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Nodulation and estimation of symbiotic nitrogen fixation by herbaceous and shrub legumes in Guinea savanna in Nigeria

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Abstract Twelve herbaceous and shrub legume species were grown in pot and field experiments in five sites representing three agroecological zones in moist savanna in Nigeria. The objectives were to: (1) assess natural nodulation of the legumes and characterize their indigenous rhizobia, (2) determine their need for rhizobia inoculation and (3) estimate the amount of N_2 fixed by each of these legumes. At 4 weeks after planting (WAP), *Crotolaria verrucosa* was not nodulated at any of the sites while *Centrosema pascuorum* had the highest number of nodules in all sites. At 8 WAP, all legumes were nodulated, with *Mucuna pruriens* having the least number of nodules and *Stylosanthes hamata* the highest. The number of nodules, however, was inversely correlated to the mass of nodules. Significant differences in nodulation of the legume species grown in the field also occurred between and within sites. *Mucuna pruriens* and *Lablab purpureus* produced more shoot and nodule biomass than the other legumes in all sites. Growth of most of these legumes responded to fertilizer application, except for *C. verrucosa* and *Aeschynomene histrix*. Except for *C. verrucosa*, average proportion of N_2 fixed was about 80% and this was reduced by about 20% with N fertilizer application. The majority of rhizobia isolates (60%) were slow growing, belonging to the *Bradyrhizobia* spp. group. Selected rhizobia isolates evaluated on *Cajanus cajan, C. pascuorum, M. pruriens* and *Psophocarpus palustris* varied from ineffective to highly effective in Leonard jar conditions. However, only growth of *M. pruriens* responded to inoculation in potted soils, whereas it was lower than that obtained with N fertilizer application. This indicated the need to screen more rhizobia in order to improve N_2 fixation and growth of legume species such as *M. pruriens* when it is introduced in soils deficient in N.

Key words *Bradyrhizobium* spp. · Effectiveness · Guinea savanna · Isolation · N fertilizer · Soil origin

Introduction

The problem facing farmers in moist savanna in west Africa is the small capacity of their soils to supply the quantities of N required for food production. Lal (1989) indicated that the available N declines rapidly once cropping is commenced. He found that available soil N declined from 0.214% (0.5 cm depth) to 0.038% after 4 years of maize cropping without *Leucaena leucocephala* hedgerows. Van Kuelen and Van Heemst (1982) reported that moist savanna soils must supply 15 kg N ha^{-1} and 2 kg P ha^{-1} for each ton of maize grain produced. Higher values have been suggested by Weber et al. (unpublished data).

This situation could be alleviated by the use of N fertilizers if it were not for their exorbitant prices and their inaccessibility to subsistence tropical farmers. There is therefore an urgent need for a cheaper and more available alternative source of N, of which biological nitrogen fixation by leguminous plants currently presents the best potential way of providing a significant contribution to the maintenance of soil fertility levels.

Herbaceous and shrub legumes are being introduced into cereal-dominated cropping systems in moist savanna zones of west Africa, where the absence of or low or poorly effective populations of indigenous rhizobia may limit N_2 fixation and therefore the capacity of these legumes to contribute to soil fertility. Sanginga et al. (1996) recently reported a large variability in nodulation and growth of *Mucuna pruriens* grown in farmers' fields in derived savanna in Benin, west Africa. Nodulation did not

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occur in 40% of the fields, indicating that some of these legumes might behave as non-fixers, depending solely on available N, if they are not effectively nodulated.

Despite the recent activity of various researchers in the moist savanna agroecological zones, there remains an enormous number of legumes whose capacity to nodulate and fix N_2 has not been determined. There is therefore the need for more detailed studies of N_2 fixation by herbaceous and shrub legumes so as to expand the number of leguminous species, not only those which contribute to soil fertility but also those used as forage for livestock feed and human consumption. The objectives of this study were: (1) to screen herbaceous and shrub legumes for efficient natural nodulation and establish the need for rhizobia inoculation in different soils, (2) to isolate, characterize and assess the infectiveness and effectiveness of rhizobia and (3) to quantify the amount of N_2 fixed by selected herbaceous and shrub legumes.

Materials and methods

Experiments were conducted in five sites located in three agroecological zones in moist savanna zones in Nigeria. These were Zaria and Kaswan-Mangani in the northern Guinea savanna (NGS), Ilorin and Patigi in the southern Guinea savanna (SGS) and Alabata and Fashola in the derived savanna (DS). The northern Guinea savanna has a mean annual rainfall of about 900 mm concentrated almost entirely in the 4 months of June to September while the southern Guinea savanna has a bimodal rainfall of about 1100 mm. The derived savanna is in the transition between forest and savanna with an average annual precipitation of 1200 mm.

Evaluation of natural nodulation

Pot experiment

Soil characteristics and preparation. Soils were collected between 0 and 15 cm depth from field plots in the five sites. Selected chemical properties determined prior to legume planting are given in Table 1. The soil was sieved (2 mm), air dried, then transferred to plastic pots (5 kg) and kept moist when necessary with deionized water to approximately field capacity. A basal fertilizer application consisting of the equivalent of 30 kg P ha^{-1} as K_2HPO_4, 60 kg K ha^{-1} as KCl and 1 ml of a combination of micronutrient solution K_2HPO_4:2.8 g; $CuSO_4 \cdot 5H_2O$:0.08 g; $ZnSO_4 \cdot 7H_2O$:0.22 g kg^{-1} soil was applied to all pots before planting. An equivalent of 75 kg N ha^{-1} as $(NH_4)_2SO_4$ was applied in the N-fertilized treatments.

Table 1 Some chemical properties of soils collected at the different sites in moist savanna zones in Nigeria

Site	pH (H_2O)	Available N (μg g^{-1} soil)	Extractable P (μm g^{-1} soil)	Total N (%)	Organic matter (%)
Alabata	6.54	3.57	5.10	0.09	1.73
Ilorin	6.01	10.29	5.36	0.05	1.48
Patigi	6.40	3.57	4.40	0.06	1.17
Zaria	5.98	3.73	8.48	0.07	2.18
Fashola	6.08	4.03	3.45	0.06	1.23
LSD (at 5.0%)	0.35	3.40	0.62	0.01	0.18

Plant species and seed preparation. Seeds of herbaceous and shrub legume species (Table 2) were surface sterilized in 30% H_2O_2 for 3 min and then rinsed several times in sterile water while *Psophocarpus palustris* seeds were surface sterilized in concentrated H_2SO_4 for 5 min before being rinsed in sterile water. The sterilized seeds were left to imbibe for 24 h after which they were pregerminated before planting. Eight seeds were sown in each pot and later thinned to two plants pot^{-1} 1 week after emergence.

Experimental design. The experimental layout was a randomized complete block design with three replicate pots per treatment. The treatments were: (1) 12 legume species and maize (used as a reference plant to estimate N_2 fixed), (2) 5 soils of different origins and (c) N treatments (with and without N fertilizer).

Harvest. Harvesting was done at 4 and 8 weeks after planting (WAP). The above-ground part was cut at the soil surface and oven dried at 60 °C to a constant weight, ground and analyzed for total N (International Institute of Tropical Agriculture 1989). The roots in each pot

Table 2 Early nodulation (nodule number pot^{-1}) of legume species grown in soils collected from five sites in the moist savanna zones of Nigeria at 4 weeks after planting

Plant species	Site					
	Alabata	Ilorin	Patigi	Zaria	Fashola	Mean
Stylosanthes hemata[a]	3	16	19	39	0	15
Pueraria phaseoloides[a]	6	2	0	2	20	6
Chaemacrista rotundifolia[a]	17	12	12	6	0	9
Psophocarpus palustris[a]	8	0	0	0	15	5
Crotolaria verrucosa[b]	0	0	0	0	0	0
Centrosema brasilianum[a]	3	0	0	9	5	3
Pseudovigna argentea[a]	48	10	0	5	59	24
Aeschynomene histrix[a]	5	0	0	0	15	4
Cajanus cajan[b]	18	0	0	4	185	41
Centrosema pascuorum[a]	45	46	19	100	71	56
Lablab purpureus[a]	28	23	0	0	82	29
Mucuna pruriens[a]	19	0	0	0	0	4
Mean	15	8	4	14	35	

LSD 5% (1) for comparing sites = 18, (2) for comparing legumes = 29
[a] Herbaceous species
[b] Shrub species

were carefully removed, and nodules were detached, counted and also oven dried to a constant weight. Two fresh nodules per legume and per site were used for rhizobia isolation. The proportion and amount of fixed N_2 in the different plant species was determined using the total N difference method with maize as reference plant.

Field experiments

Experiments were conducted at Samaru, Zaria (NGS) and Ilorin (SGS) during 1993 and 1994. The Kaswan-Mangani (NGS) site was added in the 1994 cropping season. A randomized complete block design with four replications was used. The 12 herbaceous and shrub legume species used in the pot experiment were tested at each of the sites. A minimum plot size of 12×10 m was used. A blanket application of 15 kg P_2O_5 ha^{-1} as single superphosphate and 30 kg K_2O ha^{-1} as potassium chloride was given before legumes were planted. Plants were destructively harvested in a sampling area of 4×10 m at one end of each plot. At 16 WAP, five plants were cut at soil level, roots were carefully dug out and nodules were collected, counted and their dry weights taken. The above-ground part was also dried and weighed.

Isolation and characterization of indigenous rhizobia

Rhizobia were isolated from selected surface-sterilized nodules from each legume species growing in the different sites. Single-colony isolates were maintained in McCartney bottles on yeast extract mannitol agar (YMA) slants. Purified isolates were grown in a yeast extract mannitol broth (YMB) at 28 °C on a reciprocal shaker, characterized morphologically and their growth rate determined. Acid production was assessed on YMA to which 0.5% bromothymol blue had been added (Vincent 1970).

Authentification of isolates as rhizobia was performed by inoculating seedlings in sterile growth pouches (Weaver and Frederick 1972). Three replicates of each isolate and three uninoculated controls were arranged randomly in an isolation room at 25 °C with 16 h photoperiods at 450 µmol m^{-2} s^{-1} photosynthetically active radiation supplied by cool white fluorescent lamps. The plants were grown for 6 weeks and formation of root nodules was considered as verification that the isolates were rhizobia. The authenticated isolates were deposited in the IITA *Rhizobium* collection and they are cited here according to their IITA accession numbers.

Response of herbaceous legume species to rhizobia inoculation

Leonard jar experiment

The experiment was carried out in the isolation room using Leonard jars (750 ml) filled with washed sand. After the filled jars were autoclaved, two aseptically pregerminated *M. pruriens* seeds were planted in each. Seedlings were inoculated as indicated above for growth pouches. There were three replicate jars for each of the ten rhizobia isolates selected for each of the most important legumes. Controls included uninoculated seedlings without or with N fertilizer at 75 ppm as KNO_3. Jars were arranged in a randomized complete-block design. Plants were harvested at 6 weeks after planting. Nodulation and shoot dry weight were recorded as indicated above.

Pot experiment

The best strains selected from the Leonard jars were further evaluated in pots containing soils from Fashola and Patigi in the greenhouse. These soils were selected because of the contrasting natural nodulation of the legumes. Legumes in Fashola soils had on average the highest number of nodules and Patigi the least.

Soil and seed preparations were identical to the experiment on natural nodulation. Four legume species, *Cajanus cajan, Centrosema pascuorum, Psophocarpus palustris* and *Mucuna pruriens*, were grown in the two soils. Treatments included: (1) uninoculated control, (2) uninoculated plus N fertilizer control and (3) inoculated with rhizobia isolates. Seedlings were inoculated with a mixture of the three best rhizobia isolates from the Leonard jar experiment. The experiment was a randomized complete-block design with three replications. Plants were harvested at 8 WAP by cutting at soil level. Roots were carefully removed from the pot with water. Nodules were then separated from roots, counted and dried in a forced air oven 60 °C until constant weight. Tops were also dried and weighed.

Results

Evaluation of natural nodulation

Pot experiment

Nodulation and N_2 fixation. Early nodulation (at 4 WAP) was affected by both legume species and soil types (Table 2). *Centrosema pascuorum* nodulated in all soils and produced the highest number of nodules, while *Crotolaria verrucosa* did not nodulate at any of the sites. *Mucuna pruriens* nodulated in only one site while *Aeschnomene histrix* nodulated in two out of the five sites. On average, plants grown in Fashola soils produced significantly more nodules than any of the other soils. Those grown in Patigi soils had the fewest number of nodules.

At 8 WAP, all legumes nodulated and the number of nodules varied between 24 for *M. pruriens* and 192 for

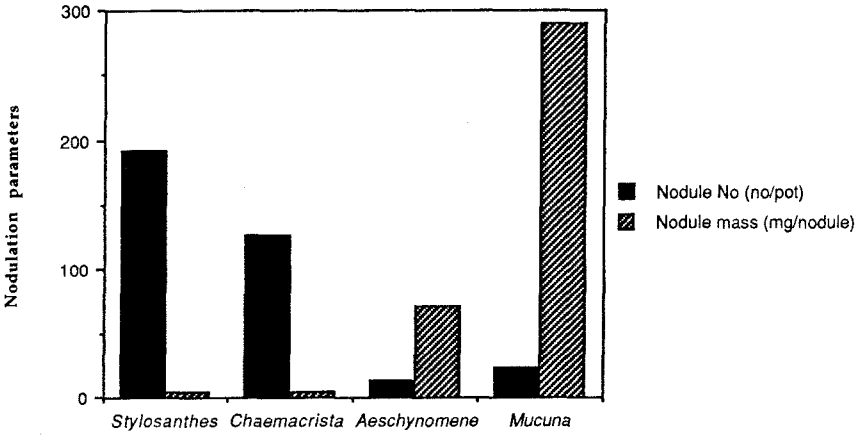

Fig. 1 Nodule number and mass of selected leguminous species at 8 weeks after planting

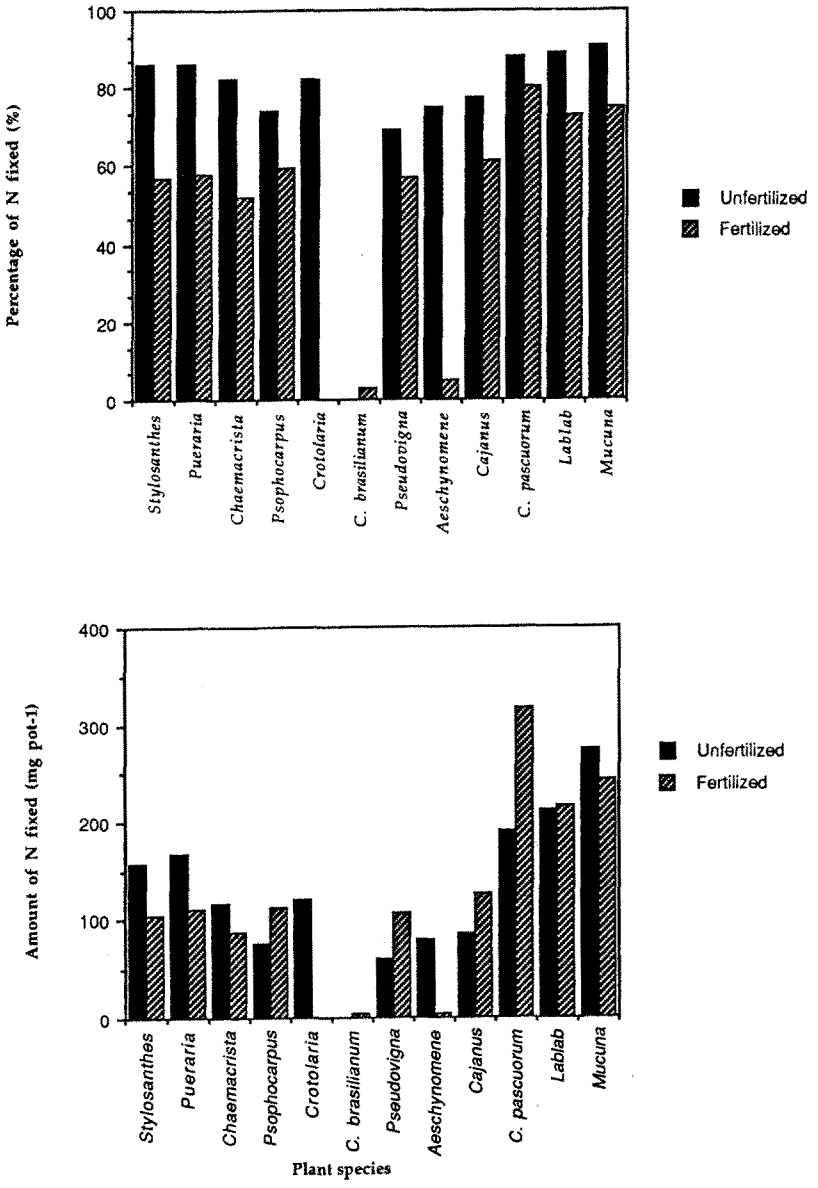

Fig. 2 Percentage and amount of N_2 fixed by herbaceous legumes grown in unfertilized and fertilized pots at 8 weeks after planting (mean value of five soil types)

Stylosanthes hemata. However, *M. pruriens* had the highest nodule specific activity as indicated by the highest nodule mass, while *S. hemata* had the lowest nodule specific activity in spite of its higher nodulation (Fig. 1).

Percentage and amount of N_2 derived from atmospheric N_2 (Ndfa) varied between legume species and were affected by N fertilizer application (Fig. 2). Percentage Ndfa ranged from 0% for *Centrosema brasilianum* to 91% for *M. pruriens* and this correlated positively with nodule mass ($r=0.91$; $P=0.05$) and amounts of N_2 fixed ($r=0.95$; $P=0.05$). Fertilizer N application reduced percentage Ndfa by an average of about 20%. For example, percentage Ndfa of *M. pruriens* was reduced from 91% to 75% and that of *Chaemacrista rotundifolia* from 82% to 52%.

Shoot dry weight and total N. Shoot dry weight of unfertilized plants ranged from 1.5 g plant^{-1} for *C. brasilianum* to 15.3 g pot^{-1} for *M. pruriens*. Most of the legume species responded to N fertilizer application except *C. verrucosa* and *A. histrix* (Fig. 3). Plants grown in Fashola soil had the highest shoot dry weight, with a 68% increase over those in Patigi soil. The interactions between legume species and soil origin were significant. For example *M. pruriens*, which had the highest biomass production in most of the soils, had a lower biomass than *Pueraria phaseoloides* in Alabata and Zaria soils. Total N was also affected by legume species and N fertilizer application and correlated significantly with shoot dry weight ($r=0.96$; $P=0.05$).

Field experiment

Nodulation. The nodule numbers in the field varied between plant species and sites (Table 3). In 1993 at Ilorin, *Lablab purpureus* had the highest number of nodules

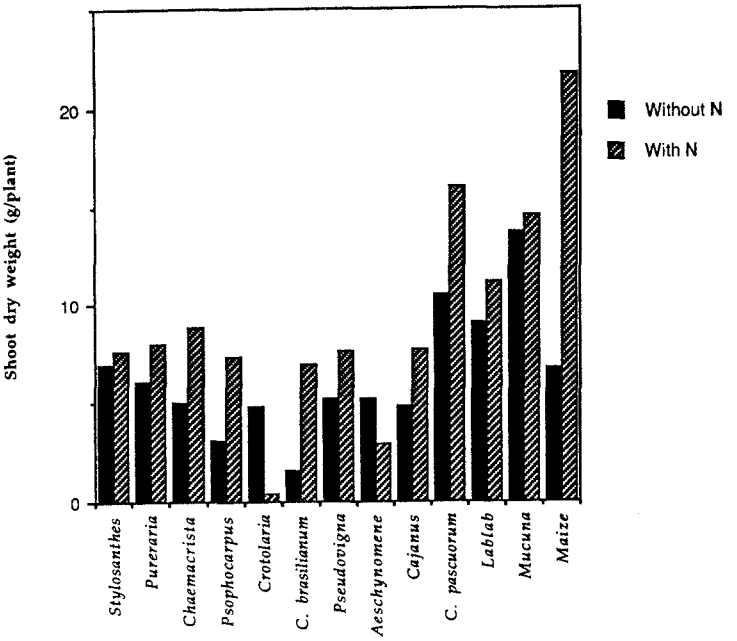

Fig. 3 Effect of N fertilizer application on shoot dry weight (g plant^{-1}) of leguminous species at 8 weeks after planting (mean values of five soil types)

Table 3 Nodulation of legume species grown in the fields at Ilorin and Zaria at 16 weeks after planting in 1993

Legume species	Nodules (no. plant^{-1})			Nodules (mg plant^{-1})		
	Ilorin	Zaria	Alabata	Ilorin	Zaria	Alabata
Chamaechrista rotundifolia[a]	2	0	3[c]	20	0	33[c]
Crotolaria verrucosa[b]	19	1	2	2990	60	0
Centrosema brasilianum	2	0	18	350	0	13
Cajanus cajan[a]	2	1	10	780	1090	35
Centrosema pascuorum[a]	32	13	28	1810	730	15
Lablab purpureus[a]	109	2	9	1660	1070	53
Mucuna pruriens[a]	8	4	16	3940	1630	90
LSD 5%	82	7	4	934	780	22

[a] Herbaceous species
[b] Shrub species
[c] Measurements taken at 4 weeks

(109) while *C. rotundifolia, C. cajan* and *C. brasilianum* had only two or three nodules per plant. Nodule numbers at Zaria were lower, ranging from 0 (*C. brasilianum*) to 13 for *C. pascuorum*. In 1994, the highest number were recorded for *L. purpureus* in both sites while *C. rotundifolia* did not nodulate in Ilorin and Zaria. Variation in nodulation between legume species also occurred in Kaswan-Mangani, and the two *M. pruriens* varieties (white and black seeds) had the highest number of nodules while *C. pascuorum* did not nodulate (Table 4).

Nodule numbers were not significantly correlated to their weight. On average the two *Mucuna* varieties had the highest nodule fresh weight followed by *L. purpureus*. The nodule weight was also influenced by site and year of growth. In 1994, nodules produced in Kaswan-Mangani were heavier than those produced in the other two sites. Those in Ilorin had the lowest weight.

Shoot dry weight. On average, there were significant differences in shoot dry weight between the legume species, which were influenced by site and year (Table 5). In 1993, *M. pruriens* varieties produced more biomass than any of the legumes at both Zaria and Ilorin. In 1994, results were different at Ilorin, with *C. rotundifolia* and *M. pruriens* (white seeds) having the highest shoot dry weight. Among the *M. pruriens* varieties, that with white seeds had more biomass in Ilorin and Zaria but not in Kaswan-Mangani. In this last site, *C. verrucosa* had an appreciable biomass followed by the two *M. pruriens* varieties.

Isolation and characterization of rhizobia

Sixty percent of rhizobia isolates authenticated in growth pouches were slow-growing and alkali producers while the rest were fast-growing and acid-producing bacteria. Their

Table 4 Nodulation of legume species grown in the fields at 16 weeks after planting in 1994

Legume species	Nodules (no. plant^{-1})				Nodules (mg plant^{-1})			
	Ilorin	Zaria	Kasuwan	Alabata	Ilorin	Zaria	Kasuwan	Alabata
Chamaechrista rotundifolia[a]	0	0	9	1	0	0	1190	11
Crotolaria verrucosa[b]	0	3	7	14	0	50	1310	36
Centrosema brasilianum[a]	0	4	22	2	0	30	360	29
Cajanus cajan[b]	1	12	9	22	10	70	600	320
Centrosema pascuorum[a]	15	41	0	12	130	250	0	31
Lablab purpureus[a]	101	58	13	12	110	1380	770	133
Mucuna pruriens[a] (black)	3	7	27	3	40	450	3710	61
Mucuna pruriens[a] (white)	9	18	28	ND	80	1840	4540	ND
LSD 5%	34	24	25	19	90	830	1600	163

[a] Herbaceous species
[b] Shrub species

Table 5 Shoot dry weight (g plant^{-1}) of legumes grown in the fields at Ilorin and Zaria and Kasuwan-Magnani at 16 weeks after planting in 1993

Legume species	1993		1994		
	Ilorin	Zaria	Ilorin	Zaria	Kasuwan
Chamaechrista rotundifolia[a]	7	5	102	7	69
Crotolaria verrucosa[b]	25	33	88	13	77
Centrosema brasilianum[a]	2	11	23	7	48
Cajanus cajan[b]	22	46	28	33	58
Centrosema pascuorum[a]	32	71	57	16	7
Lablab purpureus[a]	28	33	68	63	51
Mucuna pruriens[a] (black)	111	93	69	51	58
Mucuna pruriens[a] (white)	ND	ND	94	87	52
Means	32	42	66	34	53
LSD 5%	35	38	45	16	30

[a] Herbaceous species
[b] Shrub species

mean generation time ranged from 2–3 to 5–7 days for the fast- and slow-growing bacteria, respectively.

Response of selected legumes to rhizobia inoculation

Leonard jar experiment

Mucuna pruriens was used as an example of the host plant response to inoculation in the Leonard jar. The ten rhizobia selected from growth pouches formed nodules on *M. pruriens* seedlings. Mean nodule number, nodule dry weight and shoot dry weight of the inoculated plant varied significantly between rhizobia isolates (Table 6). Shoot dry weight of *M. pruriens* obtained in three rhizobia isolates (41 E, 27 E2 from IITA and 45C from Ilorin) was significantly higher than that of plants fertilized with N. These rhizobia isolates were then used for the pot experiment using soils collected from the field experiments at Fashola and Patigi.

Pot experiment

Nodulation. There were significant differences in nodule number between the legume species and their responses to inoculation. In both Patigi and Fashola soils, uninoculated plants with or without N had on average more nodules than inoculated ones except for *C. pascuorum* in Fashola soils (Table 7). The same pattern was observed for nodule fresh weight, whereas inoculated *M. pruriens* had a higher fresh weight than any other legume.

Shoot dry weight. Shoot dry weight was generally increased by the addition of N fertilizer (Table 7). Nitrogen fertilizer increased shoot dry weight (average of the four legumes) by 40–55% and 30–36% when compared to inoculated and uninoculated treatments in the Patigi and Fashola soils, respectively. However, there were significant interactions between N source and soil origin for some of the legume species. The best response to N fertilizer and rhizobia inoculation was obtained by *M. pruriens* in the Patigi soil and not at Fashola.

Table 6 Effect of rhizobial strains isolated from *Mucuna pruriens* growing in different soils on nodule number and dry weight of *Mucuna pruriens* grown in plastic pouches

Rhizobia isolate/origin	Nodule number (no. plant^{-1})	Nodule dry weight (g plant^{-1})	Shoot dry weight (g plant^{-1})	Relative effectiveness (%)
1. 27 E_1 (IITA)	3	2	0.942	11
2. 27 E_2 (IITA)	4	6	1.189	41
3. 44 A (IITA)	127	69	0.447	–48
4. Yamrat 2	25	47	0.434	–49
5. 41 E (IITA)	3	2	1.240	47
6. Alabata 2	15	7	0.478	–44
7. 47 A (Fashola)	63	40	0.833	–2
8. Alabata 1	12	41	0.800	–6
9. 45 C (Ilorin)	59	60	1.095	29
10. 55 A3 (Fashola)	55	34	0.384	–55
11. Control	0	0	0.383	–55
12. N fertilizer	0	0	0.846	0

Table 7 Effect of inoculation and N fertilizer on shoot dry weight (g plant^{-1}) and nodule number (no. plant^{-1}) of selected leguminous species grown in Patigi and Fashola soils at 8 weeks after planting

Plant species	Patigi			Fashola		
	Uninoculated	Inoculated	N fertilized	Uninoculated	Inoculated	N fertilzed
Shoot dry weight						
Cajanus cajan[b]	19	19	20	17	18	22
Centrosema pascuorum[a]	26	26	24	22	21	27
Mucuna pruriens[a]	15	29	48	25	23	26
Psophocarpus palustris[a]	23	16	29	20	16	23
LSD 5% (1)	5 (2)	7 (3)	7			
Nodule number						
Cajanus cajan[a]	25	37	10	57	12	70
Centrosema pascuorum[a]	171	160	181	111	159	101
Mucuna prurients[a]	25	23	51	16	24	48
Psophocarpus palustris[a]	118	22	100	148	38	93
LSD 5% (1)	17 (2)	20 (3)	45			

LSD 5%: (1) for comparing sites, (2) for comparing N sources, (3) for comparing legume species.
[a] Herbaceous species
[b] Shrub species

Discussion

Our study has shown that variation in nodulation and N_2 fixation exists between herbaceous and shrubs legumes introduced into the moist savanna zones of Nigeria. This depended to a large extent on the type of legume and the site. Except for one herbaceous legume *C. brasilianum*, the proportion of N_2 fixed was about 80%, and was comparable to the values reported for pasture legumes (Cadisch et al. 1989), showing that these legumes can contribute to the overall N economy of moist savanna cropping systems. The estimates of amount of N_2 fixed also varied widely, ranging from 10 mg to 280 mg N pot^{-1} and depended mainly on the dry matter yield of the legume. Larger amounts of N_2 fixation were therefore found where conditions were favorable for growth and biomass production. This implies that biomass production should be the first criterion for maximizing N_2 fixation in moist savanna cropping systems and this should then be coupled to the potential of the legume to fix N_2.

Mucuna pruriens and *C. pascuorum*, which were the highest N_2 fixers (averaging 250 mg N pot^{-1} each), also responded to N fertilizer. Other researchers have reported a growth response of *M. pruriens* to N fertilizer (Akobundu et al., unpublished data). This indicates that the potential of indigenous rhizobia for N_2 fixation was probably not fully exploited. This had also been illustrated by the lack of growth response of selected legumes to rhizobia inoculation. Only growth of *M. pruriens* responded to inoculation in Patigi soil and its shoot dry weight was inferior to that with N fertilizer treatment. This suggests the need to screen more rhizobia in order to improve N_2 fixation and growth of legumes such as *M. pruriens*, especially when it has to be introduced in N-deficient soils. However, the mixture of rhizobia inoculant used in this experiment was not subjected to rigorous selection in soil competition with native strains (Halliday 1984). Despite the limited record of success in this experiment, there remains considerable scope for strain improvement in the future. Attempts to select adapted strains for important her-

baceous and shrubs legumes will be made directly in fields containing large populations of indigenous and compatible rhizobia. This approach has been successfully applied for *Phaseolus vulgaris* in Colombia (CIAT 1990) and tree legumes such as *L. leucocephala* in Nigeria (Sanginga et al. 1989).

Most legumes nodulated freely, and a large variability in number and weight of nodules was found. In this study, 340 nodules were characterized as rhizobia according to Vincent (1970) and 40% were fast-growing and acid-producing bacteria, belonging to *Rhizobium* spp. according to the recent classification. Sylvester-Bradley et al. (1990) indicated that rhizobial strains isolated from nodules of *Centrosema* species such as *C. brasilianum* and *C. pubescens* used in this experiment are invariably *Bradyrhizobium* strains, although ineffective nodules can be formed when *Centrosema* is inoculated in the laboratory with fast-growing isolates (Trinick 1980). Sixty percent of rhizobia isolates from species studied here, e.g. *Pueraria phaseoloides, S. hamata* and *M. pruriens*, were slow-growing and alkali producers. Although our results are based on only limited cultural tests (growth rate and acid or alkaline production on bromothymol YMA), we suggest that the rhizobia of most herbaceous and shrub legume species used in this study belong to the slow-growing group *Bradyhizobium* spp. (Giller and Wilson 1992). However, as shown in the literature, research on rhizobia of tropical leguminous herbaceous and shrubs legumes especially in tropical Africa lags far behind that on grain legumes and even woody legumes (Dreyfus and Dommergues 1981; Sanginga et al. 1989). The interest in the introduction of rhizobia of herbaceous and shrub legumes in moist savanna agroecological zones of Africa has arisen only recently, probably because of the current search for an alternative to N fertilizer. Further studies such as those done by Sylvester-Bradley (1984), Date (1977) and Bushby et al. (1986) on the classification of the commonly used pasture legumes according to their responses to inoculation with standard strains of rhizobia from culture collections should be conducted in order to give guidance about the need to inoculate.

It is useful to know if a legume is likely to nodulate when introduced into a new region. An idea of whether the legume is highly specific or widely promiscuous in its nodulation will give an indication of this. Until we have a full knowledge of the rhizobia present in moist savanna agroecological zones of Africa has arisen only recently, probably because of the current search for an alternative to N fertilizer. Further studies such as those done by Sylvester-Bradley (1984), Date (1977) and Bushby et al. (1986) on the classification of the commonly used pasture legumes according to their responses to inoculation with standard strains of rhizobia from culture collections should be conducted in order to give guidance about the need to inoculate.

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