BIOLOGICAL NITROGEN FIXATION BY *Adesmia bicolor* AND *A. macrostachya*, POTENTIAL FORAGE SPECIES FOR ARID AND SEMI-ARID ENVIRONMENTS

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SUMMARY

In South American arid regions cattle feed lacks during winter. Adesmia bicolor *and* A. macrostachya *are promising forage species because of their growth habit and adaptability to dry winters and N poor soils. Information about their biological nitrogen fixation (BNF) efficiencies is needed. The aim of this work is to determine their BNF efficiencies, nodule morpho-anatomy and spatio-temporal distribution, in symbiosis with rhizobia. In a greenhouse, both species were seeded and irrigated with nutritive solution: "Without N"; "With N"; and "Inoculated seeds with nutritive solution without N". Nodules morpho-anatomy and spatio-temporal distribution; shoot-leaf, root, and nodule dry matter and N content were analyzed. The studied species showed a mean BNF efficiency of ~60%, which is a relatively high efficiency considering that alfalfa shows a 61% efficiency when grown in central Argentina. This attribute is desirable in wild species with forage potential in arid environments.*

FIJACIÓN BIOLÓGICA DE NITRÓGENO DE *Adesmia bicolor* Y *A. macrostachya*, POTENCIALES ESPECIES FORRAJERAS PARA AMBIENTES ÁRIDOS Y SEMIÁRIDOS

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RESUMEN

En regiones áridas de Sudamérica hay escasez de alimento para el ganado durante el invierno. Adesmia bicolor *y* A. macrostachya *son prometedoras como forraje por sus hábitos de crecimiento y adaptabilidad a inviernos secos y suelos pobres en N. Es esencial disponer de información de sus eficiencias de fijación biológica de N (BNF). El objetivo del presente trabajo es determinar sus eficiencias de BNF, morfo-anatomía y distribución espacio-temporal de los nódulos en simbiosis con rizobios. En un invernadero, ambas especies fueron sembradas y regadas con solución nutritiva: "Sin N"; "Con N"; y "Sin N-semillas inoculadas". Se analizaron la morfo-anatomía y distribución espacio-temporal de nódulos, peso seco y contenido de N de tallos-hojas, raíces y nódulos. Las especies mostraron una eficiencia de BNF media de 60%, la cual es alta considerando que alfalfa presenta un 61% cuando crece en Argentina central. Este atributo es deseable en especies silvestres con potencial forrajero en ambientes áridos.*

Introduction

Arid and semi-arid climates comprise about one third of the total land area (Johnson *et al*., 1981). These zones were previously considered as economically unimportant; however, during the last three decades their economic and agricultural utilization has emerged as a critical element in maintaining and improving the food supply for the expanding world population (Zahran, 1999; Howieson *et al*., 2008).

Most of the arid and semi-arid regions of South America (southern Brazil, Uruguay, and central Argentina) have extreme environmental conditions, such as dry and windy winters and low N content soils (Tedesco *et al*., 2000; Scheffer-Basso *et al*., 2001a, 2002; Bianco, 2002; Speroni and Izaguirre, 2003; Barreto Dias *et al*., 2004; Veneciano *et al*., 2005). In these regions, many summer forage species are used, but there is a lack of cattle feed in winter. During these months, food supplement is not viable due to its high cost. Consequently, as commercial forage species do not grow, large areas remain unused for cattle production (Döbereiner, 1997; Veneciano *et al*., 2005). Therefore, the search is focused on wild winter species with high nutritional quality that are already adapted to local soil and climatic conditions (Tedesco *et al*., 2000; Scheffer-Basso *et al*., 2001a; Speroni and Izaguirre, 2003; Barreto Dias *et al*., 2004).

Legume species are vital in ecosystems characterized by N poor-soils (Howieson *et al*., 2008). In these ecosystems, biological N_2 fixation (BNF) is the major form

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FIXAÇÃO BIOLÓGICA DE NITROGÊNIO DE *Adesmia bicolor* E *A. macrostachya*, POTENCIAIS ESPÉCIES FORRAGEIRAS PARA AMBIENTES ÁRIDOS E SEMIÁRIDOS

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RESUMO

Em regiões áridas da América do Sul há escassez de alimento para o gado durante o inverno. Adesmia bicolor *e* A. macrostachya *são prometedoras como forragem por seus hábitos de crescimento e adaptabilidade a invernos secos e solos pobres em N. É essencial dispor de informação de suas eficiências de fixação biológica de N (BNF). O objetivo do presente trabalho é determinar suas eficiências de BNF, morfo-anatomia e distribuição espaço-temporal dos nódulos em simbioses com rizóbios. Em uma estufa, ambas espécies foram plantadas e regadas com solução nutritiva: "Sem N"; "Com N"; e "Sem N-sementes inoculadas". Analisaram-se a morfo-anatomia e distribuição espaço-temporal de nódulos, peso seco e conteúdo de N de caule-folhas, raízes e nódulos. As espécies mostraram uma eficiência de BNF média de 60%, a qual é alta considerando que alfafa apresenta 61% quando cresce na Argentina central. Este atributo é desejável em espécies silvestres com potencial forrageiro em ambientes áridos.*

of N input and it represents a renewable source for agriculture (Borreani *et al*., 2003; Tabacco *et al*., 2003; Athar, 2005; Teixeira *et al*., 2006; Armas *et al*., 2008). The significance of rhizobia of wild legumes is based on their symbiotic N_2 fixation capacity and on other activities in the soil that eventually improve fertility and plant productivity (Zahran, 2001).

Even though most wild legumes that grow in central Argentina have a spring-summer growth habit, some of them develop in the fall and fructify in early spring. Among the last ones, some *Adesmia* species should be highlighted because they produce nutritive leaves, fruits and seeds for ruminants (Maestri *et al*., 2002). Studies have been carried out to domesticate many of them in different countries, such as New Zealand (Dodd and Orr, 1995), Brazil (Scheffer-Basso *et al*., 2000; Tedesco *et al*., 2000; Scheffer-Basso *et al*., 2001a, b, c; 2002; Barreto Dias *et al*., 2004; Dias *et al*., 2004), Uruguay (Coll and Zarza, 1992), and Argentina (Bianco and Kraus, 2005; Veneciano *et al*., 2005).

One of the most important characteristics that should be known in a species that is being domesticated or introduced in an environment with arid or semi-arid conditions is its efficiency to fix N_2 in symbiosis with rhizobia. Studies have been carried out with different *Adesmia* species and the results are controversial. Burkart (1952) and Date and Halliday (1980) included them among the ineffective species in response to *Rhizobium*. Scheffer-Basso *et al*. (2000, 2001b) found that *A. araujoi* has low BNF efficiency, though it develops excellent nodulation. Vidor and Neto (1992) observed that *A. tristis* produces many nodules also, but they did not study its BNF efficiency. On the other hand, Scheffer-Basso *et al*. (2001b) determined that *A. latifolia* shows an acceptable effectiveness (37%) compared to plants supplemented with mineral N after 65 post-germination days. Golluscio *et al*. (2006) observed that *A. volckmanni* has ineffective nodules because of leghemoglobin absence, although they believed that the study should continue.

There are two species of *Adesmia* that are particularly promising as forage in Argentina, Brazil, and New Zealand, *A. bicolor* and *A. macrostachya*, because of their growth habit and high adaptability to arid and semi-arid conditions (Coll and Zarza, 1992; Dodd and Orr, 1995; Weberling *et al*., 2002; Barreto Dias *et al*., 2004; Bianco and Kraus, 2005; Veneciano *et al*., 2005). However, information related to their BNF efficiency is scarce. Coll and Zarza (1992) inferred that *A. bicolor* has a high N fixation efficiency due to the high dry matter accumulation obtained when it grows on sandy soils with low N content. Nothing is known about *A. macrostachya*.

Therefore, it is necessary to know the quantity of N that *A. bicolor* and *A. macrostachya* are able to fix in symbiosis with native rhizobia in order to evaluate their potential use as forage species in the arid and semiarid regions of South America. The objective of this work was to determine nodule morpho-anatomy, nodule spatial and temporal distribution, dry matter accumulation, and N content of *A. bicolor* and *A. macrostachya* in symbiosis with *Rhizobium* native strains.

Materials and Methods

Seeds of *Adesmia bicolor* and *Adesmia macrostachya* were collected at the Universidad Nacional de Río Cuarto, Córdoba, Argentina (33º05'S, 64º20'W). They were mechanically scarified and planted in Leonard jars (Weaver and Frederick, 1982). Three treatments were applied:

Treatment 1. Plants without N: Sterile vermiculite as substrate, irrigated with modified Munns nutritive solution without N.

Treatment 2. Plants with N: Sterile vermiculite as substrate, irrigated with modified Munns nutritive solution with N.

Treatment 3. Inoculated plants: Sterile vermiculite as substrate, seeds inoculated with wild rhizobia (each species had a specific inoculant), irrigated with modified Munns nutritive solution without N.

The modified Munns nutritive solution without N contained $34g{\cdot}l^{-1}$ KH_2PO_4, $123g{\cdot}l^{-1}$ $MgSO_4{\cdot}7H_2O$, $65g{\cdot}l^{-1}$ K_2SO_4, $0.1g{\cdot}l^{-1}$ $CaSO_4{\cdot}2H_2O$, $1.4g{\cdot}l^{-1}$ $FeCl_3{\cdot}6H_20$, $1.7g{\cdot}l^{-1}$ Na_2H_2EDTA, $0.75g{\cdot}l^{-1}$ KCl, $124mg{\cdot}l^{-1}$ H_3BO_3, $67mg{\cdot}l^{-1}$ $MnSO_4{\cdot}H_2O$, $46mg{\cdot}l^{-1}$ $ZnSO_4{\cdot}7H_2O$, $10mg{\cdot}l^{-1}$ $CuSO_4{\cdot}5H_2O$ and $2mg{\cdot}l^{-1}$ H_2MoO_4; whereas the nutritive solution with N had the same chemical composition plus $101g{\cdot}l^{-1}$ KNO_3 and $133g{\cdot}l^{-1}$ $(NH_4)_2SO_4$ (Weaver and Frederick, 1982).

One rhizobia inoculant specific for each species was obtained from nodules produced by the corresponding plants growing *in situ* at the same location. The nodules were superficially sterilized and macerated. After that, bacteria were cultured in yeast extract agar with congo red media until purification. Native rhizobia were counted in order to know the number of viable microorganisms present in the inoculant (Vincent, 1970). In the greenhouse, each strain obtained from the isolations was confirmed in its ability to develop nodules in the corresponding host plants.

Five seeds were planted in each Leonard jar. After emergence, only one plant was left. Each pot constituted one experimental unit (EU), so that the total number of EU was 27. These plants were kept at optimal environmental conditions (constant temperature of 25ºC and photoperiod of 12h) in order to determine the potential BNF efficiency. This exploratory study is to be followed by studies under arid and semi-arid conditions in the greenhouse and in the field.

At 150, 270, and 360 days after germination, the aerial parts (leaves, shoots, flowers, and/or fruits) of three plants corresponding to each treatment were destructively sampled. The samples were dried at 55°C during seven days, and weighed in an analytical balance to determine aerial dry matter. At the same sampling dates, the root systems of the same three plants were also collected. In the samples corresponding to Treatment 3 (inoculated plants), nodules were counted on main and lateral roots. After that, the nodules were separated from the roots, dried at 55°C during seven days, and weighed to obtain root and nodule dry matter. The effectiveness of BNF was calculated with the following formula (Gibson, 1980):

$$\text{BNF Eff. (\%)} = \frac{\text{(symbiotic RDM + symbiotic ADM)}}{\text{(mineral N RDM + mineral N ADM)}}$$

where BNF Eff.: biological N_2 fixation effectiveness, symbiotic RDM: root dry matter in inoculated plants (Treatment 3), symbiotic ADM: aerial dry matter in inoculated plants (Treatment 3), mineral N RDM: root dry matter obtained in plants with N (Treatment 2,) and mineral N ADM: aerial dry matter in plants with N (Treatment 2).

Nitrogen obtained from biological fixation was determined in aerial, root, and nodule dry matter after Kjeldahl digestion following the potentiometer method (HANNA-pH 211 with 0.1 mV sensitivity and NH_4^+ electrode; Bremner and Mulvaney, 1982).

Nodules were also sampled for anatomical studies. They were fixed in FAA (ethanol:acetic acid:formaldehyde, 90:5:5), dehydrated in an ethyl alcohol-xylol series, and embedded in Histowax. Cross sections were cut at 10µm and stained with safranin-fast green (Johansen, 1940). Histological slides were analyzed and photographed with an Axiophot Zeiss microscope with Axiovision software and an Axiocam HRC Zeiss camera.

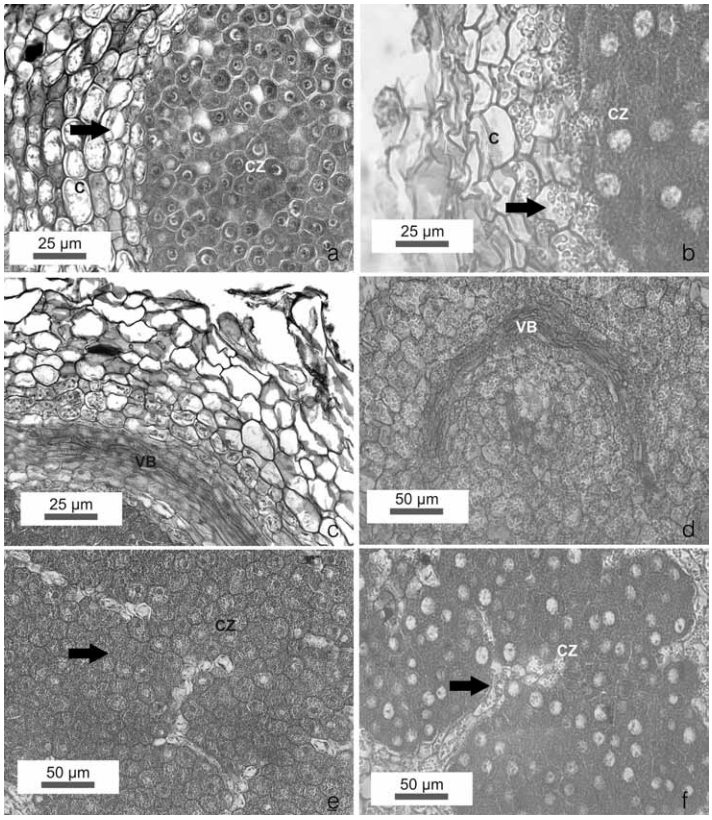

Figure 1. Nodule cross sections. a: *Adesmia bicolor* showing cortex and central zone. The arrow indicates parenchymatic sheath. b: *Adesmia macrostachya* showing the same zones. The arrow indicates parenchymatic sheath with starch. c: Vascular bundle in the inner nodule cortex of *A. bicolor.* d: Vascular bundle of *A. macrostachya*. e: Nodule central zone of *A. bicolor.* The arrow indicates cells infected with bacteroids. f: Nodule central zone of *A. macrostachya.* The arrow indicates uninfected cells with starch. C:= cortex, CZ:= central zone, VB: vascular bundle.

The experiment had a completely randomized design with three replications. Results were analyzed by analysis of variance (ANOVA) and Kruskal Wallis nonparametric ANOVA, using INFOSTAT 2005/P.1 (Universidad Nacional de Córdoba, Argentina; Steel and Torrie, 1988; Ott, 1993). When the analysis of variance showed significant differences among treatments, the least significant differences test (LSD test) was applied to make comparisons among means (Steel and Torrie, 1988; Ott, 1993).

Results

Plant growth stages in three sampling dates differed between species. *Adesmia bicolor* was in vegetative stage during the 360 days that the experiment lasted, whereas *Adesmia macrostachya* was in vegetative stage at 150 days post-germination, in flowering stage at 270 days, and in fructification stage at 360 days post-germination.

Morpho-anatomical characteristics of nodules

Typical mature nodules of *A. bicolor* and *A. macrostachya* were spherical, grew alone or in groups, and had determinate growth. The size of all nodules belonging to one plant was uniform. In nodule cross sections, two regions were observed, cortex and central zone (Figure 1). The first could be subdivided in both species into cortical parenchyma, vascular bundles, and a parenchymatous sheath. The cortex was constituted by ten cell layers. The outer four or five ones had isodiametric cells with thin walls and no visible nucleus, and they sloughed off during growth (Figures 1a and b). The inner five or six layers showed similar characteristics, and contained abundant starch. Vascular bundles were embedded in the inner cortex (Figures 1c and d).

The nodule central zone was undivided and round in both species. In *A. bicolor* this zone was occupied by parenchyma, with isodiametric cells, and nucleus usually evident and centrally located. More than 90% of them were infected with bacteroids (Figure 1e). On the other hand, the central zone of *A. macrostachya* was also occupied by parenchyma, but its cells were infected in a lower proportion than in the other species. Non-infected cells were isodiametric with thin walls, showed no visible nucleus, and contained abundant starch granules (Figure 1f).

Spatio-temporal distribution of nodules

In both species, the total number of nodules increased

TABLE I
NUMBER OF NODULES ON *Adesmia bicolor* AND *A. macrostachya* (N= 3) IN MAIN, LATERAL, AND ADVENTITIOUS ROOTS AT THREE SAMPLING DATES

Days after germination	Nodule number (mean ±SEM)				
	Adesmia bicolor			*A. macrostachya*	
	Main root	Lateral roots	Adventitious roots	Main root	Lateral roots
150	9.67 ±6.11 a	3.33 ±2.89 a	0.00	46.33 ±25.70 a	51.33 ±54.27 a
270	167.00 ±109.42 a	101.33 ±52.60 a	24.00 ±41.57 a	105.00 ±58.81 a	295.00 ±429.97 a
360	259.67 ±181.45 ab	354.00 ±170.01 b	45.00 ±56.93 a	244.33 ±145.11 a	333.00 ±124.58 a

Different letters at the same sampling date in each species indicate significant differences among root types according to Fisher LSD test (α>0.05).

as the plant grew (Figure 2). In *A. bicolor*, the mean total number of nodules at 270 days post-germination increased 2248.69% with regard to the previous sampling date (150 days). After 360 days, the increase was 234.5% compared to the previous one (270 days). From first to last sampling dates, the total number increased 5273.08%. *A. macrostachya* behaved in a similar way but the increase in nodule number was not so marked, because the plants developed nodules earlier than in *A. bicolor* (150 days post-germination). From the first sampling date (vegetative growth stage) to the second one (flowering growth stage), *A. macrostachya* nodule number increased 307.19%. From the second to the third sampling date (fructification growth stage) it increased 192.44%. The total increase was 591.16%.

Spatial distribution of nodules on the root system differed between species. At the first two sampling dates, most *A. bicolor* nodules developed on the main root (Table I). At 150 days after germination, nodule total number was 13, 10 on the main root and 3 on lateral roots. At 270 days post-germination, the total number of nodules was 292, 167 developed on the main root, 101 on lateral roots, and 24 on adventitious ones, whereas one year after the experiment started more nodules were produced on lateral roots than on the main root; the total number of nodules was 659, 260 on the main root, 354 on lateral roots and 45 on adventitious ones.

On the other hand, nodules of *A. macrostachya* developed mainly on lateral roots from the beginning to the end of the experiment. At 150 days after germination, the total number of nodules was 98, 47 developed on the main root and 51 on lateral roots. At 270 days after germination, there were 400 nodules, 105 of them on the main root and 295 distributed on lateral roots. After one year of experiment, 577 nodules were produced, 244 on the main root and 333 on lateral roots.

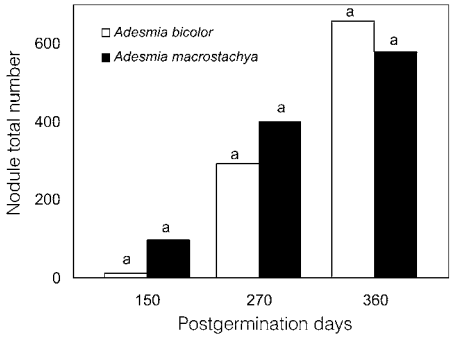

Figure 2. Nodule total number at three different sampling dates (150, 270 and 360 days post-germination). Same letters in each sampling date indicate non significant differences among treatments according to Fisher LSD test (α>0,05).

Even though there were some differences between mean total number of nodules of both species, they were not statistically significant (at 150 days: p= 0.14, 270 days: p= 0.74, and 360 days post-germination: p= 0.64; Figure 2). There were no significant differences in mean number of nodules on the main or lateral roots between both species or each sampling date either (on main root: at 150 days, p= 0.07; at 270 days, 0.44; and at 360 days, 0.91; on lateral roots: at 150 days, p= 0.20; at 270 days, 0.50; and at 360 days, 0.88).

Dry matter

Table II shows dry matter accumulation in aerial, root, and nodule parts of both species. At 150, 270, and 360 days post-germination, there were no statistically significant differences among *A. bicolor* aerial dry weight of the two treatments that had plants alive (plants with N -Treatment 2- and inoculated plants - Treatment 3; with p= 0.08, 0.21 and 0.21, respectively). Furthermore, at the same sampling dates, root dry matter showed no significant differences between treatments either (p= 0.07, 0.16 and 0.20, respectively). Nodule dry matter showed significant differences among

TABLE II
AERIAL, ROOT, AND NODULE DRY MATTER ACCUMULATION OF *Adesmia bicolor* AND *A. macrostachya* (N= 3) AT THREE DIFFERENT SAMPLING DATES

Days after germination	Treatment	*Adesmia bicolor* (g)			*A. macrostachya* (g)		
		Aerial	Root	Nodule	Aerial	Root	Nodule
150	Plants without N	0.005 ±0.002 a	0.005 ±0.001 b	0.000	0.010 ±0.002 a	0.008 ±0.002 a	0.000
	Plants with N	0.050 ±0.040 b	0.011 ±0.004 ab	0.000	0.200 ±0.240 a	0.090 ±0.120 a	0.000
	Inoculated plants	0.020 ±0.010 ab	0.008 ±0.001 a	0.002	0.130 ±0.090 a	0.030 ±0.020 a	0.020
270	Plants without N*	--	--	--	--	--	--
	Plants with N	8.350 ±0.792 a	0.540 ±0.390 a	0.000	5.640 ±1.690 b	1.120 ±0.280 b	0.000
	Inoculated plants	1.550 ±0.98 a	0.130 ±0.120 a	0.200	0.340 ±0.080 a	0.080 ±0.020 a	0.080
360	Plants without N	--	--	--	--	--	--
	Plants with N	7.690 ±3.120 a	1.750 ±1.640 a	0.000	9.050 ±5.360 a	2.800 ±1.300 a	0.000
	Inoculated plants	4.970 ±0.78 a	0.290 ±0.010 a	0.340	1.870 ±0.740 a	0.360 ±0.210 a	0.330

Different letters in the same column and same sampling date indicate significant differences among treatments according to Fisher LSD test (α >0,05).
* This treatment does not have data at 270 and 360 days post-germination because plants without N die due to its absence.

sampling dates (p= 0.002). Plants belonging to plants without N -Treatment 1- died because of N absence.

Aerial and root dry matter of *A. macrostachya* showed no significant differences among treatments at 150 and 360 days post-germination (aerial: p= 0.36 and 0.08, root: p= 0.37 and 0.07, respectively), but they were significantly different at 270 days after germination (aerial: p= 0.006, root: p= 0.004). There were significant differences in nodule dry matter also among sampling dates in this species (p=0.01). These differences arose at 360 days post-germination, as the first two sampling dates were not significantly different.

A. bicolor effectiveness of BNF was 40.32% at 150 days, 23% at 270, and 59.19% at 360 days after germination (vegetative stage), whereas *A. macrostachya* effectiveness was 62% at 150 days (vegetative stage), 7.4% at 270 days (flowering stage), and 21.6% at 360 days post-germination (fructification stage).

Comparing only inoculated plants -Treatment 3- from both species, it could be observed that aerial, root, and nodule dry matter at the first two sampling dates did not show significant differences (150 days after germination: aerial p= 0.25, root p= 0.10, nodule dry weight p= 0.10; 270 days post-germination: aerial p= 0.10, root p= 0.65, and nodule dry matter p= 0.70). However, at 360 days aerial dry matter showed significant differences between the species (p= 0.01), but root (p= 0.50) or nodule dry matter did not (p= 0.90).

Nitrogen content

Nitrogen content in aerial, root, and nodule dry matter corresponding to both species increased all along the year of the experiment (Table III). At 150 days post-germination, it could not be determined because the sample size was too small and the technique was not sensible enough. At 270 days, *A. bicolor* Inoculated plants -Treatment 3- aerial dry matter showed 52% of N and *A. macrostachya* ones 45,3% with regard to plants with N -Treatment 2- aerial dry matter. These differences were statistically significant (p= 0.03 for both species). However, at 360 days, there were no significant differences between the treatments that have plants alive: *A. bicolor* inoculated plants -Treatment 3- showed 97% of N content and *A. macrostachya* inoculated plants -Treatment 3- showed 93% of N content in the aerial dry mass in comparison to plants with N -Treatment 2- (p= 0.69 and 0.72, respectively). Root N content behaved in a similar way, except at 270 days post-germination, when *A. bicolor* did not show significant differences (p= 0.54).

Nitrogen content of inoculated plants -Treatment 3- was similar in *A. bicolor* and *A. macrostachya* as there were no significant differences in aerial, root or nodule N content at 270 and 360 days post-germination (aerial: p= 0.19 and 0.07, root: p=0.38 and 0.39, nodule: p=0.80 and 0.40, respectively).

TABLE III
AERIAL, ROOT, AND NODULE N CONTENT OF *Adesmia bicolor* AND *A. macrostachya* (N= 3) AT THREE DIFFERENT SAMPLING DATES

Days after germination	Treatment	*Adesmia bicolor* (g. 100 g sample^{-1})			*A. macrostachya* (g. 100 g sample^{-1})		
		Aerial	Root	Nodule	Aerial	Root	Nodule
150	Plants without N	ND	ND	ND	ND	ND	ND
	Plants with N	ND	ND	ND	ND	ND	ND
	Inoculated plants	ND	ND	ND	ND	ND	ND
270	Plants without N*	--	--	--	--	--	--
	Plants with N	2.23 ±0.24 b	1.08 ±0.61 a	0.00	1.50 ±0.26 b	1.99 ±0.20 b	0.00
	Inoculated plants	1.23 ±0.49 a	0.79 ±0.46 a	2.40 ±1.43	0.68 ±0.37 a	1.11 ±0.33 a	2.30 ±0.16
360	Plants without N	--	--	--	--	--	--
	Plants with N	2.57 ±0.21 a	1.78 ±0.14 a	0.00	1.86 ±0.26 a	1.29 ±0.05 a	0.00
	Inoculated plants	2.51 ±0.15 a	1.47 ±0.20 a	2.41 ±0.09	1.73 ±0.54 a	1.31 ±0.24 a	2.15 ±0.48

Different letters in the same column and same sampling date indicate significant differences among treatments according to Fisher LSD test (α>0.05).
* This treatment does not have data at 270 and 360 days post-germination because "Plants without nitrogen" die due to N absence.

Discussion

Even though there is a high degree of variability in the way nodule morphology and anatomy of *Adesmia* are described in the literature (Rothschild, 1967; Scheffer-Basso *et al*., 2000), this study established that *A. bicolor* and *A. macrostachya* develop determinate nodules and, in cross section, they are only divided in two regions: cortex and central zone. The novel finding was that *A. macrostachya* accumulates abundant starch in inner cortical cells and central zone, whereas *A. bicolor* only accumulates it in the inner cortex. This characteristic was not found in other *Adesmia* species analyzed by Rothschild (1967).

Most nodules of *A. bicolor* were found on the main root. Therefore, this species resembles *A. capitellata*, where almost all nodules developed on the main root, whereas other species, like *A. araujoi*, developed nodules only on lateral roots (Scheffer Basso *et al*., 2000). At the end of the vegetative stage, nodules of *A. bicolor* started to develop on first degree lateral roots. On the other hand, *A. macrostachya* can be considered as an intermediate case as it had ~50% of its nodules on lateral roots from the start of the experiment.

Even though leguminous species that acquire N only through symbiosis usually decrease their growth rates due to morphological alterations (Cassman *et al*., 1980), *A. bicolor* and *A. macrostachya* still maintained a relatively high biomass accumulation during the vegetative stage. Furthermore, these species can be considered as highly effective to fix N in symbiosis with rhizobia. The analyzed species showed a mean BNF efficiency of ~60% at vegetative stage, which is a relatively high efficiency considering that alfalfa shows 61% when it grows in central Argentina (Perticari, 2001). According to Vance (2002), alfalfa is one of the most efficient forage legume. Among all *Adesmia* species studied so far (Burkart, 1952; Coll and Zarza, 1992; Scheffer-Basso *et al*., 2000, 2001a), *A. bicolor* and *A. macrostachya* would be the most efficient ones. This attribute is highly desirable in wild species with forage potential in environments under arid and semi-arid conditions.

On the other hand, during flowering and fructification, *A. macrostachya* dry matter ac-

cumulation decreased because flower and fruit development and ripening demanded an important quantity of N. In order to reach growth rates close to potential ones, individuals should stay in vegetative stage or, otherwise, satisfy their N requirements from the soil, although this latter situation is hard to achieve in a N poor-environment.

In conclusion, during the vegetative stage, *A. bicolor* and *A. macrostachya* fixing N in symbiosis with native rhizobia obtain enough amounts of this nutrient in order to grow and develop in a similar way as do plants supplemented with mineral N. Therefore, these species could be considered as highly beneficial in arid and semiarid environments, because of their potential input of N and organic matter to the ecosystem during winter. As results are encouraging, this study should continue with experiments in the field and in a greenhouse under dry conditions.

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