.1 ± 36.7	30.4 ± 40.8	42.7 ± 40.7	38.6 ± 43.6
Other Poaceae	10.8 ± 25.5	6.6 ± 19.6	5.0 ± 17.0	5.2 ± 18.2	8.3 ± 25.6	9.5 ± 23.7	2.2 ± 13.3
Other Leguminosae	0.5 ± 2.3	0.1 ± 1.0	0.2 ± 1.2	2.8 ± 14.6	0.5 ± 6.0	1.5 ± 10.8	0.5 ± 3.1
Other species[1]	27.2 ± 35.2	27.6 ± 36.3	36.3 ± 35.9	20.2 ± 31.9	15.6 ± 33.0	6.7 ± 20.8	7.5 ± 26.7

[1]Species not mentioned in this table nor belonging to any of the families mentioned in this table.

the latter largely because these were rainy season ephemerals that senesced during the dry season (Table 2). During the rainy season, contribution of Poaceae to the Caatinga herbaceous layer was greater than during the drier months (Figure 1).

The annual native legume *M. martii* is highly palatable to ruminants (Ydoyaga-Santana et al. 2011) and had its greatest contribution to the rangeland during the rainy season, persisting only until the early dry season (Table 2). "Other Leguminosae" also occurred infrequently throughout the survey periods. Among these, *B. cheilantha*, despite not fixing atmospheric N, is a valuable forage and is palatable to ruminants (Moreira et al. 2006; Martinele et al. 2010; Ydoyaga-Santana et al. 2011). In January 2011, *C. sonderianus* (Euphorbiaceae) was frequent (Table 2).

Buffel grass, "other Malvaceae" and the "other species" components were the most plentiful throughout the year. Using multivariate analysis of the first principal components, these formed isolated groups apart from the other components, explaining 91% of total variation (Figure 3).

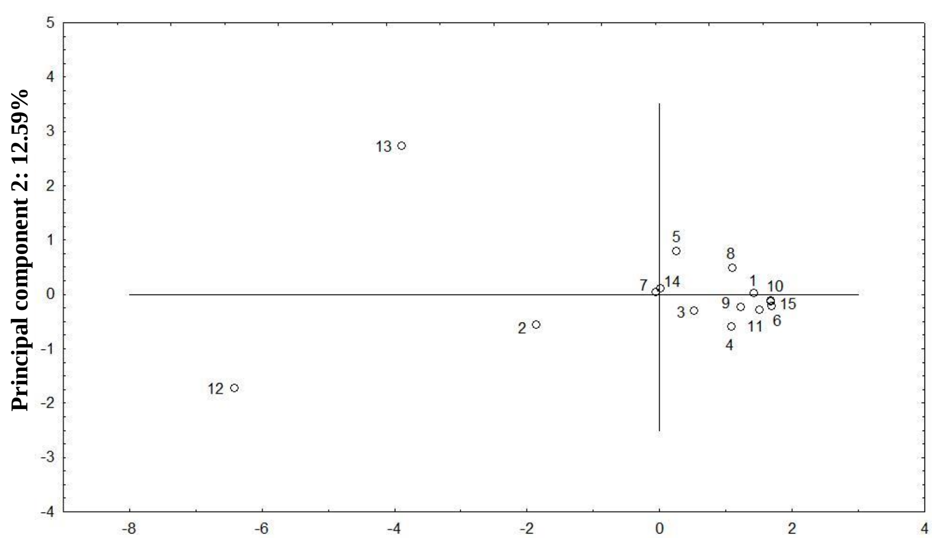

Figure 3. Projection of dissimilarity between 15 components as a percent of total botanical composition of a Caatinga pasture at Serra Talhada, PE, Brazil, with the components 13, 2 and 12 explaining 91% of total variation. Legend: (1) *M. tomentosa*, (2) *C. ciliaris*, (3) *U. mosambicensis*, (4) *C. pyramidalis*, (5) *D. teres*, (6) *M. tenuiflora*, (7) *C. sonderianus*, (8) *M. martii*, (9) *A. pyrifolium*, (10) *I. asarifolia*, (11) Cactaceae, (12) other Malvaceae, (13) other species, (14) other Poaceae, (15) other Leguminosae.

Diets of sheep

In the botanical composition of sheep diets, "other dicotyledons" predominated comprising 59.6% of the total dry matter in feces, on average, while Poaceae represented 30.5%. The Malvaceae, also dicotyledons, comprised 9.9% of dietary composition (Figure 4).

The similarity between some anatomical patterns of grasses and dicotyledons often makes species identification difficult in fecal samples. Some species, however, could be definitively identified, namely: *C. ciliaris*, *U. mosambicensis* and *M. repens* with stomata in parallel and hooks; *C. pyramidalis*, *A. pyrifolium*, *M. martii* and *B. cheilantha* with non-parallel stomata and long-hair trichomes; and *C. phyllacanthus* with glandular trichomes (Figure 5). It is noteworthy that the methodology used is simpler and less costly than the use of fistulated animals.

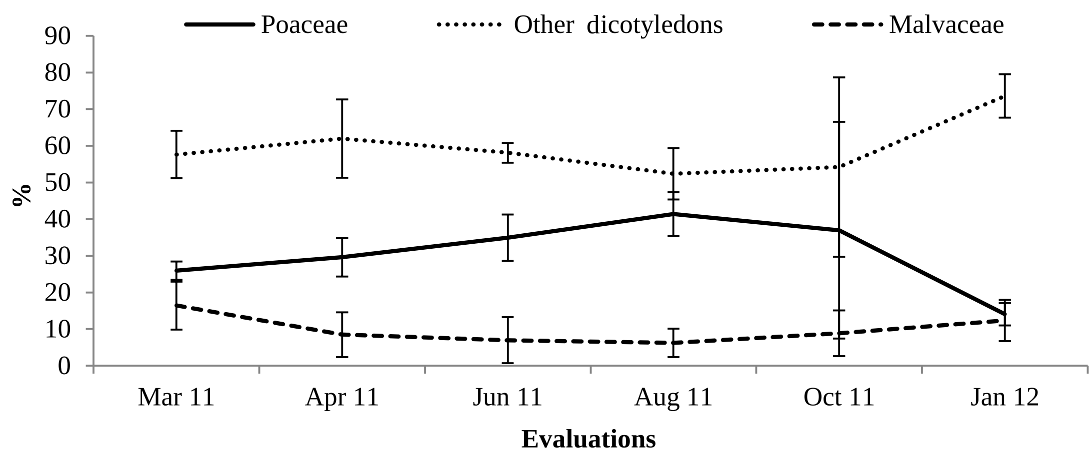

Figure 4. Seasonal dietary botanical composition, based on fecal samples, of sheep grazing thinned Caatinga at Serra Talhada, PE, Brazil.

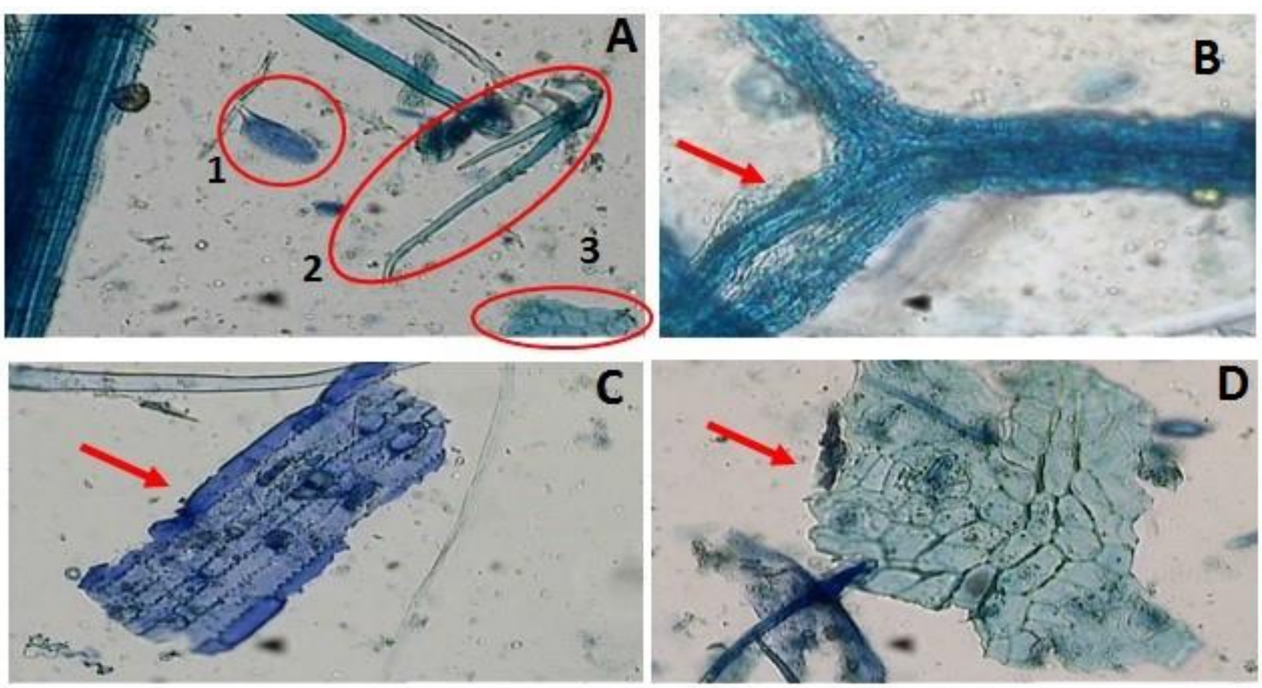

Figure 5. Slides of plant epidermal fragments from Brazilian Caatinga, obtained from sheep feces, at 10x objective: (A1) hooked *C. ciliaris*; (A2) Malvaceae star-shaped hair; (A3) dicotyledonous cuticle; (B) non-parallel dicotyledonous leaf rib network (arrow); (C) epidermal fragment of *C. ciliaris* (arrow); (D) epidermal fragment of *B. cheilantha* (arrow).

Selectivity indexes indicated that Poaceae and "other dicotyledons" were preferred by the sheep over other plants, with "other dicotyledons" replacing grasses in the diet from the beginning of the dry season (June 2011) (Figure 6). The phenological state of Poaceae (mature standing hay) and availability of tree (up to 273-cm height) and shrub (up to 175-cm height) foliage (comprised mainly of unreached leaves) fallen to the ground ("litter"), contributed to this result. Malvaceae were not preferred by animals (SI<1.0) (Figure 6) throughout the experiment; in other words, their contribution to the pasture was greater (Table 2) than their contribution to the sheep diets.

According to multivariate analysis of relationships between pasture botanical composition and sheep diets, the only component with dissimilarity between botanical composition of pasture and diet was the Malvaceae, whereas "other dicotyledons" and Poaceae formed distinct groups according to Tocher grouping, indicating intergroup dissimilarity (selectivity) (Figure 7).

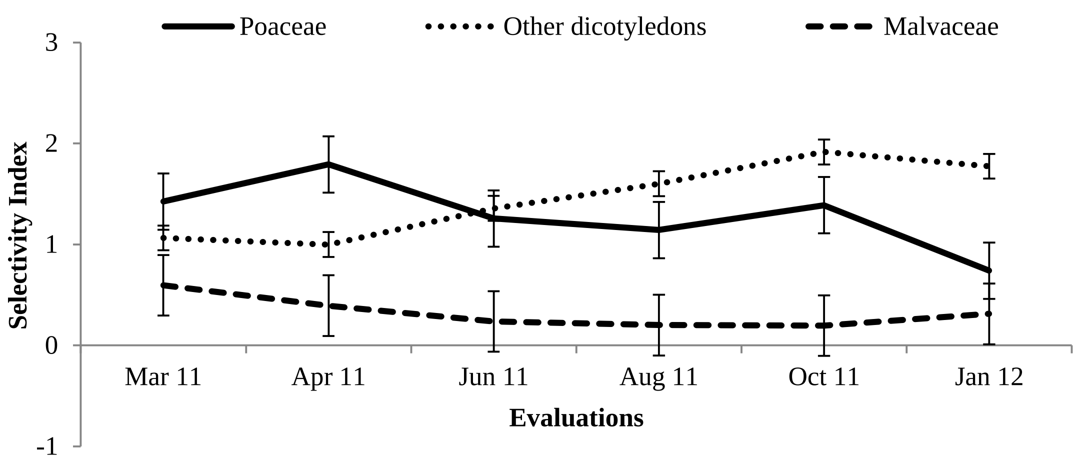

Figure 6. Selectivity indexes of sheep grazing Caatinga rangeland during different times of the year at Serra Talhada, PE, Brazil.

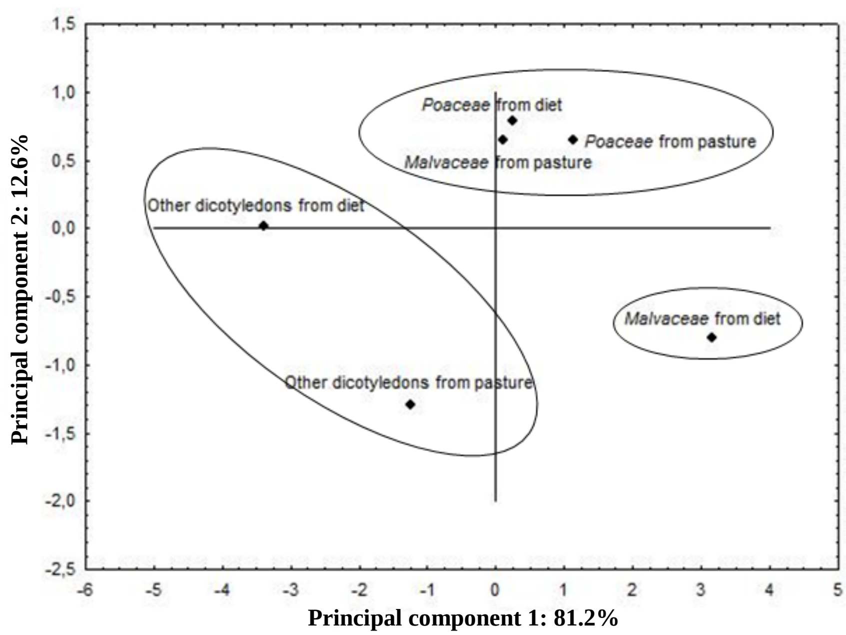

Figure 7. Projection of sheep diet dissimilarity and cluster among botanical components in Caatinga rangeland in which the first components explained 93.8% of the dissimilarity.

Discussion

This study confirms that the Caatinga ecosystem is complex, containing numerous species with multiple uses in managed systems, mainly agroforestry (Giulietti et al. 2004; Araújo Filho 2006; Gariglio et al. 2010). Some species are toxic to mammals, e.g. *I. suffruticosa*, containing the alkaloid indospicine (Tokarnia et al. 2000); *M. tenuiflora*, which may cause embryo mortality and poor bone formation; and *F. humboldtiana* that promotes photosensitization (Riet-Correa and Méndez 2007).

The Caatinga diversity (Table 1) under sheep grazing was similar to that reported by Moreira et al. (2006) in the same region, who identified 67 species in samples collected during the rainy season. In another study by Ydoyaga-Santana et al. (2011) in rangeland grazed by cattle in Serra Talhada, 41 plant species and 24 families were recorded during the rainy season, with Leguminosae, Euphorbiaceae and Poaceae predominating. Factors such as the irregularity of rainfall and reduced forage mass can promote change in the botanical composition of the pasture and increase bare soil, which may contribute to the persistence of species with low forage value and less selected by animals (Tothill 1987). Bailey and Brown (2011) reported that in semi-arid rangelands, forage growth is delayed by moisture deficit rather than defoliation.

Poaceae are of great importance to people in the Caatinga because they are largely preferred by grazing ruminants (Moreira et al. 2006; Santos et al. 2008; Martinele et al. 2010; Ydoyaga-Santana et al. 2011). According to Sousa et al. (2007) one could speculate that the Poaceae would be more resilient in this rangeland if soil phosphorus and soil organic matter were greater than the concentrations found in this study (11.8 mg/dm^3 and 30 g/kg, respectively). For *M. martii*, Moreira et al. (2006) reported values of 1.6% (March) to 2.2% (June) of the Caatinga botanical composition in Serra Talhada, Pernambuco and Ydoyaga-Santana et al. (2011) found values of 3.5% (February) to 3.9% (July) in similar rangeland. According to Freitas et al. (2007), in degraded Caatinga, *C. sonderianus* and *M. tenuiflora* are often locally abundant.

Botanical composition of sheep diets in rangeland will vary according to species available, forage mass or density and sheep thirst (Newman et al. 1994; Parsons et al. 1994; Sankhyan et al. 2001; Albuquerque et al. 2008), all factors heavily influenced by seasonal climatic conditions. However, it is noteworthy that, in our study, the botanical composition of the diet showed limited variation when comparing dry and rainy seasons.

The percentage of herbaceous dicotyledons in the diet reached as high as 70%, confirming the findings of Kirmse (1984) in studies on diets of sheep and goats in Caatinga at Ceará, Brazil. The preference for other dicots in this study is reflected in the crude protein concentrations in the diet, which varied from 8.9 to 12.9% of dry matter (Oliveira et al. 2015).

Poaceae constituted 20–45% of the diet in this study. In contrast, Santos et al. (2008) observed that the contribution of grasses to sheep diets, as determined by esophageal fistula sampling, was low and ranged from 2.5 to 19.7% from September 2004 to July 2005 in Caatinga, located in Sertânia, Pernambuco. Other species ranged from 75.4 to 94% during the same period. The authors concluded that the high contribution by dicotyledons was due to senescence and high nutritional value with up to 17.2% crude protein and 64.6% potential degradability of dry matter (Santos et al. 2009). Small ruminants may also spend more time browsing in the more elevated strata of the pasture depending on the structural characteristics of the rangeland (Pfister et al. 1988). Araújo Filho et al. (1998) summarized several studies conducted in the Caatinga and observed that the contributions of woody species to sheep diets averaged 32.3 and 48.5% in the rainy and dry seasons, respectively.

Araújo Filho et al. (1996), studying the botanical composition of sheep diets in Ceará Caatinga, reported that grasses contributed from 23.5 to 25.0%, while dicotyledons contributed from 75.7 to 76.5%, resulting in similar selectivity indexes ranging from 82.8 to 93.4% for grasses and from 80.5 to 83.2% for dicotyledons. Santos et al. (2008) observed that the selectivity index for sheep in Caatinga indicated preference for dicotyledons from September 2004 to July 2005, as compared with our results which show a preference for broad leaf plants from July to January.

Conclusions

This study has demonstrated that this thinned Caatinga rangeland vegetation was floristically diverse with some species of known forage value. Despite the presence of numerous plant families, 52% of these were represented by only a single species, i.e. this Caatinga has low flora diversity.

Botanical composition of sheep diets varied throughout the year and consisted mainly of forbs and woody browse. While grasses were preferred by sheep during the rainy season, “other dicotyledons” were preferred from the beginning of the dry season.

The Malvaceae, despite their strong presence in the pasture, were generally largely avoided by the animals, possibly due to low palatability caused by, among other factors, hirsute leaves. Systematic removal of Malvaceae and incorporation of high-quality grasses and legumes by farmers into this Caatinga rangeland seem advisable to increase carrying capacity. The trend for sheep to selectively graze on herbaceous species could result in a steady disappearance of the best forage species in that stratum unless grazing of pastures is properly managed.

The microhistological technique used to determine botanical composition of the sheep diets under grazing in the Caatinga seemed a robust and reliable assessment methodology, which avoided significant modification to animal behavior as can result with other methodologies, such as fistulae. However, this technique does not allow the identification of a considerable portion of the diet to the species level; e.g. "other dicotyledons" always comprised more than 50% of the diet. Unfortunately, we are unaware of any techniques which can identify individual species in the diet without using fistulae. Further studies, however, with different techniques, e.g. F-NIRS and n-alkanes, are needed to validate our findings.

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Research paper

Efectividad de la inoculación de hongos micorrízicos arbusculares en dos leguminosas forrajeras cultivadas en dos tipos de suelo

Effectiveness of inoculation of two forage legumes grown on two soil types with arbuscular mycorrhizal fungi

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Resumen

En Bauta, provincia de Artemisa, Cuba, se realizaron sendos experimentos con el objetivo de evaluar la respuesta de las leguminosas forrajeras: stylo (*Stylosanthes guianensis*) y siratro (*Macroptilium atropurpureum*) a la inoculación con hongos micorrízicos arbusculares (HMA) en suelos Vertisol y Nitisol. Los tratamientos consistieron en inóculos con las especies de HMA: *Funneliformis mosseae*, *Glomus cubense* y *Rhizoglomus intraradices* más un testigo sin inocular, dispuestos en un diseño de bloques al azar con arreglo factorial 4 x 2 y 4 repeticiones. Los inóculos fueron aplicados al momento de la siembra mediante el recubrimiento de las semillas. Como variables de respuesta fueron evaluados el rendimiento de las leguminosas, la frecuencia e intensidad de la colonización micorrízica, el número de esporas de HMA en la rizosfera y las concentraciones de macronutrientes en la biomasa aérea. Se encontró una respuesta positiva de ambas leguminosas a la inoculación; no obstante la efectividad de las especies de HMA varió en función del tipo de suelo. *Rhizoglomus intraradices* fue más efectiva respecto a la frecuencia e intensidad de la colonización micorrízica y para mejorar el estado nutricional y el rendimiento de las leguminosas en el Vertisol, mientras que *G. cubense* lo fue en el Nitisol. Se requiere de estudios para identificar cuáles factores de suelo determinan el comportamiento de los HMA.

Palabras clave: Fertilidad del suelo, *Macroptilium atropurpureum*, Nitisol, nutrición mineral, *Stylosanthes guianensis*, Vertisol.

Abstract

In Bauta, Artemisa province, Cuba, 2 field experiments were conducted in Nitisol and Vertisol soils to evaluate the response of the pasture legumes, stylo (*Stylosanthes guianensis*) and siratro (*Macroptilium atropurpureum*), to inoculation with arbuscular mycorrhizal fungi (AMF). In each experiment, inocula with the AMF species *Funneliformis mosseae*, *Glomus cubense* and *Rhizoglomus intraradices* were applied, and there was a control without inoculation. A randomized block design with a 4 (inocula) x 2 (legume species) factorial arrangement was used giving 8 treatments and 4 replications. AMF inocula were applied at sowing by the seed-coating method, and legume yield, the frequency and intensity of mycorrhizal root colonization and macronutrient concentrations in aboveground biomass were evaluated. The legumes responded positively to AMF inoculation, but the effectiveness of the AMF species depended on the soil type. *Rhizoglomus intraradices* was more effective with respect to the frequency and intensity of mycorrhizal root

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colonization and in improving the nutritional status and yield of legumes grown in the Vertisol, whereas *G. cubense* was more effective for those grown in the Nitisol. Studies are required to identify the soil factors that determine the effectiveness of AMF.

Keywords: *Macroptilium atropurpureum*, mineral nutrition, Nitisol, soil fertility, *Stylosanthes guianensis,* Vertisol.

Introducción

Las leguminosas forrajeras son un recurso importante para la alimentación de los rumiantes por su alto contenido de proteína y minerales, y su alta digestibilidad. Sin embargo, la baja fertilidad de los suelos que se dedican a la ganadería en Cuba (Lok 2015), así como la escasa disponibilidad en cantidades suficientes de fertilizantes para satisfacer los requerimientos nutritivos de las leguminosas forrajeras debido a razones económicas, limitan sus rendimientos y valor nutritivo y reducen su presencia en las pasturas. La escasez de fertilizantes y la creciente necesidad de adoptar tecnologías amigables con el medio ambiente, son una oportunidad para la búsqueda de nuevos modelos agrícolas basados en el manejo eficiente del sistema suelo-pastura-animal, con el propósito de lograr la sostenibilidad de la producción ganadera.

Dentro de esos modelos se incluye el manejo de las asociaciones micorrízicas, por sus potencialidades para mejorar la productividad y a la vez, reducir las necesidades de fertilizantes de las especies forrajeras (Carneiro et al. 2011; Castillo et al. 2014). En los agroecosistemas de pasturas, los beneficios de los hongos micorrízicos arbusculares (HMA) están estrechamente ligados al aumento de la absorción de elementos minerales y agua presentes en el suelo, a través de una red de hifas interconectadas que incrementan el volumen de suelo que exploran las raíces, mejoran su estructura y facilitan el acceso de las plantas a los nutrientes que se encuentran en formas menos asimilables (Zhang et al. 2012; Klabi et al. 2014).

Cuando las pasturas poseen bajas cantidades de propágulos micorrízicos nativos o los HMA existentes en el suelo no son capaces de establecer una simbiosis efectiva, la inoculación de cepas adaptadas a las condiciones ambientales donde se desarrollan los pastos puede proporcionar considerables beneficios. No obstante, estos solo se conseguirán después de una selección controlada del hongo o del consorcio de hongos, que demuestre el más alto nivel de compatibilidad funcional y ecológica para el sistema suelo-planta (Pellegrino et al. 2011; Öpik y Moora 2012).

Verbruggen et al. (2013) consideran que el éxito de la inoculación micorrízica depende, no solo de los genotipos de plantas y hongos, sino también de las condiciones del ambiente. Aunque la influencia del suelo en los genotipos de HMA es aún pobremente entendida, algunos autores reconocen que el suelo impone una fuerte presión de selección sobre estos microorganismos (Oehl et al. 2010; Pellegrino et al. 2012). Rivera et al. (2007), a partir de la recopilación de la información de numerosos estudios realizados en Cuba para evaluar el efecto de la aplicación de inóculos de HMA con diferentes especies fúngicas en cultivos agrícolas de interés económico, concluyeron que el tipo de suelo y posiblemente su fertilidad asociada son los factores que más influyen en la efectividad de las especies evaluadas. Sin embargo, en relación con las leguminosas forrajeras la información disponible es escasa.

Debido a la complejidad de los agroecosistemas de pasturas, el estudio de los factores ambientales que influyen en el funcionamiento de la simbiosis es una herramienta que contribuye al éxito de la inoculación con HMA. Teniendo en cuenta esta hipótesis el presente trabajo tuvo como objetivo evaluar la efectividad de la aplicación de inóculos con diferentes especies de HMA en leguminosas forrajeras cultivadas en 2 tipos de suelo.

Materiales y Métodos

El trabajo se realizó en áreas de producción de forrajes de la Empresa Pecuaria Genética (EPG) Niña Bonita, localizada en el municipio de Bauta, provincia de Artemisa, Cuba, donde fueron seleccionados 2 sitios con diferentes tipos de suelo, los cuales se clasificaron como Vertisol Stágnico Mólico y Nitisol Ferrálico Líxico, según la Base Referencial Mundial del Recurso Suelo (IUSS 2007).

En ambos sitios, antes de realizar los experimentos, los suelos fueron utilizados con pasturas de guinea (*Panicum maximum* cv. Likoni) durante 10 años, con un sistema de uso de 1 a 3 días de ocupación y 30 a 55 días de descanso, según la época de año, y una carga animal entre 1.5 y 2 unidades de ganado mayor por hectárea. Durante ese período, las pasturas se explotaron en condiciones de secano y no recibieron aplicaciones de fertilizantes.

Para la caracterización de los suelos, en cada sitio se tomaron 10 muestras compuestas por el método de zig-zag a la profundidad de 0–20 cm, para determinar

(Paneque et al. 2011): pH (H_2O) por potenciometro (1:2.5, relación suelo:agua), materia orgánica por el método de Walkley y Black, fósforo (P) asimilable por el método de Oniani (extracción con H_2SO_4 0.05 mol/L), cationes intercambiables con NH_4Ac 1 mol/L a pH 7, Ca y Mg por complejometría y Na y K por fotometría de llama.

Los experimentos se realizaron desde mayo de 2013 hasta abril de 2014. Durante ese período, según observaciones realizadas en la EPG Niña Bonita, las precipitaciones alcanzaron un total de 1,233 mm, del cual el 77.8% correspondió a la época lluviosa (mayo a octubre).

En cada sitio se realizó un ensayo de campo con 8 tratamientos, así: 3 inóculos de HMA (la cepa INCAM-2 de *Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler; la cepa INCAM-4 de *Glomus cubense* Y. Rodr. & Dalpé; y la cepa INCAM-11 de *Rhizoglomus intraradices* (N.C. Schenck & G.S. Sm.) Sieverd., G.A. Silva & Oehl) más un testigo sin inocular (factor A); y 2 leguminosas forrajeras: stylo (*Stylosanthes guianensis* cv. CIAT 184) y siratro (*Macroptilium atropurpureum* cv. Siratro) (factor B), en un diseño de bloques al azar con arreglo factorial 4 x 2 y 4 repeticiones. Las cepas de HMA procedían de la colección del Instituto Nacional de Ciencias Agrícolas (INCA). Las parcelas constituyeron la unidad experimental y tenían un área total de 28 m^2 y un área útil experimental de 21 m^2.

El suelo se preparó con una secuencia de laboreo consistente en roturación (arado), grada, cruce (arado) y grada, a intervalos aproximados de 20 días entre cada una. La siembra de las leguminosas se hizo en surco continuo (chorrillo) en mayo de 2013, en surcos separados 70 cm a una dosis de 8 kg/ha de semilla total, equivalente a 2 kg/ha de semilla pura germinable, tanto para stylo como para siratro, a una profundidad de 1.5 cm.

En ambos experimentos, la inoculación se realizó recubriendo las semillas, para lo cual se sumergieron en una pasta fluida, elaborada mediante la mezcla de una cantidad de inóculo sólido equivalente a 10% del peso de las mismas (800 g) y 300 mL de agua (Fernández et al. 2001). Una vez recubiertas las semillas y solidificado el inóculo, se procedió a la siembra, sin aplicación de fertilizantes.

Los inóculos utilizados se multiplicaron en un sustrato arcilloso esterilizado en autoclave a 120 °C por 1 hora durante 3 días, con el uso de *Brachiaria decumbens* cv. Basilisk como planta hospedera. Cada inóculo utilizado contenía 35 esporas de la especie de HMA a evaluar por gramo de sustrato, así como cantidades abundantes de fragmentos de raicillas e hifas.

En total se realizaron 4 cortes de la biomasa aérea de las plantas a una altura de 10 cm de la superficie del suelo: el primero 120 días después de la siembra y los demás a intervalos de 60 y 90 días, dependiendo de la producción de biomasa aérea, ya que los experimentos se realizaron en condiciones de secano. En cada corte se pesó la masa fresca (MF) de la parte aérea de las plantas que ocupaban el área de medición de cada parcela; para el efecto se tomaron muestras de 200 g y se secaron en una estufa con circulación forzada de aire a 70 °C durante 72 horas, para determinar el porcentaje de masa seca (MS), así como las concentraciones de N, P y K en el forraje (Paneque et al. 2011). El rendimiento de MS se estimó a partir del rendimiento de MF y el porcentaje de MS.

En cortes alternos, de cada parcela se tomaron 10 sub-muestras de raíces y de suelo de la rizosfera a una profundidad de 0–20 cm, mediante el empleo de un cilindro metálico de 5 cm de diámetro y 20 cm de altura. Los puntos de muestreo se distribuyeron equidistantes y separados a 10 cm de los surcos. Las muestras obtenidas fueron homogenizadas para formar una muestra compuesta por parcela, de la cual se extrajo 1 g de raicillas para tinción y clarificación (Rodríguez et al. 2015). Se evaluaron la frecuencia de colonización micorrízica, que expresa el grado de ocupación de las raicillas por los HMA, mediante el método de los interceptos (Giovanetti y Mosse 1980); la densidad visual o intensidad de la colonización, según Trouvelot et al. (1986); y el número de esporas en la rizosfera, mediante el tamizado y decantado por vía húmeda de dichas estructuras y su observación en microscopio (Herrera et al. 1995).

Para determinar en cada suelo la efectividad de la inoculación en el incremento del rendimiento de MS de las leguminosas, se utilizó el índice de eficiencia (IE, %). Este se calculó mediante la fórmula siguiente (Siqueira y Franco 1988):

$$\mathrm{IE}\ (\%) = \left[\frac{\mathrm{MS\ (t/ha)\ tratam.\ inocul.} - \mathrm{MS\ (t/ha)\ testigo}}{\mathrm{MS\ (t/ha)\ testigo}}\right] \times 100$$

El procesamiento estadístico de los datos se realizó mediante el análisis de varianza bifactorial y cuando existieron diferencias entre tratamientos, se utilizó la prueba de rangos múltiples de Duncan (1955) a $P<0.05$. A los valores promedio de los análisis de los suelos y a la variable índice de eficiencia de las cepas de HMA se le estimó el intervalo de confianza de las medias a $\alpha=0.05$ (Payton et al. 2000). Todas las variables cumplieron los supuestos de normalidad y homogeneidad de varianzas, por lo que en todos los casos se analizaron los datos originales (Vásquez 2011). Para el análisis de datos se utilizó el programa SPSS Statistics 21 (IBM 2012).

Resultados

Suelos

En el Cuadro 1 se presentan las principales características químicas de los suelos. El Vertisol presentó pH neutro, contenidos medios de materia orgánica, altos tenores de P asimilable y K intercambiable, así como una alta capacidad de intercambio de bases. El Nitisol presentó un pH ligeramente ácido, contenidos medios de materia orgánica, bajos tenores de P asimilable y K intercambiable, y baja capacidad de intercambio de bases.

Ambos suelos son arcillosos; sin embargo, la diferencia en su capacidad de intercambio de bases está dada por el tipo de arcilla. En el Vertisol predominan las arcillas de retículo 2:1, mientras que en el Nitisol, las de retículo 1:1 (IUSS 2007).

Cuadro 1. Características químicas de los suelos (0–20 cm).

Suelo	pH	MO (%)	P (mg/100 g)	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	CCB
				($cmol_c$/kg)				
Vertisol	7.2	4.7	5.6	33.2	6.9	0.55	0.46	41.1
± *IC*	0.3	0.43	0.5	2.7	0.6	0.09	0.12	2.23
Nitisol	6.3	3.3	0.8	9.7	2.2	0.15	0.26	12.3
± *IC*	0.2	0.31	0.2	0.8	0.3	0.02	0.07	0.76

MO, materia orgánica; CCB, capacidad de intercambio de bases; *IC*, intervalo de confianza (α=0.05).

Materia seca

En ambos suelos se encontró una respuesta positiva de las leguminosas a la inoculación, ya que todos los inóculos incrementaron el rendimiento en relación con el testigo sin inocular (Cuadro 2). No obstante, en cada condición edáfica se encontró un inóculo que produjo el mayor rendimiento. El mejor comportamiento para las leguminosas en el Vertisol correspondió a *R. intraradices*, mientras que en el Nitisol correspondió a *G. cubense*. No se observó interacción entre los tratamientos para el rendimiento de MS.

En la Figura 1 aparece el índice de eficiencia de los inóculos de HMA, el cual refleja el incremento porcentual del rendimiento de las leguminosas en relación con el testigo sin inocular. Esta variable mostró un comportamiento similar al rendimiento, es decir, aunque en ambos suelos los inóculos de HMA incrementaron la producción de biomasa por unidad de superficie, aquellos que produjeron los mayores efectos no fueron los mismos en cada condición edáfica.

En el Vertisol, el inóculo con *R. intraradices* alcanzó los mayores índices de eficiencia, tanto para stylo como para siratro, mientras que en el Nitisol los mayores resultados se obtuvieron con *G. cubense*. No obstante los inóculos más efectivos no presentaron los mismos índices de eficiencia en ambas condiciones edáficas, ya que en el Vertisol, *R. intraradices* alcanzó cerca de 42%, mientras que en el Nitisol, el inóculo con *G. cubense* presentó valores cercanos a 55%.

Cuadro 2. Efecto de los inóculos de HMA y de las especies de leguminosas en el rendimiento de MS de las leguminosas (t/ha) en dos suelos diferentes.

Inóculo/Leguminosa	Vertisol	Nitisol
Inóculo		
Testigo	5.80c	4.13c
G. cubense	7.10b	6.39a
F. mosseae	7.15b	5.15b
R. intraradices	8.25a	5.11b
EE	0.13**	0.11**
Leguminosa		
Stylo	7.19	5.08
Siratro	7.30	5.31
EE	0.10	0.08

Promedios con letras diferentes en cada columna difieren en forma significativa (P<0.05), según la prueba de Duncan. EE: error estándar.

Variables fúngicas

El efecto de los inóculos en las variables que caracterizan el funcionamiento micorrízico se evaluó a través de los indicadores: frecuencia de colonización, intensidad de la colonización o densidad visual, y contenido de esporas en la rizosfera. No se encontró interacción entre los tratamientos, aunque estos tuvieron un efecto significativo en el comportamiento de tales variables (Cuadro 3).

Los inóculos produjeron niveles de colonización micorrízica, densidad visual y contenidos de esporas en la rizosfera mayores que los alcanzados en el testigo sin inocular. Esto refleja el bajo nivel de ocupación radical de los HMA residentes, pero los mayores efectos en el Vertisol y Nitisol se obtuvieron, en ese orden, con los inóculos de *R. intraradices* y *G. cubense*.

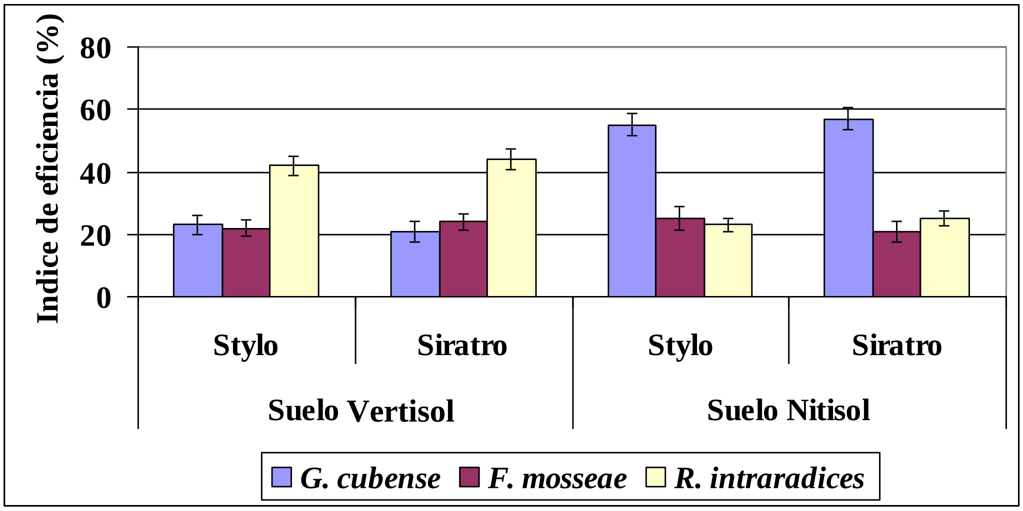

Figura 1. Índice de eficiencia de los inóculos con diferentes especies de HMA en cada leguminosa y tipo de suelo. Las barras verticales representan el intervalo de confianza de las medias; la ausencia de sobreposición de los intervalos de confianza indica diferencia significativa (α=0.05).

Cuadro 3. Efecto de los inóculos de HMA y de las leguminosas en las variables fúngicas evaluadas en dos suelos diferentes.

Inóculo/Leguminosa	Vertisol			Nitisol		
	Colonización (%)	Densidad visual (%)	Esporas/50 g de suelo	Colonización (%)	Densidad visual (%)	Esporas/ 50 g de suelo
Inóculo						
Testigo	15.7c	1.15c	160c	12.5c	1.13d	175d
G. cubense	24.0b	1.33b	314b	43.2a	2.35a	385a
F. mosseae	23.5b	1.31b	304b	30.5b	1.40c	314b
R. intraradices	42.7a	2.47a	441a	29.8b	1.51b	253c
EE	2.0**	0.02**	16**	0.3**	0.02**	9**
Leguminosa						
Stylo	27.6	1.58	307	29.2	1.58	277
Siratro	26.9	1.54	303	28.7	1.60	287
EE	1.4	0.02	11	0.2	0.02	7

Promedios con letras diferentes en cada columna difieren en forma significativa (P<0.05), según la prueba de Duncan. EE: error estándar.

Concentraciones de macroelementos

En el Vertisol, los inóculos incrementaron las concentraciones de N en la biomasa de la parte aérea de las leguminosas, y de N, P y K en el Nitisol, pero al igual que en las variables anteriores, su comportamiento fue diferente en cada condición edáfica (Cuadro 4). Los mayores contenidos de estos nutrientes se encontraron con los tratamientos que contenían *R. intraradices* en Vertisol y *G. cubense* en Nitisol. No se encontró interacción entre los tratamientos para las concentraciones de N, P y K en la biomasa de la parte aérea.

Cuadro 4. Efecto de los inóculos de HMA y las especies de leguminosas en las concentraciones de N, P y K (g/kg) en la biomasa aérea de las leguminosas en dos suelos diferentes.

Inóculo/Leguminosa	Vertisol			Nitisol		
	N	P	K	N	P	K
Inóculo						
Testigo	16.9c	2.20	13.9	14.1c	1.16c	12.4c
G. cubense	23.2b	2.14	13.7	25.4a	2.05a	14.2a
F. mosseae	22.2b	2.19	14.0	16.1b	1.78b	13.3b
R. intraradices	26.3a	2.16	13.8	16.4b	1.73b	13.2b
EE	0.4**	0.06	0.3	0.2**	0.05**	0.1**
Leguminosa						
Stylo	21.1	2.12	13.9	17.9	1.66	13.2
Siratro	23.2	2.21	13.7	18.0	1.69	13.3
EE	0.3	0.04	0.2	0.1	0.04	0.1

Promedios con letras diferentes en cada columna difieren en forma significativa (P<0.05), según la prueba de Duncan. EE: error estándar.

Discusión

La respuesta de las leguminosas a la inoculación mostró una alta dependencia micorrízica de ambas especies y también una mayor efectividad de los inóculos aplicados, en relación con los HMA residentes, para mejorar la nutrición e incrementar la producción de biomasa aérea de ambas especies. Estos resultados sugieren que en las condiciones del ensayo el manejo de la simbiosis micorrízica arbuscular vía inoculación debe ser tenida en cuenta como una práctica agronómica efectiva para mejorar la productividad de las especies forrajeras, tal como se recomienda para otros cultivos agrícolas (Rivera et al. 2007).

En este sentido, varios autores coinciden en que la introducción de especies de HMA seleccionadas puede ser una opción de manejo deseable e incluso necesaria, en los casos en que los HMA residentes no sean lo suficientemente efectivos para producir los beneficios deseados (Verbruggen et al. 2013; Cavagnaro et al. 2014; Oliveira et al. 2014).

Llama la atención el hecho de que en ambos suelos se haya logrado una inoculación efectiva con cantidades bajas de inóculos (600 g/ha), los cuales también aportaron cantidades bajas de esporas por hectárea. Sin embargo, la presencia en el inóculo de abundantes fragmentos de raicillas e hifas procedentes de la planta hospedera, conjuntamente con el método de inoculación empleado, pudo haber garantizado que además de las esporas, otros propágulos micorrízicos viables también quedaran en íntimo contacto con las semillas de las leguminosas, facilitando la colonización de sus raíces desde el momento de la germinación. Además, la escasa abundancia de HMA residentes, que se presume al observar la frecuencia e intensidad de la colonización y el número de esporas en la rizosfera de las plantas que no fueron inoculadas, también pudo haber facilitado la acción de los inóculos.

La ausencia de interacción entre las leguminosas y los inóculos de HMA que se observó en todas las variables, tanto en el Vertisol como en el Nitisol, indica que estos tuvieron un comportamiento similar en un mismo tipo de suelo; es decir, el inóculo que resultó más efectivo en un suelo, lo fue para ambas especies de leguminosas. Este comportamiento parece confirmar la baja especificidad entre especie de HMA y planta hospedera de las asociaciones micorrízicas (Öpik y Moora 2012; Verbruggen et al. 2012).

Cuando se comparan los resultados obtenidos en ambos suelos se observa que los inóculos tuvieron un comportamiento diferente, ya que el que produjo los mayores efectos en las variables fúngicas y el rendimiento y estado nutricional de las plantas no fue el mismo en cada condición edáfica. El inóculo con *R. intraradices* resultó el más efectivo para las leguminosas en el Vertisol, mientras *G. cubense* lo fue para las leguminosas cultivadas en el Nitisol. Estos resultados indican la existencia de alguna relación entre la efectividad de la cepa y el tipo de suelo, en la respuesta de ambas leguminosas a la inoculación de HMA. Según Verbruggen et al. (2013) el éxito de la inoculación micorrízica arbuscular depende no solo de las particularidades genéticas de ambos simbiontes, sino también de las condiciones del ambiente, en el cual el suelo juega un papel determinante para el establecimiento y la persistencia de los HMA introducidos. En este contexto, al evaluar los efectos de la inoculación micorrízica arbuscular en una amplia gama de suelos y cultivos con diferentes ciclos de crecimiento y requerimientos nutricionales, Rivera et al. (2015) concluyeron que en comparación con la especificidad del cultivo, el factor suelo determina en mayor grado la efectividad de las especies de HMA introducidas.

Al evaluar los índices de eficiencia de los inoculantes, también se pudo comprobar que el inóculo que contenía *R. intraradices* fue más efectivo para incrementar el rendimiento de stylo y siratro en el suelo Vertisol, mientras que el formulado con *G. cubense* lo fue para ambas leguminosas en el suelo Nitisol. Sin embargo, el inóculo con *R. intraradices* en el Vertisol presentó un índice de eficiencia menor que el inóculo con *G. cubense* en el Nitisol, lo que pudo ser debido a una disminución de la efectividad de la inoculación en aquel suelo, en función de su mayor fertilidad.

Se conoce que la efectividad de la simbiosis micorrízica se reduce a medida que aumenta la disponibilidad de nutrientes, ya que la entrega de los recursos del suelo a la planta hospedera a través de los HMA va perdiendo importancia (Antunes et al. 2012; Grman y Robinson 2013). No obstante, estos resultados también indican que los beneficios de la inoculación de las leguminosas forrajeras con HMA pueden extenderse a los suelos de mayor fertilidad y no solo a aquellos de baja fertilidad.

El efecto positivo de la inoculación micorrízica arbuscular en las leguminosas estuvo relacionado con su contribución al mejoramiento del estado nutricional de las plantas. Este resultado confirma lo observado por González et al. (2012) y Crespo Flores et al. (2014) cuando introdujeron especies seleccionadas de HMA en esquemas de biofertilización para leguminosas forrajeras. El hecho de que con los inóculos que resultaron más efectivos en cada condición edáfica se hayan alcanzado los mayores valores de frecuencia e intensidad de la colonización, indica que las plantas tuvieron un mejor acceso a los recursos del suelo a partir de la formación de mayores cantidades de estructuras micorrízicas, lo cual evidentemente contribuyó al incremento de las concentraciones de los macronutrientes en la biomasa de la parte aérea y en el rendimiento.

No obstante, el hecho de que el inóculo con *R. intraradices* no incrementara las concentraciones de P y K en la biomasa de las leguminosas en el Vertisol, sugiere que los contenidos de ambos elementos en este suelo fueron suficientes para satisfacer los requerimientos de las plantas. Se debe tener en cuenta que, a diferencia del Nitisol, el Vertisol contenía altos tenores de P asimilable y K intercambiable (ver Cuadro 1). Además, las concentraciones de P y K en la biomasa observadas en este suelo fueron semejantes a las encontradas por Lopes et al. (2011) y Dong et al. (2013), respectivamente, en plantas de stylo y siratro bien abastecidas de K.

Rivera et al. (2007) al evaluar la contribución de inóculos de HMA a la nutrición de diferentes cultivos, observaron que la simbiosis, más que favorecer la absorción de uno u otro elemento, se comportó como un mecanismo que permitió a las plantas obtener sus requerimientos nutricionales, en dependencia de sus propias necesidades y de la disponibilidad de los mismos en el sistema. Otros autores también han arribado a conclusiones similares (Maiti et al. 2011; An Dong et al. 2013).

Conclusión

A partir de los resultados obtenidos en este trabajo, se concluye que: (1) mediante la inoculación con especies efectivas de HMA es posible mejorar el estado nutricional y el rendimiento de las leguminosas forrajeras stylo y siratro, y (2) que la efectividad de la inoculación parece estar más relacionada con el tipo de suelo que con la especie de planta. No obstante, otros estudios deberán conducirse para definir cuáles propiedades del suelo determinan el comportamiento de las especies de HMA.

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Research paper

Grazing behavior and intake of goats rotationally grazing Tanzania-grass pasture with different post-grazing residues

Comportamiento y consumo de forraje de cabras en pastoreo rotacional del pasto Tanzania con diferentes niveles de forraje residual

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Abstract

This study aimed to evaluate intake and ingestive behavior of goats rotationally grazing Tanzania (*Panicum maximum* cv. Tanzânia 1) pastures with 2 levels of post-grazing residue. The experimental area consisted of 1.2 ha of Tanzania pasture divided into 12 paddocks (24 areas), managed under 2 post-grazing residues: low green (leaf + stem) herbage mass (GHM) post-grazing (LR, approximately 1,500 kg/ha GHM); and high GHM post-grazing (HR, approximately 3,000 kg/ha GHM). Each paddock was grazed for 3 consecutive days (D1, D2, D3) followed by 33 days rest and evaluated from October 2005 to April 2006. Animal behavior (grazing time, bite rate and bite size/weight) was evaluated on each grazing day. While goats spent more time grazing on LR than HR (P=0.02), bite rate did not differ between treatments or among days (P=0.31) and averaged 26.5 bites/min. In contrast, bite weight was greater in HR (0.15 g/bite) than in LR (0.12 g/bite), and decreased from D1 to D3 (P<0.001). Absolute dry matter intake of goats was greater in the HR (2.19 kg/d) than the LR (1.89 kg/d) treatment; however, differences were not significant (P>0.05) when intake was determined on a body weight or metabolic weight basis. Our findings are consistent with the general assumption that bite weight is a trade-off between quantity and quality of the herbage mass and is the main determinant of animal performance. More studies are needed to determine animal performance on the various treatments and to determine management strategies to provide a desirable balance between animal weight gain and pasture stability.

Keywords: Animal behavior, foraging, grazing systems, *Megathyrsus maximus*, plant - animal relations.

Resumen

En el estudio se evaluaron el comportamiento o hábito de pastoreo y el consumo del pasto Tanzania (*Panicum maximum* cv. Tanzânia 1) por cabras en pastoreo rotacional manejado con 2 niveles de residuos pospastoreo. El área experimental consistió en 1.2 ha de la pastura divididas en 12 potreros (24 áreas) que se manejaron con 2 niveles de residuos pospastoreo: baja masa de forraje verde (hoja + tallo) pospastoreo (LR, aproximadamente 1,500 kg de masa verde/ha); y alta masa de forraje verde pospastoreo (HR, aproximadamente 3,000 kg de masa verde/ha). Cada potrero fue pastoreado por 3 días continuos (D1, D2, D3), seguido por 33 días de descanso en el período octubre de 2005 a abril de 2006. Cada día de pastoreo se evaluó el comportamiento animal (tiempo de pastoreo, tasa de bocados y tamaño de bocados/peso). Los resultados mostraron que las cabras pasaron más tiempo pastando en LR que en HR (P=0.02) pero la tasa de bocados,

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con un promedio de 26.5 bocados/minuto, no difirió entre los tratamientos ni entre días (P=0.31). En contraste, el peso de los bocados fue mayor en HR (0.15 g/bocado) que en LR (0.12 g/bocado) y disminuyó del D1 al D3 (P<0.001). El consumo absoluto de materia seca fue mayor en el tratamiento HR (2.19 kg/d) que en LR (1.89 kg/d); sin embargo, las diferencias no fueron significativas (P>0.05), cuando el consumo se determinó con base en el peso corporal de las cabras o el peso metabólico. Nuestros resultados son consistentes con la hipótesis general de que el peso de un bocado refleja un compromiso entre la cantidad y la calidad del forraje y que es el principal determinante del rendimiento animal. Se necesitan más estudios para determinar el rendimiento animal en los diferentes tratamientos y para definir las estrategias de manejo para proporcionar un equilibrio deseable entre la ganancia de peso de los animales y la estabilidad de la pastura.

Palabras clave: Comportamiento animal, *Megathyrsus maximus*, relación planta - animal, sistemas de pastoreo.

Introduction

Due to their anatomical and physiological characteristics coupled with their acceptance of a wide range of forages, goats generally are quite adaptable to varying situations and are productive even in areas with low production potential. As a result, they are used widely throughout the world in native pasture systems but not necessarily in cultivated pasture systems. With the intensification of goat production, an understanding of goat behavior on cultivated tropical pastures is needed to define management practices that will allow effective forage intake and productivity.

According to Burns and Sollenberger (2002), several factors related to tropical forages, such as the amount of available forage, ease of prehension and chemical composition, can affect the voluntary intake of ruminants during grazing. Sward structure is generally characterized by height, bulk density and distribution of the morphological components (leaf and stem) of the canopy. In contrast with C3 grasses, the C4 grasses, such as Tanzania grass, *Panicum maximum* (now: *Megathyrsus maximus*) cv. Tanzânia 1, can accumulate high forage levels in tropical management systems with high percentages of stem and low bulk density, which can restrict the process of bite formation (Benvenutti et al. 2006). Consequently, the relationship between intake and animal performance is mainly influenced by sward structure, which can vary in tropical pastures depending on forage mass prior to and after grazing (Pedreira et al. 2005; Carnevalli et al. 2006). On the other hand, animals can alter grazing behavior in response to changes in sward structure by modifying the following variables: grazing time, bite rate and bite size.

A previous study with goats has demonstrated that increasing Tanzania-grass sward height (from 30 to 90 cm) resulted in increased forage and leaf mass, but decreased forage intake of the animals, so that 50-cm sward height was suggested as optimal (Ribeiro et al. 2012). Conversely, Rodrigues et al. (2013) observed that a pre-grazing sward height above 100 cm did not reduce the intake of the pasture by goats, attributing this fact to the high mass of green leaf, which constituted 76% of total forage dry matter. Even though management strategies based on sward height have been investigated with goats, inconsistent results indicate that height might not be the appropriate criterion on which to base management of tropical pasture.

In tropical pastures, post-grazing residue directly affects the potentially grazed strata as a consequence of changes in herbage bulk density (Difante et al. 2009), the main component of tropical grass sward structure that influences intake (Stobbs 1973b). Nonetheless, the appropriate post-grazing residue depends on the desired objective and type of animal being used in the system (Difante et al. 2009).

This study evaluated the intake and ingestive behavior (grazing time, bite rate, bite size/weight) of goats grazing pastures of Tanzania grass subjected to different rotational grazing intensities characterized by 2 amounts of post-grazing residue. This should assist in developing pasture management strategies to provide a desirable balance between individual animal performance and area productivity.

Materials and Methods

All animal procedures in this experiment were approved by the São Paulo State University's Institutional Animal Care and Use Committee.

The experiment was conducted at Jaboticabal, São Paulo, Brazil (21°15'22'' S, 48°18'58'' W; 595 masl), which experiences a tropical climate with dry winters and hot and rainy summers (Aw). During the experimental period (October 2005–April 2006), mean monthly temperature and rainfall were, respectively: 23.2, 24.6, 24.9, 25.1, 25.2, 25.3 and 24.9 ºC; and 135.4, 184.7, 138.7, 358.5, 81.2, 128.0 and 59.6 mm for October, November and December 2005, and January, February, March and April 2006.

Soil of the area under analysis was a clay oxisol (Soil Survey Staff 2010). According to pre-experimental soil analysis [65 ± 4% base saturation (V), 18 ± 5 mg P_{resin}/dm^3, 1 ± 0.3% K in Cation Exchange Capacity, CEC], 3,600 kg of a highly reactive dolomitic lime, 800 kg of simple superphosphate (8% P) and 5,300 kg of potassium chloride (50% K) were applied differentially (on a per-paddock basis, over the entire experimental area), so that soil was corrected to obtain high fertility levels: 80% V; 30 mg P/dm^3; 5% K in CEC. The experimental area consisted of a 1.2 ha Tanzania-grass (cv. Tanzânia 1) pasture that was divided into 12 paddocks (990 m^2), which were further subdivided into sub-paddocks and grazed rotationally in a 36-d cycle (3 days of grazing followed by 33 days of rest; Figure 1). Each sub-paddock was allocated to 1 of 2 treatments: low green (leaf + stems) herbage mass post-grazing (LR, approximately 1,500 kg of dry green herbage mass, GHM/ha); or high GHM post-grazing (HR, approximately 3,000 kg GHM/ha). Immediately after grazing, each paddock was fertilized with 40 kg urea (46% N)/ha and 33 kg K/ha, as potassium chloride (50% K).

On 31 October 2005, paddocks were mowed to about 45 cm for staging and standardization and the area was left ungrazed for 36 days. A 36-day pre-experimental grazing cycle was then introduced, during which stocking rate was adjusted regularly in each paddock, according to the visual analysis of the previous grazing day, to achieve a target post-grazing residue, according to future treatment.

Data were analyzed in a randomized block design using repeated measures (plots: high and low GHM post-grazing; sub-plots: cycles 11 January–15 February 2006, 16 February–23 March 2006, 24 March–28 April 2006; sub-sub-plots: days 1, 2 and 3 in an area). Grazing was carried out using F1 Boer x Saanen goats (approximately 18 months of age and 31.7 ± 5.7 kg body weight). Animals were permitted to graze between 07.00 and 19.00 h and were confined to pens during the night. The grazing strategy consisted of rotational stocking with stocking rate adjusted according to the put-and-take method to achieve the particular GHM post-grazing (Bransby and Maclaurin 2000).

Daily grazing times, including idle time plus rumination, were monitored in 4 tracer animals per treatment during 3 grazing cycles (sub-treatments) for the 3 days of occupation (sub-sub-treatments). Observations were made every 10 minutes during which time the types of activities executed by the grazing animals were recorded. At the same time, bite rate was determined by counting the number of bites in two 3-minute evaluations in both morning and evening, using a counter and a chronometer.

Bite weight was measured using 2 esophageal-fistulated (OF) goats each day in the areas being grazed. Early in the morning, cannulae of fistulated animals were removed and cotton collection bags attached to them. One OF goat was put into each area for 15 minutes, then they were swapped over for 15 minutes in a sequential manner: treatment/area grazing was reversed each day (or rather, the first paddock of the first treatment grazed by the first animal on the first day was the first paddock of the first treatment grazed by the second animal on the second day).

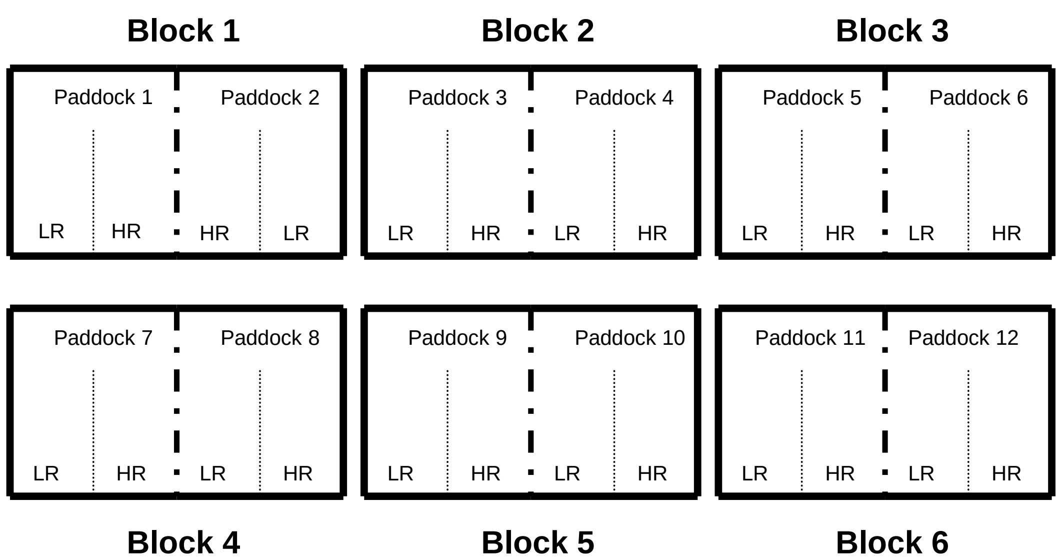

Figure 1. Arrangement of experimental area with pastures grazed rotationally, with 3 days grazing followed by 33 days rest. LR = low green herbage mass (GHM) post-grazing, approximately 1,500 kg/ha; HR = high GHM post-grazing, approximately 3,000 kg/ha.

During the 15-minute grazing, bites per animal were counted and the extrusa was collected. Extrusa samples were packed in plastic bags, weighed, placed in thermal boxes and stored at -18 °C. The samples were then thawed, dried, ground and analyzed for dry matter (DM; AOAC 1990; method number 930.15), ash (AOAC 1990; method number 920.39), indigestible neutral detergent fiber (NDF; Van Soest et al. 1991), indigestible acid detergent fiber (ADF) and lignin (Van Soest 1963).

Forage intake was estimated using an external marker technique in the same 4 tracer animals per treatment. A total of 1.2 g of chromium oxide (Cr_2O_3) was orally administered twice a day, starting 13 days before collection. Feces were collected twice a day (07.00 and 19.00 h) during the last 6 days of supplying the marker. Immediately after collection, samples were frozen, dried, ground and analyzed for DM, ash, NDF, ADF and lignin, following previously described procedures, and chromium as described by Williams et al. (1962).

Feces produced was estimated by the formula:

[1]

$$\text{Feces produced (g DM/d)} = \frac{\text{weight of } Cr_2O_3 \text{ supplied (g/d)}}{\text{concentration of } Cr_2O_3 \text{ in feces (g/g DM)}}$$

Hand plucked samples were collected daily in each pasture during the last 6 days of supplying the marker. The herbage was removed at approximately 10 locations per pasture based on the direct observation of number and type of bites of goats, which were grazing concomitantly. DM digestibility of hand-plucked samples (Bonnet et al. 2011) was calculated by the in vitro technique (Tilley and Terry 1963). Dry matter intake (DMI) was calculated from the production of feces and DM digestibility, according to the following equation:

[2]

$$\text{DMI (g DM)} = \frac{\text{feces produced (g DM/d)} \times 100}{(100 - \text{digestibility of DM})}$$

Owing to the difficulty in calculating intake of animals during each day of grazing using the marker technique, the relative intake was estimated by multiplying the variables of ingestive behavior (bite weight, bite rate and grazing time) and proportional intake was determined as a percentage of the highest intake per treatment.

Immediately before (pre-) and after (post-) grazing in the sampling paddocks, herbage mass was determined by harvesting material from 0.25 m^2 quadrats placed at 4 representative points, determined through visual appraisal within the experimental unit. Forage was then taken to the laboratory and the morphological components (leaf, stem and dead material) were separated manually. Components were dried in a forced-air oven at 55 °C for 72 h and the morphological composition of the harvested forage calculated.

Sward height was measured with a 1.5 m rule, as the mean height at 5 representative sites within each area.

Data were analyzed as a complete randomized block design with repeated measurements in time using the Mixed procedure of SAS version 9.2 (SAS Institute Inc., Cary, NC, v.9.2). The model included the fixed effects of treatments (1 d.f.), grazing cycle (2 d.f.), days of occupation (2 d.f.) and their interactions (4 d.f.) and the random effects of blocks (5 d.f.). Double error structures (UN@AR(1); UN@CS; UN@UN) were evaluated and the most adequate structure was chosen, following Bayesian Information Criterion (BIC). When no double error structure was convergent, only the error structure for the sub-sub-plot (days) was chosen by the above-mentioned criterion. The error structure of the sub-plot (Cycles) was CS (Compound Symmetry - matrix default of MIXED procedure). When significant, means were compared using Fisher´s protected LSD (DIFF option of LS MEANS). Significance was declared at P<0.05.

Results

Table 1 depicts the forage structural characteristics. Dry herbage mass (HM), GHM, dry stem mass (S), dry leaf mass (L), leaf:stem ratio (L:S) and height were lower in LR than in HR treatment (P<0.05). Increasing grazing time in a paddock (from Day 1 to Day 3) resulted in decreasing HM, GHM and L but did not affect S and dead material mass (D). In the LR treatment, the height decreased by 54% during grazing (from 57 cm at entry on Day 1 to 26 cm post-grazing), whereas in the HR treatment, the height decreased only 40% (from 61 cm on Day 1 to 37 cm post-grazing). Mean values for post-grazing GHM of treatments LR and HR were 2,006 ± 222 kg/ha and 3,133 ± 222 kg/ha, respectively (Figure 2).

Differing herbage mass on the 2 treatments affected goat behavior and intake with longer grazing time spent on the treatment with low post-grazing residue (P<0.02, Table 2). Time spent grazing on the third day was greater than on the first day on both a percentage basis and actual minutes (P=0.03).

Table 1. Mean (± s.e.; DM basis) total herbage mass (HM), green herbage mass (GHM), leaf (L), stem (S), dead material (D), leaf:stem ratio (L:S) and height of Tanzania pasture rotationally grazed by goats to 2 post-grazing heights (LR and HR).

	HM	GHM	L	S	D	L:S	Height
			(kg/ha)			ratio	(cm)
Treatment[1] (T)							
LR	5,520±362b[3]	3,078±204b	1,555±128b	1,522±92b	2,442±177	1.0±0.06b	38.2±1.0b
HR	6,173±362a	3,776±204a	2,085±128a	1,691±92a	2,397±177	1.3±0.06a	47.2±1.0a
P	0.006	<0.001	<0.001	0.01	0.63	0.001	<0.001
Day[2] (D)							
1	6,940±382a	4,361±242a	2,780±158a	1,581±98	2,579±242	1.8±0.07a	59.0±1.4a
2	6,152±382b	3,685±209b	2,062±130b	1,623±98	2,466±208	1.3±0.05b	45.4±1.0b
3	5,478±382c	3,090±209c	1,422±130c	1,667±98	2,387±221	0.9±0.08c	35.2±1.0c
Post-grazing	4,814±382d	2,569±204d	1,016±121d	1,553±98	2,244±193	0.6±0.03d	31.2±0.8d
P	<0.001	<0.001	<0.001	0.46	0.47	<0.001	<0.001
Interaction (P) TxD	0.23	0.12	0.06	0.35	0.79	0.08	0.01

[1]LR = low green herbage mass (GHM) post-grazing, approximately 1,500 kg/ha; HR = high GHM post-grazing, approximately 3,000 kg/ha.
[2]Day of grazing in the area.
[3]Means within columns and parameters followed by different letters differ at P<0.05 by Fisher's test.

Figure 2. Representation of post-grazing mass of treatments LR (low green herbage mass) and HR (high green herbage mass.

Table 2. Mean (± s.e.) grazing time, bite rate (BR) and bite weight (BWe) of F1 Boer x Saanen goats rotationally grazing Tanzania pasture (*Panicum maximum* cv. Tanzânia 1) with 2 levels of post-grazing residue (LR and HR).

	Grazing time[1]		BR	BWe
	(%)	(min)	(bite/min)	(g OM/bite)
Treatment[2] (T)				
LR	75±0.01a[4]	489±9a	27±1.9	0.124±0.004b
HR	71±0.01b	462±9b	26±1.9	0.155±0.004a
P	0.03	0.02	0.31	<0.001
Day[3] (D)				
1	71±0.01b	460±9b	27±1.9	0.184±0.005a
2	73±0.01ab	476±9b	27±1.9	0.133±0.005b
3	75±0.01a	491±9a	26±1.9	0.102±0.008c
P	0.03	0.03	0.30	<0.001
Interaction (P) T x D	0.22	0.30	0.65	0.82

[1]Time spent grazing daily: presented as % of time in the area and as actual minutes.
[2]LR = low green herbage mass (GHM) post-grazing, approximately 1,500 kg/ha; HR = high GHM post-grazing, approximately 3,000 kg/ha.
[3]Day of grazing in the area.
[4]Means within columns and parameters followed by different letters differ at P<0.05 by Fisher's test.

Bite weight of goats on the HR treatment was greater than on the LR treatment (Table 2) and bite weight decreased progressively with number of days in an area (P<0.001). On the other hand, bite rate did not differ among treatments (P=0.31) or grazing days (P=0.30).

Chemical composition of extrusa was not influenced by treatments (Table 3). However, NDF and ADF percentages in extrusa on the first grazing day in an area were lower than on the second and third grazing days.

Absolute DMI (kg/d) of goats was greater in the HR than in the LR treatment (P<0.05); however, differences were not significant (P>0.05) when intake was determined on a body weight (BW) or metabolic weight basis (Table 4). Using markers to estimate intake did not allow precise estimation of variation in forage intake as time in an area increased. While daily DMI can be overestimated using the variables of ingestive behavior (bite weight x bite rate x grazing time), to evaluate the impact of occupation day on pasture intake, we estimated DMI using these variables and calculated intake as a proportion of the highest intake per treatment (Figure 3). Dry matter intake, calculated using this methodology, decreased 25% between the first and second grazing day and 20% between the second and third day in an area. No interaction occurred between post-grazing residue levels and grazing days.

Table 3. Mean (± s.e.) acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin (LIG) and dry matter (DM) concentrations in extrusa of F1 Boer x Saanen goats rotationally grazing Tanzania (*Panicum maximum* cv. Tanzânia 1) pasture with 2 levels of post-grazing residue (LR and HR).

	ADF	NDF	LIG	DM
		(g/kg DM)		(g/kg)
Treatment[1] (T)				
LR	370±10	730±5	60±8	110±4
HR	380±10	730±5	70±8	110±4
P	0.28	0.23	0.18	0.15
Day[2] (D)				
1	360±10b[3]	710±5b	60±8	110±4
2	380±10a	740±5a	60±7	110±4
3	380±10a	740±5a	60±9	100±4
P	0.004	<0.001	0.84	0.32
Interaction (P) T x D	0.33	0.55	0.31	0.44

[1]LR = low green herbage mass (GHM) post-grazing, approximately 1,500 kg/ha; HR = high GHM post-grazing, approximately 3,000 kg/ha.
[2]Day of grazing in an area.
[3]Means within columns and parameters followed by different letters differ at P<0.05 by Fisher's test.

Table 4. Mean (± s.e.) dry matter intake of F1 Boer x Saanen goats rotationally grazing Tanzania (*Panicum maximum* cv. Tanzânia 1) pasture with 2 levels of post-grazing residue (LR and HR).

Treatment[1]	Dry matter intake (kg/d)	(g/d $BW^{0.75}$)	(% BW)
LR	1.89±0.11b[2]	98.8±6.0	3.7±0.2
HR	2.19±0.11a	106.1±6.0	3.9±0.2
P	0.01	0.23	0.44

[1]LR = low green herbage mass (GHM) post-grazing, approximately 1,500 kg/ha; HR = high GHM post-grazing, approximately 3,000 kg/ha.
[2]Means within columns followed by different letters differ at P<0.05 by Fisher's test.

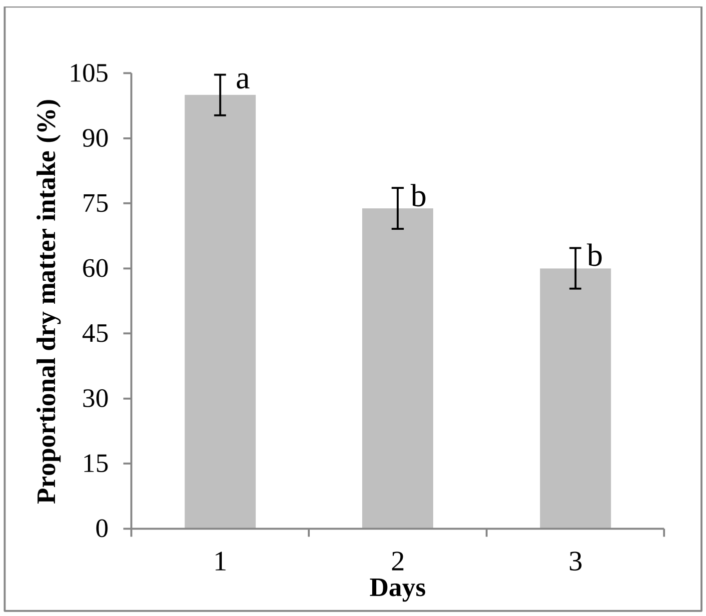

Figure 3. Estimated daily dry matter intake of goats (calculated using grazing time, bite number and bite weight), as a proportion (%) of the highest intake during 3 days of grazing, on rotationally grazed Tanzania (*Panicum maximum* cv. Tanzânia 1) pasture. Columns with different letters differ at P<0.05 by Fisher's test.

Discussion

This study has provided valuable information on the influence of management conditions of a tropical pasture on intake and ingestive behavior of goats. According to Burns and Sollenberger (2002), several forage variables, such as the amount of available forage, ease of prehension and chemical composition, can affect the voluntary intake of ruminants during grazing. This study has demonstrated the significant influence that forage mass exerts on the ingestive behavior of goats grazing tropical Tanzania grass (*Panicum maximum* cv. Tanzânia 1) and has provided evidence of why this occurs.

During daylight hours (07.00−19.00 h) when goats had access to pasture, 70–74% of the time was spent grazing. Solanki (1994) observed that goats in the field showed a diurnal pattern of grazing, preferring to graze during the morning and rest at other times; thus one might infer that the management used in this study did not unduly disturb the normal behavior of goats.

Daily intake of grazing goats is a function of bite rate and bite size (the amount harvested by an animal per bite), which depends on type of pasture (Stobbs 1973a; 1973b). We observed a strong relationship between bite weight and grazing time, with animals increasing grazing time to offset reduction in bite weight as supply of leaf in a pasture decreased, as occurred in the LR treatment or time spent in a particular area increased. Consistent with our results, Agreil and Meuret (2004) showed that, on temperate pastures, instantaneous intake rate of goats was affected more by bite mass than by bite frequency or rate, with bite weight varying between 0.01 and 1.72 g DM.

This study indicated no effect of plant structure on bite rate, corroborating the findings of Solanki (1994). As a result, animals had to graze longer to satisfy appetite as availability of leaf in the pasture declined and bite size was reduced but bite rate remained constant. In short pasture, Orihuela and Solano (1999) reported that the time spent foraging by grazing goats increased significantly as forage availability decreased. As pasture height increased above 50 cm, Ribeiro et al. (2012) observed a reduction in bite rate as searching for and handling bites in those pastures was more difficult than in shorter pastures or because leaves were sparse in the higher canopy strata. In our study, maximum pasture height was 59 cm, and the increase in the number of bites to satisfy appetite occurred in the shorter pasture by an increase in grazing time. Since the latter occurred when forage availability decreased, bite rate failed to respond to treatments and grazing days.

Ruminants adjust bite dimensions according to the force or effort involved in biting and the cost/benefit of consuming the bite (Benvenutti et al. 2006). Therefore, animals select forage that contains a high leaf:stem ratio because of the low physical resistance of leaves to being grazed (Flores et al. 1993). This preference for low-tensile resistance forage can be confirmed by a decrease in leaf percentage and leaf:stem ratio as animals spent more days in an individual area. As a result, ingestive behavior changed with grazing time in a particular area or with different pasture availability (Table 2), where both availability of leaf and leaf:stem ratio differed (Table 1). This type of behavior could be more relevant in goats due to their anatomical and physiological characteristics, which support feed selection (Van Soest 1994).

A decline in bite weight was proportional to a decrease in leaf mass and increase in stem percentage. However, when forage mass was low (treatment with low post-grazing mass), goats were forced to consume a higher percentage of stem, as evidenced by increasing ADF and NDF concentrations in extrusa as number of days grazing a particular area increased. This is due to lack of options with an increase in grazing time and a decrease in bite size. Estimates of daily intake showed a reduction in DMI as time in an area increased from 1 day to 3 days (Figure 3). As mentioned before, increases in time spent grazing failed to compensate for the decrease in bite size, resulting in lower intake in conditions with low forage availability. Therefore, our findings are consistent with the general assumption that bite weight is a function of trade-offs between quantity and quality of the herbage mass as well as configuration of the pasture.

We observed that goats grazed in a uniform manner (Figure 4), from top to bottom, through successive layers in contrast to the behavior of cattle and sheep, which tend to graze deeper resulting in a less evenly grazed sward (Del Pozo et al. 1998). Since the goats harvested forage from all layers of the sward, only minor height variation occurred within the area. Del Pozo and Osoro (1997) reported the same behavior in temperate pastures. These findings suggest that goats behave differently from other ruminant species when grazing tropical or temperate pastures.

The familiarity of animals with the observer should be taken into account when conducting studies of this nature. Similar to all ethological surveying, presence of the observer(s) should produce minimal disturbance to normal animal behavior. Our goats were familiar with humans and the 7-day adaptation period between the observer and the animal was designed to minimize interference with normal animal behavior. We consider this was achieved and our data truly reflect what would occur in a commercial situation. Agreil and Meuret (2004) evaluated the periods for goats and sheep to become familiar with an observer and verified that, whereas sheep took 45 days, goats took only 5 days to adapt to human presence and behave normally during observations.

This study has indicated that pasture availability is important in determining intake and performance of goats on improved pasture of Tanzania grass. Under the rotational grazing system used, animal intake declined dramatically by day 3 of grazing in a paddock. This would have resulted in a significant reduction in animal performance over the 3-day period. Since no liveweight data are reported in this study, we are unable to indicate how serious this reduction in performance might be.

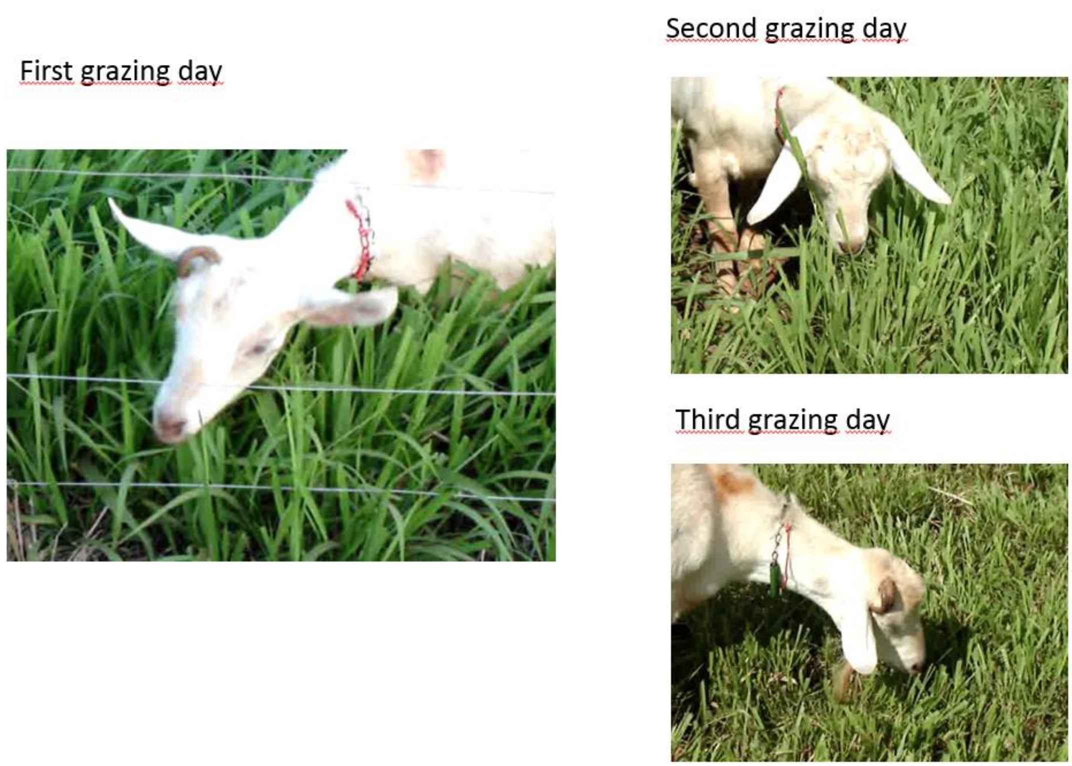

Figure 4. Representation of uniform grazing, from top to bottom, of goats during the 3 days of grazing in the paddock.

Further studies are needed where animal weight gains are measured to determine the effects of the changes in intake on animal performance. Based on the data generated it may be possible to speculate on what might be an appropriate grazing regime for utilization of Tanzania grass by goats to obtain an optimal balance between individual animal performance, production per unit area and performance of pasture.

Acknowledgments

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Research paper

Tillering of Marandu palisadegrass maintained at fixed or variable heights throughout the year

Altura de corte y macollamiento de Urochloa brizantha *cv. Marandu*

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Abstract

Satisfactory tillering is the basic attribute to ensure stability and productivity of a grass population. We aimed to develop an understanding of tillering in *Urochloa brizantha* syn. *Brachiaria brizantha* cv. Marandu (Marandu palisadegrass) maintained at constant or variable heights during the various seasons of the year and to identify defoliation strategies that optimize tillering. In an experiment conducted in Uberlândia, Minas Gerais, Brazil, 3 defoliation strategies were studied: sward kept at 30 cm during the whole year (constant height); kept at 15 cm in fall/winter, 30 cm in spring and 45 cm in summer (increasing height); and kept at 45 cm in fall/winter, 30 cm in spring and 15 cm in the summer (decreasing height). The experiment was completely randomized, with 4 replicates. The following variables were evaluated: tiller appearance (TAR), mortality (TMR) and survival (TSR) rates; the balance (BAL) between TAR and TMR; tiller population stability (TPS); and number of tillers/m^2 (NT). In winter and late spring, TAR and BAL were low, while in early spring, the sward with decreasing height showed high TAR, BAL and TPS. The NT was higher when managed with increasing height than with other height strategies. Lowering pasture height from 45 to 30 cm after the winter increased TAR in early spring. Grazing studies seem warranted to assess how these results can be reproduced under grazing and how pasture yield and quality plus animal performance compare with those under the fixed grazing height regimen.

Keywords: Defoliation, grazing management, pasture height, tillers, *Urochloa brizantha.*

Resumen

La capacidad para formar macollas es un atributo básico para garantizar la sostenibilidad y productividad de una población de plantas en una pastura. En Uberlândia, Minas Gerais, Brazil se evaluó el macollamiento de *Urochloa brizantha* (syn. *Brachiaria brizantha*) cv. Marandú manejado a alturas constantes o variables durante las diferentes estaciones del año con el objetivo de identificar las estrategias de defoliación que optimicen el macollamiento de este cultivar. Las estrategias de defoliación consistieron en: pastura a una altura de 30 cm a través del año (altura constante); pastura a 15 cm en otoño/invierno, 30 cm en primavera y 45 cm en verano (altura creciente); y pastura a 45 cm en otoño/invierno, 30 cm en primavera y 15 cm en verano (altura decreciente). El diseño del experimento fue completamente al azar, con 4 repeticiones. Las variables evaluadas fueron: tasas de aparición de rebrotes (TAR), mortalidad de rebrotes (TMR) y supervivencia de rebrotes (TSR); equilibrio (BAL) entre TAR y TMR; estabilidad de la población de rebrotes (TPS); y número de rebrotes/m^2 (NT). En invierno y hacia finales de la primavera, TAR y BAL fueron bajos, mientras que a principios de la primavera, el tratamiento decreciente mostró altos TAR, BAL y TPS. El NT fue mayor en el

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tratamiento creciente que en los demás tratamientos. La reducción de la altura de la pastura de 45 a 30 cm después del invierno aumentó la TAR a principios de la primavera. Se sugieren estudios de pastoreo para investigar cómo estos resultados pueden ser reproducidos en condiciones de pastoreo y cómo la producción y calidad de la pastura más la producción animal se comparan con aquellas bajo un régimen de pastoreo a altura fija.

Palabras clave: Altura de pastura, defoliación, manejo de pastoreo, rebrotes, *Urochloa brizantha*.

Introduction

In Brazil, it is estimated that 85% of pastures belong to the genus *Urochloa* syn. *Brachiaria* (Macedo 2004), and that, of the 190 Mha, 50 Mha are monocultures of *U. brizantha* cv. Marandu (Jank et al. 2014). Despite the widespread adoption of these pastures, adoption of adequate management practices is low, resulting in low livestock production (Ferreira et al. 2013). For Marandu palisadegrass to express its forage production potential, proper defoliation management should be adopted. Defoliation influences light interception and photosynthesis in the sward (Lara and Pedreira 2011), key processes in the development of the forage plant, and consequently in pasture production.

Tillers are the basic units of development of forage grasses (Hodgson 1990), so understanding of plant density and tiller dynamics (appearance, death and survival) are essential to comprehend the effects of defoliation management on pasture production (Giacomini et al. 2009; Fialho et al. 2012).

Continuous stocking of Marandu palisadegrass to maintain pasture at given heights has been recommended (Calvano et al. 2011). However, climatic conditions influence pasture tillering (Santos et al. 2011), such that a particular pasture height may optimize tillering in one season of the year, but not in others (Sbrissia et al. 2010). In view of this scenario, the management of a pasture should be planned seasonally to the extent that the number of stock on the farm allows flexibility.

This study aimed to develop an understanding of the tillering patterns in Marandu palisadegrass kept at constant or variable heights during the various seasons of the year and thus identify defoliation strategies to optimize tillering, and hence stability and productivity of the plant population in the pasture.

Materials and Methods

The experiment was conducted from January 2013 to March 2014 in the Forage Section, on Capim Branco Farm, at the Faculty of Veterinary Medicine of the Federal University of Uberlândia (UFU), located in Uberlândia, Minas Gerais, Brazil (18º53'19" S, 48°20'57" W; 776 masl). The climate of Uberlândia is a tropical Cwa type, with mild, dry winters and well-defined dry and rainy seasons (Köppen 1948). The average annual temperature is 22.3 ºC, varying between 19.1 and 23.9 ºC. Average annual precipitation is 1,584 mm, varying from 1,260 to 1,680 mm.

Climatic data during the experimental period were obtained at the meteorological station located approximately 200 m from the experimental area (Figures 1 and 2).

Before the experiment commenced, soil samples were collected from the 0–10 cm layer and analyzed to reveal the following chemical characteristics: pH (H_2O): 6.1; P: 9.4 mg/dm^3 (Mehlich-1); K^+: 156 mg/dm^3; Ca^{2+}: 5.5 cmol$_c$/dm^3; Mg^{2+}: 1.7 cmol$_c$/dm^3; Al^{3+}: 0.0 cmol$_c$/dm^3 (KCl 1 mol/L); effective CEC: 7.6; CEC at pH 7.0: 10.3; and base saturation: 74%. Based on these results, 21.8 kg P/ha (as single superphosphate), 60 kg N/ha (as urea) and 41.5 kg K/ha (as KCl) were broadcast on all plots in the first week of January 2013 and again on 1 January 2014.

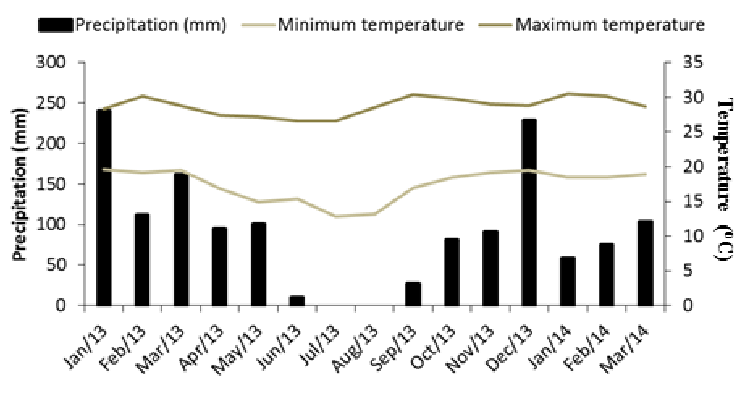


Figure 1. Monthly maximum and minimum mean daily temperatures and precipitation from January 2013 to March 2014. The seasons are: winter, July–September 2013; early spring, October 2013; late spring, November–December 2013; and summer, January–March 2014.

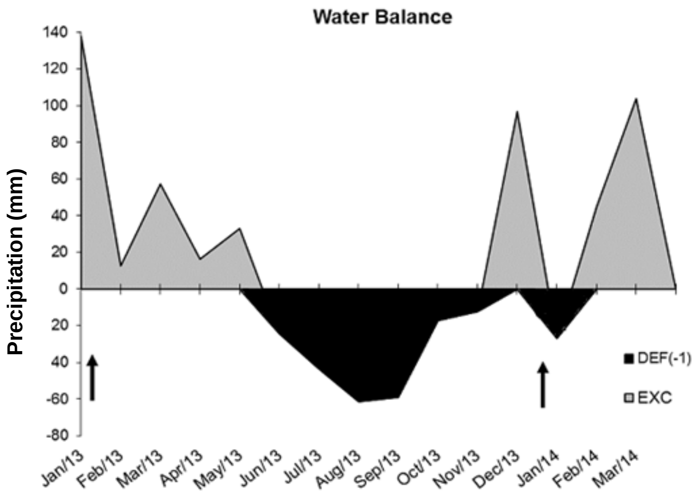


Figure 2. Water balance in the soil from January 2013 to March 2014. Arrows indicate the times when fertilizer was applied. The seasons are: winter, July–September 2013; early spring, October 2013; late spring, November–December 2013; and summer, January–March 2014. DEF (-1) = Deficit; EXC = Excess.

The experimental area consisted of 12 plots (experimental units) of 6 m^2 each, of a healthy Marandu palisadegrass established in 2000.

Three defoliation strategies for Marandu palisadegrass were evaluated: kept at a constant height of 30 cm during the entire experimental period (Sbrissia and Silva 2008); kept at 15 cm in fall and winter, 30 cm in spring and 45 cm in summer (increasing height throughout the year); and kept at 45 cm in fall and winter, 30 cm in spring and 15 cm in summer (decreasing height).

The grass kept at 15 cm in fall and winter took approximately 1 month to reach 30 cm in spring. Likewise, the pasture was not cut for about a month at the end of spring to allow it to reach 45 cm in summer. In contrast, the grass maintained at 45 cm in fall and winter was cut to 30 cm in the beginning of spring (October 2013) and to 15 cm in early summer (January 2014). By contrast, pastures managed at 30 cm in fall and winter continued under the same management until the end of the experiment.

The experimental period in which the evaluations took place was divided, based on the similar patterns of the response variables, into the following subperiods: winter (July−September 2013); early spring (October 2013); late spring (November−December 2013); and summer (January−March 2014). The experimental design was completely randomized, arranged as split plots in time, with 4 replicates.

From January 2013, cuts were made for the desired heights to be implemented in the fall and winter seasons, according to the treatments. The period January−March 2013 was considered the acclimation of the pastures to the various heights. Heights were measured once weekly in fall and winter and twice weekly in spring and summer at 10 points throughout each plot, using a graduated ruler. Excess forage above the desired height was cut and removed manually on each occasion. Thus, in each season of the year, the pastures were maintained at relatively constant heights, through mechanical defoliations.

In early July 2013, evaluation of tillering dynamics commenced in 2 areas, which were delineated with a PVC ring of 30 cm diameter (0.07 m^2) that was fixed in the soil using metal clasps. Initially, all tillers were marked with strings of the same color, and identified as the base generation (BG). Thirty days afterwards, live tillers inside the BG rings were counted and dead ones were calculated by difference. New tillers that appeared were marked with strings of a different color, counted and identified as first generation (G1), and this procedure was repeated every 30 days. From these data, tiller appearance (TAR), tiller mortality (TMR) and tiller survival (TSR) rates were calculated on a percentage basis every 30 days. TAR corresponded to the number of new tillers, multiplied by 100 and divided by the total number of existing tillers at the beginning of that period; TMR consisted of the total number of dead tillers from the previous marking, multiplied by 100 and divided by the total number of tillers in the previous generations. TSR was obtained by subtracting TMR from 100%. The balance between TAR and TMR in each period of the year was calculated by subtracting TMR from TAR. Based on the original tiller count data from the base generation, the monthly variation curves of the number of tiller generations were generated monthly, and the total number of tillers/m^2 present in the area of evaluation of tillering dynamics was also calculated.

Tiller population stability was calculated by the equation proposed by Bahmani et al. (2003):

$$FP/IP = TSR\ (1 + TAR)$$

in which FP/IP represents the current or final tiller population (FP), expressed as a percentage of the original or initial tiller population (IP) in a given period of evaluation.

For analysis of the data, the results were grouped according to the seasons of the year (winter, early spring, late spring and summer). Initially, the dataset was analyzed to check if it met the assumptions of variance analysis (normality and homogeneity). For the statistical assumptions to be met, data for the response variable 'tiller mortality rate' were transformed using the square root. The data were analyzed using the MIXED (mixed models) procedure of the SAS® (Statistical Analysis System) version 9.2 program. The variance and co-variance matrix was chosen using Akaike's Information Criterion (Wolfinger 1993). Treatment means were estimated using the LSMEANS option and compared by Student's t test at 5% probability.

Results

Tiller appearance rate (TAR) was influenced by an interaction between sward height and time of year (P=0.0021). In winter and late spring, TAR was lower than at other times of the year, and was independent of sward height. In early spring and summer, swards with decreasing height showed a higher TAR than those with constant or increasing heights (Figure 3) and within heights TAR was higher in early spring than summer.

Tiller mortality rate (TMR) was influenced by an interaction between sward height and time of year (P=0.0002). In winter and late spring, no difference was detected in TMR between defoliation strategies, but in summer, the sward with decreasing height showed a higher TMR and in early spring the sward with constant height had lower TMR (Figure 4).

Tiller survival rate (TSR) was influenced by an interaction between sward height and time of year (P<0.0002). While in winter and early and late spring, TSR did not vary between defoliation management strategies, in summer, TSR in the sward with decreasing height was lower than in those with constant and increasing heights (Figure 5).

The balance between tiller appearance and mortality rates (BAL) was influenced by an interaction between sward height and time of year (P<0.0001). In winter and late spring, BAL was lower than for other seasons and negative for all studied heights. In early spring, the sward with decreasing height achieved a higher BAL than others, while in summer, sward height did not affect BAL (Figure 6).

Tiller population stability (TPS) also was influenced by an interaction between sward height and time of year (P<0.0001). Although no difference in TPS was found between sward heights during winter and late spring, in early spring, TPS was higher in the sward with decreasing height than in those with constant and increasing heights. In summer, TPS was lower in the sward with decreasing height than in that with constant height (Figure 7). Overall, TPS was better in early spring than in other seasons.

Sward height influenced (P=0.0039) the number of tillers in Marandu palisadegrass, which showed higher values when managed with increasing height than with other height strategies (Figure 8).

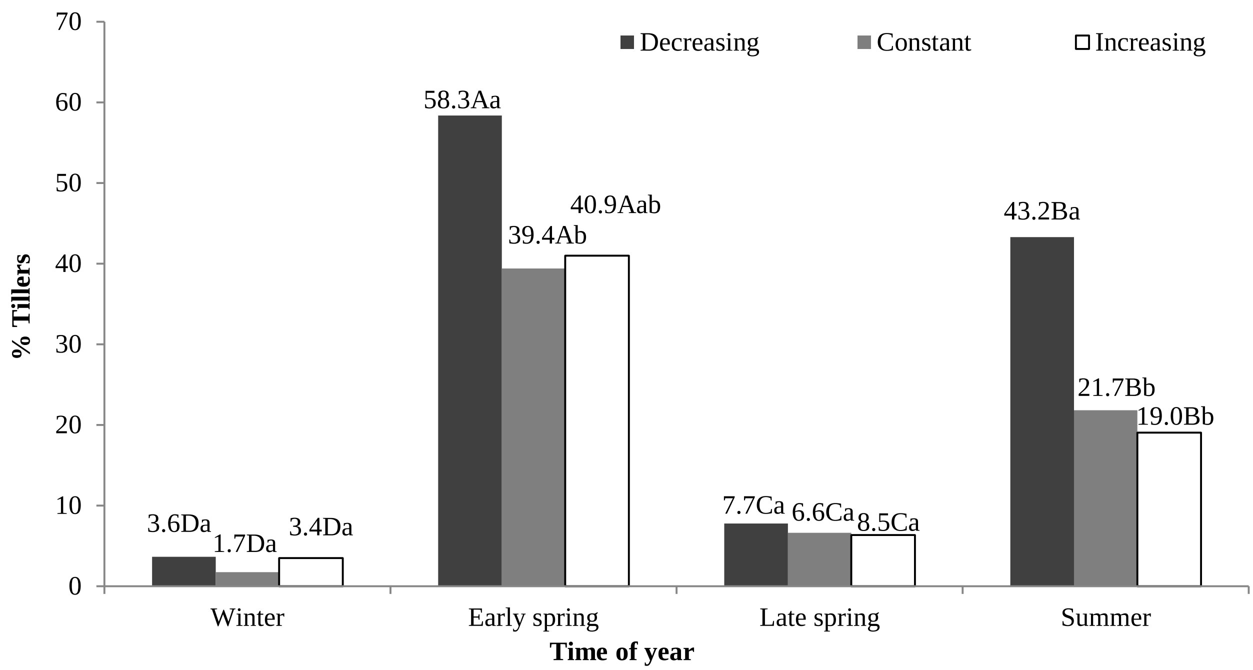


Figure 3. Effects of time of year and sward height on monthly tiller appearance rate (TAR) in Marandu palisadegrass.
Decreasing: sward at 45 cm in fall and winter, 30 cm in spring and 15 cm in summer; Constant: sward at 30 cm during the entire experimental period; Increasing: sward at 15 cm in fall and winter, 30 cm in spring and 45 cm in summer. Lower-case letters compare heights within times of the year and upper-case letters compare times of the year within heights. Where letters are different, means differ (P<0.05).

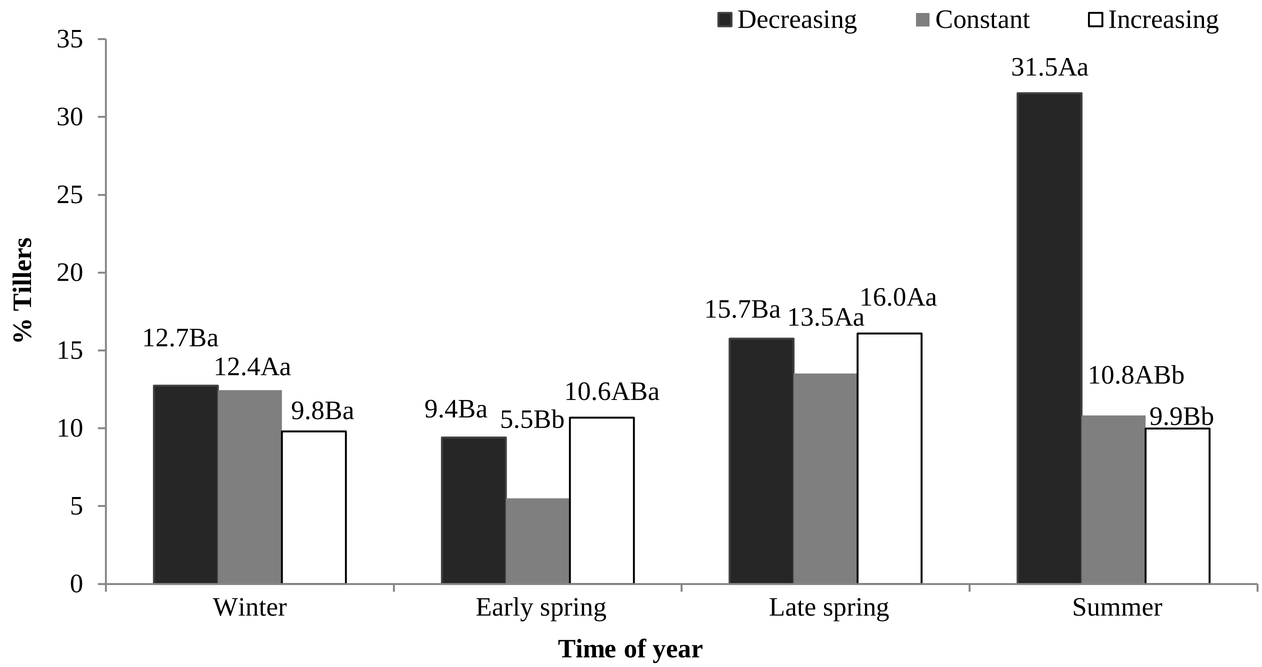


Figure 4. Effects of time of year and sward height on monthly tiller mortality rate (TMR) in Marandu palisadegrass.

Decreasing: swards at 45 cm in fall and winter, 30 cm in spring and 15 cm in summer; Constant: swards at 30 cm during the entire experimental period; Increasing: swards at 15 cm in fall and winter, 30 cm in spring and 45 cm in summer. Lower-case letters compare heights within times of year and upper-case letters compare times of year within heights. Where letters are different, means differ (P<0.05).

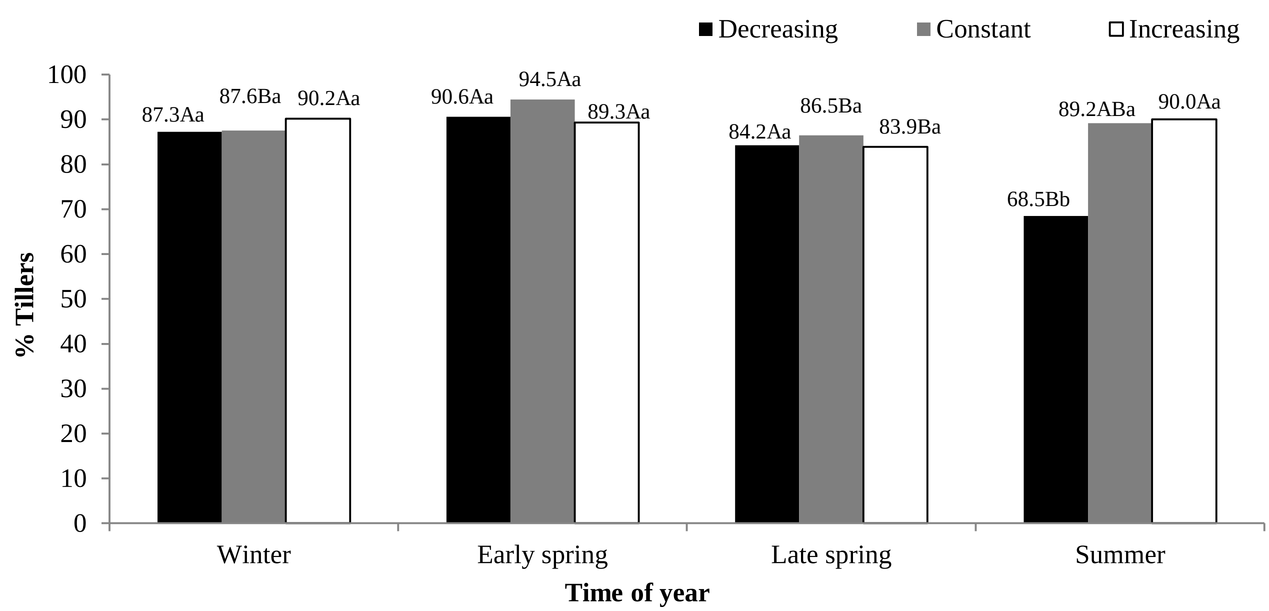


Figure 5. Effects of time of year and sward height on monthly tiller survival rate (TSR) of Marandu palisadegrass.

Decreasing: swards at 45 cm in fall and winter, 30 cm in spring and 15 cm in summer; Constant: swards at 30 cm during the entire experimental period; Increasing: swards at 15 cm in fall and winter, 30 cm in spring and 45 cm in summer. Lower-case letters compare heights within times of year and upper-case letters compare times of year within heights. Where letters are different, means differ (P<0.05).

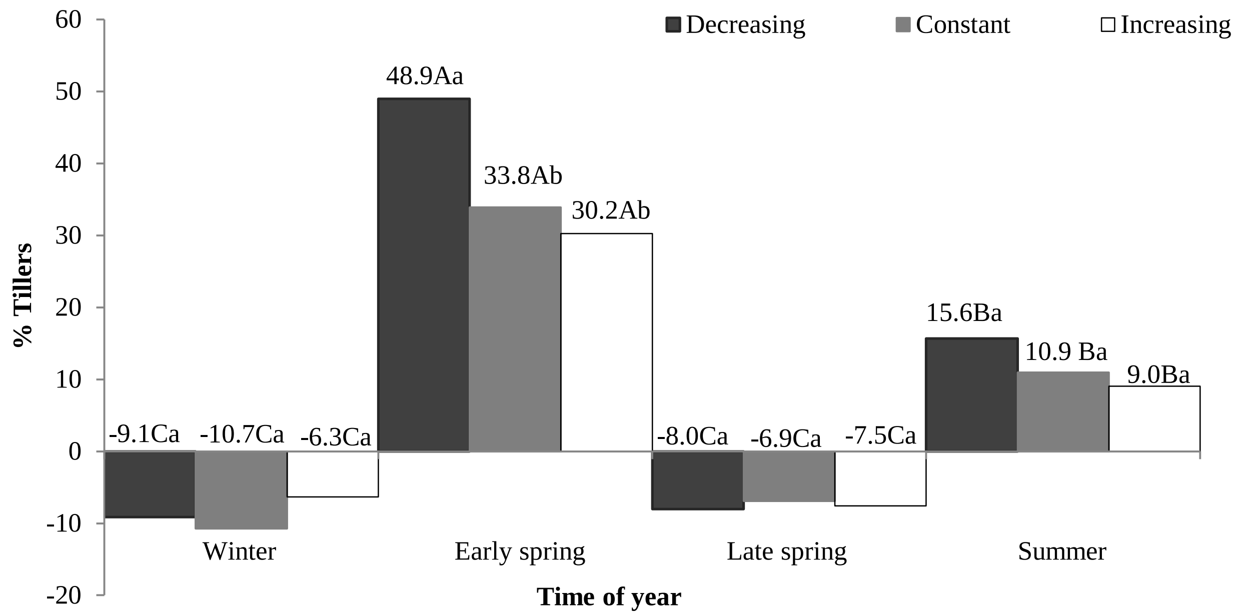


Figure 6. Effects of time of year and sward height on balance (BAL) between monthly tiller appearance and mortality in Marandu palisadegrass.

Decreasing: swards at 45 cm in fall and winter, 30 cm in spring and 15 cm in summer; Constant: swards at 30 cm during the entire experimental period; Increasing: swards at 15 cm in fall and winter, 30 cm in spring and 45 cm in summer. Lower-case letters compare heights within times of year and upper-case letters compare times of year within heights. Where letters are different, means differ (P<0.05).

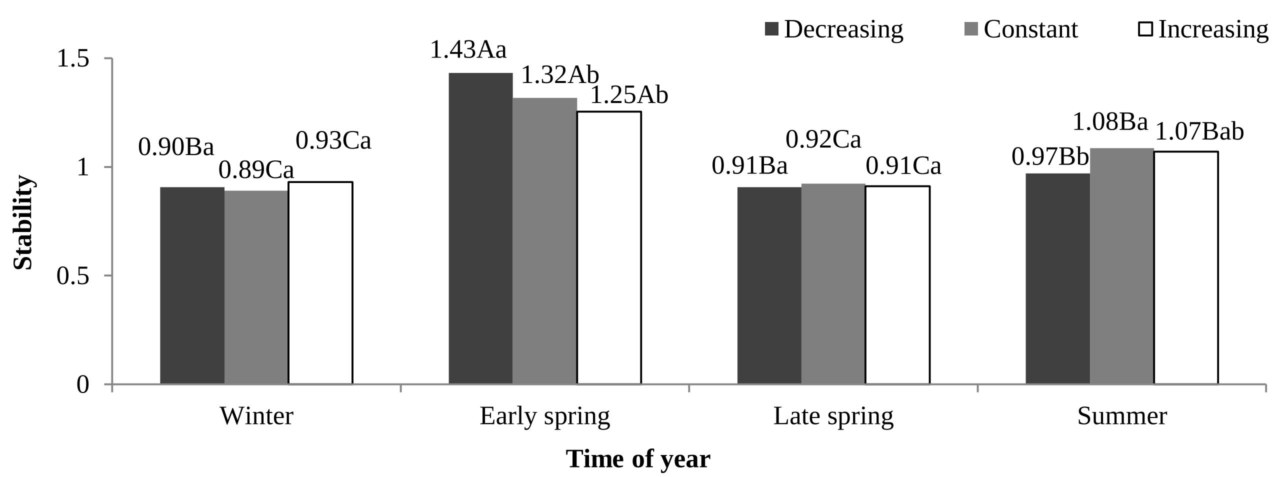


Figure 7. Effects of time of year and sward height on monthly tiller population stability (TPS) in Marandu palisadegrass.

Decreasing: sward at 45 cm in fall and winter, 30 cm in spring and 15 cm in summer; Constant: sward at 30 cm during the entire experimental period; Increasing: sward at 15 cm in fall and winter, 30 cm in spring and 45 cm in summer. Lower-case letters compare heights within times of year and upper-case letters compare times of year within heights. Where letters are different, means differ (P<0.05).

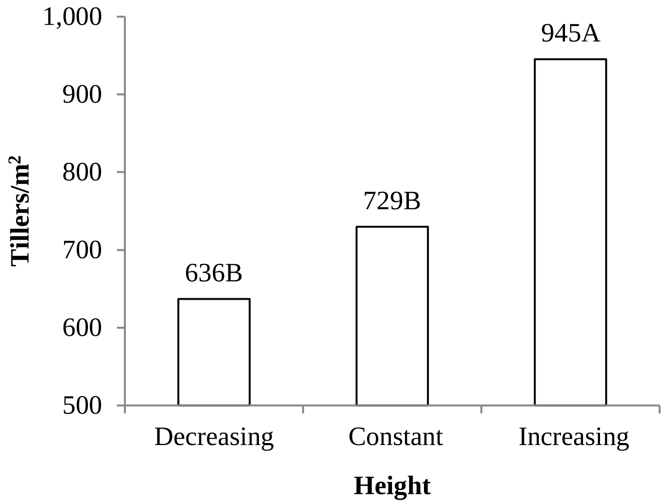


Figure 8. Effects of height management on number of tillers in Marandu palisadegrass.
Decreasing: sward at 45 cm in fall and winter, 30 cm in spring and 15 cm in summer; Constant: sward at 30 cm during the entire experimental period; Increasing: sward at 15 cm in fall and winter, 30 cm in spring and 45 cm in summer. Means followed by different upper-case letters differ (P<0.05).

Discussion

This study has shown the important interactions between height management of a Marandu palisadegrass pasture and season of year on degree of tillering in the pasture.

Climatic conditions were unfavorable for growth of the sward during winter, which explains the low TAR in this period, when low temperature, precipitation and incident radiation were experienced (Figures 1 and 2). In July and August, minimum temperatures were below 15 ºC and there was no rain (Figure 1). These environmental conditions inhibit the development of buds located at the base or lateral portions of the plant (Matthew et al. 2000). According to McWilliam (1978), the ideal temperature for growth of tropical grasses ranges from 30 to 35 ºC, while growth is severely impaired from 10 to 15 ºC.

A high TSR in winter (Figure 3) failed to offset the low TAR in this period, resulting in a negative BAL, as well as a TPS lower than 1.0. When TPS is lower than 1.0, the tiller population decreases, because the appearance of tillers is insufficient to offset their poor survival (Bahmani et al. 2003).

In early spring, the change in climatic conditions, which became more favorable for plant growth (Figures 1 and 2), stimulated the development of basal buds on new tillers, so TAR values (Figure 3) exceeded TMR levels (Figure 4). As a consequence, in early spring, BAL (Figure 6) and TPS were the highest recorded during the study.

However, tillering rates declined in late spring (Figure 3). The intense tillering in early spring increased the number of tillers in the sward, probably causing shading at the base of the plants, which possibly inhibited the development of basal buds on new tillers in late spring. As a result, BAL became negative again (Figure 6), and TPS was lower than 1.0, typical of swards with unstable tiller populations (Caminha et al. 2010).

Despite the soil water deficit (Figure 2), tillering increased from late spring to summer (Figure 3). This might be a response to the application of NPK fertilizer in January 2014, which stimulated the Marandu palisadegrass and increased the percentage of buds that developed on new tillers (Morais et al. 2006).

The higher TAR values in early spring and summer compared with winter (Figure 3) support the results of Santos et al. (2011), who obtained low TAR in winter and high TAR in spring and summer in a *Brachiaria decumbens* cv. Basilisk pasture managed under continuous grazing by cattle.

The high TAR in early spring indicates renewal of tillers, which demonstrates an increased percentage of younger tillers in the sward. These tillers have higher leaf appearance and elongation rates (Paiva et al. 2011), in addition to having better morphological composition and nutritive value (Santos et al. 2010). In addition, they are more responsive to nitrogen fertilization, which is appropriate for improving the performance of a grazing animal.

The response pattern of TPS observed in this study (Figure 7) corroborates that obtained by Sbrissia et al. (2010), who found TPS values slightly lower than 1.0 in Marandu palisadegrass pastures kept at 30 cm at times when environmental conditions were least favorable for growth. As environmental conditions improved in spring, the TPS values increased.

Since it showed lower forage mass, the sward kept at a lower height in fall and winter (increasing height) was expected to have a lower demand for growth factors like light and water, which would improve the water and carbon balance in plants at times when these resources were limited (Taiz and Zeiger 2012). Thus, the sward with increasing height would have low senescence and reduced dead tissue at the base of plants, thereby allowing light to penetrate to basal buds, a predisposing factor for tillering (Martuscello et al. 2009). However, this hypothesis was not confirmed in this study, possibly due to the increased shading within the sward with the increasing height in early spring from 15 to 30 cm.

Moreover, the increased shading within the sward as height increased in early spring might have elevated the allocation of nutrients and photoassimilates to older tillers as opposed to formation of new tillers, which would mean higher energy requirements by the plant (Langer 1963; Briske et al. 1996).

In contrast, the greater tillering in early spring in the sward with decreasing height might have been stimulated by its rapid lowering from 45 to 30 cm. This could have resulted in sudden increased incidence of light at the base of the sward, and consequently stimulated tillering (Figure 3). As a result BAL (Figure 6) and TPS (Figure 7) were higher in the sward with decreasing height than in swards with constant and increasing heights in early spring.

The same physiological response might explain the higher TAR in summer in the sward with decreasing height compared with the other defoliation strategies (Figure 3), as this pasture was lowered in late spring from 30 to 15 cm, which broke apical dominance by removing apical meristems of existing tillers.

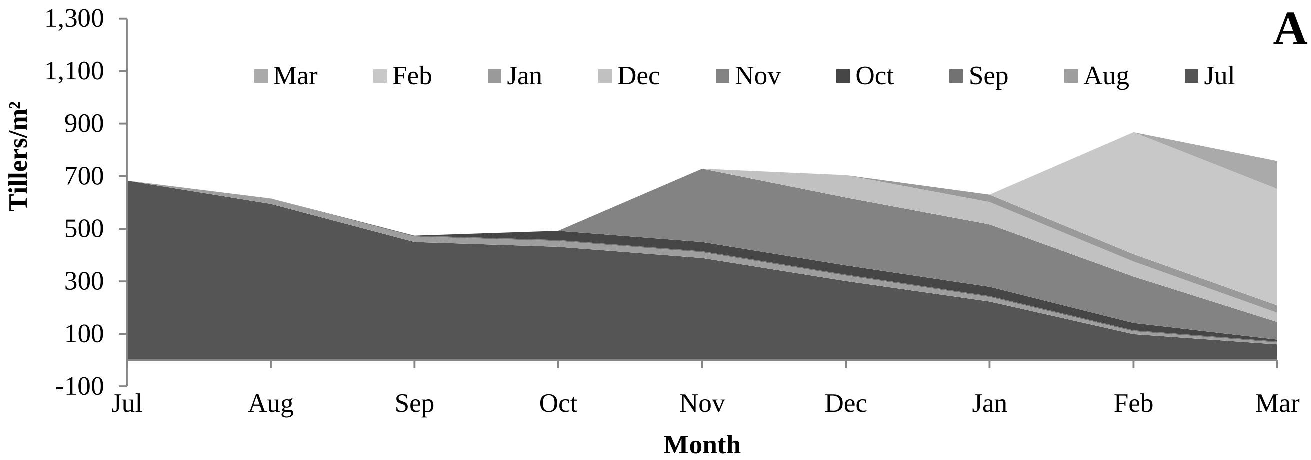
The higher TMR in summer, with consequent reduction of TSR in the sward with decreasing height, may be a function of the reduction in height of pasture eliminating the apical meristem of many tillers, which resulted in their deaths (Matthew et al. 2000). The decreasing height strategy obviously produced a higher tiller turnover rate with a more positive balance (BAL) than other sward management strategies in times of active growth (early spring and summer, Figure 6).

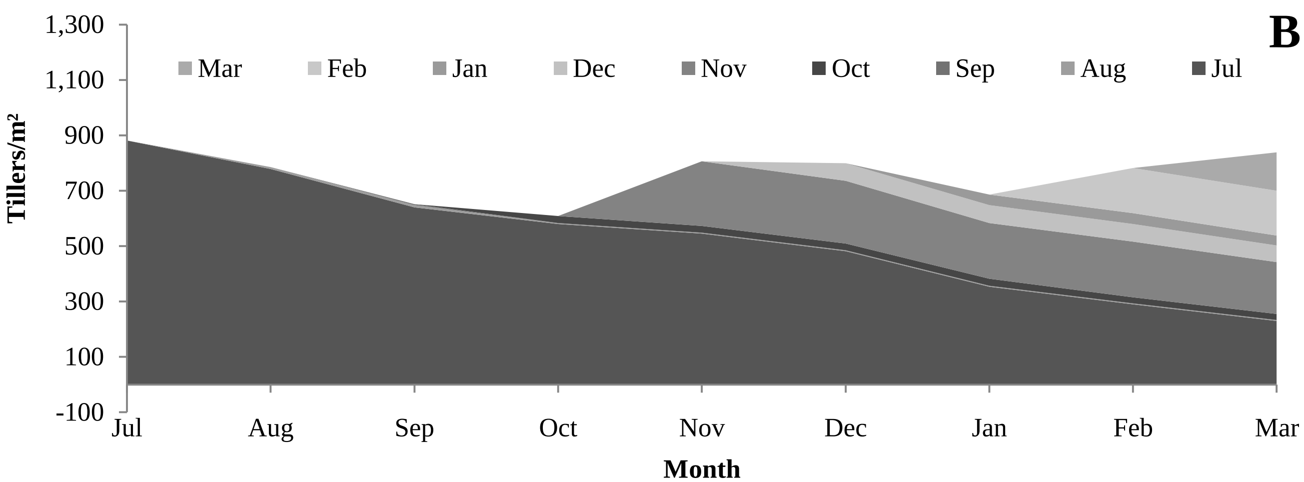
For swards with constant and increasing heights, the highest TAR occurred in early spring (Figure 3), whereas the highest TMR was found in late spring (Figure 4). This response pattern demonstrates that, when climatic conditions are again favorable for grass growth, i.e. in spring, the plant primarily supports the emergence of new tillers, which then triggers the death of old tillers. This can be a strategy of Marandu palisadegrass to ensure the stability of the tiller population during transition periods between seasons.

The number, i.e. density, of tillers was higher in the sward with increasing height than in those with constant and decreasing heights (Figure 8). Lowering the sward to 15 cm from the summer of 2013 (acclimation period) and during the fall and winter, i.e. 6 months before the commencement of observations, probably promoted the emergence of tillers, as the summer is a period with favorable climatic conditions and the weather in the fall is still not completely restrictive to plant growth (Figures 1 and 2). When pasture is kept short, there is a higher incidence of light at the base of the plant, and apical dominance of existing tillers is broken, which results in the activation of a higher number of buds, which in turn produce tillers (Difante et al. 2008; Giacomini et al. 2009; Portela et al. 2011). As a result, at the beginning of the evaluation in winter, the sward already had a large number of tillers, which remained high during the subsequent periods (Figure 9). To what extent the differences in tiller density in the various treatments at the start of the observations (approximately 700 tillers/m^2 for decreasing, 800 tillers/m^2 for constant and 1,000 tillers/m^2 for increasing) affected the outcome of the study and subsequent tiller performance we do not know.

High numbers of tillers are important for productivity, provided that their growth is not compromised, and also for the perenniality of forage plants (Matthew et al. 2000). A high tiller density also results in dense pasture, which may lead to high bite mass of grazing animals (Fonseca et al. 2013), which is desirable for increasing daily forage intake and, consequently, the performance of grazing animals.

Tillers marked in early July contributed significantly to total tiller population in the sward during winter, but this participation decreased sharply after early spring (Figure 9). Fewer tillers were produced in winter than in spring and summer. In early spring and summer, the new generations of tillers contributed most to total tiller density, which is a reflection of high tiller renewal. However, it should be noted that, even with the renewal of tillers in early spring and summer, some old tillers remained until the end of the experiment, less though at decreasing than at constant or increasing height.





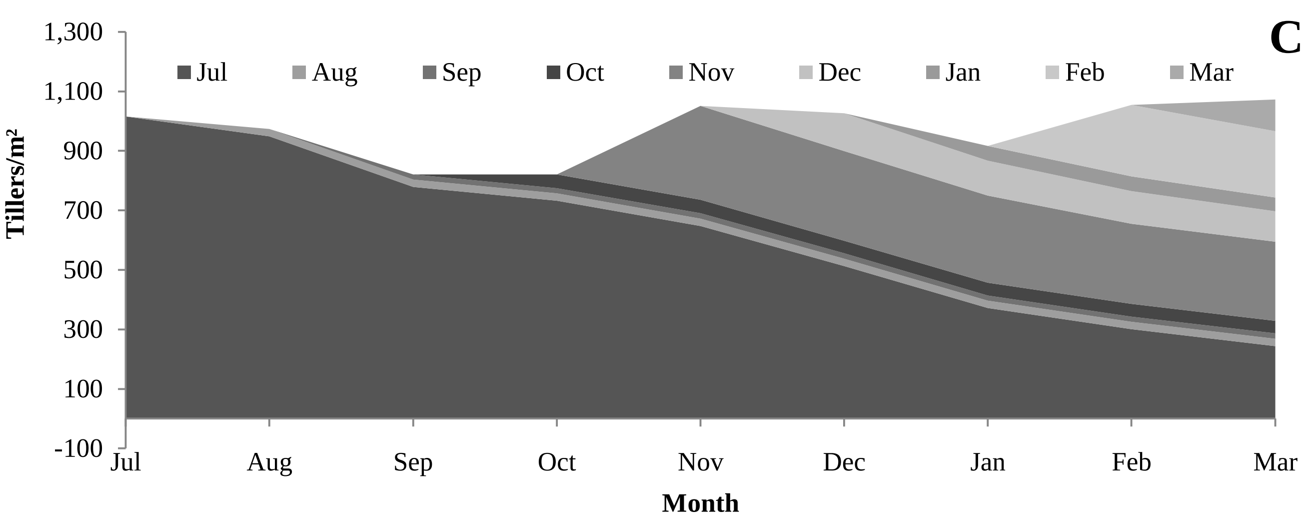


Figure 9. Tiller demographic pattern in Marandu palisadegrass kept at decreasing (A), constant (B) or increasing (C) heights during the various seasons of the year.

Decreasing: plants at 45 cm in winter, 30 cm in spring and 15 in summer; Constant: plants at 30 cm in winter, spring and summer; Increasing: sward at 15 cm in winter, 30 cm in spring and 45 cm in summer.

Conclusions

This study has shown that both grazing height of pasture and season affect tiller dynamics of *Urochloa brizantha* (syn. *Brachiaria brizantha*) cv. Marandu. Tiller appearance and stability rates were higher in early spring than in other seasons of the year and lower grazing heights stimulated appearance of new tillers.

This suggests that, for grazing management, farmers should adjust canopy height throughout the year rather than grazing at a fixed height as has been recommended in the past.

Further grazing studies seem warranted to determine whether the effects demonstrated in this experiment hold under grazing and how they compare with a fixed grazing height. How the different pasture dynamics affect pasture yield and quality and translate into animal performance should be monitored before recommendations should be made.

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Research paper

Determinants of the utilization of desho grass (*Pennisetum pedicellatum*) by farmers in Ethiopia

Factores determinantes para el uso del pasto desho (Pennisetum pedicellatum) *por productores en Etiopía*

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Abstract

A study was conducted to document how smallholder farmers in Ethiopia utilize desho grass (*Pennisetum pedicellatum*) and explain the determinants of alternative and competing uses of the grass. The study was conducted using a semi-structured questionnaire for 240 farmers in the districts of Burie Zuria and Doyogena, complemented with input from key informants and secondary data. The dependent variables tested were the use of desho grass as a feed, multipurpose uses of the grass and types of livestock fed. To test the effect of the explanatory variables on the dependent variables, separate univariate Probit models were used. Although the majority of respondents can read and write, about 23% of respondents were illiterate. The average desho grass-producing farmer in the sample owned 0.95 ha of farmland and 3.56 tropical livestock units; average household size was 6.5 people with a household head who was typically male (91% of households). Eighty percent of respondents in Burie Zuria and all respondents in Doyogena district depended solely on rain for desho grass production. Fifty-eight percent in Burie Zuria and 65% in Doyogena district applied either manure or artificial fertilizer to the grass. Weeding of desho grass was not practiced by any respondents in either district. Sixty percent of farmers used desho grass as a feed and 35% used it for more than a single purpose. Forty-two percent of farmers who fed desho grass did so to only lactating cattle, 3% fed it to small ruminants and 53% fed it to all livestock species. There were significant negative correlations ($P<0.01$) between both experience in production of desho grass and access to training in its production and utilization, and its utilization as a feed. Seventy percent of farmers in Burie Zuria and 13% in Doyogena have received training in desho grass production. To expand the utilization of the grass to as many farmers as possible, further training should be provided. A multi-faceted approach would be needed for the 23% of illiterate farmers over the 2 districts.

Keywords: Cut-and-carry, lactating animals, multipurpose, Probit models.

Resumen

En el estudio se analiza la utilización del pasto desho (*Pennisetum pedicellatum*) por pequeños agricultores en Etiopía y se explican los factores que determinan sus usos alternativos. El trabajo se realizó con la colaboración de 240 agricultores de los distritos de Burie Zuria y Doyogena mediante un cuestionario semi-estructurado, complementado con aportes de informantes clave y datos secundarios. Las variables dependientes analizadas fueron: uso del pasto como forraje; usos multipropósito; y tipo de animales que lo utilizan. Para probar el efecto de las variables explicativas

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sobre las variables dependientes, se utilizaron modelos Probit univariados en forma separada. Aunque la mayoría de los encuestados sabía leer y escribir, alrededor del 23% de los encuestados eran analfabetos. En la muestra, el productor promedio del pasto desho poseía 0.95 ha de tierra y 3.56 cabezas de ganado (TLU, tropical livestock unit) y la familia promedio consistía en 6.5 personas con una cabeza de familia típicamente masculina (91% de las familias). Para la producción del pasto, el 80% de los encuestados en Burie Zuria y todos los encuestados en el distrito de Doyogena dependían exclusivamente de las lluvias. El 58% en Burie Zuria y el 65% en el distrito de Doyogena fertilizaban el pasto con estiércol o con fertilizantes comerciales. En ambos distritos los productores en la encuesta no controlaban maleza en sus parcelas del pasto. El 60% de los agricultores utilizaba el pasto para alimentar sus animales y el 35% para más de un propósito. El 42% de los agricultores que lo usaban para alimentar su ganado lo suministraban solo a vacas lactantes, el 3% a pequeños rumiantes y el 53% a todo tipo de ganado. Se encontraron correlaciones negativas significativas ($P<0.01$) entre tanto la experiencia en la producción del pasto desho como el acceso a capacitación en producción y utilización, y su utilización como forraje. El 70% de los agricultores de Burie Zuria y el 13% en Doyogena habían recibido ese tipo de capacitación en producción del pasto. Para extender la utilización del pasto a un mayor número posible de agricultores, se sugiere intensificar las prácticas de capacitación. Para llegar a los agricultores analfabetos se requiere de un enfoque multifacético.

Palabras clave: Corte y acarreo, modelos Probit, multipropósito, vacas lactantes.

Introduction

Despite the large livestock population in Ethiopia (CSA 2015), its contribution to the national economy is below potential, owing to a range of factors including availability and quality of feed, poor genetic potential of animals for productive traits, poor health care and poor management practices (Mengistu 2006; Legesse 2008). Of these factors, the most limiting is low quantity and quality of feed (Shapiro et al. 2015). Among the recommended mitigation strategies of feed shortage in the country is the utilization of indigenous adaptable multi-purpose fodder species, e.g. desho grass (*Pennisetum pedicellatum*). This perennial grass is native to tropical Africa and widespread from West to East Africa (Leta et al. 2013). Though often considered to be a noxious weed (ISC 2015), in Ethiopia the grass was first used in Southern Nations Nationalities and Peoples' Region and is currently utilized for soil conservation practices and animal fodder in other regions of the country (Welle et al. 2006; Yakob et al. 2015). The grass has the ability to control water loss effectively and recovers rapidly after watering even under severe drought conditions (Noitsakis et al. 1996; Welle et al. 2006). Moreover, desho grass provides a small business opportunity for Ethiopian farmers (sale of cut forage and planting material) (Shiferaw et al. 2011). Desho grass can provide large amounts of fodder per unit area (30–109 t green herbage/ha/year; Heuzé and Hassoun 2015) and can be a year-round fodder for livestock (Leta et al. 2013). However, despite its abundance and expansion in different parts of the country, there is a lack of information on how farmers manage and utilize the grass.

The objective of this assessment was to document how smallholder farmers in Ethiopia utilize desho grass and explain the determinants of alternative and competing uses of the grass.

Materials and Methods

Description of study areas

The study was conducted in 2 districts purposely selected from Amhara and Southern Nations, Nationalities, and Peoples' (SNNP) Regional States of Ethiopia (Figure 1). Bure Zuria district (10°17'–10°49' N, 37°00'–37°11' E) is located in West Gojam Zone of Amhara region and covers 58,795 ha made up of 52% cropping, 6% grazing land, 25% forest and bushland, 9% wasteland, 7.5% construction and 0.5% water bodies (BZDoA 2014). The topography of the district is characterized by 76% plain, 10% mountainous, 7% undulating and 7% valleys. The district has 3 soil types: red (63%), blue (20%) and black (17%). The major portion of the district is Woinedaga/midland (77%) followed by Kola/lowland (22%) and Dega/highland (1%) with daily temperature range of 17–25 °C. The annual average rainfall is 1,000–1,500 mm. The major crops grown in the district are maize, finger millet, teff, wheat, barley, potato, pepper, onion, field pea and faba bean. The types of livestock reared in Burie Zuria district include cattle, sheep, goats, equines, chickens and bee colonies. The total area growing desho grass in the district reported for 2014 was 47.5 ha (BZDoA 2014).

Doyogena district (7°20' N, 37°50' E; 1,900–2,750 masl), located in Kembata-Tembaro zone of SNNPs

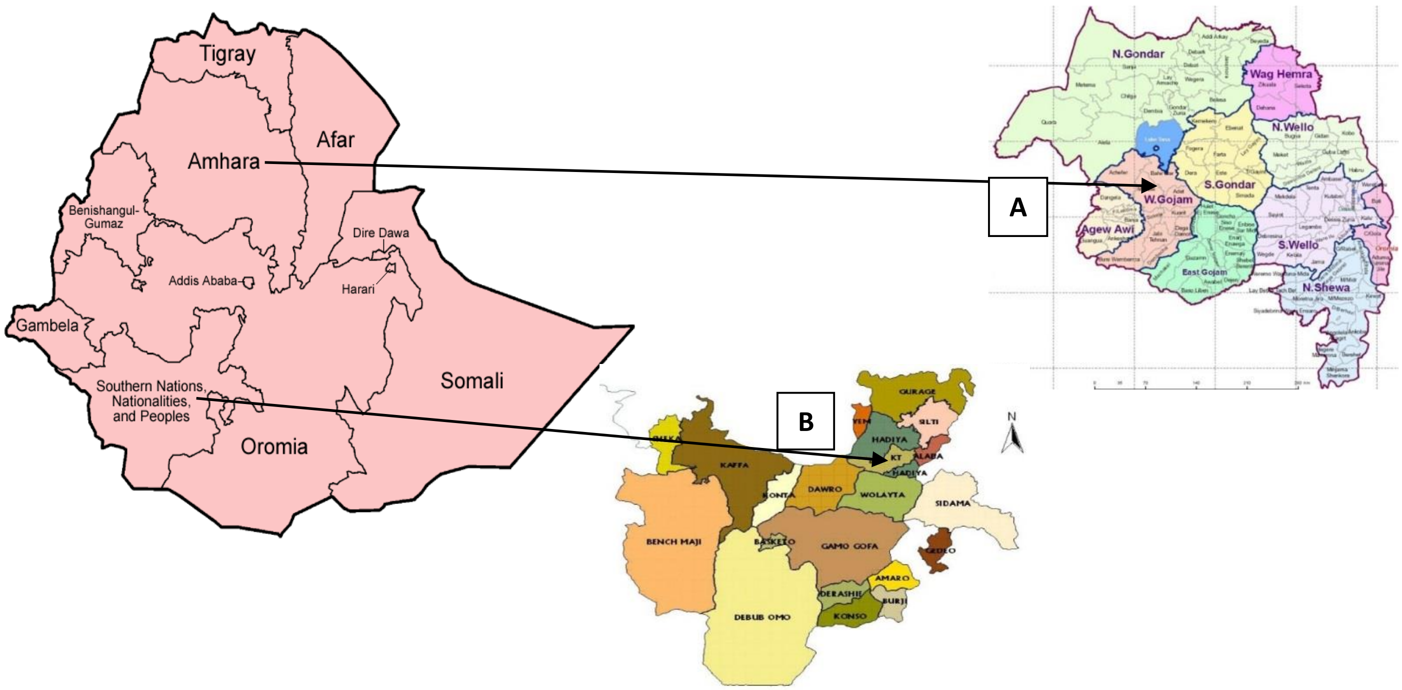

Figure 1. Map showing study districts: Burie Zuria (A), and Doyogena (B) in West Gojam and Kembata-Tembaro, respectively.

Regional State, 258 km southwest of Addis Ababa, covers a total area of 17,264 ha. About 86% of the area is used for cropping, 11.8% forest and bushes, 2% grazing land and 0.2% degraded land. The district has 2 major agro-ecologies, Dega/highland (70%) and Woyina dega/midland (30%). About 10% of the area is plain, while the remaining 90% is mountainous/hilly. It has minimum and maximum temperatures of 10 and 16 °C, respectively, and average annual rainfall of 1,400 mm (DDoA 2014). Major crops cultivated in the highlands are ensete, cabbage, potato, barley, wheat, faba bean and field pea. At low elevations, farmers also cultivate sugar cane and small areas of maize. The soil type is mostly black clay loam, rich in organic matter (InterAide 2014). Types of livestock in the district are cattle, sheep, goats, equines and poultry. The total area growing desho grass reported for 2014 was 2,790 ha (DDoA 2014).

Data collection and analysis

The study districts were selected based on desho grass production and utilization practice. From each district, 4 kebeles (kebele is the name for a local administration in Ethiopia) were selected, based on adoption of desho production and utilization. From each kebele, 30 farmers producing desho grass were randomly selected, making the total number of respondents 240. The survey was conducted using a semi-structured questionnaire completed as a personal interview with experienced and trained interviewers. A preliminary questionnaire was prepared, pretested with a group of farmers and modified before the actual data collection started. The data were complemented with information obtained from key informants, comprised of people from each kebele, including animal science and natural resource experts. Secondary data were obtained from the Office of Agriculture. Livestock holding per household was converted to standard units (Tropical Livestock Unit, i.e. 1 TLU = 250 kg) based on conversion factors set previously (ILCA 1990).

Descriptive statistics were used as a preliminary investigation procedure to gain an understanding of inherent significant socio-economic characteristics of the smallholder farmers. All data were systematically coded and analyzed using Statistical Analysis System (SAS 2007). To estimate the effect of socio-economic factors, agro-ecology and farmers' perceptions in desho grass-producing households were collected using a semi-structured questionnaire. The dependent variables tested were: the extent of use of desho grass as a feed (use as a feed, 1 = yes, 0 = no, if it is used for other purposes such as soil conservation or income sources); multipurpose aspect of desho grass (0 = single use, 1 = multiple uses as soil conservation and income sources); and preferred livestock for feeding (fed to lactating cattle, 0 = no, 1 = yes; fed to small ruminants, 0 = no, 1 = yes; fed to all livestock species, 0 = a single species, 2 = all livestock species). The dependent variables were binary in nature and independent. To accommodate this non-independence, a bivariate Probit model was used to simultaneously estimate the effect on the probability of multiple use of desho grass of the set of explanatory variables.

The model used for analysis can be expressed as:

$Y_i = x_i\beta + \varepsilon_i$ (Greene 2012)

where: Y_i is the decision vector, x_i is a vector of explanatory variables derived from household surveys, with β as the corresponding regression coefficient, and ε_i is the error term.

The factors tested (explanatory variables) were: household characteristics (head's age, education level, gender, experience in desho grass utilization, land holding, distance of the land from the homestead), agro-ecology and feed accessibility (midland vs. highlands), herd structure and access to training. The most common variables used in modeling technology adoption processes are: nature of the farming system, land tenure, resource endowment, social capital and social psychological factors (Rogers 1995; Namara et al. 2007; Salasya et al. 2007).

Results

Household characteristics of respondents

As shown in Table 1, most household heads were male (92.9%). The majority of respondents were at a mature stage of life (41–50 years, 49.4%), while the remainder were split fairly uniformly among remaining age groups. The overall educational level attained by the majority of respondents was elementary school (31.2%), while 15.9% had attended high school and a further 25.6% had no schooling but could read and write. Almost 23% were illiterate.

Table 1. Description of household heads of respondents.

Variables	No.	%
Gender		
Male	223	92.9
Female	17	7.1
Age (yr)		
18–30	26	10.8
31–40	49	20.4
41–50	115	47.9
51–60	33	13.8
>60	17	7.1
Education level		
Illiterate	58	24.2
Read and write	54	22.5
Elementary school	69	28.8
Junior	21	9.0
High school	38	15.8

Average family size was 6.29 people with 3.9 active labor units (Table 2). Each family owned on average 3.56 TLU, which were maintained on a farm size of 0.95 ha. Average experience with desho grass production was 3.05 years. The types of livestock species kept by respondents in both districts comprised cattle, sheep, goats, equines and chickens. In Burie Zuria district the mean TLU for cattle, sheep, goat, equine and poultry populations were 4.92, 0.32, 0. 27, 1.06 and 0.07 TLU, respectively, while the corresponding values for Doyogena district were 2.68, 0.28, 0.15, 0.72 and 0.04 TLU.

Table 2. Details of families, size of holding and livestock carried.

Parameter	Mean	SD
Family size (No.)	6.29	1.93
Active labor units in the family (No.)	3.90	1.80
Total livestock holding (TLU)	3.56	1.85
Experience with desho grass production (yr)	3.05	1.44
Landholding (ha)	0.95	0.69

TLU = Tropical Livestock Unit.

Feed shortage as a major livestock production constraint

Seasonal shortage of feed was the major problem raised by all respondents in both districts. In Burie Zuria district, 98.3% of respondents faced seasonal feed shortage, with 54.2% of these experiencing the problem only during the dry season, while the remaining 45.8% faced shortage in both dry and wet seasons. Similarly, in Doyogena district, 99.2% of respondents faced feed shortages, 86.3% of these in the dry season only and the remaining 13.7% in both wet and dry seasons. Feed shortage mitigation strategies employed in both districts were similar, with only the proportions differing between districts. In Burie Zuria district, strategies were: purchase feed plus use crop residues (54.2% of respondents); purchase feed only (35%); and use crop residues only (10.8%). Corresponding values in Doyogena district were 21, 44.6 and 34.4%, respectively. The graphical presentation of values for both districts is shown in Figure 2.

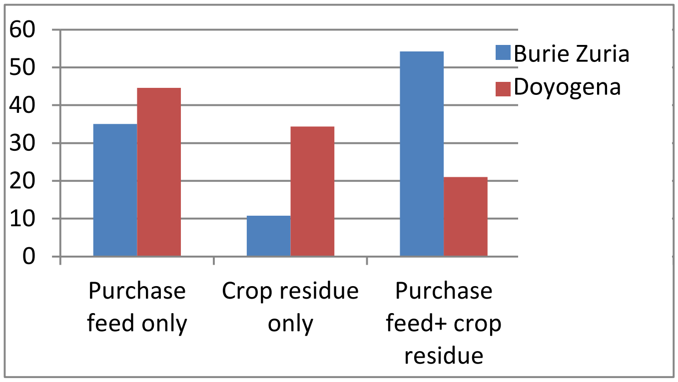

Figure 2. Feed shortage mitigation strategies of respondents in the two districts (%).

Desho grass production and management

The system of desho grass production in the study areas was determined mainly by rainfall. In Burie Zuria district 80% of respondents depended on rain for desho grass production, while 20% also had access to irrigation. In Doyogena district all respondents depended only on rain. Fertilizer application in the form of manure or artificial fertilizer is important for desho grass production (Leta et al. 2013). However, fertilizer usage was not uniform in either the application or form of fertilizer. In Burie Zuria district only 58.3% of respondents applied fertilizer to desho grass. Of the respondents using fertilizer, 75.8% applied manure, while 24.2% applied artificial fertilizer. In Doyogena district 64.5% of respondents applied fertilizers, 94.6% of them using manure and the remaining 5.4% using artificial fertilizer. In both districts weeding of desho grass was not practiced.

In both districts, the dominant form of desho grass production was as a backyard enterprise with 86.3% of respondents following this practice. This may be because the strategy is more convenient for the cut-and-carry feeding system, enabling intensive management and thus high yields in areas where land shortage is a problem. As far as harvesting of desho grass is concerned, in both districts all respondents harvested the grass at about 4 months after establishment. The frequency of cuts during the rainy season after the first harvest was: for Burie Zuria district respondents - every 2 weeks (44.7%), more than 2 weeks (20.3%) and depends on rain/moisture availability (35%); and for Doyogena - every 2 weeks (23.8%) and depends on rain/moisture availability (74.6%).

Utilization of desho grass for animal feed

Further investigation of the utilization of desho grass for animal feed (Table 3) was conducted because most respondents used it for animal feed only (60%) rather than for soil conservation and as source of income. In Burie Zuria district, use of desho grass for animal feed was much less common than in Doyogena. In Doyogena, where the grass is comparatively more utilized, the topography is mountainous and grazing is limited, so farmers would tend to cut-and-carry more. In this district, major crops are potatoes and ensete, which present fewer crop residues for livestock than in Burie Zuria (Table 3).

Table 3. Utilization of desho grass by farmers.

Parameter	No.	%
Desho grass used for 1 purpose (yes)	155	64.6
Desho grass used for 2 purposes (yes)	69	28.8
Desho grass used for multipurpose (yes)	16	6.7
Desho grass for feeding only (yes)	144	60
Desho grass for cattle (yes)	97	41.8
Desho grass for small ruminants (yes)	7	2.9
Desho grass for all animals (yes)	128	53.3
Both grazing and yard feeding (yes)	215	89.6
Feed conservation (yes)	183	81.3

The relationships between utilization of desho grass as feed and characteristics of household heads and farm parameters is indicated in Table 4. Age of the household head had no relationship (P>0.05) but there were significant negative correlations (P<0.01) between both experience in production of desho grass and training in its use, and its utilization as a feed. However, there was no significant correlation (P>0.05) between number of active laborers in a homestead and the utilization of the grass

Table 4. Relationships between utilization of desho grass as feed and characteristics of the household head and farm.

Explanatory variables	Estimate	SD	Sig. level
District	-1.34	0.40	***
Household head age (yr)			
18–30	-0.55	0.53	NS
31–40	-0.16	0.48	NS
41–50	-0.55	0.44	NS
51–60	-0.61	0.47	NS
Education level			
Illiterate	0.58	0.36	*
Read and write	0.86	0.39	*
Elementary school completed	-0.11	0.34	NS
High school graduate	-0.29	0.42	NS
Experience with desho grass (yr)	-0.20	0.08	**
Active laborers (No.)	-0.04	0.06	NS
Farm size (ha)	0.28	0.26	NS
Backyard desho production (yes)	0.29	0.32	NS
Access to training (yes)	-0.93	0.26	***
Total TLU (No.)	0.04	0.06	NS
Feeding system (grazing)	0.98	0.36	***
R^2	0.37		
No. of observations	240		

as a feed. There was no significant correlation (P>0.05) between farm size and distance of the desho grass plots from the homestead.

While there was no significant correlation (P>0.05) between livestock numbers in a farm and utilization of desho grass as a feed, the type of feeding system employed was positively and significantly correlated (P<0.01). There is a high tendency to supplement using desho grass when grazing is the main source of feed. During scarcity of desho grass (or other feed resources), farmers prefer to give the grass to lactating animals. The key informants indicated that, in comparison with other forage grasses used by them (oats, Rhodes and Napier), desho grass was the most important forage species because of its vigorous vegetative growth and high yields. All respondents from both districts had a positive impression of the feeding value of the grass as they indicated that it increases milk yield and improves growth and overall performance of animals when given fresh in the form of cut-and-carry. This elucidates that desho grass is playing a positive role in feeding of growing, fattening and lactating animals in the study district.

Number of roles of desho grass

Data for number of roles (single, dual and multiple roles) for desho grass are presented in Table 5. The non-feed roles of the grass are as soil conservation and income source by selling the grass to other farmers. While there was no significant correlation (P>0.05) between district, age and education level of household head, number of labor units in the family, size of farm and number of livestock and the number of uses for desho grass, there was a significant negative correlation (P<0.01) between experience in production of the grass and its use for only one purpose. In Burie Zuria district about 57.5% of respondents used desho grasses as a source of income by selling planting material (seedlings) to other farmers, while only 12.3% of respondents in Doyogena district earned income from selling seedlings and fresh grass, which was in line with other reports (IPMS 2010; Shiferaw et al. 2011). Desho grass was also used for land conservation purposes in both districts, as reported by other workers (Welle et al. 2006; Leta et al. 2013).

Preferred livestock to feed desho grass

The species of livestock fed desho grass are presented in Table 6. Based on respondents' information, the major species of livestock fed desho grass were used in the bivariate Probit model analysis. There was a significant correlation (P<0.01) between districts in desho grass use for lactating cattle, small ruminants and all livestock. Age of household head, educational level and number of active labor units had no significant relationship (P>0.05) with types of livestock fed desho grass. There was a significant negative correlation (P<0.01) between feed shortages and use of desho grass for lactating cattle, small ruminants and all livestock.

Table 5. Relationships between number of uses[1] of desho grass by the family and characteristics of the household head and the farm.

Explanatory variables	Single role		Dual role		Multi-role	
	Est.	SD	Est.	SD	Est.	SD
District	-0.94	0.34	0.64	0.42	0.68	0.76
Age of household head (yr)						
18–30	-0.44	0.49	0.59	0.58	0.04	0.78
31–40	-0.18	0.44	0.25	0.52	0.45	0.65
41–50	-0.36	0.40	0.73	0.49	0.03	0.57
51–60	0.24	0.44	0.68	0.52	-0.59	0.73
Education level						
Illiterate	0.34	0.35	-0.66	0.39*	0.34	0.58
Read and write only	0.24	0.37	0.35	0.40	-0.21	0.63
Elementary school completed	-0.07	0.33	0.21	0.35	-0.40	0.59
High school graduate	-0.23	0.41	0.38	0.43	-5.10	0
Experience with desho grass (yr)	-0.13	0.08*	0.09	0.09	0.19	0.15
Active laborers (No.)	-0.01	0.06	-0.03	0.06	0.08	0.11
Farm size (ha)	-0.01	0.24	0.11	0.27	0.14	0.46
Backyard desho production (yes)	0.17	0.30	-0.24	0.34	0.11	0.52
Total TLU (No.)	0.04	0.06	-0.11	0.06	0.02	0.11
R^2	0.12		0.29		0.70	
No. of observations	240		240		240	

[1]Uses of desho grass considered: livestock feed, soil stabilization and income source (sale of forage, sale of planting material).

Table 6. Relationship between livestock fed desho grass and household and farm parameters.

Explanatory variables	Lactating cattle		Small ruminants		All livestock	
	Est.	SD	Est.	SD	Est.	SD
District	0.64	0.39*	15.17	1.66***	-0.82	0.40**
Household head age (yr)						
18–30	-0.39	0.48	31.28	1.98	0.16	0.49
31–40	-0.28	0.44	32.37	3.51	0.32	0.44
41–50	-0.28	0.40	28.17	1.40	0.26	0.40
51–60	-0.76	0.44	25.79	2.53	0.59	0.44
Education level of household head						
Illiterate	0.43	0.37	7.38	1.81	-0.65	0.37
Read and write	0.57	0.38	6.91	1.66	-0.75	0.38
Elementary school	0.45	0.36	3.95	2.90	-0.55	0.36
High school graduate	-0.20	0.44	11.47	4.15	0.03	0.43
Active family labor (No.)	-0.03	0.06	-0.30	0.84	0.16	0.09
Feed shortage (yes)	5.85	0.33***	-0.93	0.26	-1.48	0.52***
Landholding (ha)	-0.12	0.25	-1.52	3.95	0.14	0.25
Total livestock (TLU)	-0.14	0.06**	-2.37	2.00	0.25	0.07*
Backyard desho production (yes)	-0.05	0.30	-1.80	2.42	0.00	0.30
R^2	0.24		0.993		0.31	
No. of observations	240		240		240	

Farm size had no significant correlation (P>0.05) with type of livestock species fed desho grass. There was a significant negative correlation (P<0.01) between the total livestock holding and desho grass utilization for lactating cattle and a significant positive correlation (P<0.05) between total livestock holding and desho grass utilization for all livestock. In addition to lactating cattle, desho grass was used for fattening cattle (14%) and equines (3.8%) in both districts.

Discussion

Household characteristics of respondents

The adoption of desho grass in the current study is below expectations, in comparison with findings of other studies (Mugisha et al. 2004; Salasya et al. 2007), which reported that education enhances the use of agricultural technologies because better educated farmers have more opportunity to acquire and process information as well as understand the technical aspects of new technologies. As the literacy level of respondents in the current study is better than the findings of various authors from different parts of Ethiopia (Eba 2012; Mekuriaw and Asmare 2014; Wondatir and Mekasha 2014), this kind of population should be more amenable to technology adoption.

Farm size is an important factor which normally determines the adoption of improved forages (Yami and Markel 2008). Desho grass is here considered as improved forage because it requires allocation of resources such as land, fertilizer and labor in addition to management practices. With regard to farmland, size of farm (0.95 ha) was comparable with the findings of Yayeh et al. (2014), who reported that rural land holding was 0.98 ha for Debre Markos District, Amhara National Regional State. However, farms were smaller than reported by Amare (2006) and Admasu (2008), where average farm sizes were 3.28 and 2.55 ha per household in Amhara and Southern Nationalities Regional States, respectively. Moreover, average farm size was much lower than the national average holding size of 1.6 ha reported by FAO (2008), which may in turn affect improved forage production in both districts. On small farms, a greater percentage of the available area may be required to provide food for the family, leaving less to grow forage for livestock.

With the small farm size, total TLU/household of Burie Zuria (5.46) was lower than the 9.87 (Solomon 2006) and 7.73 TLU (Yayeh et al. 2014) for Dejen and Debre Markos districts, respectively, Amhara National Regional State. The 3.37 TLU/household in Doyogena district was also smaller than the 5.45 TLU reported by Admasu (2008) for Alaba district with average farm size of 2.55 ha, Southern Nations, Nationalities, and Peoples' Regional State, Ethiopia.

Feed shortage as livestock production constraint

The fact that the major problem of livestock production in both districts was shortage of feed in both dry and wet seasons was not surprising as feed shortage is a common feature in many African countries. Both quality and quantity of feed were insufficient as observed in previous studies by other workers in different parts of the country (Tolera 2007; Fetsum et al. 2009; Tegegne and Asefa 2010), a situation which is aggravated by expansion of crop production and increase in livestock numbers. Under these circumstances, planting of improved grasses to produce higher yields per hectare should reduce the feed deficit and improve the quality of available feed. Size of farm has a very important role in ensuring adequate supplies of feed for livestock, as the primary consideration must be providing food for the families on the farm.

Desho grass production and utilization

For maximum or optimal production, desho grass should be managed properly in terms of weeding, fertilizing, harvesting and utilization. It was gratifying to find that in both districts all respondents started harvesting desho grass when it was about 4 months of age, which is the recommended initial harvesting stage (Göhl 1981). However, 35% (Doyogena) and 42% (Burie Zuria) of farmers did not fertilize their grass, which raises the question of how these farmers utilize their livestock manure. The major difference in utilization of desho grass for fodder in the 2 districts may be related to the different crops grown. In Burie Zuria district crop production is a major activity, especially cereals, and crop residues are a high potential source of feed. However, in Doyogena, major crops are potatoes and ensete, which provide fewer crop residues for livestock. Therefore, desho grass is comparatively more utilized under a cut-and-carry system in Doyogena, as the topography is mountainous and grazing is limited.

The absence of correlation between age of household head and utilization of desho grass for animal feed indicates that all age groups appreciate the advantages of using the grass for this purpose. This contrasts to some degree with earlier reports by other workers (Adesina and Zinnah 1993; Fufa and Hassan 2006) that increasing age of a farmer reduces the probability of using new agricultural technologies.

The significant negative correlation ($P<0.01$) between experience in production of desho grass and its utilization as a feed indicates that growing desho grass for a longer time has provided the experience to utilize it for other purposes like soil stabilization. Similar results have been found by other workers (Welle et al. 2006). It is also significant that about 70% of respondents from Burie Zuria had received training in production and utilization of desho grass, while only 13% had received similar training in Doyogena. Both longer experience with the grass and access to training would have equipped the farmers in Burie Zuria with the knowledge to adopt a more flexible approach to its usage. This suggests that a concerted effort should be mounted, especially with farmers in Doyogena, to provide training and information on production and utilization of this grass. This would need to be a multi-faceted approach, as the illiterate farmers could not take advantage of printed material.

The absence of any significant correlation ($P>0.05$) between number of active laborers in a household and the utilization of desho grass as a feed suggests that the use of the grass is not necessarily labor-intensive or the areas on which desho grass is produced are limited, so usage for stock does not demand much labor input. Non-significant relationships ($P>0.05$) between livestock numbers in a household and utilization of desho grass as a feed may be due to the low livestock units per household in the 2 districts, which averages 3.56 TLU.

Number of roles for desho grass

The absence of any relationship between district, age of household head, and education level and the number of uses for desho grass might be due to the fact that the grass was produced on very small plots of land, which may be used for one or more purposes. This implies that within each district desho producers observed the production systems being used by others and largely adopted a similar approach. Desho grass production and utilization is a relatively new experience and most of the respondents have similar understanding about the roles of the grass. Where experience and training are minimal, the perception of farmers is low, they are not aware of the range of beneficial roles of desho grass, and use it largely for only one purpose.

Preferred livestock to feed desho grass

The non-significant ($P>0.05$) relationship between age of household head, educational level and number of active labor units and type of cattle fed desho grass suggests that the grass is used for the cattle with highest nutritional needs, i.e. lactating animals, regardless of other factors. Moreover, desho grass utilization does not need high literacy level or special knowledge, as all farmers would be aware from experience that these groups of animals need the best feed. This fact overrides all other considerations.

There was a significant negative correlation ($P<0.01$) between feed shortage and use of desho grass for lactating cattle, small ruminants and all livestock. This might be due to the use of other feeds such as crop residues to satisfy basal dietary requirements because desho grass was used to supplement the basal diet by all respondents. Lack of significant correlation (P>0.05) between farm size and type of species fed desho grass could be associated with the fact that the grass was not planted on large areas of land as a trade-off with other forages or crops. Households in the highland areas tend to use desho grass to feed livestock more than those in the midland area. The higher the dependence on grazing, the greater the likelihood of the grass being used as a feed. The low accessibility to feeds in the highlands (possibly due to limitations of grazing), vulnerability of soil towards erosion and high density of livestock per household, create a higher potential for desho grass to be utilized as both fodder and for soil conservation in the highlands.

Conclusions

Provision of training on the use of desho grass is important to promote production and use of this grass as an avenue to generate income and for soil conservation in addition to animal fodder. This has the potential for capacity building, which would contribute to the sustainable use of desho grass in the future. Research on why multipurpose use is not more common in highland areas would clarify if this approach is necessary. To fully exploit the potential of desho grass, further research is needed on its agronomic characteristics, plus evaluation at the laboratory and animal production levels under a range of conditions.

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Short Communication

New species, nomenclatural changes and recent taxonomic studies in the genus *Stylosanthes* (Leguminosae): An update

Especies nuevas, cambios nomenclaturales y recientes estudios taxonómicos en el género Stylosanthes *(Leguminosae): Una actualización*

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Abstract

Since the last taxonomic overview during the 1982 *Stylosanthes* Symposium in Townsville, Australia, 10 new species: *S. falconensis*, *S. longicarpa*, *S. maracajuensis*, *S. nunoi*, *S. quintanarooensis*, *S. recta*, *S. salina*, *S. seabrana*, *S. vallsii* and *S. venezuelensis*; and 1 botanical variety: *S. guianensis* var. *pauciflora*; have been validly described. Furthermore, 2 nomenclatural changes have been proposed, both being elevations of botanical varieties to the rank of species: *S. gracilis* and *S. rostrata*. In the major taxonomic databases, The Plant List and GRIN, the taxonomic status ("accepted" vs. "synonym" vs. "unresolved") of some of these new taxa, however, differs. In addition, this paper reports on *Stylosanthes* names that can be found in the post-symposium literature but have not been validly published, and on recent regional studies of *Stylosanthes* taxonomy. Suggested research needs as perceived by non-botanists include an updated *Stylosanthes* monograph and taxonomic studies within the *S. guianensis*, *S. hamata* and *S. scabra* species complexes.

Keywords: Taxonomy, validly published names.

Resumen

Desde la última sinopsis taxonómica del género *Stylosanthes* realizada en 1982 con ocasión del Simposio sobre *Stylosanthes* llevado a cabo en Townsville, Australia, se han descrito válidamente 10 nuevas especies y una variedad botánica: *S. falconensis*, *S. longicarpa*, *S. maracajuensis*, *S. nunoi*, *S. quintanarooensis*, *S. recta*, *S. salina*, *S. seabrana*, *S. vallsii* y *S. venezuelensis*, y la variedad botánica: *S. guianensis* var. *pauciflora*. Por otra parte, se han propuesto 2 cambios nomenclaturales; los mismos son elevaciones de variedades botánicas al rango de especie: *S. gracilis* y *S. rostrata*. No obstante, existen diferencias entre las principales bases de datos taxonómicos, The Plant List y GRIN, con respecto al estatus taxonómico ("aceptado" vs. "sinónimo" vs. "sin resolver") de algunos de estos nuevos taxones. Adicionalmente, en el artículo se presentan una serie de nombres, los cuales no han sido válidamente publicados, pero sí aparecen en la literatura posterior al Simposio de 1982, y un listado de recientes estudios regionales sobre taxonomía de *Stylosanthes*. Las sugerencias sobre necesidades de investigación incluyen, según la percepción de investigadores no botánicos, una monografía actualizada del género *Stylosanthes* y estudios sistemáticos dentro de los complejos taxonómicos de *S. guianensis*, *S. hamata* y *S. scabra*.

Palabras clave: Nombres válidamente publicados, taxonomía.

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Introduction

Stylosanthes Sw. is an important tropical and subtropical forage plant genus (Chakraborty 2004). To date, more than 80 *Stylosanthes* species have been described (IPNI 2014). Nine of them are or were of economic importance (Chakraborty 2004; Cook et al. 2005): *Stylosanthes capitata* Vogel, *S. fruticosa* (Retz.) Alston, *S. guianensis* (Aubl.) Sw., *S. hamata* (L.) Taub., *S. humilis* Kunth, *S. macrocephala* M.B. Ferreira and Sousa Costa, *S. scabra* Vogel, *S. seabrana* B.L. Maass and 't Mannetje and *S. viscosa* (L.) Sw.

Swartz (1788) described the genus and included 2 species, *Stylosanthes procumbens* Sw., nom. illeg. (≡ *S. hamata*) and *S. viscosa*. Vogel (1838) prepared the first revision of *Stylosanthes*; therein he divided the genus into 2 sections and listed 15 species. Mohlenbrock (1957) monographed *Stylosanthes* across its full geographic range, listing 25 species. However, the material that was available to him more than half a century ago was limited in terms of specimen numbers and origins. In a subsequent taxonomic overview presented at the International Symposium on *Stylosanthes* held in November 1982 in Townsville, Australia, Mannetje (1984) listed 24 unambiguous, 5 problematic and 5 doubtful species; this overview was complemented by a study of some Brazilian *Stylosanthes* species (Costa and Ferreira 1984). With these 2 chapters on taxonomy in the symposium book (Stace and Edye 1984), it became evident that the genus presents considerable taxonomic difficulties.

There is no doubt that taxonomy of some *Stylosanthes* species is unclear and names might prove to be synonyms of other described species. However, this paper is not about the identification of synonyms vs. correct names, which should rather be the subject of a comprehensive taxonomic revision of the genus. Instead, our objective is to inform non-botanists about: (1) new validly published taxa; and (2) nomenclatural changes since the 1982 International Symposium. The term "nomenclatural changes" refers here to new combinations, i.e. when a new name, based on a legitimate, previously published name (= the so-called basionym of the species), is published (McNeill et al. 2012). In addition, we present: (3) a list of new variety and species names occasionally found in post-symposium publications, which, however, should not be used because they are invalid; and (4) a brief account of recent taxonomic *Stylosanthes* studies with a regional focus (country, state).

With this update we pretend to contribute to: (1) stimulate continuing interest in the genus; and (2) clarify and prevent potential confusion related to the use of species names.

Methodology

The study is based on analyses of botanical *Stylosanthes* literature partly accessed online from the Biodiversity Heritage Library (www.biodiversitylibrary.org). Literature not available online was obtained from the library of the Royal Botanic Gardens, Kew. New names and combinations and standard abbreviations of plant name authorities were obtained from the International Plant Names Index (www.ipni.org). For information on the taxonomic status of new names and combinations, 2 taxonomic databases were accessed: The Plant List (TPL, www.theplantlist.org) and GRIN, the US Department of Agriculture – ARS Germplasm Resources Information Network (https://npgsweb.ars-grin.gov/gringlobal/taxonomybrowse.aspx).

TPL is a taxonomic database produced through the collaboration of mainly the 2 world-leading research centers for legume taxonomy and nomenclature, Royal Botanic Gardens, Kew and Missouri Botanical Garden, where botanists are in charge of maintaining and updating the respective databases, ILDIS and TROPICOS. Algorithms are used for detecting and resolving conflicting opinions resulting from data coming from such different sources. When this leads to uncertainties, a taxonomic status will be presented as "unresolved". The TPL database is not updated regularly (TPL 2013). On the other hand, the GRIN database is monitored and regularly updated by botanists of the USDA National Germplasm Resources Laboratory (GRIN 2016). It follows a more pragmatic approach: when taxonomic differences arise, GRIN would generally be guided by current usage, if nomenclature of a specific case adheres to the international rules of the "Melbourne Code" (McNeill et al. 2012). We chose TPL and GRIN because of the particular expertise in legume taxonomy of the institutions contributing to these databases.

New species and botanical varieties

Since 1982, 10 new species and 1 botanical variety name have been described within the genus *Stylosanthes* (Table 1, first part): *S. falconensis* Calles and Schultze-Kr., *S. longicarpa* Brandão and Sousa Costa, *S. maracajuensis* Sousa Costa and van den Berg, *S. nunoi* Brandão, *S. quintanarooensis* Gama and Dávila, *S. recta* Vanni, *S. salina* Sousa Costa and van den Berg, *S. seabrana*

B.L. Maass and 't Mannetje, *S. vallsii* Sousa Costa and van den Berg, *S. venezuelensis* Calles and Schultze-Kr. and *S. guianensis* var. *pauciflora* M.B. Ferreira and Sousa Costa. However, though validly published, i.e. in accordance with international nomenclatural rules as specified in the "Melbourne Code" (McNeill et al. 2012), not all new taxa are currently equally accepted by the scientific community (Table 1). For example, *S. nunoi* and *S. maracajuensis* are accepted by both TPL and GRIN, whereas *S. venezuelensis* and *S. salina* are treated as unresolved by TPL and as accepted by GRIN.

These discrepancies among databases are the result of different species concepts. A good example is the case of *S. seabrana*; Maass and Mannetje (2002) compiled morphological, karyotypic and molecular evidence to describe a new species, *S. seabrana*, which was subsequently accepted by both TPL and GRIN. Under this name, the species has found entrance in tropical forage legume research worldwide and 2 commercial cultivars of *S. seabrana* were released in Australia (Cook et al. 2005). However, Vanni and Fernandez (2011), based on morphological and karyotypic characteristics, concluded that *S. seabrana* should be treated as a synonym of *S. scabra*. While TPL follows Maass and Mannetje (2002) and continues to accept the species as *S. seabrana*, GRIN now follows the broader concept of Vanni and Fernandez (2011) and treats it as a synonym of *S. scabra*.

Table 1. New taxa, nomenclatural changes and taxonomic status within the genus *Stylosanthes*, during period 1982–2014.

Taxon	Taxonomic status[1]		Reference	Distribution	Related species[2]	Comments in the original description
	TPL	GRIN				
New taxa						
S. guianensis var. *pauciflora*	----	Synonym of *S. guianensis* var. *guianensis*	1	Brazil, Venezuela	*S. guianensis* var. *guianensis*	• Tolerant of anthracnose and drought • Late-flowering; inflorescences few-flowered
S. nunoi	Accepted	Accepted	2	Brazil	*S. hippocampoides*	• Collected in Cerrado vegetation on sandy, very infertile soil
S. longicarpa	Unresolved[3]	Accepted	3	Brazil	*S. nunoi*	• No comments reported
S. recta	Unresolved	Accepted	4	Paraguay	*S. macrosoma*	• Collected in Chaco vegetation on sandy soils
S. salina	Unresolved	Accepted	5	Mexico	*S. viscosa*	• Collected in mangrove ecosystem, in sandy, salty fields • Annual
S. seabrana	Accepted	Synonym of *S. scabra*	6	Brazil	*S. scabra*	• Collected in Caatinga vegetation, in areas with low rainfall and long dry season • Collected on medium- to heavy-textured soils
S. maracajuensis	Accepted	Accepted	7	Brazil	*S. sericeiceps*, *S. leiocarpa*, *S. viscosa*	• Collected in shallow soils over rocky outcrops and within rock cracks
S. venezuelensis	Unresolved	Accepted	9	Venezuela	*S. scabra*	• Collected in shady environments
S. quintanarooensis	Unresolved	Accepted	10	Mexico	*S. calcicola*, *S. macrocarpa*, *S. mexicana*	• Collected on sandy soils
S. vallsii	Unresolved	Accepted	11	Brazil	*S. maracajuensis*, *S. leiocarpa*	• Collected on shallow basaltic lithosols, Paraná state
S. falconensis	Unresolved	Accepted	12	Venezuela	*S. hamata*	• Collected at 900−1,200 masl, low-montane deciduous dry tropical forest
Nomenclatural changes						
S. rostrata	Unresolved	Accepted	8	Argentina, Uruguay	*S. hippocampoides*	• Elevation to the species rank from *S. gracilis* var. *rostrata*
S. gracilis	Synonym of *S. guianensis*	Accepted	13	South and Central America	*S. guianensis*	• Elevation to the species rank from *S. guianensis* var. *gracilis*

[1]Information at December 2015. [2]According to information provided in the respective original publication. [3]Unresolved names are those to which a status of "accepted" or "synonym" cannot be assigned. References: (1) Brandão et al. 1985; (2) Brandão 1991; (3) Brandão and Costa 1992; (4) Vanni 1995; (5) Costa and van den Berg 2001; (6) Maass and Mannetje 2002; (7) Costa and van den Berg 2003; (8) Vanni 2009; (9) Calles and Schultze-Kraft 2009; (10) Gama-López and Dávila 2009; (11) Costa and van den Berg 2009; (12) Calles and Schultze-Kraft 2010a; (13) Calles and Schultze-Kraft 2010b.

Nomenclatural changes

Two nomenclatural changes are presented in the second part of Table 1.

Burkart (1939) reported on an Argentinian botanical variety, *Stylosanthes gracilis* var. *rostrata* Burkart. Recently, Vanni (2009) found reasons to elevate this variety to the rank of species as *Stylosanthes rostrata* (Burkart) Vanni. Until now, however, there has been no consensus on the taxonomic status of this name (Table 1).

Stylosanthes gracilis Kunth was originally described by Kunth (1823) as a species, but subsequently treated as a synonym of *S. guianensis* (Mohlenbrock 1957) or as a variety of *S. guianensis* (Vogel 1838; Mannetje 1977, 1984). Ferreira and Costa (1979) suggested elevating the epithet *gracilis* to species rank but it was only recently that sufficient taxonomically relevant evidence to re-establish *S. gracilis* at species level was collected (Calles and Schultze-Kraft 2010b). However, again there is no consensus on the taxonomic status (Table 1).

Invalid *Stylosanthes* names in the post-symposium literature

In Table 2 we mention several names of *Stylosanthes* taxa that have not been validly published or even not been published at all, which, however, can occasionally be found in post-symposium (i.e. post-1982) publications. Since these names have not been validly published, they should be considered as "nomina nuda" [(singular: nomen nudum: any new taxon published without a description or diagnosis; McNeill et al. 2012; Art. 38)]. These names should not be used in scientific literature; in particular cases where this cannot be avoided, such a name should be set in inverted commas and it should be made clear that the name has not been validly published.

Recent regional studies on *Stylosanthes* taxonomy

Although there has been a slow-down in botanical research on *Stylosanthes* during the past 3 decades, several regional studies on the taxonomy of the genus have been carried out recently (Table 3). The areas covered are several Brazilian states, Mexico and Venezuela.

Research needs

We see the following taxonomic research issues as particularly relevant in the interest of non-botanists working with this important forage legume genus and, in particular, its economically most important species:

- A revision of the genus *Stylosanthes* is urgently required. Costa's (2006) dissertation provides such an opportunity, although condensation of the 470-page thesis to produce an international publication would be a prerequisite.
- There are many discrepancies around the so-called "*Stylosanthes guianensis* species complex". Mannetje (1984) recognized 6 botanical varieties within *S. guianensis* [i.e. the varieties *gracilis* (Kunth) Vogel ex Benth., *intermedia* (Vogel) Hassl., *robusta* 't Mannetje, *marginata* Hassl., *longiseta* (Micheli) Hassl. and *dissitiflora* (Robinson and Seaton) 't Mannetje], while Costa and Ferreira (1984) treated the first 5 varieties as species (i.e. *S. gracilis*, *S. campestris* M.B. Ferreira and Sousa Costa, *S. grandifolia*, *S. acuminata* M.B. Ferreira and Sousa Costa and *S. longiseta* Micheli, respectively). At present, there are considerable uncertainties regarding the taxonomic status of these taxa and therefore a revision of this complex, including the newly described variety *pauciflora*, is urgently needed. We suggest that such studies be based on molecular analyses such as the evidence provided by Santos-Garcia et al. (2012).
- In the genus *Stylosanthes*, *S. scabra* is a particularly polymorphic species. This is also indicated by the suggestion made by Vanni and Fernandez (2011) that *S. seabrana* should actually be considered as a synonym of *S. scabra*. In view of the significance of *S. scabra* as a forage species, we suggest that it merits closer examination in order to identify morphological, molecular and karyotypic characteristics that might allow separating distinct groups that could be described as infraspecific taxa (e.g., botanical variety, subspecies) within an eventual "*S. scabra* species complex".
- Likewise, further examination of the variability in *S. hamata*, also an economically very important species, is required, with eventual description of the tetraploid forms as a separate taxon (see comment on "*S. hemihamata*" in Table 2).

We suggest that in the aforementioned taxonomic studies conventional morphological analyses using herbarium material be complemented with molecular analyses, making use of the large *Stylosanthes* germplasm collections held in the major tropical forage genebanks at CIAT (International Center for Tropical Agriculture) in Cali, Colombia, the Australian Pastures Genebank in Adelaide, Australia, and ILRI (International Livestock Research Institute) in Addis Ababa, Ethiopia.

Table 2. Invalid *Stylosanthes* names found in recent literature (period 1982–2014).

Name	Reference examples	Comments
S. grandiflora M.B. Ferreira & Sousa Costa nom. nud.	Kazan et al. 1993 Vander Stappen et al. 1999 Fortuna-Perez et al. 2011	There is no description of a *Stylosanthes* species under this name. Thus the name has not been validly published and consequently is a nomen nudum. We assume this is a misspelling of *S. grandifolia* M.B. Ferreira and Sousa Costa.
S. guianensis var. *vulgaris* M.B. Ferreira & Sousa Costa	Gillies and Abbott 1996 Vieira et al. 1997	The naming of this variety (Ferreira and Costa 1979) did not follow international nomenclatural rules (McNeill et al. 2012; Arts. 24.3 and 26.1). Consequently, the name was not validly published and should not be used. The correct name for this taxon is *S. guianensis* var. *guianensis* (see Table 1).
S. hemihamata nom. nud.	Maass and Sawkins 2004 Gama-López et al. 2007	Following an earlier suggestion of H. Stace (pers. comm. 1984), Maass and Sawkins (2004) proposed the name *S. hemihamata* for tetraploid *S. hamata* such as the cultivars Verano and Amiga. However, the species has never been described and consequently the name is a nomen nudum.
S. pseudohumilis Gama & Dávila nom. nud.	Gama-López et al. 2007	Gama-López et al. (2007) referred to *S. pseudohumilis* as a new species described in an MSc thesis (Gama López 2006). However, names published in independent non-serial work (e.g. post-graduate theses) are not effectively published (McNeill et al. 2012; Art. 30.8). Consequently the name is a nomen nudum.
S. tehuacanensis Gama & Dávila nom. nud.	Gama-López et al. 2007	Gama-López et al. (2007) referred to *S. tehuacanensis* as a new species described in an MSc thesis (Gama López 2006). However, names published in independent non-serial work (e.g. post-graduate theses) are not effectively published (McNeill et al. 2012; Art. 30.8). Consequently the name is a nomen nudum.
S. guianensis var. *occidentalis* Gama & Dávila nom. nud.	Gama-López et al. 2007	Gama-López et al. (2007) referred to *S. guianensis* var. *occidentalis* as a new botanical variety described in an MSc thesis (Gama López 2006). However, names published in independent non-serial work (e.g. post-graduate theses) are not effectively published (McNeill et al. 2012; Art. 30.8). Consequently the name is a nomen nudum.

Table 3. Regional studies on *Stylosanthes* taxonomy, period 1982–2014.

Country	State	Reference	Comments
Brazil	Ceará	Sousa et al. 2003	Seven species are reported, none being endemic to this state.
	Mato Grosso do Sul	Costa et al. 2008	Seventeen species are reported, including *S. maracajuensis*, which is endemic to this state.
	Roraima	Medeiros and Flores 2014	Seven species are reported, none being endemic to this state.
	São Paulo	Fortuna-Perez et al. 2011	Nine species are reported, none being endemic to this state.
Mexico	Countrywide	Gama-López et al. 2007	This Bioversity International booklet contains a taxonomic synopsis of Mexican *Stylosanthes* and includes 11 species and 2 varieties of *S. guianensis*. *Stylosanthes macrocarpa*, *S. mexicana* and *S. subsericea* are reported as endemic to Mexico. Three taxa mentioned in this booklet are not validly published (see Table 2).
Venezuela	Countrywide	Calles and Schultze-Kraft 2010c	Eleven species are reported, including *S. falconensis*, *S. sericeiceps* and *S. venezuelensis*, which are endemic to Venezuela.

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