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Evaluation of symbiotic properties and nitrogen contribution of mucuna to maize grown in the derived savanna of West Africa

N. Sanginga[1,4], B. Ibewiro[1], P. Houngnandan[2], B. Vanlauwe[1], J.A. Okogun[1], I.O. Akobundu[1] and M. Versteeg[3]

[1]*International Institute of Tropical Agriculture ITA, Oyo Road PMB 5320,Ibadan, Nigeria,* [2]*Institut National des Recherche Agricoles du Benin (INRAB), BP 884 Cotonou, Republique du Benin and* [3]*IITA, Station de Recherche du Benin, BP 08-0932 Cotonou, Republique du Benin.* [4]*Corresponding author: c/o L.W. Lambourn 8 Co, 26 Dingwall Road, Croydon CR9 3 EE, UK*

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Abstract

The severity and increase of the *Imperata cylindrica* constraint as a weed, the decline of the traditional fallow systems as a means of soil fertility management and the lack of inorganic fertilizer appear to have created opportunities for adoption of mucuna (*Mucuna pruriens*) technology by smallholder farmers in some areas in the derived savanna of West Africa. What is not known, however, is the extent to which the establishment and N contribution of mucuna in these areas depend on symbiotic properties such as effective nodulation and mycorrhizal infection. Short term surveys carried out in 34 farmer's arable fields located in four different sites in the derived savanna, southern Benin, West Africa, together with results of greenhouse and field experiments showed that mycorrhizal infection rate of mucuna ranged from 2 to 31% and correlated positively with nodulation and shoot dry matter production. Nodulation occurred in 79% of the fields with numbers of nodules ranging from 0 to 135 plant^{-1}. Mucuna responded both to inoculation and N fertilizer in degraded soils but growth response depended on the rhizobia strains and mucuna varieties. Mucuna accumulated in 12 weeks about 313 kg N ha^{-1} as either a sole crop or 166 kg N ha^{-1} when mixed/intercropped with maize, respectively. Across all cropping systems it derived an average of 70% of its N from atmospheric N_2 (estimates made by the ^{15}N isotope dilution method), representing 167 kg N ha^{-1} per 12 weeks in the field. Mucuna interplanted with maize obtained a greater proportion of its nitrogen (74%) from fixation than did mucuna grown alone (66%) suggesting that competition for soil N influences the proportion of nitrogen fixed by mucuna. The total amount of N_2 fixed per hectare was, however, reduced significantly by intercropping mucuna with maize. A preceding mucuna crop provided a maize yield equivalent to 120 kg N kg ha^{-1} of inorganic N fertilizer.

Introduction

Low soil fertility due to N and P deficiencies and low soil organic matter content seems to be an overriding constraint and a major limiting factor to food production in the moist savanna zones in West Africa. For example, the reduction of fallows from 6 years to 2 years has resulted in a yield decline of cassava in some humid or sub-humid areas from 11 t ha^{-1} to less than 2 t ha^{-1} (FAO, 1992). With maize and sorghum, Weber et al. (1994) suggested that the soil must supply 60 kg N and 30 kg P ha^{-1} in plant available form for each ton of grain produced. Van Keulen and Van Heemst (1982) have reported lower values of 15 kg N ha^{-1} and about 2 kg P ha^{-1} for each ton of grain produced. The deficiency of N and P in soils could be alleviated by the use of fertilizers if they were available at prices that the smallholder farmers can afford. By contrast with chemical fertilizers, use of biological means such as N_2 fixation by leguminous plants to improve soil fertility may be an attractive and practicable alternative.

Herbaceous legumes are currently proposed for use in the moist savanna cropping systems undergoing degradation (COMBS, 1992). It is often assumed that they will fix N_2 freely and therefore not depend on available soil N. These legumes, however, might behave as non-fixers and depend solely on available N if they are not effectively nodulated.

Mucuna (*Mucuna pruriens*) is prominent among the herbaceous legumes currently being promoted in the moist savanna of West Africa for use as green manure for soil fertility improvement and weed control (Akobundu, 1987; Versteeg and Koudokpon, 1990). Technologies such as the repeated use of mucuna short fallows have been adopted to some extent by farmers who have seriously degraded fields in the derived savanna of the Mono province in Benin.

In the other parts of the world (United States, India, South Asia and Latin America) mucuna soil-improving effects are well documented (Buckles, 1994). In contrast, no quantitative data are available in West Africa on the amount of N_2 fixed by mucuna and its contribution to associated or succeeding crops. Russell (1936) cited by Buckles (1994) reported numerous studies with mucuna in crop rotations aimed at intensifying shifting agricultural systems in West Africa. Research done in Nigeria indicated that maize yields after mucuna were considerably increased and that soil fertility was maintained for 8 years with mucuna rotations compared to 4 years for shifting cultivation systems. This result has prompted researchers in West Africa to regard mucuna cultivation as one way to intensify production systems, especially in degraded lands.

Mulongoy and Akobundu (1990) conducted experiments with a number of leguminous cover crops including *Calopogonium mucunoides*, *Centrosema pubescens*, *Mucuna pruriens* var. *utilis*, *Psophocarpus palustris* and *Pueraria phaseoloides* for their potential in the live mulch system. Annual dry matter yields of these legumes ranged between 1.5 and 7.5 t ha^{-1}, with N yields ranging from 30 to 300 kg ha^{-1} per year. The real impact of the system on soil conservation and sustainability was shown by maize response to residual effects of the live mulch systems with *P. palustris* and *C. pubescens*. Without fertilizer N, maize yield averaged less than 1.5 t ha^{-1} while yield in the live mulch plots without fertilizer N exceeded 3. 0 t ha^{-1}. However, N accumulation and equivalence methods used by these authors do not differentiate between the contribution of soil and biological N_2 fixation, so reliable data on the amount of N_2 fixed and made available to succeeding crops are not available.

Information is sparse on the basics of mucuna-rhizobium symbiosis and our knowledge of the rhizobia of herbaceous legumes lags far behind that of the grain legumes. More research is needed to improve existing mucuna-rhizobium and vesicular arbuscular mycorrhizal (VAM) symbioses and to assess how they are affected by abiotic and biotic factors and their contribution to mucuna systems. This paper examines possibilities and approaches for increasing N_2 fixation and its contribution to N status of succeeding or associated crops.

Materials and methods

Short term survey of nodulation and mycorrhiza

This investigation was carried out in 34 farmers' fields at 4 different locations (Zouzouvou, Tchi, Eglimé and Niaouli) in the derived savanna of the Mono and Antlantic provinces of the Republic of Benin. The major site characteristics are given in Table 1. The fields were selected according to their degree of degradation, cropping history and length of fallow periods.

Soil sampling and plant harvest

Plant sampling was done in each plot using a quadrat measuring 0.50×0.50 m. For each field 2 to 4 positions were sampled depending on the size of the field (0.1 to 1.2 ha). Mucuna shoots were cut at soil level and weighed fresh. Two soil cores (0-20 cm) were taken in each quadrat with a 6.0 cm diameter soil auger. Roots were collected (0-20 cm) in each quadrat, put in a plastic bag and taken to the station where they were washed on a sieve with 1 mm openings. Nodules were removed and counted and their fresh weight taken. Two nodules were selected for rhizobia isolation according to their large size (above 0.5 cm diameter). Mycorrhizal infection was rated on fresh roots using the method of Giovanetti and Mosse (1980).

Soil chemical properties

Twenty soil cores (0-20 cm) were randomly collected in each field. The 20 cores were bulked and passed through a sieve with 2 mm openings. Soil available N (NO_3^- and NH_4^+) was determined on a subsample collected from each plot and preserved in a cooler (approximately 4 °C) before KCl extraction. Other soil chemical analyses included total soil organic C, extractable P (Bray 1) and pH (IITA, 1989).

Table 1. Selected site characteristics in 34 farmers' fields in Zouzouvou, Eglimé Tchi and Niaouli, southern Benin Republic

Parameters	Sites			
	Zouzouvou	Eglimé	Tchi	Niaouli
pH(H_2O)	6.31(5.61-6.78)	5.87(4.55-6.25)	6.59(6.16-7.40)	4.73(4.35-5.11)
Total Org. Carbon (%)	0.62(0.34-1.05)	0.90(0.62-1.25)	1.19(1.13-1.21)	0.29(0.28-0.30)
Extractable P ($\mu g\ g^{-1}$)	12.20(3.58-44.40)	5.03(0.79-13.20)	0.42(0.38-0.54)	23.01(16.48-29.53)
Available NO_3 ($\mu g\ g^{-1}$)	0.71(0.21-1.47)	1.12(0.52-1.85)	1.80(1.08-3.39)	0.70(0.68-0.72)
Available NH_4 ($\mu g\ g^{-1}$)	0.71(0.23-1.48)	0.81(.56-1.02)	2.26(0.98-2.39)	0.57(0.46-0.68)
Soil Order (USDA)	Oxisols	Alfisols	Vertisols	Oxisols
Number of fields	12	16	4	2

Values in brackets are ranges (minimum and maximum).

Enumeration of rhizobia

Rhizobia populations nodulating mucuna in selected fields in each site were enumerated by the most-probable-number (MPN) method (Alexander, 1965) using plastic pouches (Weaver and Frederic, 1972). Soil for the MPN assays was taken from the sub-sample collected for soil chemical analyses. A 5-fold dilution series with four replicates per dilution was used (Woomer et al., 1988). *Mucuna pruriens* var. *utilis* was the legume host to enumerate rhizobia. Seeds were surface sterilized in 2% sodium hypochlorite for 2 min., rinsed and planted two per pouch. Plants received Jensen's solution (Vincent, 1970) as required. The pouches were incubated at 28 °C under daylight fluorescent tubes (450 μmol $m^{-2}\ s^{-1}$) with 16h photoperiods. Nodulation was assessed between 21 and 35 days after inoculation.

Isolation and characterisation of indigenous rhizobia

Rhizobial strains were isolated according to Vincent (1970) from two selected surface sterilized nodules from mucuna species growing at the different locations of the Mono province in Benin and in southwestern and northern Guinea savanna in Nigeria. 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).

Authentication of isolates as rhizobia was performed by inoculating seedlings grown in plastic pouches with rhizobia isolates containing approximately 10^8 cells per mL. The plants were grown in the isolation room for 6 weeks and formation of root nodules was considered as verifcation that the isolates were rhizobia. The authenticated isolates were deposited in the IITA *Rhizobium* collection and they are herein cited according to their IITA accession number.

Response of Mucuna *sp. to rhizobia inoculation and contribution of N to maize*

This experiment was carried out in an isolation room, in the greenhouse and finally in the field.

Identification of effective rhizobia strains

The experiment was carried out in an isolation room, in Leonard jars (750 mL) filled with sand (washed with conc. HCl). After the filled jars were autoclaved, two aseptically pre-germinated mucuna seeds were planted in each jar. Seedlings were inoculated as indicated above. There were 3 replicate jars for each of the rhizobia isolates. Uninoculated seedlings without or with N fertilizer at 75 mg L^{-1} (equivalent to 56 mg per jar) as KNO_3 served as controls. Jars were arranged in a randomized complete-block design. Plants were harvested at 6 weeks after planting (WAP). Nodulation and shoot dry weight were recorded.

Response of Mucuna *sp. to rhizobia inoculation*

Pot experiment. The best four rhizobia strains selected from the Leonard jar experiment were further evaluated in pots containing soils collected from a field (0-20 m depth) grown to mucuna or to *Imperata cylindrica* at Ijaiye, a derived savanna site in southwestern Nigeria. The soil was sieved to pass a 2 mm opening screen and 5 kg soil was used per pot. *Mucuna pruriens* var. *utilis* and *Mucuna pruriens* var. *cochinchinensis* were grown

in the two soils. Treatments were (i) uninoculated control, (ii) uninoculated plus N fertilizer control and (iii) inoculated with rhizobia isolates. The experiment was a randomized complete block design with 3 replications. Plants were harvested at 8 WAP by cutting the top at soil level. Roots were carefully washed from the soil in running tap water. Nodules were then separated from roots, counted and dried in a forced air oven at 60 °C until constant weight. Tops were also dried as described above and weighed.

Field experiment. The trial was established in April 1994 on an *Imperata cylindrica* infested field at Ijaiye near Ibadan. *Mucuna* was established into slashed *I. cylindrica* which was allowed to regrow. A split plot design with 3 mainplot treatments (17.5 m×18 m) consisting of uninoculated control, N fertilization, (90 kg N ha^{-1}) and rhizobia inoculation of mucuna was used. The subplot treatment (8 m×3 m) was intercropping maize in mucuna or *I. cylindrica* plots. Sole maize plots were also included as controls. There were 4 replications per treatment. Each of these systems was established with a broadcast dressing of P as single superphosphate and K as muriate of potash, each at 30 kg of P or K ha^{-1} before planting maize. Nitrogen was applied at 90 kg ha^{-1} as urea in the N treatment plots in 3 split applications at 2, 4 and 6 WAP of maize. *Mucuna pruriens* var. *utilis* was introduced at 5 WAP to reduce competition and smothering of the maize plants. Mucuna seeds were inoculated as appropriate at planting with broth cultures containing approximately 10^7 cells per mL of a mixture of three rhizobia strains selected from the pot experiment.

Microplots (2.50 m^2) were marked out in each plot. Enriched $(^{15}NH_4)_2SO_4$ with 5 atom % ^{15}N was applied in the microplots at a rate of 10 kg N ha^{-1} at 2 split applications of 5 kg N ha^{-1} in the first (May to August 1994) and second (September to November 1995) seasons. The isotope dilution method (Fried and Middelboe, 1977) was used to estimate the proportion and amount of N_2 fixation. Maize and *I. cylindrica* were used as reference plants to calculate N_2 fixation. Plants were harvested at 4, 8 and 12 WAP. Number and weight of the nodules and biomass yields of mucuna were collected as indicated above. Maize yield was determined in each treatment at 12 WAP.

Contribution of N to maize

Another field experiment was carried out earlier (Van der Meersh, 1992; Vanlauwe, unpubl.) to examine the effect of alley cropping and mucuna fallows on total N and yield of maize. The experimental design and treatments are described in previous reports (Van der Meersh, 1992). In this paper we only report the maize data collected in mucuna plots.

Table 2. Number of rhizobia per g of soil for mucuna grown in farmer's fields in four different sites in derived savanna in the Mono Province, southern Benin

Plot history	Locations			
	Zouzouvou	Tchi	Eglimé	Niaouli
Imperata fallow	7±4	22±6	0	0
Adjacent field	2±1	7±2	0	0
Mucuna field	7±2	108±19	0	7±2

Table 3. Number of rhizobia per g of soil as affected by plot history for mucuna grown in five selected farmer's fields at Zouzouvou in derived savanna Mono province, Benin

Plot history	Field numbers					Means
	1	2	3	4	5	
Imperata	15	1	0	8	12	7±3
Mucuna	10	5	5	9	6	6±2
Adjacent field	5	2	2	0	0	2±1
Means	10±2	3±1	4±3	6±2	6±3	

Statistical analyses

Analysis of variance (ANOVA) was carried out using Statistical Analysis Systems (SAS) to determine treatment and interaction effects. When a significant treatment effect was found then the least significant difference (LSD) was calculated in order to compare treatment means.

Results

Short term survey on nodulation and mycorrhiza

Rhizobia density

The average densities of indigenous rhizobia capable of nodulating mucuna varied between locations and were affected by the plot history (Table 2). No rhizobia infective for mucuna were detected at Eglime and Niaouli except in the mucuna field in the latter location. Rhizobia populations in Tchi were higher than in the other 3 sites especially in the mucuna plot where their numbers were above 100 rhizobia per g of soil.

Table 4. Evaluation of some symbiotic and growth parameters of mucuna in 34 farmer's fields at Zouzouvou, Eglimé, Tchi and Niaouli in derived savanna, southern Benin

Sites	Number of fields	Nodule number (No. quadrant^{-1})	Nodule weight (g plant^{-1})	Mycorrhiza (%)	Shoot fresh weight (g quadrant^{-1})
Zouzouvou	12	27(0-135)	2.78(0-7.09)	9(2-28)	459(120-1200)
Eglimé	16	19(0-73)	2.16(0-7.96)	17(10-26)	490(200-880)
Tchi	4	65(17-122)	3.94(0.60-12.40)	15(11-20)	396(30-780)
Niaouli	2	25(2-47)	4.80(0.07-10.10)	21(14-31)	559(400-820)
LSD 5%		23	NS	11	NS*

Values in brackets are ranges (minimum and maximum).
NS*: not significant.

Results in Table 3 illustrate the large differences in rhizobia population obtained between and within farmers' fields in Zouzouvou. The numbers of rhizobia ranged between zero and 15 cells per g of soil depending on the plot history and the fields. Plots infested by *I. cylindrica* and those grown to mucuna had higher numbers of rhizobia cells than fields that were adjacent to the two plots. Other crops such as maize or cotton were grown in the adjacent plots.

Nodulation

Nodulation was not observed in 7 out of the 34 fields. The number of nodules per plant varied between 0 and 135 (Table 4) and were on average more related to shoot dry weight ($r = 0.54$, $p < 0.05$, $n = 72$) than the weight of nodules ($r = 0.41$, $p < 0.05$, $n = 72$). However the coefficient of correlation between the number of nodules and shoot dry weight was affected by locations (Table 5) being higher for Zouzouvou ($r = 0.68$); Niaouli ($r = 0.61$) and Eglimé, ($r=0.57$) than for Tchi ($r=0.29$, not significant at $p<0.05$). On average, the two nodulation parameters (number and weight of nodules) correlated significantly ($r= 0.47$, $p<0.05$).

Vesicular arbuscular mycorrhiza (VAM)

VAM fungi infection rate of mucuna varied between 2 and 31% among fields (Table 4). There were significant correlations between VAM and shoot dry weight ($r = 0.51$, $p= 0.05$: $n = 70$) in three of the sites except at Niaouli but also between VAM and nodule weight at Zouzouvou (Table 5).

Shoot biomass

Plant shoot biomass varied between fields in the four locations (Table 4). Mucuna growing in Niaouli had, on average, a higher shoot weight than that in the other locations. However, its maximum fresh weight was significantly lower than that obtained in Zouzouvou. When the minimum fresh biomass was considered, plants in Tchi produced 18, 33 and 75 times less biomass than those in Zouzouvou, Eglimé and Niaouli, respectively.

Isolation and characterization of rhizobia

Nodules of mucuna grown in the different locations in Benin and Nigeria yielded two types of rhizobia based on physiological and morphological characteristics. Forty percent of rhizobia isolated from mucuna root nodules were fast growing and acid producers while the rest were slow growing and alkaline producers. Mean generation time of the isolates ranged from 2-3 to 5-7 days for the fast and slow growing bacteria, respectively. However, only the majority of the slow growing rhizobia consistently formed nodules on mucuna growing in plastic pouches in the isolation room.

Response of Mucuna *sp. to rhizobial inoculation*

Identification of effective rhizobia strains

All authenticated isolates selected from growth pouches formed nodules on *Mucunu pruriens* var. *utilis*. Mean nodule number, nodule dry weight and shoot dry weight of the inoculated plants varied significantly between rhizobia isolates (Table 6). Shoot dry weight of mucuna obtained by four rhizobia isolates (IRM_4, IRM_5, IRM_{10} and IRM_{13}) was similar or 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 experiment at Ijaiye.

Table 5. Coefficient of correlation between growth, nodulation and some soil chemical characteristics on the growth of mucuna in farmer's fields at Zouzouvou (n=48), Eglimé (n=60), Tchi (n=16) and Niaouli (n=8)

	Zouzouvou			Eglimé			Tchi			Niaouli		
	SDW	NoNo	NW	SDW	NoNo	NW*	SDW	NoNo	NW	SDW	NoNo	NW
Shoot dry weight (SDW)	-	0.68**	0.57**	-	0.57**	0.49**	-	0.29	0.39	-	0.61	0 .54
Nodule number (NoNo)	-	-	0.42**	-	-	0.65**	-	-	0.34	-	-	0.23
Mycorrhiza	0.62**	ND	0.58**	-0.38*	ND	0.15	-0.52*	ND	0.48	0.17	ND	0.43
NO_3	-0.01	-0.10	-0.04	0.16	0.075	0.02	-0.03	-0.008	-0.15	0.16	-0.68**	0.50
NH_4	0.01	-0.16	-0.19	-0.08	-0.02	-0.01	-0.39	-0.69**	-0.65**	-0 .17	0.38	0.67*
C	0.01	-0.03	-0.02	-0.13	0.04	0.02	0.13	-0.33	0.04	0.09	-0.09	-0.43
P	-0.07	-0.15	-0.04	0.04	-0.02	-0.02	0.19	-0.08	-0.27	-0.24	0.15	0.49
pH	0.21	0.25	0.23	-0.16	0.01	0.01	-0.54*	-0.22	-0.43	0.05	-0.39	-0.63*

*Significant at p=0.05
**Significant at p=0.01.
NW = Nodule weight.
ND=Not determined.

Table 6. Screening of *Mucuna* rhizobia isolates for symbiotic effectiveness (%) in Leonard jars

Rhizobia isolates Accession No	Origin	Nodule No	Nodule dry weight (mg plant^{-1})	Shoot dry weight (g plant^{-1})	Symbiotic* effectiveness(%)
Control		0	0	0.400	40
N fertilizer		0	0	0.980	100
IRM_1^{**}	Zozouvou	8	7	0.786	80
IRM_2	Zozouvou	33	10	0.683	69
IRM_3	D_2IITA	27	14	0.622	63
IRM_4	Zouzouvou	26	20	0.683	69
IRM_4	Tchi	17	5	0.907	92
IRM_5	Tchi	6	10	1.032	105
IRM_6	Eglimé	13	3	0.830	84
IRM_7	Eglimé	5	4	0.665	67
IRM_8	Niaouli	6	11	0.672	68
IRM_9	Niaouli	13	21	0.431	43
IRM_{10}	Niaouli	32	136	1.186	121
IRM_{11}	Yamrat	8	9	0.840	85
IRM_{12}	Yamrat	23	28	0.771	78
IRM_{13}	Yamrat	5	8	0.859	87
IRM_{14}	Ijaiye	10	953	0.756	77
IRM_{15}	Ijaiye	68	390	0.557	56
IRM_{16}	Ijaiye	14	6	0.639	65
IRM_{17}	Ijaiye	15	24	0.571	58
LSD 5%		29	6	0.29	

*=Symbiotic effectiveness = percentage of inoculated plants over N fertilizer control.
**=IRM=IITA Rhizobia for *Mucuna*.

Response of Mucuna *to rhizobia inoculation*

Pot experiment: Nodulation. There were no significant differences in nodule number between mucuna varieties and inoculation treatment (data not shown). For both *Mucuna pruriens* var. *utilis* and var. *cochinchinensis*, uninoculated plants had, on average

Table 7. Effect of *Rhizobium* inoculation and N fertilizer on shoot dry weight (g plant^{-1}) and nodule fresh weight (g plant^{-1}) of *Mucuna pruriens* var. *utilis* and var. *cochichinensis* grown in soils collected from Imperata and mucuna fields at Ijaiye, southwestern Nigeria

	Soil origin							
	Imperata cyclindrica				*Mucuna*			
	var. *utilis*		var. *cochichinensis*		var. *utilis*		var. *cochichinensis*	
Inoculation treatment	SDW*	NFW**	SDW	NFW	SDW	NFW	SDW	NFW
Uninoculated	23.17	1.33	27.07	1.47	21.32	0.22	18.93	0.02
N fertilizer	25.19	0.89	26.29	1.60	19.49	0.08	21.08	0.18
Rhizobia IRM_4	22.22	1.15	24.19	1.08	20.81	0.13	23.01	0.03
Rhizobia IRM_5	25.89	1.57	26.26	1.35	20.43	0.31	20.73	0.01
Rhizobia IRM_{10}	25.91	2.02	24.04	0.71	20.70	0.02	20.32	0.35
Rhizobia IRM_{13}	25.56	1.43	23.68	0.76	19.69	0.03	21.39	0.02
Means	24.70	1.40	25.25	1.16	20.41	0.13	20.91	0.10
LSD 5%[a]	2.49	0.15						
[b]	1.86	0.48						

[a]For comparing plant varieties.
[b]For comparing plant inoculation treatments.
*SDW=Shoot dry weight.
**NFW=Nodule fresh weight.

more, nodule numbers than inoculated ones. However, nodule fresh weight of inoculated *M. pruriens* var. *utilis* was increased by rhizobia strains IRM_{10} while that of *M. pruriens* var. *cochinchinensis* was not affected by rhizobia inoculation. (Table 7)

Shoot dry weight. Shoot dry weight response of mucuna to rhizobia inoculation and N fertilizer depended on variety and soil origin (Table 7). Response to N fertilizer was obtained only by *M. pruriens* var. *utilis* in soil originating from a *I. cylindrica* field while that of *M. pruriens* var. *cochinchinensis* responded to N in soil from a *M. pruriens* var. *utilis* field.

Shoot growth response to inoculation occurred only in plots where the two mucuna varieties responded to N fertilizer. Rhizobia strains IRM_5, IRM_{10} and IRM_{13} induced higher shoot dry weights of *M. pruriens* var. *utilis* grown in *I. cylindrica* soil but they were totally ineffective on *M. pruriens* var. *cochinchinensis* in that soil. *Mucuna pruriens* var. *cochinchinensis* responded to inoculation with IRM_4 (ineffective in *I. cylindrica* field) when grown in soil collected from a *M. pruriens* field.

Field experiments: Biomass production. Biomass production of mucuna during the cropping season is shown in Figure 1. When grown as sole crop, total dry biomass production at 12 WAP were 9.2, 7.7 and 8.7 t ha^{-1} for inoculated, uninoculated and N fertilized treatments, respectively. Biomass production was reduced by 44%, 25% and 56% for inoculated, uninoculated and N fertilizer treatments, respectively, when mucuna was intercropped with maize. Sole cropped mucuna accumulated in its above ground biomass an average of 313 kg N ha^{-1} by 12 WAP (Table 8). This amount was reduced to an average of 166 kg N ha^{-1} when mucuna was interplanted with maize.

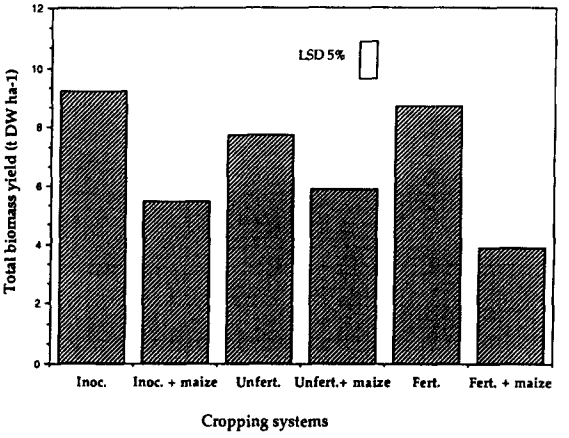

Figure 1. Effect of N fertilization, rhizobia inoculation and different cropping systems on mucuna bioma reduction in the field at Ijaiye at 12 weeks after planting.

Nodulation and N_2 fixation. Nodulation of mucuna was significantly reduced by N fertilization (Fig. 2). Nodule numbers were significantly increased by rhizo-

Table 8. Proportion and amount of N derived from atmospheric N_2 (Ndfa) by *Mucuna pruriens* using *Imperata cylindrica* as reference plants in the field at Ijaiye

Treatments	Isotope dilution			N difference method	
	Total N (kg N ha^{-1})	N fixed (%)	N fixed (kg N ha^{-1})	N fixed (%)	N fixed (kg N ha^{-1})
No fert. *Mucuna* alone	325	69	224	77	252
Fert. *Mucuna* alone	302	64	193	64	195
Inoc. *Mucuna* alone	310	64	198	76	238
No fert. *Mucuna* + maize	188	74	139	56	106
Fert. *Mucuna* + maize	117	63	74	42	50
Inoc. *Mucuna* + maize	190	86	163	56	108
Means	239	70	167	62	158
LSD 5%	128	19	116	ND	ND

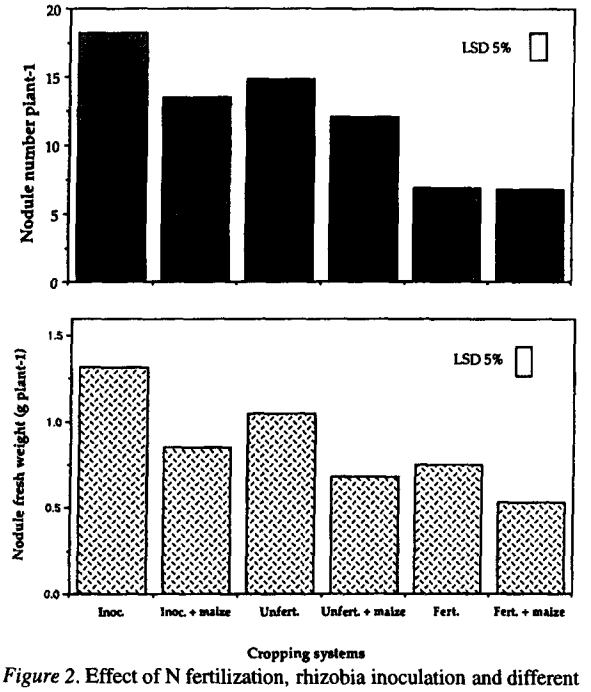

Figure 2. Effect of N fertilization, rhizobia inoculation and different cropping systems on mucuna nodule number and nodule fresh weight in the field at Ijaiye.

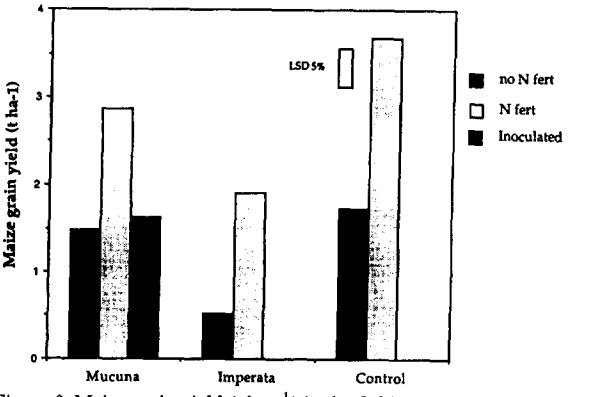

Figure 3. Maize grain yield (t ha^{-1}) in the field at Ijaiye as affected by N fertilization and different cropping systems at the first season.

bia inoculation in sole mucuna but not in a mixture of mucuna and maize. Nodule fresh weight was increased by inoculation in both treatments. When inoculated mucuna was intercropped with maize, nodule fresh weight and nodule numbers were reduced by 34% and 27%, respectively.

Across all cropping systems the proportions of N accumulation due N_2 fixation by mucuna measured by the ^{15}N isotope technique averaged 70%, equivalent to the amount of 167 kg N ha^{-1} during the 12 weeks of growth (Table 8). The proportion of N_2 fixed was increased when mucuna was intercropped with maize. When grown alone, mucuna derived about 66% of N from N_2 fixation representing 205 kg N ha^{-1}, whereas in mixed cropping with maize it derived 74% from fixation or 125 kg N ha^{-1}. The proportions of N_2 fixed estimated by the total N difference method were similar to those calculated by the isotope dilution method except in the mixed cropping systems. There were no significant differences in the proportions and amounts of N_2 fixed between uninoculated and inoculated mucuna monocultures. Inoculated mucuna mixed with maize, however, fixed a higher % and greater amount of N_2 than uninoculated mucuna. Nitrogen fertilizer at the rate of 90 kg ha^{-1} reduced the quantity of N_2 fixed by 27% and 15% in inoculated and uninoculated mucuna grown in mixed cropping systems, respectively.

Maize yield. Nitrogen fertilization significantly increased maize grain yield as compared to N fertilizer or when the legumes were inoculated with rhizobia

Table 9. Effect of mucuna and fertilizer application on maize shoot and N biomass, and grain yield in the field at IITA, derived savanna, southwestern Nigeria in 1992. Mucuna was established in 1986, 1987, 1989 and 1991 and maize was rotated in 1988, 1990 and 1992

Treatments	Seasons		
	1989		1992
	Biomass (kg ha^{-1})[b]	Total N (kg ha^{-1})	Grain yield (kg ha^{-1})
Control plots			
Without fertilizer	8,638	88.5	330
With fertilizer[a]	15,746	172.2	1430
Mucuna plots			
Without fertilizer	11,083	106.6	1380
With fertilizer	17,148	192.9	2340

[a]Fertilizer: 120 kg ha^{-1} N, 90 kg ha^{-1} P_2O_5 and 30 kg ha^{-1} K_2O.
[b]Fresh weight of above ground biomass including stover and complete cobs.

(Fig. 3). Grain yield was higher in inoculated mucuna than in unfertilized treatments but the difference were not significant. Maize grain yield averaged 2 t ha^{-1} in mucuna cover crop systems and were significantly higher than yields under *I. cylindrica*.

Contribution of N to maize

Data collected after three seasons of mucuna in 1992 show that maize grown after mucuna had 4 times more yield than that obtained in the plot continuously cropped to maize without N fertilizer (Table 9). Similar results were obtained for total N yield and biomass in 1989.

Discussion

How much N_2 does mucuna fix and contribute to an associated or succeeding crop? Estimates carried out using the isotope dilution method indicated that on average 70% of the mucuna total N is derived from N_2, fixation when other nutrient limitations are removed. These estimates are similar to those reported for herbaceous legumes such as *Pueraria phaseoloides* in some countries in Latin America and south Asia (Cadisch et al., 1989). Using this approach we calculated that inputs from N_2 fixation were on average 167 kg N ha^{-1} at 12 WAP. These quantities are similar to those we usually get in alley cropping systems and justify the use of mucuna as a source of N in degraded cropping systems. In 40% of the fields, surveyed in the Mono province it was possible to find well nodulated plants and these might have achieved high rates of N_2 fixation as indicated above. However, more commonly either a few or no nodules were found on the root systems, generally, due to the lack of rhizobia or to the presence of inefficient and compatible rhizobia. Thus, the actual contribution of N_2 fixation even with mucuna in many cases may be small. More research is needed to optimize or maximize the benefits of N_2 fixation in mucuna cropping systems especially if these are to be adopted by smallholder farmers. Simple measurements of N accumulation in mucuna will provide a useful guide to the amount of N_2 fixation over short time periods if the proportion of N_2 fixed is known. Larger amounts of N_2 fixation are generally found when the legume produces a lot of biomass.

When mucuna was mixed with maize its biomass production and amount of N_2 fixed per hectare were reduced. This observation could be biased, because the measurement took place rather early, i.e. 12 WAP, when the maize was just harvested, but when the mucuna is still in full growth. At later stages, e.g. 20 WAP, there are no differences any more in biomass production between sole and previously relay-cropped mucuna (Versteeg, pers. commun.). It is therefore recommended to take more measurements until the end of the complete growing cycle of mucuna when the biomass difference will be much smaller and probably insignificant. Very probably the amount of accumulated N and fixed N_2 would also have been quite similar in both sole and intercropped mucuna at 20 WAP. Unlike the

reduction observed in total amount of N_2 fixed, mucuna interplanted with maize obtained a greater proportion of its N from fixation, suggesting that competition for soil N between maize and mucuna influences the proportion of nitrogen fixed by mucuna. However, results in Table 9 show N gains for maize from a preceding mucuna crop in terms of equivalent fertilizer N. Mucuna provided a maize grain yield equivalent to that with 120 kg fertilizer N ha^{-1}. When used in a rotation system, mucuna may thus contribute significant amounts of N to the system and reduce the requirements for fertilizers considerably, especially for smallholder farmers.

It is useful to know if mucuna is likely to nodulate when introduced into a new area. An idea of whether mucuna is highly specific or widely promiscuous in its nodulation will give an indication of its need for rhizobia inoculation. Until we have a full knowledge of the rhizobia present in the derived savanna zones where mucuna is being adopted, it will not be possible to resolve this question. Our experiments with growth pouches and Leonard jars indicated that mucuna was infected both by fast and slow growing rhizobia. However, only nodules formed by the slow growing alkaline producer isolate were effective in fixing N_2, as shown by the increase in dry matter of inoculated mucuna as compared to that of uninoculated plants with or without N fertilizer (Table 7). Some authors have noted that a few tree legumes such as *L. leucocephala* were also nodulated by both fast and slow growing rhizobia strains (Dreyfus and Dommergues, 1981; Sanginga et al., 1989) but here only the fast growing rhizobia were effective in fixing N_2.

Sylvester-Bradley et al. (1990) indicated that rhizobial strains isolated from nodules of *Centrosema* species such as *C. brasilianum* and *C. pubescens* are invariably *Bradyrhizobium* strains although ineffective nodules can be formed when *Centrosema* is inoculated in the laboratory with fast growing isolates (Trinick, 1980). Some other rhizobia isolates from herbaceous legume species e.g. *Pueraria phaseoloides*, *Stylosanthes hamata* have also been classified as *Bradyrhizobium* (Giller and Wilson, 1991).

Although our results are based on only limited cultural tests (growth rate and acid or alkaline production, and response to inoculation) we suggest that effective rhizobia nodulating mucuna belong to *Bradyrhizobia* sp. Further studies, such as those done by 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 demonstrate the need to inoculate.

Potential benefits from inoculation and assessment of the need to inoculate were illustrated by both the pot and field experiments (Tables 7 and 8). The pot experiment with the two mucuna species showed that it is difficult to identify strains that consistently give inoculation responses. With *M. pruriens* var. *utilis*, grown in soil from an *I. cylindrica* field, growth of the plants was stimulated by addition of N fertilizer and rhizobia inoculation, but *M. pruriens* var. *cochinchinensis* failed to respond to both added N and rhizobia inoculation. The behavior of *M. pruriens* var. *utilis* in the soil from the mucuna plot was similar to that of *M. pruriens* var. *cochinchinensis* in *I. cylindrica* soil. These results indicate that N_2 fixation can be improved through exploitation of the enormous genetic variability that exists between bacterial strain and host genotypes but they also emphasize the interaction of biological and non-biological factors in modifying the expected symbiotic response.

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References

Alexander M 1965 Most-probable-number method for microbial populations. *In* Methods of Soil Analysis. Part 2. Chemical and Microbiological properties. Eds. C A Black and D D Evans. American Society of Soil Science, USA.

Akobundu I O 1987 Weed Science in the Tropics: Principles and Practices. John Wiley and Sons, Chichester, UK. 522p.

Buckles D 1994 Velvetbean: A "new" plant with a history. CIMMYT Internal Document. CIMMYT, Mexico, D.F 21p.

Bushby H V A, Date R A and Sundran J 1986 *Rhizobium* collections and their assessment. *In* Forages in Southeast Asian and South Pacific Agriculture. Eds. G J Blair, D A Ivory and T R Evans. pp 133–140. ACIAR, Canberra.

Cadisch G, Sylvester-Bradley R and Nösberger J 1989 ^{15}N based estimates of nitrogen fixation by eight tropical forage-legumes at two levels of P:K supply. Field Crops Res. 22, 181–208.

Collaborative Maize Base Systems COMBS 1992 Soil fertility improvement and weed suppression through legume based

technology. An approach to technology targeting. Collaborative Research paper No. 1. IITA, Ibadan, Nigeria.

Date R A 1977 Inoculation of tropical pasture legumes. *In* Exploiting the Legume-Rhizobium Symbiosis in Tropical Agriculture. Eds. J M Vincent, A S Whitney and J Bose. University of Hawaii College of Tropical Africulture Special Publication No. 145, pp 293–311.

Dreyfus B L and Dommergues Y R 1981 Nodulation of Acacia species by fast- and slow growing tropical strains of *Rhizobium*. Appl. Environ. Microb. 41, 97–99.

FAO 1992 Annual report 1992. FAO, ROME, Italy.

Fried M and Middelboe V 1977 Measurement of amount of nitrogen fixed by a legume crop. Plant and Soil 47, 713–715.

Giavonnetti M and Mosse B 1980 An evaluation of methods for measuring vesicular-arbuscular infection in roots. New Phytol. 84, 489–500.

Giller K E and Wilson K J 1991 Nitrogen Fixation in Tropical Cropping systems. CAB International Wallingford, UK. 313p.

IITA 1989 Analytical manual. IITA, Ibadan, Nigeria.

Mulongoy K and Akobundu I O 1990 Agronomic and economic benefits of nitrogen contributed by legumes in live-mulch and alley cropping systems. *In* Nitrogen Fixation: Achievements and objectives. Eds. P M Gresshoff, L E Roth, G Stacey and W E Newton. pp 625–632. Chapman and Hall, New-York.

Sanginga N, Mulongoy K and Ayanaba A 1989 Effectivity of indigenous rhizobia for nodulation and early-nitrogen fixation with *Leucaena leucocephala* grown in Nigerian soils. Soil Biol. Biochem. 21, 231–235.

Sylvester-Bradley R, Souts S M and Date R A 1990 Rhizosphere biology and nitrogen fixation of Centrosema. *In* Centrosema-Biology, Agronomy and Utilization. Eds. R Schultze-Kraft and R J Clements. pp 151–174. CIAT, Cali Colombia.

Trinick M J 1980 Relationships amongst the fast growing rhizobia of *Lablab purpureus*, *Leucaena leucocephala*, *Mimosa* spp., *Acacia farnesiana*, *Sesbania grandiflora* and their affinities with other rhizobia groups. Bacteriol. 49, 39–53.

Van Keulen H and van Heemst H D J 1982 Crop response to the supply of macronutrients. Agric. Res. Rep. 916 (Versl. Landbk. Onderz,) Pudoc, Wageningen, the Netherlands.

Van der Meersch M K 1992 Soil fertility aspects of alley cropping systems in relation to sustainable agriculture. Diss. de Agricultura. Leuven, Belgium. 179 p.

Vincent J M 1970 A manual for the Practical study of Root - Nodule Bacteria. IBP Handbook No. 15. Blackwell, Oxford, UK.

Versteeg M V and Koudokpon V 1990 Mucuna helps control *Imperata cylindrica* in southern Benin. West African Farming Systems Research Network (WAFSRN) Bull. 7, 7–8.

Weaver R W and Frederick L R 1972 A new technique for most-probable-number counts of rhizobia. Plant and Soil 36, 219–222.

Weber G, Smith F and Manyong V M 1995 System dynamics and definition of research domains for the northern Guinea savanna of West Africa. Agric. Ecos. Environ. (*In press*).

Woomer P, Singleton P W and Bohlool B B 1988 Ecological indicators of native rhizobia in tropical soils. Appl. Environ. Microbiol. 54, 1112–1116.

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