Comparative Studies of Nitrogen Fixing Potential of *Desmodium ramississimon* and *Vigna unquiculata* for Soil Fertility Management

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Summary

The occurrence of large numbers of legume species in the tropics with potentials for nitrogen fixation could be exploited to supply nitrogen, if they can be integrated into the farming system. The N_2 – fixing potential of a native herbaceous leguminous species namely, Desmodium ramississimon *(Dm) and grain legume,* Vigna unquiculata *(Cp) were studied in the green house and field, on three types of soil.*

In both situations, nodulation was influenced by the soil type. Nsukka soil, which had sandy texture, highest level of available phosphorus among the soils investigated in the study and moderate level of other plant nutrients (Mg and K) enhanced nodulation, which supported N-fixation. Soil type also influenced the quantity of N accumulated by each species, but had no effect on nitrogen concentration in the different plant parts. Desmodium ramississimon *had higher nodule weight and accumulated more nitrogen and fixed more N_2 than* Vigna unquiculata *in the three soils. The mean nodule dry weights were in the ranges of 61.6-239.2 mg/plant for Dm in the three soils as compared to the range 3.2-31.4 mg/plant for Cp. Symbiotic dependence of DM varied with soil type ranging from 63.62% in Adani soil to 88% in Nkpologu soil, whereas Cp had the least symbiotic dependence value. These trends were confirmed in the field thereby indicating that* Desmodium ramississimon *had greater N_2-potential than the cultivated legume studied.*

Résumé

Etudes comparatives de la capacité de fixation de l'azote par *Desmodium ramississimon* et *Vigna unquiculata* pour la gestion de la fertilité du sol

L'existence d'un grand nombre d'espèces de légumineuses qui présentent une bonne capacité de fixation de l'azote atmosphérique, dans les tropiques peut être exploitée pour remplacer l'utilisation d'engrais azotés, s'il est possible d'intégrer ces légumineuses dans les systèmes de production agricole. Des essais ont été réalisés en serre et au champ sur trois types de sol afin de comparer la capacité de fixation de l'azote de deux légumineuses: une plante de couverture, Desmodium ramississimon *(Dm), et une plante principalement cultivée pour ses graines comestibles,* Vigna unquiculata *(Cp). Dans les deux cas, le type de sol a eu un effet significatif sur la formation des nodules. Pour le sol de Nsukka, caractérisé par la texture sableuse, une haute teneur en phosphore disponible comparativement aux autres sols étudiés, une teneur moyenne en autres éléments (Mg et K), la formation de nodules a connu une augmentation significative et a permis une fixation importante d'azote. Le type du sol a également influencé la quantité d'azote accumulée d'une manière significative pour chaque espèce mais sans toutefois montrer de différence significative sur sa concentration dans différentes parties de la plante. Pour les trois types de sol,* Desmodium ramississimon *avait un poids élevé en nodules et fixait une quantité plus importante d'N_2 par rapport à* Vigna unquiculata*. Dans les trois types de sols, les poids secs moyens des nodules se situaient entre 61,6-239,2 mg/plante pour Dm et entre 3,2-31,4 mg/plante pour Cp. Quant à la dépendance symbiotique de Dm, elle variait de 63,62% dans les sols d'Adani à 88% dans les sols de Nkpologou tandis que cette dépendance était la plus faible pour Cp. Ces tendances ont été confirmées lors des essais réalisés en champ montrant que* Desmodium ramississimon *avait une capacité plus élevée de fixation de N_2 comparativement à la légumineuse à graines étudiée.*

Introduction

Nitrogen required by plants is commonly obtained from either mineralization of native nitrogen in the soil, from the application of fertilizers or through nitrogen fixation by both leguminous plants and non-symbiotic

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nitrogen fixers (16). Legumes have been used to enhance soil fertility because of their ability to fix atmospheric nitrogen through symbiotic association with Rhizobia. Of all known biological systems that fix atmospheric nitrogen, a *Rhizobium* symbiosis is the most elaborate and efficient (10). It contributes the greatest quantity of nitrogen to the ecosystem and therefore, influences crop production more than any other nitrogen fixing system. A *Rhizobium* symbiosis contributes up to 20% of the annual total of fixed nitrogen by all biological systems (13). The *Rhizobium* symbiosis has, therefore, been more intensely studied than all other biological nitrogen fixing systems because of its singular importance in the nitrogen balance of ecosystems.

Industrial sources of nitrogen are expensive. Most farmers in developing countries cannot easily afford using fertilizers because of their cost. Hence, nitrogen importance in farming systems of the technologically less developed countries of the tropics. Moreover, the use of nitrogenous fertilizers has posed some problems such as pollution of fresh waters in developed countries. Apart from direct use of elemental nitrogen gradual release of nitrogen from residues enables more efficient use of nitrogen by subsequent non-legume crop (3).

Legumes occur through out the world but the largest numbers of genera and species are found in the tropics. Members of the three subfamily *Papillinoideceae* have the highest (93%) nodulating species followed by *Minosoideae* (87%) and *Caesalpinoidecaeae* with only 23% nodulating species. Ironically, it is also in the tropics that the greatest demand for nitrogen occurs due to weather and soil factors, which combine to make nitrogen availability very short-lived. Although tropical agriculture incorporates many leguminous species, the full potential of these species as sources of cheap nitrogen has not been intensely exploited. In traditional farming systems of the tropics, bush fallows are relied upon to recycle nutrients and restore soil fertility and soil organic matter, which are lost during several cycles of cropping without fertilizer application.

Natural bush regrowth often contains a high proportion of leguminous species, which contributes to the restoration of soil fertility. Little is known, however, about the quantities of nitrogen contributed by the legumes to the nitrogen economy of these fallows. Proper utilization of legumes in agriculture requires fairly detailed knowledge of the quantities of nitrogen fixed by the plants under different conditions. Managed or planted fallows can dramatically reduce the length of cultivation cycle and provide an intermediary practice between shifting cultivation and continuous cultivation. For instance, fallow under Kudzu (*Pueraria phaseoloides*) a nitrogen fixing creeper for 1-2 years, has the same restorative effect as 25 years of forest fallows (*14*). There are good indicators that selection of herbaceous fallow legumes with high dry matter yield, nitrogen fixing potential and moderate decomposition rate, can reduce the duration of fallow drastically. This will be to the advantage of the peasant farmers where increasing population density exerts pressure on the use of land with the consequent need to decrease the fallow period.

Justification for choosing *D. ramississimon* and *V. unquiculata* for the study.

A survey of the distribution of herbaceous legumes in fallow lands of the derived savanna conducted by Ezedinma *et al.* (6), showed that *Desmodium* and *Vigna* genera were found at all the invistigated sites, whereas abrus was found at only two sites.

Based on this premise, it was decided that species belonging to *Desmodium* and *Vigna* genera should be chosen. *Desmodium ramississiman* is a representative of species with forage qualities which is quite available in the experimental area. Nodulation of *Desmodium* is profilic and the amount of N_2 fixed quite considerable amounting to 320–360 kg N/ha/yr. As *Vigna unquiculata* occurred in all the sites studied, it was interesting to have a more accurate idea of its possible contribution to the nitrogen supply in local soils. Therefore, the nitrogen- fixing potential of wild and cultivated legume species were evaluated with a view to using them in planted fallow.

Materials and methods

Two separate experiments were conducted, namely a green house experiment using three soil types and two-legume species and a field experiment conducted during the rainy season using the same legume species as in the green house experiment. A preliminary screening to select the wild legume species used was conducted before the start of the experiments and selection of the wild legume species was based on the following observed criteria:

(a) The general growth habit of the plant, that is whether erect, spreading or climbing.
(b) Relative growth rate.
(c) Ability to nodulate without inoculation.
(d) Amount and viability of available seeds. The legume species considered in the preliminary screening were *Abrus canescens, Vigna micranta.*

The main characteristics of the two species finaly chosen are as follows:

(1) *Desmodum ramississimon* (G. Don). This plant has spreading growth habit, high seed viability, good establishment, comparatively high dry matter yield, and abundant and well-distributed nodules along the whole root system including adventitious roots. It is annual with indeterminate growth, flowering and fruiting continuous with first flowers appearing at 8 weeks.

Vigna unquiculata. This plant has spreading growth habit, high viability of seeds, good establishment, good dry matter yield and nodules are found on main root and its branches. It is an annual with interminate growth habit, flowering and fruiting are continuous for a fairly short period with first flowers appearing at 6 weeks.

The green house study

The legume species used were given the following designations, *Vigna unquiculata* (CP) *Desmodium ramississimon* (DM). The three soils (15 cm soil depth) used were Nsukka soil collected from the Faculty of Agriculture farm, University of Nigeria, Nsukka, the other soils were collected from Nkpologu and Adani. The characteristics of the soils are as shown in table 1. Although from the results of the particles size analysis the Adani soil was classified as loam, it has very poor physical properties (poor water infiltration and high puddling capacity). This was probably because the silt and clay fractions accounted for 62% of the weight of the soil. The soil, therefore, behaved more like a clay soil.

The treatment combinations of the species and the three soils in this experiment were as follows:

CPNS = CP + Nsukka
CPNK = CP + Nkpologu
CPAD = CP + Adani
DMNS = DM + Nsukka
DMNk = DM + Nkpologu
DMAD = DM+ Adani
Control = CO (no crop).

The experimental design was a randomized complete block with three replications and five harvest periods from the three locations.

The three soils were air-dried in the green house and crushed to pass through a 2 mm sieve. Four kilograms of each soil were weighed into 28 cm diameter non-perforated black polythene bags. Three bags without any crops growing in them were included to serve as controls and to correct for any N that may be introduced in the water used to grow the plants and possible activities of free-living N_2 fixers. The legume seeds were scarified in concentrated H_2SO_4 for one week and left to sprout in petri-dishes. After sprouting, they were transplanted at the rate of five seedlings per bag and later thinned to two per bag at two weeks after transplanting. The grain legume seeds were planted unscarified into the bags at the rate of five seeds per bag on the same day the wild legume seeds were scarified. One week after seedling emergence, the plants were thinned down to two plants per bag.

Harvesting

The first harvest was taken at 4 weeks for cowpea and all subsequent harvests were taken at 2 weeks interval till the last harvest at 16 weeks. At each harvest the following observations were carried out.

Table 1

Characteristics of the three soils used in the green house study and the field experiment

Soils	PH				Exchangeable Cations			Available	Particle size Analysis			Textural Class
	KCl	H_20	Organic C	total N	Ca	Mg	K	P	Sand	Silt	Clay	
			– % —		mg/kg	mg/kg —		ppm		%—		
Nsukka	4.16	4.65	0.51	0.056	210.00	55.00	17.50	5.00	90	6	4	Sand
Nkpologu	4.05	4.76	0.57	0.043	100.00	25.00	20.00	4.00	84	4	12	Loamy sand
Adani	4.20	4.43	0.39	0.063	325.00	225.00	32.50	2.00	38	48	14	Loam
Field Block 1	4.31	4.86	0.87	0.071	162.00	21.00	15.41	10.00	72	8	20	Sandy clay loam
Field Block 11	4.05	4.80	0.87	0.074	168.00	23.00	16.06	5.50	72	8	20	Sandy clay loam
Field Block 111	4.08	4.68	1.1	0.084	156.00	240.00	15.82	8.00	72	8	20	Sandy clay loam
Field Block IV	4.06	4.76	1.02	0.081	181.00	21.00	18.03	8.00	68	6	26	Sandy clay loam

i. Inspection of nodules for the presence of leghemglobin (pink coloration).
ii. Separation of nodules from roots and enumeration.
iii. Complete excavation of roots from the soil and separation of each plant into leaves, stems, roots and fruits.
iv. Collection of soil sample from each bag for N determination.

The separated plant materials and nodules were properly bagged, labeled and dried to a constant weight at 65 ˚C (using a forced draft oven) and weighed. The samples were ground with a stainless steel mill, and stored in polythene bags for N determination.

Analytical procedure

Soil

The particle size analysis was done using (1) hydrometer method, while soil pH was measured in a suspension with a soil/water and soil/0.1N KCl ratio of 1:2.5 using a Beckman pH- meter. Soil total N, Organic matter, available P and exchangeable P were determined by the Kjeldahl method, nitrogen determinations for the soil were done using the microkjeldahl method. Carbon determination was by the Walkley and Black (17) method, Bray's method (II), Bray and Kurtz (2) and flame photometry, respectively. Calcium and magnesium were determined by atomic absorption spectrophotometry.

Plant materials

All the plant components collected at harvest were analysed for total N using the regular microkjeldahl procedure.

Determination of the amount of N fixed symbiotically

The amount of apparent N fixed was estimated using the following equation:

N accretion= Total N in the system at harvest – (seed N+ initial soil N+N change in soil control) (9). Where Total N in the system is equivalent to: soil N + plant N (N in leaves, stems, root nodules and fruits). The N content is the N percentage of the component multiplied by the dry weight. For the wild legume species, *Desmodium* seed weight was so small (0.002/g) that N from this source was negligible. There was no need to correct for this N source.

Statistical analysis

All data were analysed using the analysis of variance (8, 15). All values were compared using Fisher's least significant difference at 5% levels of probability.

The field experiment

The field experiment was conducted during parts of rainy season of 1989 (May -October) at a site in the University of Nigeria Nsukka farm that had not been cropped for four years, the characteristics of the soil were as given in table 1 and the soil was very low in N. The same legume species used in the green house study were grown.

Field layout

The field experiment was laid out in a randomized complete block design with four blocks. Each block (22.75 m x 8.25 m) composed of five plots each measuring (9.55 m x 7.60 m) and each having one of the species used. The blocks were separated by a distance of 1.5 m and plots within the block by 1 m. Each plot had 6 rows of plants sown at a spacing of 75 cm between and within row. Scarified seeds of the wild legume species were planted out in seed boxes and the seedlings transplanted at 2 weeks of age. The grain legume seeds were planted on the same day the wild legume seeds were planted, weeding was done at intervals of two weeks.

Harvesting

The first harvest was taken at 4 weeks for cowpea and all subsequent harvests were taken at intervals of 2 weeks till the end of the fifth harvest period at 12 weeks after transplanting.

For *Desmodium*, the first harvest was at 12 weeks due to delay in plant establishment and nodule initiation. The experimental design was a randomized complete block with three replications. There were (7) treatments as follows:

So	Soil alone (Control)
So + Cpl	Soil + *V. unquiculata* leaves
So + Cps	Soil + *V. unquiculata* stems
So + Cpr	Soil + *V. unquiculata* roots
So + cpl	Soil + *V. unquiculata* leaves
So + Dml	Soil + *D. ramississimon* leaves
So + Dms	Soil + *D. ramississimon* stems
So + Dmr	Soil + *D. ramississimon* roots

Results and discussion

Green house experiment

Nitrogen fixation and some parameters for assessing its efficiency.

(1) Nodule weight and number

Table 2 shows the mean dry weights (mg/plant) of nodules of CP and DM. At each harvest period, nodules were visually inspected for the presence of legbemoglobin. Most of the nodules had pink coloration (an indication of effectiveness), which decreased with increasing nodule age. In all the

Table 2

Mean dry matter of legumes as well as number of nodules as influence by plant age

Treatment	Dry matter yield Leaf	Stem	Root	Nodule	Total legumes Components	Nodule number/plant
	g/plant	g/plant	g/plant	mg/plant	g/plant	
CPNS	3.71	2.18	1.27	200.80	7.16	45.11
CPNK	1.54	1.05	0.67	86.00	3.26	26.06
CPAD	1.70	0.78	0.45	46.00	2.93	31.39
DMNS	6.21	7.06	2.53	239.20	15.80	391.95
DMNK	2.71	3.16	1.53	86.68	7.40	244.80
DMAD	2.26	2.49	0.86	61.60	5.61	103.11
FLSD (0.05) (CP and Dm)	0.79	0.53	0.30	0.80	2.61	0.14

legume species, soil type affected nodule weight.
Nodulation was highest in Nsukka soil and for each species, not significantly different between Nkpologu and Adani. Nodule numbers and weight per plant for CP recorded in this experiment fall within the ranges observed by Eaglsham *et al.* (4). *Desmodium* had higher nodule weight in all the three soils although the magnitude of the difference depended on soil condition. High performance of DM is likely to be due to superior genetic potential to nodulate.
The highest number of nodules was observed in Nsukka soil and least in Adani soil. Nodule weight is a very important parameter that contributes to nitrogen fixation. In a study conducted by Miller *et al.* (11) using three cowpea genotypes, nodule weight was the major factor contributing to N_2 fixation activity in both green house and field experiments while number was important mainly through correlation with nodule weight.
Figure 1 shows the pattern of nodules mass accumulation of cultivated and wild legumes respectively.

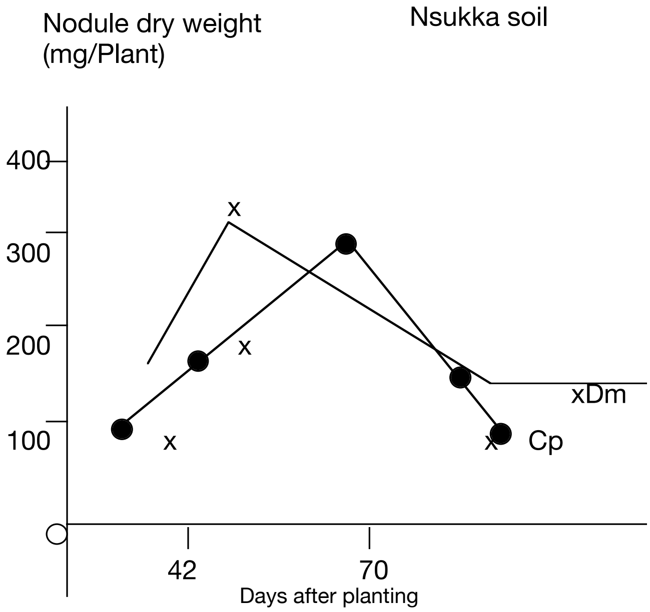

Figure 1: Green house experiment, nodule mass with time for cowpea and Desmodium in three different soils.

In Nsukka soil, all the legume species had clearly defined peaks of nodule weight after which there were marked declines. Cowpea reached its peak at 56 DAP in Nsukka soil and at 70 DAP in Nkpologu and Adani soils. *Desmodium* reached its own peak at 84 DAP in Nsukka and Nkpologu with no clear peak in Adani. Maximum nodulation occurred in CP after flowering.

Time of peaking of nodule number in CP, was influenced by soil type. Nodule population pattern was more variable within species than nodule mass, thus suggesting that numbers may be more sensitive than weight indifferent soil conditions.

Nitrogen fixation
Table 3 shows the mean values of symbiotically fixed N_2 (mg/plant) in the legume species. Soil type influenced the amount of N_2 fixed by each species.

Table 3
Mean values of symbiotically fixed N_2 in the green house as influenced by plant age

	Legume Species	
Soils	Cp mg/plant	DM
Nsukka	108.62	173.74
Nkpologu	37.56	91.98
Adani	23.34	56.15

F-LSD 0.05 (CP and Dm)= 0.32

All the species fixed maximum N_2 in Nsukka soil. There were no significant differences in amount of N_2 fixed in Nkpologu and Adani by CP while DM fixed significantly more N_2 in all the three soils. For example in Nsukka soil, DM fixed 173.7 mg N_2/plant and CP 108.62 mg N_2/plant. The superiority was also manifested in the other two soils. The amount of N_2 fixed by leguminous plant varies considerably among species and is influenced by several factors.
However, when a legume consistently shows higher levels of N_2–fixation under different growing conditions, it might be concluded that it has superior N_2 fixation potential. Among the several factors that determine the quantity of N_2 fixed by a legume plant, dry matter yield is very important (5, 7, 12, 18). Therefore, most of the unfavourable factors retarding plant yield

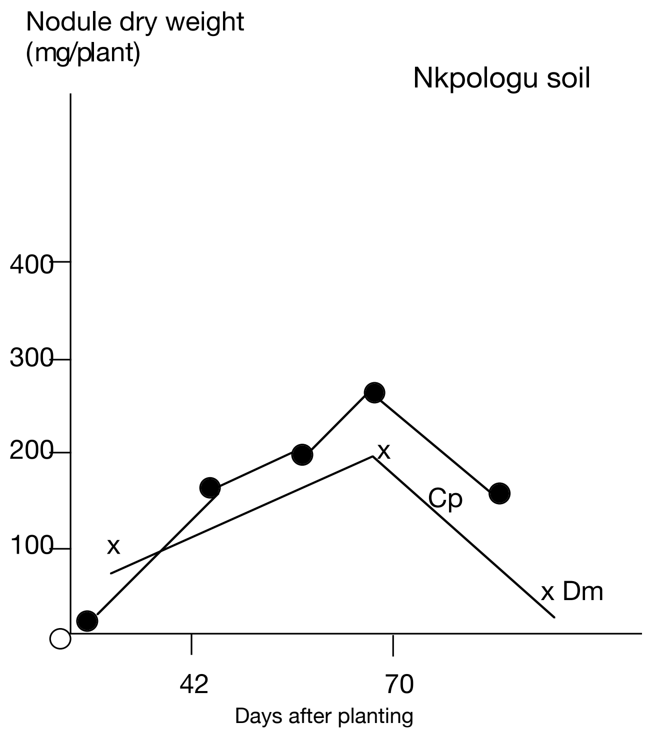

Figure 2: Green house experiment, nodule mass with time for cowpea and Desmodium in three different soils.

invariably also reduced N_2 fixation. The rate of N_2–fixation was strongly influenced by plant age and soil type but the pattern varied with species although it was sigmoidal. The highest level of N_2 fixation was attained after flowering as shown in figure 3.

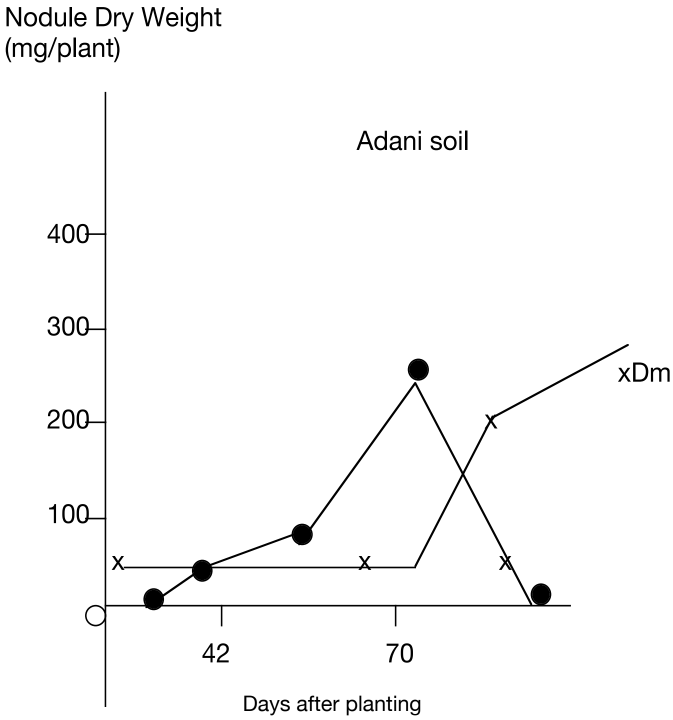

Figure 3: Green house experiment, nodule mass with time for cowpea and Desmodium in three different soils.

Field experiment

Some parameters of assessing N_2–fixation
Number and weight of nodules

Table 4 shows the dry weight of nodules (g/plant) of the legume species *Desmodium* had significantly the highest mass of nodules (8.50 g/plant), while CP had 1.51 g/plant. In the green house experiment, DM also had higher nodule mass than CP in each of the three soils.

From the green house results of this study and the work of Whiteman (19) the amount of N_2 fixed is a function of nodule mass and since DM had the highest nodule mass, it meant that DM had the greatest genetic potential for fixing N_2. *Desmodium* had the unique ability to form nodules on adventitious roots and stolons and this characteristic contributed significantly to the very high nodule mass.

The green house nodule yields represented only 8.0 and 2.81 percent of the yields of the field grown CP and DM, respectively. The very low value of nodule yield in the green house of only 2.8% of field value for DM could be due to the stress imposed by restricted soil volume, DM grown in the polythene bags formed only very little adventitious roots and stolons on which nodules would form since the spreading branches did not come into much contact with soil while in table 4, *Desmodium* produced the highest numbers of nodules, which differed significantly from CP.

Conclusion

The results of the green house and field experiments suggest strongly that *Desmodium* had greater N_2-fixing potential than CP. *Desmodium* fixed greater amount of N_2, had higher nodule weight.

There is very strong evidence that the wild legume species, *Desmodium ramississimon* has higher N_2–fixing potential than CP, therefore this wild legume species should be better adapted for building up the N–status of the soil than CP- grain legume studied.

In addition, the spreading growth habit (forms very good ground cover), unique ability to nodulate on adventitious roots and stolons, long vegetative growth period all combine to make *Desmodium* a good species to be incorporated into our farming systems as a fallow legume species.

Table 4

Mean dry matter yield of legumes as well as number of nodules for plant as a function of plant age (field)

Legume Species	Leaf	Stem	Root	Nodul	Fruit	Total components	Number of nodules per plant
			g/plant				
Cowpea	86.89	79.81	18.25	1.51	51.39	237.89	521.67
Desmodium	109.01	136.9	321.60	8.50	0.00	276.04	562.78
FLSD (0.05)	NS*	NS*	0.58	2.76	0.10	NS*	0.17

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