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Nodulation and nitrogen fixation in three wild legumes of the derived savannah of Nigeria

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The nodulation and N fixation in 3 wild legumes was investigated under greenhouse conditions between the 6th and 18th week after planting. In *Cassia rotundifolia* Pers. the mean nodule number ranged between 9 and 12 (nodule dry weight 2 to 294 mg), in *Vigna micrantha* Harms between 3 and 56 (nodule dry weight 12 to 153 mg) and in *Tephrosia candida* Dc between 12 and 71 (nodule dry weight 7 to 150 mg). The legumes exhibited significant differences in nodule morphology. In all 3 legumes there were significant correlations ($r = 0.83$ to 0.93) between nodule weight and root weight. A significant correlation ($r = 0.94$ and 0.77) was found between the nodule weight and N content in the shoot only for *Cassia* and *Tephrosia*. N concentrations in the legumes and N uptake differed among the legumes. The N gains by the soil decreased with increasing age of the trial plants.

1. Introduction

Tropical legumes have been reported to occur in large numbers (Keay et al., 1964) but their role in biological nutrient cycling between the components of the ecosystem has not been fully exploited within the tropics.

Legume shrubs as cover crops during fallow have been reported to increase the soil level of nitrogen (Gethin-Jones, 1942; Orchard and Darby, 1956; Wilson et al., 1982). Okigbo (1977) summarized the role of legumes in maintaining soil fertility. Mongi and Huxley (1979) stressed the importance of legume trees and shrubs in maintaining soil fertility since they offer low cost alternatives in developing countries. The role and potential of legumes in alley cropping, live mulching, and rotation systems in humid and sub-humid tropical Africa have been generally highlighted by Mulongoy and Kang (1986). Also the usefulness of tropical tree and shrub legumes in pasture improvement and management has been widely studied (Moore, 1962; Morison, 1971; Shanker et al., 1976; Russo, 1986; Clatworthy et al., 1986).

The aim of this study was to observe and compare nodulation and nitrogen fixation through *Rhizobium* root nodule symbiosis without inoculation in three wild legumes, namely *Vigna micrantha* Harms, *Cassia rotundifolia* Pers., and *Tephrosia candida* Dc, collected from some parts of the derived savannah of Nigeria. A knowledge of their potentials for nitrogen fixation and the possible use of these potentials in improving agricultural and pasture lands are the key considerations.

2. Material and methods

A surface soil sample was obtained from a section of the University of Nigeria, Nsukka, farm centre.

2.1. *Routine soil analysis*

Mechanical analysis was done using the BOUYOUCOS (1951) hydrometer method; the pH was determined in water and 0.1 N KCl with a soil to a solution ratio of 1 : 1 using a BECHMAN pH-meter. The % organic carbon was determined by the WALKLEY-BLACK (1934) method. The total nitrogen was determined according to the regular KJELDAHL method. Available P was determined using the BRAY No. 1 method (BRAY and KURTZ, 1945). The field capacity was determined using a laboratory modification of the method described by PETERS (1965). Some of the properties of the soil sample used are presented in table 1.

Table 1. Some properties of the soil sample used

pH KCl	pH H_2O	Sand (%)	Silt (%)	Clay (%)	Total N (%)	Org. C (%)	Org. matter (%)	Av. P μgP/g	K^+ me/100 g	CEC me/100 g
4.1	5.1	72	6	22	0.075	0.99	1.71	3	0.08	24.1

2.2. *Soil sample treatment and planting*

4 kg of soil, air-dried and sieved through an 8 mesh sieve, were placed in plastic pots, watered to field capacity, and allowed to stand for 2 days. The seeds of the 3 legumes were scarified using concentrated H_2SO_4 and pregerminated in petri dishes for 6 days, after which the sprouted seedlings were carefully transplanted into the prepared pots, and allowed to develop in the greenhouse. Pots were arranged ina randomized complete block (RCB) design with 3 replications. Normal watering of the pots to maintain the soil at field capacity was done by gently sprinkling water on the surface of the soil, the whole pot being weighed.

The plants were thinned to 3 per pot after 2 weeks of stand establishment. The first harvest was done after 6 weeks of transplanting, and subsequent harvest were done at three week intervals terminating at the 18th week. The data obtained at each harvest were subjected to analysis of variance (ANOVA) procedures and the treatment differences were evaluated by DUNCAN's Multiple Range Test (DMRT).

3. Results and discussion

3.1. *Plant morphology*

Morphologically, the three legumes possessed primary and secondary root systems but differed in their shoot morphology. *Cassia rotundifolia* is a sub-woody herb with prostrate branches, paired sessite and long spreading leaflets with yellow flowers. *Vigna micrantha* possesses slender pubescent stems with lobulate leaflets and pale yellow flowers. *Tephrosia candida* is a woody shrub with compound pinnate leaf arrangement.

3.2. Dry matter yield

Table 2 shows the mean separation for differences among the legumes with respect to time of harvest (age) on the root and shoot dry weights. The dry matter yields of *Cassia* and *Tephrosia* increased with age, but those of *Vigna* decreased with age from 12 weeks following some fungal attack on this plant resulting in severe leaf loss.

Table 2. Mean dry matter yields (g) of shoots and roots as affected by age of plant (time of harvest)

Plant part	Legume species	Time of harvest (weeks)				
		6	9	12	15	18
Shoots	*Cassia rotundifolia*	0.99**	3.77e	6.47d	8.11c	10.06b
	Vigna micrantha	2.19f	3.29ef	3.53ef	3.14ef	1.56g
	Tephrosia candida	1.36g	4.0e	7.74c	9.49b	14.24a
Roots	*Cassia rotundifolia*	0.16e	0.63cd	1.01bc	1.3bc	1.85b
	Vigna micrantha	0.35de	0.78cd	1.03bc	1.36bc	0.5de
	Tephrosia candida	0.27de	0.84cd	1.84b	2.72a	3.45a

** Means with the same letter for shoot or root are not significantly different at the 1% level of probability.

3.3. Nodule yield

The three legumes differed in their nodule yield (table 3). Nodules on *Cassia* were either irregular or oblong in shape, small in size (about 12.5 mm^2) and darkish brown in colour, and located entirely on the lateral roots. For *Vigna*, the nodules were equally small in size (about 6 mm^2), appearing black with grey stripes, oval in shape, and universally distributed along lateral roots with 3–4 clustered at the top 2 cm of the primary root. Nodules on *Tephrosia* were found only on the lateral roots. The nodules were cylindrical corraloid in shape when fully developed with few appearing flat but lobed, and sizes averaged 18 mm^2. They all appeared yellowish brown in colour. Ming-Mao et al. (1986) surveyed N_2-ase activities of 19 species of tree legumes and noted variations among them in terms of nodule numbers, location, shapes, sizes, and colour with *Tephrosia candida* nodules located on lateral roots, of cylindrical or branched shape, and light yellow in colour. The nodulation pattern of *Prosopis ceneraria* and *Prosopis juliflora* studied by Basak and Guyal (1975) showed that the number of nodules per plant was higher on lateral roots than on tap roots. The contribution of nitrogen to the soil through symbiosis depends on the effective nodulation of the legume host concerned as manifested in the number of effective nodules formed and the weight of the nodules. Chen and Thornton (1940) noted that nodule weight is an index of effectiveness partly depending on the particular bacterial strain plant variety interaction as well as on the volume of root materials formed. In this study, these legumes gave a significant positive correlation ($p = < 0.05$) between root weight and nodule weight (*Cassia* $r = 0.93$; *Vigna* $r = 0.83$; *Tephrosia* $r = 0.93$). Thus in the natural environment where the development of the roots is not restricted by the area as observed in the pots, root proliferation and growth will encourage the formation of more nodules if effective strains of rhizobia are present in the soil and the environmental conditions are suitable for both the legume host and the *Rhizobium* species.

Field observations are needed to determine the range of nodulation by these legumes before valid conclusions can be drawn on their nodulation efficiency. Nodulation of these legumes in the greenhouse might have been affected by the temperature which ranged between 30 and 35 °C. Evidence exists to show that for tropical legumes, nodu-

Table 3. Mean nodule numbers and nodule weights as affected by age of plant (time of harvest)

Nodule yield	Legume species	Time of harvest (weeks)				
		6	9	12	15	18
Number of nodules	*Cassia rotundifolia*	9e**	96c	101.3c	131.3b	181.7a
	Vigna micrantha	10.7e	5.3e	56.7d	53.7d	3.3e
	Tephrosia candida	12.7e	70.7d	51.3d	71.3d	59.3e
Nodule dry weights (mg)	*Cassia rotundifolia*	1.50e	83.1cd	97.97c	180.33b	293.77a
	Vigna micrantha	12.97d	26.7d	144.10bc	153.47b	45.00d
	Tephrosia candida	7.43d	13.3d	103.76c	150.27b	140.60bc

** Means with the same letter for number of nodules or for nodule weight are not significnatly different at the 1% level.

lation is favoured at root temperature between 24 °C and 30 °C, because temperature has a controlling effect on the main stages in the formation and functioning of nodules (GIBSON, 1971). EZEDINMA (1970) reported that soil temperature is one of the key factors in the natural nodulation of uninoculated legumes in the humid tropics.

3.4. Nitrogen concentrations

The total nitrogen content of shoots, roots, nodules, and soil was determined after each harvest as shown in table 4. Nodule nitrogen concentrations varied with respect to age and species of the legume between 4.76 % and 6.7 %. Differences among legumes constitute the first factor to be considered in nitrogen assimilation and general fixation. Other parameters include the age of the plant and soil conditions. From this study, it is generally noted that the age of plants is not a regulating factor in the nitrogen concentration of the nodules.

Table 4. Mean nitrogen concentration (%) in shoots roots, and nodules as well as soil as affected by the age of plant (time of harvest)

N concentration (%)	Legume species	Time of harvest (weeks)				
		6	9	12	15	18
Shoots	*Cassia rotundifolia*	3.96a**	1.87f	1.55h	1.45h	1.11i
	Vigna micrantha	2.76c	2.04e	2.56d	1.91f	1.72g
	Tephrosia candida	2.90b	1.87f	1.87f	1.58h	1.22i
Roots	*Cassia rotundifolia*	1.51b	1.14c	1.12c	1.45bc	1.39bc
	Vigna micrantha	1.65b	1.71ab	1.84a	1.82a	2.1a
	Tephrosia candida	1.93a	1.43bc	1.56b	1.94a	1.71ab
Soil	*Cassia rotundifolia*	0.089b	0.089b	0.084bc	0.083bc	0.078c
	Vigna micrantha	0.096a	0.088b	0.083bc	0.084bc	0.080c
	Tephrosia candida	0.096a	0.102a	0.082bc	0.084bc	0.078c
Nodules+	*Cassia rotundifolia*	ND	6.70	5.32	6.16	5.32
	Vigna micrantha	ND	5.60	6.44	5.6	5.80
	Tephrosia candida	ND	6.70	5.60	4.76	5.32

** Means with the same letter for N concentration in shoot or root or soil are not significantly different at the 1% level.

+ ANOVA was not calculated for N concentration in nodules because the replicate values of samples were bulked for determination of N

ND No determination

In all legumes, the N concentrations in the shoots decreased significantly with age in response to an increased build-up of lignin and cellulose materials in the plant tissue. Root N concentrations exhibited some irregular trends. Soil N concentration changed significantly with age for all legumes, but the differences were not significant. Generally, however, all the pots gained some nitrogen compared with the N level in the soil at the start of the experiment (table 1) although the N gained was decreasing with the age of the plant. Presumably, the rate of plant uptake was greater than the rate of fixation and addition of N to the soil. The small increases in the soil N concentration as a result of cultivating these legumes in pot culture are signifi nt results and could be attributed mainly to nitrogen fixation through rhizobium root nodule symbiosis. Small increases in soil N gains ranging from 0.01 % to 0.06 % were observed by WILSON et al. (1982). However, it is necessary to consider all aspects of N accession processes to ascertain the overall nitrogen gains in the natural environment. One area of research interest is the residual effect of forage and wild legumes in our farming systems, because some quantity of nitrogen will result from root and nodule decay (HENZELL and VALLIS, 1977).

3.5. *Nitrogen uptake*

The nitrogen uptake (the product of nitrogen concentrations and dry matter yield of shoots, roots, nodules, and whole plant) is tabulated in table 5. The parameters tested differed among the legumes.

There were significant positive correlations between the shoot N uptake and nodule weight for *Cassia* ($r = 0.77$) and *Tephrosia* ($r = 0.91$). This indicates some efficiency in the fixation process. On the other hand, the correlation between the N uptake of shoots and the nodule weight of *Vigna* was low ($r = 0.39$) due to foliage loss from the fungal attack observed in this legume.

Table 5 Mean nitrogen uptake (mg) by the shoots, roots, nodules and whole plant as affected by age of plant (time of harvest)

Plant part	Legume species	Time of harvest (weeks)				
		6	9	12	15	18
Shoots	*Cassia rotundifolia*	38.68ef**	70.49de	101.26cd	117.23c	111.67cd
	Vigna micrantha	59.31e	65.04de	89.75d	60.08e	26.76f
	Tephrosia candida	39.52ef	74.62de	114.63b	150.38b	174.37a
Roots	*Cassia rotundifolia*	2.37f	7.19f	11.28ef	18.92d	24.03c
	Vigna micrantha	5.83f	13.05e	18.79d	24.89c	10.57ef
	Tephrosia candida	5.20f	12.02ef	28.81c	52.75b	58.93a
Nodules	*Cassia rotundifolia*	ND	5.56d	5.21d	11.11b	15.62a
	Vigna micrantha	ND	1.49e	9.22bc	8.59c	2.61e
	Tephrosia candida	ND	0.89e	5.81d	7.51c	7.48cd
Whole plant	*Cassia rotundifolia*	13.77d	27.74cd	39.24c	49.08bc	50.43bc
	Vigna micrantha	21.70cd	26.50cd	39.27c	31.19cd	15.15d
	Tephrosia candida	14.90d	29.17cd	59.75b	70.09ab	80.26a

** Means with the same letter for each plant part or for whole plant are not significantly different at the 1% level.

ND No determination

4. Conclusion

This study has indicated the potentials of some legumes in the nitrogen economy of the derived savannah. In terms of the overall contribution of nitrogen, all the legumes studied appear promising, and *Tephrosia candida* seems best due to a higher foliage yield. Thus these legumes should be considered for use as green manures, cover crops, and in fallow lands for nutrient recycling.

It is hoped that more interest will be aroused for further research under different tropical conditions. A considerable proportion of the research should be carried out in the field and emphasis placed on the use of these legumes, especially *Tephrosia* species, in the protection and nutrient cycling of our agricultural and pasture lands.

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T. O. IBIA und N. N. AGBIM: Knöllchenbildung und Stickstoffbindung bei drei wildwachsenden Leguminosenarten in der nigerianischen Savanne

Unter Gewächshausbedingungen wurden bei drei Wildleguminosenarten die Knöllchenentwicklung und N-Fixierung im Zeitraum 6. bis 18. Woche nach dem Auspflanzen der Sämlinge analysiert. Die durchschnittliche Knöllchenanzahl lag bei *Cassia rotundifolia* PERS. zwischen 9 und 12 (Knöllchenmasse: 2 bis 294 mg), bei *Vigna micrantha* HARMS zwischen 3 und 56 (Knöllchenmasse: 12 bis 153 mg) und bei *Tephrosia candida* DC zwischen 12 und 71 (Knöllchenmasse: 7 bis 150 mg). Die Wurzelknöllchen wiesen wirtspezifische morphologische Unterschiede auf. Es bestand bei allen drei Leguminosenarten eine signifikante Korrelation ($r = 0,83$ bis 0,93) zwischen Knöllchen- und Wurzelmasse. Eine signifikante Korrelation ($r = 0,94$ und 0,77) zwischen Knöllchenmasse und N-Gehalt im Sproß wurde nur bei *Cassia* und *Tephrosia* ermittelt. Artspezifische Unterschiede gab es bei der N-Aufnahme und im N-Gehalt der Leguminosen. Die N-Anreicherung im Boden verminderte sich mit zunehmendem Alter der Versuchspflanzen.

T. O. IBIA и N. N. AGBIM: Клубочкообразование и связывание азота у 3 дикорастущих видов бобовых в нигерианской саванне

В условиях теплицы в течение 6–18 недель после высадки рассады у трёх видов дикорастущих бобовых изучалось развитие клубочков и фиксация азота. Среднее число клубочков составило у *Cassia rotundifolia* PERS. 9–12 при массе клубочков от 2 до 294 мг, у *Vigna micrantha* HARMS – от 3 до 56 клубочков при массе их от 12 до 153 мг и у *Tephrosia candida* DC – от 12 до 71 клубочке при массе их от 7 до 150 мг. Корневые клубочки в зависимости от хозяина имели морфологические различия. В случае трёх видов бобовых были обнаружены достоверные коэффициенты корреляции $r = 0,83$ до 0,93 между массой клубочков и массой корней. Достоверная корреляция ($r = 0,94$ и 0,77) между массой клубочков и содержанием азота в побеге была получена только для *Cassia* и тефрозии. Видозависимая разница наблюдалась в усвоении азота и содержании его в бобовых. Накопление азота в почве уменьшалось с увеличением возраста подопытных растений.

T. O. IBIA et N. N. AGBIM: Formation de tubercules et fixation de l'azote dans des espèces de légumineuses agrestes de la savane nigériane

Dans des conditions de serre ont été analysées, dans trois espèces de légumineuses agrestes, la formation de tubercules et la fixation de l'azote, dans la periode de la 6^{e} à la 18^{e} semaine après la plantation des semis. Le nombre moyen de tubercules était pour *Cassia rotundifolia* PERS. entre 9 et 12 (masse de tubercules: 2 à 294 mg), pour *Vigna micrantha* HARMS entre 3 et 56 (masse de tubercules: 12 à 153 mg), et pour *Tephrosia candida* DC entre 12 et 71 (masse de tubercules: 7 à 150 mg). Les tubercules des racines montraient des differences morphologiques spécifiques de l'hôte. Il y avait pour toutes les trois espèces de légumineuses une corrélation significative ($r = 0,83$ à 0,93) entre masse de tubercules et masse de racines. Une corrélation significative ($r = 0,94$ et 0,77) entre masse de tubercules et teneur en N dans la pousse n'a été trouvée que pour *Cassia* et *Tephrosia*. Il y avait

des différences spécifiques de l'espèce quant à la fixation de N et la teneur en N des légumineuses. L'enrichissement de N dans le sol a diminué parallèlement à l'âge croissant des plantes expérimentales.

T. O. IBIA y N. N. AGBIM: Formación de tubérculos y fijación de nitrógeno en tres variedades de leguminosas silvestres de la sabana nigeriana

Bajo condiciones de invernáculo se analizaron en tres variedades de leguminosas silvestres el desarrollo de tubérculos y la fijación de N en el período de la 6ª hasta 18ª semana después de plantadas las plántulas. El número medio de los tubérculos se situaba en *Cassia rotundifolia* PERS. entre 9 y 12 (masa de los tubérculos: 2 hasta 294 mg), en *Vigna micrantha* HARMS entre 3 y 56 (masa de los tubérculos: 12 hasta 153 mg) y en *Tephrosia candida* DC entre 12 y 71 (masa de los tubérculos: 7 hasta 150 mg). Los tubérculos radicales mostraron diferencias morfológicas específicas según el huésped. Existió en todas las tres variedades de leguminosas una correlación significativa ($r = 0{,}83$ hasta 0,93) entre la masa de los tubérculos y la de la raíz. Una correlación significativa ($r = 0{,}94$ y 0,77) entre la masa de los tubérculos y el contenido de N en el vástago se determinó únicamente en *Cassia* y *Tephrosia*. Había diferencias específicas relacionadas a la variedad en la absorción de N y el contenido de N en las leguminosas. La concentración de N en el suelo se iba reduciendo con la edad de las plantas de ensayo.