Artículo Científico

Quantifying the nitrogen fixed by *Stylosanthes*

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Introduction

In established pastures, nitrogen is the main limiting nutrient to improving and sustaining pasture production on a long-term basis, although other nutrients, especially P, are often more limiting during pasture establishment. As in other systems, a pasture system gains N through applied fertilizer; atmospheric-nitrogen fixation by legumes, grasses, or algae; dust blown in from eroded areas; nitrate produced in thunderstorms; or, in areas close to industries, deposits of pollutants (O'Connor, 1983). Discarding N fertilization as a man-made process and thus limited to certain conditions, N_2 fixation by legumes is by far the most important natural process of N input to any agricultural system.

Legumes form a symbiotic relationship with N_2-fixing bacteria, of which the predominant group is rhizobia (Young, 1996). The legumes shelter and provide the bacteria with photosynthates in the nodules formed on their roots. The bacteria, in their turn, reduce atmospheric N_2 to assimilable forms, exporting ammonium directly to the plant. Research data suggest that tropical forage legumes can meet the requirements for balancing the N cycle of grazed pastures (Cadisch et al., 1994; Thomas, 1995). Unfortunately, their use by farmers is still limited, and more research is needed on promising new genera and varieties that would have a significantly positive impact on pastures and animal nutrition, thus becoming more readily accepted.

The main characteristic of a pasture forage legume that is important to pasture sustainability is the amount of N_2 it can fix. This quantity depends partly on inherent genetic characteristics of both components of the legume-rhizobium association, and partly on the environment in which they grow. If the affinity between the two partners is low, legume performance will be affected, reducing N_2 fixation. Most tropical forage legumes form effective symbiotic relationships with a wide range of soil-resident strains of rhizobia, but this genus of bacteria is known to possess different degrees of specificity for legumes such as *Centrosema* (Behling Miranda, 1995). Cadisch et al. (1989) have also shown that low P and K availability limits forage legume growth and significantly reduces the amount of N_2 fixed.

The genus *Stylosanthes*, native to South America, encompasses 41 species, 25 of which are from Brazil alone. The genus is well adapted to low fertility, acid soils under a wide variety of climatic conditions, while providing high-quality fodder (Schultze-Kraft et al., 1984). Because most of the 50 million hectares of pure-grass pastures in central Brazil are degraded, introducing legumes such as *Stylosanthes* could be one way of efficiently restoring pastures. A program to evaluate and select new *Stylosanthes* varieties adapted to the area around Campo Grande, MS, is being conducted by the National Center for Research on Beef Cattle (CNPGC) of the Brazilian Agricultural Research Corporation (EMBRAPA). One objective of the study is to provide new varieties with a high potential for N_2 fixation with native rhizobia, considering the importance of this element for pasture reclamation and sustainability.

We describe an experiment in which the potential for N_2 fixation of five germplasm materials of four species of *Stylosanthes* was assessed, using the natural ^{15}N-abundance technique.

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Material and methods

The experiment was set up on an acidic, low fertility, quartz-sandy (quartzipsamment) soil of central Brazil, in Agropecuária Ribeirão, Chapadão do Sul, MS (19° S and 52° W). The climate is characteristically bimodal, with an annual average rainfall of 1800 mm, peaking in October and in May. Temperatures average 25 °C in the wet season and 20 °C in the dry season.

Soil was mechanically prepared and fertilized with 300 kg/ha of lime, 200 kg/ha of simple superphosphate, and 50 kg/ha of potassium chloride. Plots of 2 x 5 m, with four rows spaced at 0.5 m, were established for each treatment in a randomized complete block design, with four replicates containing the following species:

- *Stylosanthes capitata*: an advanced line under evaluation for commercial release ('Multilinha') and accessions 1082, 1173, 1466, and 1469;

- *Stylosanthes guianensis*: the commercial variety Mineirão and accessions 1468, 1557, 1585, and 1586;

- *Stylosanthes macrocephala*: the commercial variety Pioneiro and accessions 1507, 1508, 1582, and 1587;

- *Stylosanthes scabra*: accessions 1490, 1493, 1498, 1536, and 1538.

According to several trials (unpublished data), these varieties and accessions are the most promising for their respective species and are currently being evaluated at three sites within Brazil.

The equivalent of 4 kg/ha of scarified seed of each accession was planted in November 1996, at the beginning of the rainy season. Because these species form effective symbiotic relationships with a wide range of *Bradyrhizobium* spp., commonly found in these soils, no inoculation was made. Plant shoots were harvested in 1997, during March, October, and December, and in March 1998. At each harvest, because of the species' different growth habits, shoots within one square meter of the two central rows of each plot were cut at a height of 10 cm for *S. capitata* and *S. macrocephala*, and at 30 cm for *S. guianensis* and *S. scabra*. Harvested material was dried at 65 °C to constant weight, and dry matter (DM) production measured. Shoot material from the fourth harvest was finely ground and analyzed for its total N content and natural ^{15}N enrichment, using a CN Auto-analyzer (Roboprep) coupled to a Mass Spectrometer "Europe 20/20" (Europe Scientific, Crewe, UK). At the same time, shoots of a weed (*Cyperus rotundus*) and grasses (*Brachiaria decumbens*, *Paspalum plicatulum*, and *Aristida capillacea*) growing in the same area were harvested and analyzed for their natural ^{15}N enrichment. These plants were used as reference for quantifying the N_2 fixed by the legumes.

Calculations for N_2 fixation were made, using the ^{15}N natural abundance method, as proposed by Amarger et al. (1979), and which employs the following equation:

$$\%\ N_2\ fixation = \left(\frac{\delta\,^{15}N\ nonfixing\ reference\ -\ \delta\,^{15}N\ fixing\ legume}{\delta\,^{15}N\ nonfixing\ reference\ -\ B}\right) x\ 100$$

Where

$$\delta\,^{15}N\ (\%) = \left[\left(\frac{^{15}N/^{14}N\ sample}{^{15}N/^{14}N\ standard}\right) - 1\right] x\ 1000$$

The standard was atmospheric N, which, by definition, has a $\delta\,^{15}N$ equal to zero. *B* is the $\delta\,^{15}N$ value of the N_2-fixing legume when grown with N_2 as the sole source of N. In the absence of measured values, *B* was assumed to be zero for all species.

Results and discussion

On the average, accessions of *S. capitata* produced significantly more DM ($P < 0.01$) than accessions of *S. guianensis* (Table 1). The latter, in turn, produced similarly to *S. macrocephala*. Accessions of *S. scabra* produced the least DM at all harvests, showing poor performance (Figure 1) during both rainy (first harvest) and dry periods (second and third harvests). The difference among the four species was accentuated only at the fourth cut (second rainy period), where accessions of *S. capitata* outyielded the other species.

All species had established well, using the resources made available through initial fertilization and soil preparation. By the fourth harvest, when soil nutrient availability had probably dropped and the species were thus stressed, the accessions of *S. capitata* showed sustained high DM production. Average total N accumulation (Table 2) by the *S. capitata* accessions was also significantly higher (204 kg/ha N) than that of the other species (117, 107, and 53 kg/ha N for *S. guianensis*, *S. macrocephala*, and *S. scabra*, respectively), and may partly explain *S. capitata*'s higher DM production. The higher DM production suggests that *S. capitata* accessions were able to maintain a more effective symbiosis under stress and were thus fixing larger amounts of N, which should be reflected in a higher percentage of fixed N_2.

The measured $\delta\,^{15}N$ values confirm the hypothesis (Table 2). Compared with the non-N_2-fixing controls, all

Table 1. **Dry matter production (kg/ha) of four species of *Stylosanthes* at four harvests. (Each value is the mean of production of five germplasm materials for each species.)**

Species	Harvest date				Mean
	1 Mar 97	10 Oct 97	17 Dec 97	12 Mar 98	
S. capitata	5289 b*	3475 def	2836 efg	7153 a	4688 A
S. guianensis	5045 b	4339 bcd	2138 gh	4644 bc	4041 B
S. macrocephala	5328 b	3802 cde	1926 gh	4406 bcd	3866 B
S. scabra	2199 gh	2725 fg	1620 h	2056 gh	2150 C

* Values followed by the same small letter among harvests or capital letter in the mean, do not differ (Tukey, $P < 0.05$).

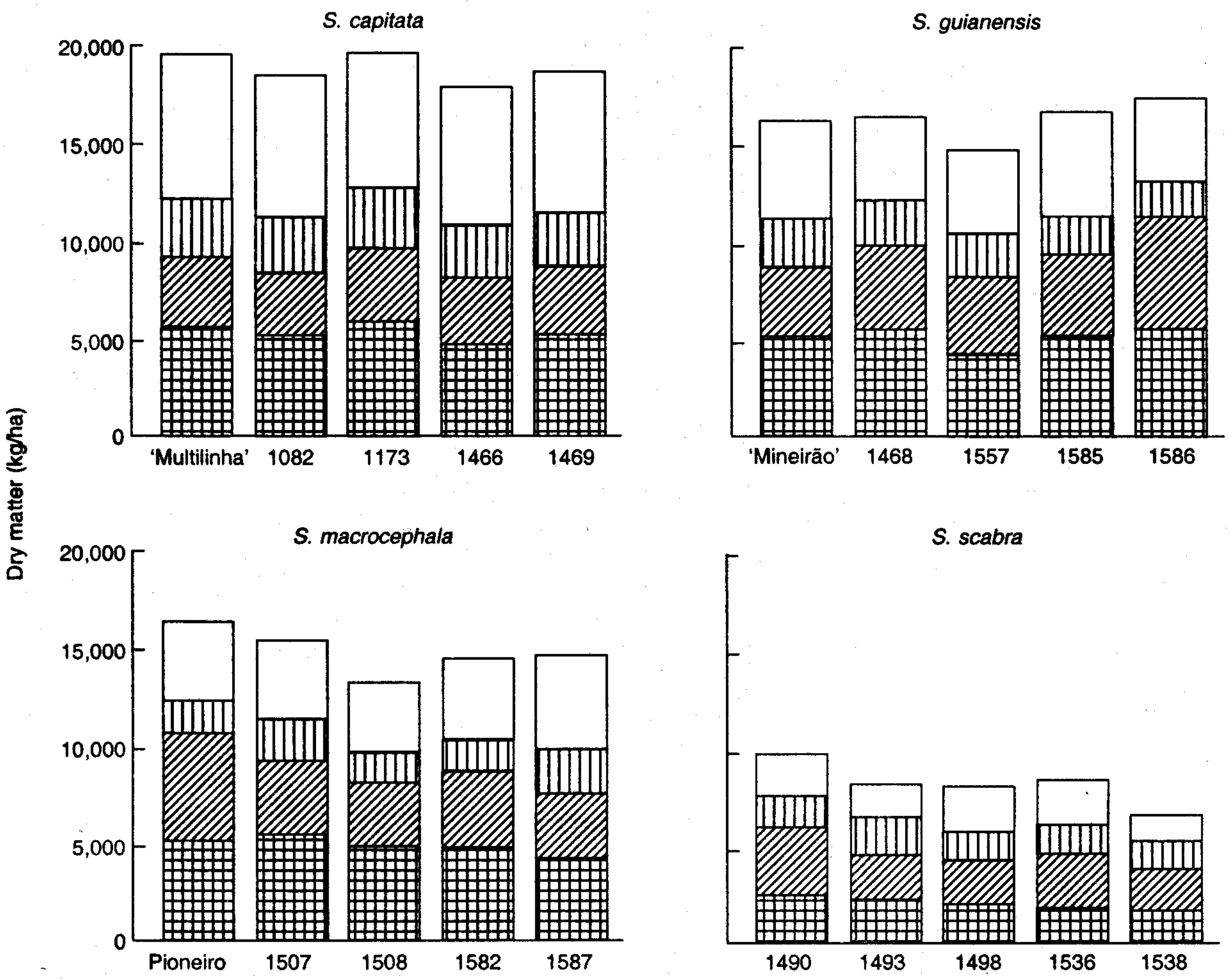

Figure 1. *Dry matter production (kg/ha) of five germplasm materials of each of four species of* Stylosanthes *harvested on 12 March 1998 (□), 17 December 1997 (▥), 10 October 1997 (▨), and 1 March 1997 (▦).*

legumes had significantly lower δ ^{15}N values, suggesting that the legumes were less dependent on soil N, because of inputs from atmospheric N_2 fixation. Calculations, using the proposed equation, indicate that, on the average, 73% to 88% of the plant's total N was fixed by *S. capitata* accessions; 74% to 79% by *S. macrocephala* accessions; 68% to 79% by *S. guianensis* accessions; and 52% to 70% by *S. scabra* accessions. These values closely agree with N_2 fixation estimates from the Colombian savannas, where *S. capitata* CIAT 10280 was among the legumes with the highest proportion of N derived from fixation (76%-87%) and *S. guianensis* (from CIAT) showed a lower fixation ability at 63%-75% (Cadisch et al., 1989).

Table 2. **Total nitrogen, δ ^{15}N, percentage of N_2 fixed, and amount of N obtained from N_2 fixation and from soil by different germplasm materials of *Stylosanthes*.**

Species	Variety or accession	δ ^{15}N	Total N (kg/ha)	Fixed N (%)[a]	N absorbed (kg/ha) Fixation[a]	Soil
S. capitata	'Multilinha'	0.45	204	88 (86-91)	179	24
	1082	0.46	212	85 (85-90)	178	34
	1173	0.62	201	83 (84-90)	167	34
	1466	1.01	194	73 (67-79)	141	53
	1469	0.65	208	83 (79-86)	171	36
Mean		0.64	204	82	167	30
S. guianensis	'Mineirão'	1.00	130	73 (68-79)	95	34
	1468	1.03	108	72 (67-78)	78	30
	1557	1.02	105	72 (67-79)	76	28
	1585	0.77	127	79 (75-84)	102	24
	1586	1.17	115	68 (62-75)	78	36
Mean		1.00	117	73	86	24
S. macrocephala	Pioneiro	0.80	113	78 (74-83)	88	24
	1507	0.88	89	76 (72-82)	68	21
	1508	0.96	105	74 (69-80)	78	27
	1582	0.89	118	76 (71-81)	89	28
	1587	0.78	109	79 (75-84)	88	21
Mean		0.86	107	77	82	24
S. scabra	1490	1.26	53	66 (59-74)	36	17
	1493	1.12	46	70 (64-76)	34	12
	1498	1.29	59	65 (58-73)	40	19
	1536	1.29	65	65 (58-73)	42	22
	1538	1.79	41	52 (42-62)	22	19
Mean		1.35	53	64	35	18

a. Calculated, using the average δ ^{15}N measured in *Cyperus rotundus* (3.20) *Brachiaria decumbens* (3.28), *Paspalum plicatulum* (4.77), and *Aristida capillacea* (3.09). Values in parentheses are ranges obtained by using individual δ ^{15}N values of the different reference plants.

However, *S. capitata* 10280 grew poorly in Colombia and did not fix as large quantities of N as did the accessions used in our experiment.

The four reference plants used in this study showed δ ^{15}N values of 3.20, 3.28, 4.77, and 3.09 for *C. rotundus, B. decumbens, P. plicatulum,* and *A. capillacea,* respectively. Because we did not know which of these reference plants was the most suitable, N_2 fixation estimates were presented as ranges derived from the variation of these enrichments (Boddey et al., 1990). The respective N_2 fixation ranges presented in Table 2 have an uncertainty value of about 15%. Further research is needed to obtain more precise information about these legumes' N_2-fixation ability by finding out their respective *B* values. Because legume shoots depend entirely on N_2 fixation, they often become slightly depleted, leading to a possible overestimate of N_2 fixation (Cadisch et al., 1993).

Within each species, the accessions took up a similar amount of N from the soil (Table 2), except for the *S. capitata* accession 1466, which took up almost double the amount of its companions, while presenting a lower percentage of fixed N_2. This result does not appear to be caused by sampling or analytical errors because values of all four randomized samples fell closely to each other. Such an ability of absorbing soil N therefore deserves further attention. Although no measurements of available soil N were made, *B. decumbens*, for example, is known to absorb as much as 50 kg/ha N during a similar season and from a similar soil. Thus, accession 1466 is able to exploit available soil N more fully than other accessions by virtue, probably, of an extended root system or a more efficient recycling of underground N.

During the 1998 rainy season, legumes fixed substantial amounts of N_2. This was particularly true for the *S. capitata* accessions, which fixed more than 140 kg/ha N in 4 months. The N_2 fixation values measured for all species and accessions show the *Stylosanthes* species' potential for accreting such an important nutrient as N. Considering the broad extension of tropical pastures in Brazil, and the costs of N fertilizers, the use of grass-legume pastures is desirable, not only for consumption by grazing animals, but also for adding N to the soil. Tropical grasses are known to add continuously high C-to-N ratio materials into the soil, which leads to the immobilization of N and a buildup of recalcitrant organic matter (Robbins et al., 1989; Robertson et al., 1997). The presence of a N_2-fixing legume that adds N-rich material to the soil may help keep a positive N balance in the soil by providing N inputs and a more efficient recycling of fixed N through higher residue quality (Cadisch et al., 1994).

However, these measurements were made with pure stand of legumes, where competition with grasses or animal influences was absent. In a mixed sward, competition between legume and grass for water, light, and nutrients such as phosphorus, potassium, calcium, and magnesium may directly reduce the rate of N_2 fixation by the legume, or affect its agronomic performance (Haynes, 1980). Furthermore, competition for available mineral N in the soil may increase the dependence of legumes. Thomas et al. (1997) found that *S. capitata* obtained 85% (instead of 100%) of its N from N_2 fixation when grown in association with *B. dictyoneura*. More, similar, evaluations of mixed pastures thus need to be carried out.

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Resumen

En un suelo arenoso con alto contenido de cuarzo de los Cerrados de Brasil se realizó un ensayo con el objeto de medir la fijación del nitrógeno por varias accesiones de *Stylosanthes macrocephala*, *S. guianensis*, *S. capitata* y *S. scabra*. Los tratamientos fueron distribuidos en bloques completos al azar con cuatro repeticiones. Los cortes de las plantas se realizaron en marzo, octubre y diciembre de 1997. En marzo de 1998 se realizó un corte adicional en el que se midió el contenido total de N y del isotopo N^{15}. Simultáneamente, se cosecharon plantas de *Cyperus rotundus* y de las gramíneas *Brachiaria decumbens*, *Paspalum plicatulum* y *Aristida capillacea* que crecían en la misma área y se determinó su contenido para ^{15}N natural. Estas plantas fueron utilizadas como patrones de comparación en la cuantificación del N fijado por las leguminosas, según la técnica de la abundancia natural del N^{15}. Los resultados mostraron que, en promedio, las accesiones de *S. capitata* produjeron significativamente más materia seca ($P < 0.01$) que *S. guianensis*, que presentó producción semejante a *S. macrocephala*. En las accesiones de *S. scabra* se observaron las producciones más bajas de MS, entre las especies evaluadas. El contenido total de N fue similar en las cuatro especies evaluadas. En relación con las plantas utilizadas para comparación, las accesiones de *S. capitata* fijaron entre 73% y 88% del contenido de N total; para las accessiones de *S. guianensis*, estos valores variaron entre 68% y 79%; para las de *S. macrocephala* entre 74% y 79% y para las de *S. scabra* entre 52% y 70%. El N fijado, en el promedio, 4 meses después de la siembra, fue 167, 86, 82 y 35 kg N/ha para *S. capitata, S. guianensis, S. macrocephala* y *S. scabra,* respectivamente.

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