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Applied Soil Ecology 3 (1996) 215-224

Applied Soil Ecology

Persistence and effectiveness of rhizobia nodulating promiscuous soybeans in moist savanna zones of Nigeria

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Accepted 7 November 1995

Abstract

The symbiotic performance of promiscuous soybeans depends upon the population size, effectiveness and survival of indigenous or introduced rhizobia in the field. A pot experiment was conducted using soils collected from 13 farmers' fields located in two agroecological zones (northen and southern Guinea savanna) in the moist savanna of Nigeria to determine the relationships between growth response to previous rhizobial inoculation and the indigenous rhizobial populations. At each farmer's field, soil was collected in plots which were planted the previous year (1993) to: (1) maize, (2) soybean cv. Bossier, (3) soybean cv. TGX 1456-2E and (4) soybean cv. TGX 1660-19F. The soybean cultivars TGX 1660-19F and TGX 1456-2E were either uninoculated or inoculated with an enriched population of a mixture of local rhizobial strains. Four test plants: soybean cultivars TGX 1660-19F (slightly promiscuous), TGX 1456-2E (highly promiscuous), Bossier (non-promiscuous) and cowpea (typically promiscuous) were planted in pots containing soils from the above field treatments. Previous inoculation increased shoot dry matter production by an average of 32% over the uninoculated controls in 94 of the 312 (30%) legume inoculations and farmers' field combinations while the indigenous rhizobia were more effective than the introduced ones in 103 combinations (33%) with an average of 20% increase compared to previous inoculation treatments. Previous inoculation increased biomass yield of both promiscuous and non-promiscuous soybean varieties. The response to previous inoculation treatments was farmers' fields dependent and inversely related to the numbers of rhizobia in the soil. Soil rhizobial population ranged from 0 to > 400 cells g^{-1} soil and response to inoculation often occurred when numbers of indigenous rhizobia were fewer than 10 cells g^{-1} soil. Numbers of indigenous rhizobia were generally lower or below detection limit in soils previously cropped to maize. These results indicate a relationship between rhizobia cell counts and promiscuous soybean responses, which may be used to indicate under which conditions inoculation will be beneficial to a farmer.

Keywords: Bradyrhizobia; Guinea savanna; Indigenous rhizobia; Inoculation; Most probable number; Promiscuous nodulation

1. Introduction

In 1978, the International Institute of Tropical Agriculture (IITA) soybean program targeted the improvement of biological nitrogen fixation (BNF) for soybean through a breeding program to develop

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0929-1393/96/$15.00
SSDI 0929-1393(95)00089-5

'promiscuous' soybean varieties that nodulate with indigenous soil rhizobia thus eliminating the need for inoculation. The program was based on selection of progeny from crosses between Asian varieties with good nodulation in local soils (using visual score for nodule mass) and American varieties with favorable agronomic traits. From that period IITA has identified several more effective promiscuous soybean varieties screened from 400 soybean accessions for nodulation in Nigeria without rhizobial inoculants. Currently, smallholder farmers in Nigeria and Zambia are widely adopting 'free nodulating soybean cultivars'.

However, there is still a major question that needs to be answered: are indigenous rhizobia clearly meeting the yield potentials of these promiscuous soybean varieties? Few studies have been conducted to characterize the rhizobial strains nodulating promiscuous soybean. Bromfield and Roughley (1980) indicated that promiscuous soybeans are not nodulated by the same rhizobia as cowpea when grown in the same soil. This has further been confirmed by Eaglesham (1985) and recently by Abaidoo (personal communication) suggesting that soybean nodulation can be poor even in soils where cowpeas are abundantly nodulated. Responses of these promiscuous soybeans to inoculation have also been reported (Okereke and Eaglesham, 1993) indicating that the initial nodulation with indigenous bacteria is poor in comparison to the plant's capacity for nodulation.

Work by Eaglesham (1985) concentrated on ecological aspects and on the quantitative limitations of the soil rhizobial populations. The effectiveness of the indigenous strains does not seem to be as often addressed. It is possible that the nodulation scoring system used so far by soybean breeders at IITA (Kueneman et al., 1984) is not well correlated with the symbiotic effectiveness of those nodules. Knowledge on the indigenous rhizobia nodulating promiscuous soybeans is limited and their symbiotic properties may differ between locations. It may be impossible to produce a soybean which will nodulate effectively with indigenous rhizobia in more than a few trials. This study was conducted to determine how the response of promiscuous soybeans to rhizobia inoculation is related to the population sizes of indigenous rhizobial in 13 sites in two different agroecological zones in moist savanna. A second purpose was to evaluate the influence of a previous crop on the effectiveness and persistence of introduced or indigenous rhizobia.

2. Materials and methods

In 1993, Abaidoo et al. (unpublished) conducted field trials in 17 farmers' fields within approximately 50 km of Zonkwa and Zaria; both sites located in two agroecological zones in moist savanna in Nigeria. Zaria lies in the northern Guinea savanna ecological zone with a mean annual rainfall of about 900 mm concentrated almost entirely in the 4 months, June to September. Zonkwa is situated in the southern Guinea savanna zone with a mean rainfall of about 1200 mm. Selected physico-chemical characteristics of the two sites are given in Table 1.

Table 1
The range of selected site characteristics of Zaria and Zonkwain, two agroecological zones in the moist savanna of Nigeria

Parameters	Northern Guinea Savanna Zaria	Southern Guinea savanna Zonkwa
Annual rainfall (mm year^{-1})	900	1200
pH (KCl)	5.76 (4.28–6.24)	5.20 (4.85–5.75)
Soil organic carbon (%)	0.50 (0.30–0.60)	0.50 (0.34–0.80)
NO_3 (μg g^{-1} soil	1.19 (0.48–0.84)	3.14 (2.35–4.96)
NH_4 (μg g^{-1} soil)	0.70 (1.06–1.59)	1.11 (.54–1.84)
N (NO_3 + NH_4)	1.89 (1.00–2.07)	4.25 (2.89–6.01)
Extractable P (μg g^{-1} soil)	18.33 (8.63–40.87)	14.31 (6.05–25.17)
Clay content	10 (8–12)	14 (12–16)
Sand content	48 (39–56)	69 (67–71)
Silt content	43 (37–49)	19 (17–21)

Mean values given.

The experimental design in 1993 was a randomized complete block design with the following treatments replicated four times: (1) maize; (2) soybean cv. Bossier (non-promiscuous); (3) Soybean TGX 1456-2E (highly promiscuous); (4) Soybean cv. TGX 1660-19F (moderately promiscuous). The soybean TGX cultivars had four treatments: (i) no rhizobia, no inorganic N; (ii) no rhizobia, inorganic N; (iii) local rhizobia, no inorganic N and (iv) *Bradyrhizobium japonicum* (NifTAL), no inorganic N. Plot sizes were 6 m long with four rows 60 cm apart giving approximately 600–700 m^2 per site.

In 1994, pot experiments were conducted in the greenhouse to assess survival and effectiveness of introduced rhizobia and their relation to the remaining population of indigenous rhizobia in the soil.

2.1. Site selection, soil sampling and preparation

Soils from six farmers' fields in Zonkwa and seven farmers' fields in Zaria were collected and used for the pot experiment. The field cropping histories are described in Table 2. Twenty cores were randomly collected between 2 cm and 20 cm depth in each plot representing a treatment. The 20 cores were bulked and later passed through a 6.5 mm screen. The screened soil was placed in plastic kegs for storage before use after a subsample had been taken for chemical analyses and rhizobial population counts. For the pot experiment, bulk soil was mixed with sand, three parts placed into 5 kg pot and fertilized with the equivalent of 30 kg P ha^{-1} as KH_2PO_4 and 60 kg K ha^{-1} as muriate of potash and 1 ml kg^{-1} of a combination of micronutrients (Vincent, 1970).

2.2. Experimental design

The pot experiment was a randomized complete block design using six farmers' fields from Zonkwa and seven from Zaria. Legumes used were: soybean cv. Bossier, soybean cv. TGX 1456-2E, soybean cv. TGX 1660-19F and cowpea. Soils selected from the previous 1993 plots were: (1) maize; (2) soybean cv. Bossier, (3) uninoculated or inoculated promiscuous soybean cv. TGX 1660-19F and (4) uninoculated or inoculated promiscuous soybean cv. TGX 1456-2E. Each treatment was replicated three times. Due to the large number of pots, the experiment was divided into two halves, the first half was conducted with the Zonkwa 1, 4, 5, 6 sites and Zaria 15, 16, 17 sites from 10 May to 21 June 1994 and the second half with the Zonkwa 8 and 10 and Zaria 18, 20, 21, 22 from 8 July to 1 September 1994. The entire experiment was conducted in the greenhouse at IITA, Ibadan in Nigeria. The mean temperature varied between 25°C and 32°C and the relative humidity between 70% and 80%.

2.3. Enumeration of rhizobia

Rhizobial populations for each plant test were enumerated by the most-probable-number (MPN) method using plastic pouches (Weaver and Freder-

Table 2
Cropping history of fields used for trial of soybean contributions (1993–1994) at Zaria and Zonkwa in two agroecological zones in the moist savanna zones of Nigeria

Number	Zone	Village-Farmer	1992	1991	1990	1989	1988	1987	1986	1985	1984
1	Zonkwa	Mabulu-B.Nuhu	Y	S	S	G/M	Mz	S	G/M	S	F
4	Zonkwa	Mabulu-Adamu	M	Mz	S	Sb/G	Mz				
5	Zonkwa	Kurfi-B Sam	M	M	M	M	M	M	M	M	M
6	Zonkwa	Kurfi-B Sam	Mz	S	Mz	Mz	Mz	Mz	Mz	Mz	Mz
10	Zonkwa	Karami-Gade	S/Mz	S/Sb	S/Mz						
16	Zaria	Kayawa-Alh.Idi	Mz	G	Mz	G	Mz	G	Mz	G	Mz
17	Zaria	Kayawa-Abu	F	F	F	F	F	F	F	F	F
20	Zaria	Saboline-M.Bassa	Mz	Mz	Mz	Mz	Mz	Mz	Mz	Mz	Mz
21	Zaria	Saboline-M.Bassa	Mz	Mz	Mz	Mz	Mz	Mz	Mz	Mz	Mz
22	Zaria	A.B.U.	Sb	C							

S, Sorghum; M, Millet; Mz, Maize; G, Groundnut; Sb, Soybean; Y, Yam; C, Cotton; F, Fallow.

ick, 1972). Soil for the MPN assays was taken from the subsample collected from the previous 1993 treatments and farmers' fields combinations and 100 g was used for the initial dilutions. A five-fold dilution series with four replicates per dilution was used (Woomer et al., 1988). Cowpea was the legume host to enumerate *Bradyrhizobium* spp. Soybean cv. Bossier was used for *Bradyrhizobium japonicum* and cultivars TGX 1660-19F and TGX 1456-2E possibly for *Bradyrhizobium* spp. Plants received Jensen's solution (Vincent, 1970) as required. The pouches were incubated at 28°C under daylight fluorescent tubes and nodulation was assessed between 21 and 35 days after inoculation. Analysis of variance was performed on the numbers of rhizobia per gram of oven-dry soil (100°C, 24 h) and after log transformation of these data. A constant of 1 was added to all data before log transformation to avoid computer calculation of log 0 for soils where no rhizobia were detected.

2.4. Seed preparation

Seeds of the four test plants were surface sterilized in 2% sodium hypochlorite for 2 min, rinsed and planted (six per pot) and thinned to three after germination. Pots were regularly watered with deionized water and maintained at field capacity.

2.5. Harvest

Plants were harvested 8 weeks after germination. Tops were cut at soil level, dried at 70°C and weighed. The root systems of three replicates were cleaned by washing over 1 mm mesh screen. Nodules were removed and counted and their fresh weight taken. Two selected nodules were used for rhizobia isolation (Vincent, 1970).

2.6. Soil N availability

Soil available N (NO_3 and NH_4) was determined on composite soil samples collected in mid-July 1993 (after the first rains) from each plot and preserved in a cooler (approximately 4°C) before KCl extraction (IITA, 1989)

2.7. Determination of response to inoculation

Persistence and effectiveness of rhizobia after one introduction was assessed through a model (Thies et al., 1991) that relates the improvement in legume growth (herein referred to as response) to the population sizes of introduced or indigenous rhizobia. Response to inoculation for each test plant and farmers' field combinations was defined as $R = 100\ (I_s - I_0)/I_0$ where R = response to inoculation, I_s = average shoot dry weight in previous inoculated plots, and I_0 = average shoot dry weight in previous uninoculated treatments.

2.8. Statistical analysis

Statistical analyses were done using SAS (Statistical Analysis Systems Institute Inc., 1989). Correlation and analysis of variance for a randomized complete block design with three replications was done using 'PRO CORR' and 'PROC GLM' to determine the statistical differences between the treatments and their interactions. Specific pair-wise comparisons of treatment levels were done using the Least Significant Difference (LSD) at $P = 0.05$. The two batches of the pot experiments were combined for the analysis as there were no statistical differences between the time of planting and its interactions with the other factors.

3. Results

3.1. Rhizobia enumeration

The population sizes of indigenous rhizobia nodulating promiscuous soybean varieties were studied (Table 3). The numbers of rhizobia per gram of soil were not significantly influenced by the plant cultivars and locations. However, they varied between farmers' fields (Table 3). Rhizobia populations were generally higher in soils from Farmers' Fields 16 and 17 in Zaria and Farmers' Fields 4 and 5 in Zonkwa than in the other fields at the two locations. Soils which had maize as a previous crop had the lowest number of rhizobia in both locations (Table 4) while soils previously cropped to Bossier had a greater

Table 3
Numbers of rhizobia per gram of soils nodulating promiscuous soybean TGX 1660-19F and TGX 1456-2E grown in soils collected from different selected sites at Zonkwa and Zaria

Cultivars/Locations	Sites				Means
Zonkwa	Site 1	Site 4	Site 5	Site 6	
TGX 1456	1.02	1.47	1.17	0.45	1.03
TGX 1660	0.91	1.82	1.27	0.43	1.11
Means	0.96	1.64	1.22	0.44	1.07
Zaria	Site 15	Site 16	Site 17	Site 22	
TGX 1456	0.33	1.09	1.43	0.40	0.81
TGX 1660	0.57	1.49	1.89	0.38	1.03
Means	0.45	1.29	1.66	0.39	0.92
LSD 5%	0.33				

Numbers, $\log_{10}$ rhizobia + 1; mean values of previous crop treatments given.

Table 4
Numbers of rhizobia per gram of soils nodulating promiscuous soybeans as affected previous soybean and maize treatments at Zaria and Zonkwa locations

Previous crop treatments	Locations		
	Zonkwa	Zaria	Means
Maize	0.90	0.40	0.65
Bossier	1.37	1.22	1.79
TGX 1660-19FI$_u$	0.81	0.89	0.85
TGX 1660-19FI$_s$	1.05	1.02	1.04
TGX 1456-2EI$_u$	0.83	1.06	0.95
TGX 1456-2EI$_u$	1.52	1.09	1.31
Means	1.08	0.94	
LSD 5%	0.35		

Numbers, $\log_{10}$ rhizobia + 1; mean values of different sites given.

rhizobial population than those which had TGX 1660-19F and TGX 1456-2E as previous crops. Numbers in previously inoculated plots were higher than those in uninoculated ones but differences were statistically significant only in plots previously grown to TGX 1456-2E in Zonkwa.

3.2. Nodulation

Nodulation of soybean cultivars and cowpea was related to the size of indigenous rhizobial population. The coefficients of correlation between rhizobial populations and nodule weight ($r = 0.51$; $P = 0.05$; $n = 136$) and nodule numbers ($r = 0.24$; $P = 0.05$; $n = 136$) were highly significant.

The numbers of nodules per plant varied between 44 and 117 and were dependent on the farmers' fields (Fig. 1). Nodule weight ranged from 0.56 g plant^{-1} to 4.08 g plant^{-1} between farmers' fields and was more closely related to shoot dry weight than to the number of nodules. On average, cowpea had more nodules than soybean cultivars (Table 5). Amongst the soybean cultivars Bossier had a lower number of nodules than the two promiscuous soybeans TGX 1660-19F and 1456-2E. However, the number of nodules and mass were inversely related ($r = -0.97$, $P = 0.05$) with cowpea having less nodule mass than soybean cultivars. As with the

Table 5
Effect of previous crop treatments on nodulation and shoot dry weight of test plants grown in pots containing soils collected from Zonkwa and Zaria

	Shoot dry weight per plant (g)					Nodule numbers per plant				
	1660	1456	Bossier	Cowpea	Means	1660	1456	Bossier	Cowpea	Means
Maize	10.02	9.34	7.87	9.75	9.25	68	78	47	97	72
Bossier	10.30	10.41	9.23	11.06	10.25	79	93	68	96	84
TGX 1660-19FI$_u$	10.77	9.99	8.41	10.76	9.98	95	96	84	96	93
TGX 1660-19FI$_s$	10.18	9.92	8.93	10.45	9.87	68	74	59	89	73
TGX 1456-2EI$_n$	10.47	10.08	7.88	10.15	9.65	86	94	63	100	96
TGX 1456-2EI$_s$	10.17	9.88	9.46	9.12	9.66	69	75	62	78	71
Means	10.32	9.94	8.63	10.22		78	85	64	92	
LSD 5% [a]	0.46					6				
LSD 5% [b]	0.35					8				

Mean values across sites given; [a] comparing previous treatments; [b] comparing cultivars.

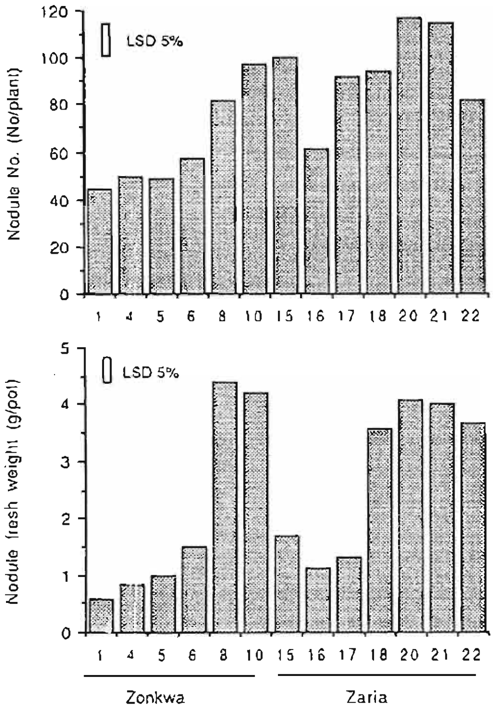

Fig. 1. Effect site (farmers' fields) and locations and nodulation of legumes (mean values of promiscuous soybeans and cowpea) grown in soils from Zaria and Zonkwa in moist savanna zones of Nigeria.

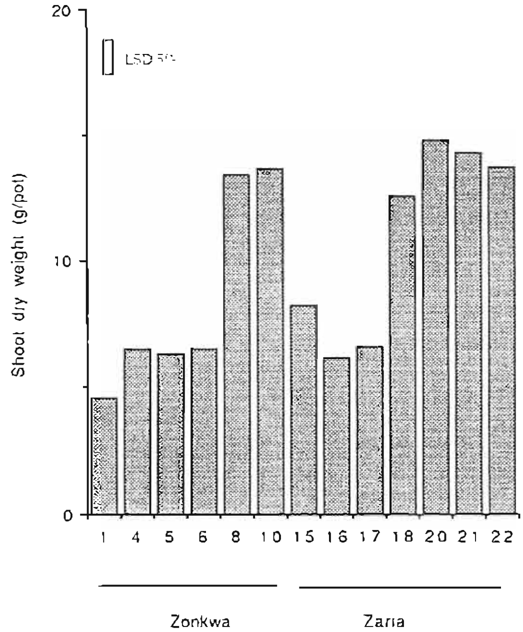

Fig. 2. Effect site (farmers' fields) and locations and shoot dry weight of legumes (mean values of promiscuous soybeans and cowpea) grown in soils from Zaria and Zonkwa in moist savanna zones of Nigeria.

rhizobial populations, plants grown in soil previously collected from maize plots had a lower number of nodules than plants grown in previous soybean plots, except those in previously inoculated treatments.

3.3. Response to inoculation

Shoot dry weight ranged from 4.56 to 13.61 g plant^{-1} at Zonkwa and between 6.13 and 14.74 g plant^{-1} at Zaria, respectively (Fig. 2) but this was related to the previous crops and their respective

Table 6
Frequency of significant shoot dry biomass increase due to inoculation with introduced rhizobia or due to indigenous rhizobia for six sites in Zonkwa and seven sites in Zaria

	Frequency									
	Zonkwa [a]					Zaria [b]				
	1660	1456	Bossier	Cowpea	Total	1660	1456	Bossier	Cowpea	Total
Inoculated	10	11	14	4	39	12	11	22	10	55
Uninoculated	16	6	13	13	48	9	15	17	14	55
No increase	11	19	9	19	58	21	16	3	18	58
Percentage increase (%)										
					Mean					Mean
Inoculated	28	24	80	25	39	26	20	37	19	26
Uninoculated	21	16	24	18	20	18	16	25	18	19
Means	25	20	52	22		22	18	31	19	

[a] 144 combinations; [b] 168 combinations.

treatments (Table 5). Plants grown in soil previously grown to maize had lower shoot dry weight, although statistically not different from those grown in soil from promiscuous soybean TGX 1456-2E, either inoculated or uninoculated. Plants grown in soil collected from previous Bossier and TGX 1660-19F plots had the highest shoot dry matter yields.

Response to inoculation as evaluated by total shoot dry matter of inoculated plants over uninoculated ones depended on the plant host, farmers' fields and the previous treatments (Table 6). Previous inoculation increased shoot dry matter by an average of 33% above the uninoculated in 94 of 312 (30%) of the plant hosts and farmers' field combinations. Of those plant host and farmers' field combinations that responded to previous inoculation, the response frequency was higher in Bossier followed by the promiscuous soybeans TGX and cowpea.

Previously uninoculated plants had higher shoot dry matter than the inoculated ones in 103 cases out of 312 (33%). However, the 20% increase in shoot dry weight due to indigenous rhizobia was lower than that of previous inoculated treatments.

The frequency of shoot dry weight increase due to indigenous rhizobia was also higher with Bossier followed by cowpea and TGX soybean varieties. In 115 combinations uninoculated and inoculated plants gave similar shoot dry matter yields.

Response to inoculation also varied between farmers' fields and locations. Inoculation response to previous inoculated treatments was less frequent in Zonkwa than in Zaria but the percentage shoot dry matter increase due to inoculation was higher in Zonkwa (39%) than in Zaria (26%) location.

3.4. Relationship between rhizobial number and response to inoculation

Shoot dry weight response of host plant to rhizobial inoculation was inversely related to the number

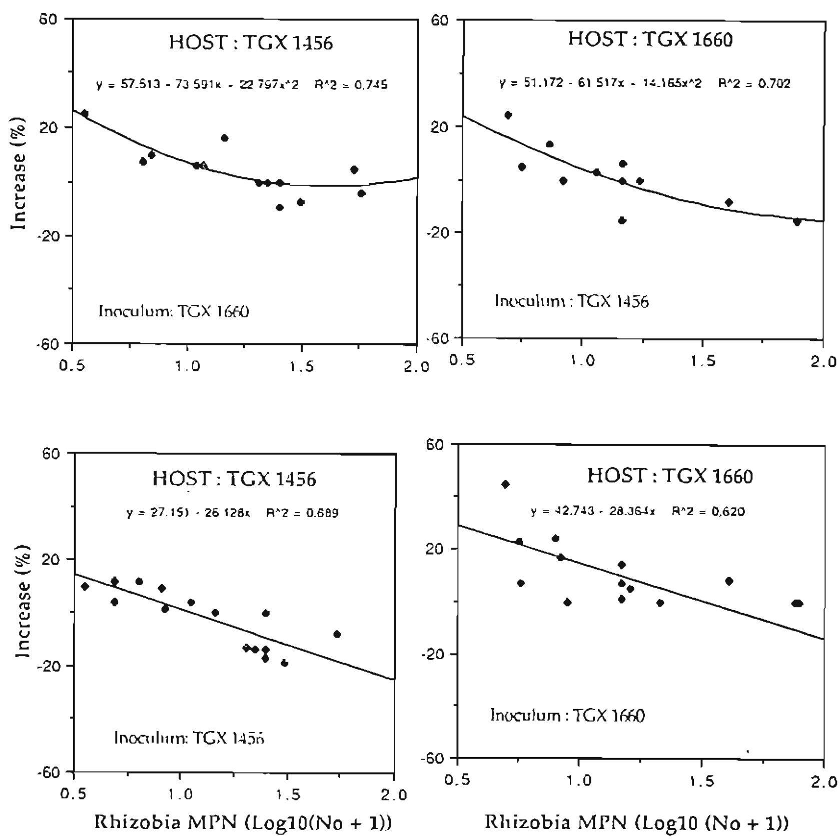

Fig. 3. Relationships between numbers of indigenous rhizobia and legumes TGX 1660-19F and TGX 1456-2E inoculation responses.

of indigenous rhizobia. The regression equations describing these relationships for promiscuous soybean cultivars are shown in Fig. 3. On average, when < 10 rhizobia cells g^{-1} soil were present, previous inoculation increased shoot dry weight between 5% and 25% for TGX 1456-2E and between 3% and 45% for TGX 1660-19F. Little or no response was obtained when the numbers of indigenous rhizobia were > 10 cells g^{-1} soil except in a few cases such as that of cultivar TGX 1456-2E grown in previous inoculated TGX 1660-19F treatments (data not shown).

4. Discussion

Rhizobial population sizes varied between farmers' fields and were influenced by cropping sequences and rhizobial inoculation. Native populations of rhizobia were frequently higher in terms of numbers and effectiveness in soils collected in soybean plots (Tables 3 and 4) or, e.g. Farmers' Field 4 (Table 2) or, when a legume such as groundnut was in the field in the previous years, e.g. Farmers' Fields 4 and 16 (Table 2). Populations of rhizobia nodulating promiscuous soybeans grown in soils from maize plots (Table 4) or from fields that had cereals in their cropping history (see Table 2; Farmers' Fields 5, 6, 15 and 22) were insufficient and therefore did not meet the crop's N requirements through symbiosis. This was illustrated by the lower nodulation and growth of uninoculated soybeans (Table 5) and the frequent response of promiscuous soybeans to rhizobial inoculation in soils with previous maize cropping. This shows that symbiotic N requirements of promiscuous soybeans can be met only if sufficient numbers of effective rhizobia are available to the crop either through inoculation or exploiting the existing populations is effective.

The importance of cropping sequence on N_2 fixation has been demonstrated by several authors (Peoples et al., 1995). Whenever a particular legume has been long established in an agricultural system or is considered as a traditional crop, it is likely that there will be adequate numbers of indigenous rhizobia for nodulation (Thompson et al. (1991). But beyond this generalisation it is difficult, as shown in this study, to predict situations where inoculation might not be necessary. Singleton et al. (1992) indicated that even the notion that it is unnecessary to inoculate promiscuous legumes in tropical soils may be flawed. This might be the case with IITA promiscuous soybeans. It is imperative, therefore, that prior to promoting promiscuous soybean varieties without inoculation (i) the present symbiotic potential of rhizobia in soils targeted for soybean production be sufficient; or (ii) the symbiotic potential of existing rhizobial populations be improved with proper cultural managements such as repeated cultivation of soybean.

The results of our experiment indicate that response to inoculation depended on the number of invasive rhizobia and it occurred only when the population was < 10 rhizobia cells g^{-1} soil. The relationship between rhizobial population and inoculation observed in our study (Fig. 3) have been reported by Thies et al. (1991) who found that a hyperbolic model best described the relationships between rhizobial population size and percentage increase in shoot dry weight of promiscuous soybean cultivars due to inoculation. However, differences between cultivars occurred in our study depending on the source of rhizobia inoculants (Fig. 3)

From the results presented in this paper, a rough recommendation would be to inoculate promiscuous soybeans where MPN population estimates are < 10 rhizobia cells g^{-1} soil. If this recommendation is applied to our observed results, inoculation would have been recommended for 94 out of 312 plant hosts, previous treatment and farmers' field combinations, representing 30%. But no inoculation would have been recommended for about 70% of plant tests, previous treatments and farmer's field combinations where the previous uninoculated treatments gave equal or higher shoot dry weight than previously inoculated plants, showing the higher effectiveness of indigenous rhizobia population or adaptation of these cultivars to the native rhizobial populations.

When assessing whether or not inoculation is required, soybean planters should take into account not only factors related to rhizobial population size and its effectiveness but also available N in the soil (Thies et al., 1991). In some cases, lack of response to inoculation occurred even when there were < 10 rhizobia g^{-1} soil. This could be attributed to some other soil factors such as high available N and low P soil. In sites such as Zonkwa 8 and 10 where available N was above 5 ug g^{-1} soil (data not shown), soybeans did not respond to rhizobial inoculation but had higher shoot dry biomass. As indicated by Thies et al. (1991) inoculation response is dependent upon there being a demand for fixed N by the soybean or cowpea. When soil N is insufficient to meet crop N demand, inoculation response is dependent upon whether the sum of available soil N plus N_2 fixed by the indigenous or introduced rhizobial population is sufficient to meet the N demand. In these trials, an indigenous rhizobial population in excess of 10 cells g^{-1} soil was sufficient to achieve shoot dry weight yield not significantly different from those of previously inoculated plants, except when the rhizobial populations were mostly ineffective. As indicated by Turk et al. (1993), the MPN assay can help identify areas (on a regional basis) where introduced rhizobia by inoculation or present indigenous rhizobia in the soil are likely to result in improved soybean performance. However a precautionary measure should be always taken. The MPN assay should be used in association with an effectiveness test for cultivars that are promiscuous for nodulation but relatively specific for effectiveness in their rhizobial requirements.

Cultivars such as Bossier known to be specific in its nodulation (nodulating with *Bradyrhizobium japonicum*) responded more often to previous inoculation and was more improved by indigenous rhizobial population than promiscuous cultivars TGX 1456-2E and TGX 1660-19F (Table 6). Effective nodulation of Bossier grown in soils with TGX 1660-19F and TGX 1456-2E previously inoculated with *Bradyrhizobia* spp. indicates that Bossier was effectively nodulated with *Bradyrhizobia* spp. other than only *Bradyrhizobia japonicum*. This was confirmed further with nodulation of Bossier using pure culture isolated from cowpea grown in Zonkwa and Zaria soils (data not shown). The response frequency of TGX 1660-19F was lower than that of TGX 1456-2E, making it closer to the cowpea, considered as the typically promiscuous.

This probably indicates diversity in types of *Bradyrhizobia* spp. strains able to form effective nodulation with Bossier and the promiscuous soybean cultivars. However, our data could not make a clear distinction between type of rhizobia, i.e. *Bradyrhizobium* spp. of cowpea type or *Bradyrhizobium japonicum*, nodulating the two promiscuous cultivars since number of nodules formed by all soybean plants were similar. The indigenous rhizobia were relatively effective at N_2 fixation with Bossier, as a significant increase (36%) above previously inoculated treatments in Zaria and Zonkwa despiste MPN estimates of up to 100 rhizobia g^{-1} soil. Similarly, the response of TGX 1456-2E and TGX 1660-19F to indigenous rhizobia in these two locations, despite an MPN estimate of over 100 rhizobia g^{-1} soil supports our suggestion that these cultivars and Bossier could be promiscuous for nodulation but specific for effectiveness. Abaidoo (personal communication) suspected that the strains from Nigeria could be mainly soybean strains since they nodulate effectively with non-promiscuous soybean varieties. They may also be cowpea rhizobia which have probably evolved to nodulate effectively with soybean through repeated cultivation. It is clear from our observations that effective nodulation was obtained with indigenous rhizobia populations for non-promiscuous and promiscuous soybean cultivars. The question, however, remains as to the behaviour of both Bossier and promiscuous soybeans in areas where soybean cultivation has never been practised.

Acknowledgements

Thanks to B. Ibewiro for graph fitting, J. Oyedipe, and Eniola for assistance in soil collection and pot experimentation and Anthony Nwachuku for the tedious task of nodule picking, to V. Adedokun for help with the MPN assays, and O. Orowale for secretarial services.

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