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Persistence of *Rhizobium* inoculants originating from *Leucaena leucocephala* fallowed plots in Southwest Nigeria

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Ten core soil samples were collected from experimental plots at IITA, SW Nigeria that were previously inoculated with *Rhizobium* strains (IRC1045 and IRC 1050) specific for *Leucaena leucocephala* at two depths; 0–15 cm and 15–30 cm. The control soil samples were collected at similar depths from an adjacent field with no previous history of legume cultivation. Six weeks after planting of *L. leucocephala* in the soil samples in the greenhouse shoots, roots and nodules were harvested aseptically. Typing of the nodules as well as the identification of the persisting population of the introduced strains were based on the intrinsic resistance of IRC 1045 and IRC 1050 to streptomycin at 500 μg/ml and nodules were found to be made up of 100% of previously introduced strains. The potency and competitive ability of the recovered IRC 1045 and IRC 1050 were thus confirmed via the pot experiment and plant reinfection experiment in the greenhouse. At 0–15 cm and 15–30 cm depths 8.0×10^4 and 9.0×10^4 rhizobia/g of soil were recovered respectively in spite of the 10-year fallow period. Biomass production with the three woody legumes revealed Root and Shoot dry weights of the following order of magnitude *Senna siamea* > *L. leucocephala* > *Senna spectabilis*. This report showed the great potential of application of *Rhizobium* technology in low input sustainable agricultural practice and environmental pollution abatement for non-use of chemical nitrogen fertilizers.

Key words: Agriculture, *Leucaena leucocephala*, nodulation, persistence, *Rhizobium*.

INTRODUCTION

The challenge facing research on increasing food production in tropical Africa is to develop low input systems that are not only sustainable but also economically viable. Alley cropping system is one of the potential alternatives to the bush fallow system because they can sustain food production with low purchased inputs in the humid and subhumid tropics. The main value of legumes is in building up soil organic nitrogen to the level that adequate amount can be made available for future crops (Mulongoy and Van der Meersch, 1988). Inoculation with an effective and persistent *Rhizobium* strain has numerous advantages, which includes non-repeated application of nitrogen fertilizers (Sanginga et al., 1994). However, for many soil types adequate nodulation and N_2 fixation via *Leucaena leucocephala* require inoculation with the appropriate and specific

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Rhizobium strain particularly where *L. leucocephala* has not previously been cultivated (Sanginga et al., 1985; Lales et al., 1989).

Changing environmental conditions and management practices such as pruning and cutting affects the survival of inoculants and legume yields in the field (Sanginga et al., 1990; 1994). The selection of adapted inoculant strains and understanding of changes in *Rhizobium* populations as well as the factors influencing such changes would enhance the integration of *L. leucocephala* inoculants in the desired low-input farming system. *L. leucocephala* is a fast-growing ubiquitous tropical legume which has found use in agroforestry, soil improvement, land reclamation, wood and forage due to its exceptional capacity to produce biomass (Danso et al., 1992). This study presents some economic consideration on the profitability of low-input farming system relative to the use of nitrogen fertilizer and to the bush fallow system.

The objectives of the current study were to examine the persistence, potency and competitive abilities of recovered rhizobial strains in fallowed plots previously inoculated with known strains of *Rhizobium* with a view to ascertaining ecological factors responsible for the development of the observed agronomic qualities of the host plant.

MATERIALS AND METHODS

History of Sampled Field

The experiments were carried out at the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria. The soil is an Alfisol of the Egbeda series (Sanginga et al., 1988). The sandy loam soil is characterized by pH(H_20) 6.1; organic carbon, 0.85%; total nitrogen, 0.07%; cation exchange capacity (C.E.C), 5.38 milli equivalent (meg.) per 100g; available phosphorus (Bray 1), 6.46 ppm; and 360 *Leucaena* rhizobia per gram of soil.

Single strains of *Rhizobium* IRC 1045 (isolated from *L. leucocephala* grown in Fashola soil) and IRC 1050 (isolated from *L. leucocephala* grown in Ibadan) were used separately to inoculate *L. leucocephala* in the 1988 inoculation trial (Sanginga et al., 1988). These were applied to the seed as peat based inoculants using Nitracoat adhesive (Nitragin Co.) and inoculated seeds were planted in moist soil. Uninoculated and fertilizer nitrogen treatments had also been used in this trial with 150 kg nitrogen/ha of urea being applied once at planting. Two years after planting, *L. leucocephala* was harvested and assessed for its response to inoculation (Sanginga et al., 1988).

In 1992, the field was cleared and uninoculated *L. leucocephala*, *Senna siamea* and *Senna spectabilis* were sown in the previous *L. leucocephala* plots. The experiment was laid out as a split plot design with 3 replicates of the previous uninoculated, inoculated with *Rhizobium* IRC 1050 or 1045, and uninoculated plus nitrogen fertilizer as the main treatments and three woody legumes as subtreatments; the N_2-fixing *L. leucocephala* and two non- N_2 fixing plant; *S. siamea* and *S. spectabilis* giving a total of 36 plots. Each plot measured 96 m^2 (8 x 12 m) with 3 rows spaced by 2 m and a planting distance of 1 m within rows. One month after planting, 20 kg nitrogen/ha as ammonium sulphate labelled with 10-atom % ^{15}N excess, was applied onto isotope subplots (6 m^2) containing the 4 central trees, after thinning the seedlings. An adjacent field, which has never been planted with *L. leucocephala*, was used as a control.

Sample Collection: Soil samples were collected at planting time in 1998. A soil auger of 3-cm diameter was used to collect soil at depths of 0–15 and 15–30 cm. Ten cores were collected at random in each of the previous plots in the alleys and beneath trees of *L. leucocephala*, *S. siamea* and *S. spectabilis*. They were mixed into composite samples and air-dried.

Enumeration of Rhizobia: Rhizobia were enumerated by the soil dilution plate count method using yeast extract mannitol Agar (YEMA) (Vincent, 1970). This was supplemented with streptomycin at four different concentrations of 0, 125, 250 and 500 µg/ml, which served as the basis of distinguishing between the introduced and indigenous rhizobia population. The introduced strains have been reported to have intrinsic antibiotic-resistance (Sanginga, 1985). The inoculated plates were incubated at temperature between 26 and 28^oC for 48 hours. The colonies were counted with the Leica Quebec's darkfield colony Counter (Model 3328, Buffalo N Y 14420 USA).

Nodulation Assay

The persistence, effectiveness and competence of the previously introduced *Rhizobium* strains, IRC 1045 and IRC 1050 to nodulate *L. leucocephala* were tested in the pot experiments in the greenhouse using a randomized complete block design. There were a total of 36 plots in the experimental field which was replicated twice giving 72 pots in the greenhouse, 8 pots from the adjacent control field; four of the pots were receiving potassium

nitrate solution and the other four Jensen's solution (contains no nitrogen) (Vincent, 1970), thus serving as the uninoculated controls. This resulted in a total of 80 pots filled with soil samples from *L. leucocephala*, *S .siamea*, *S spectabilis* and control pots.

At six weeks after planting, the plants were excised with sterile surgical blade just at the soil level, the above-ground plant material (shoot) and the below-ground plant materials (roots and nodules) were harvested, weighed and oven-dried at 65^{o}C for 48 hours. Roots were carefully removed and examined for nodulation. Fresh nodules were counted, cleaned of soil particles, weighed and then used for strain identification. From each treatment, 10 nodules were chosen at random and typed using YEMA supplemented with streptomycin at a concentration of 500 µg/ml (Sanginga, 1985).

Plant Re-Infection Assay (The Leonard Jar Experiment): Field recovered strains of IRC 1045 and IRC 1050 were cultured in 100 ml of yeast extract mannitol broth (YMB) for testing at 26 to 28^{o}C on a rotary shaker for 9 days. The control was a blank YMB without inoculant (Vincent, 1970). The pot experiment was set in a randomized complete block design with two replicates for each treatment. One microliter of *Rhizobium* suspension (10^9 cells/ml) were introduced on the seeds per pot. There were 4 pots filled with dry-sterile sand as controls; 2 pots for nitrogen controls receiving sterile Jensen's nutrient solution (Vincent, 1970) and the other two receiving sterile KNO_3 solution containing 0.05% N. The test pots received *Rhizobium* inoculant as the source of Nitrogen (Vincent, 1970).

Six weeks after planting, the above-ground plant material (shoot) and below-ground material (root) were excised with sterile surgical blade. Roots were carefully removed and examined for nodulation. Fresh nodules were counted, cleaned of soil particles and then used for strain identification on YMA supplemented with streptomycin at 500 µg/ml (Schwinghamer and Dudman, 1973).

Statistical Analysis

Analysis of variance was performed on the log-transformed numbers of rhizobia per gram of soil. Analysis of variance (ANOVA) was also performed on data of all plant parameters measured using the SAS software package (SAS User's Guide Statistics SAS institute Inc., Cary. NC, USA).

Table 1: Influence of plant species and depth of sampling on the number of *L. Leucocephala*-rhizobia (10^4) per g of soil collected in experimental plots after 10 years of fallow.

	Depth		
Plant species	0 – 15 cm*	15 – 30 cm*	Mean
Inoculated plots			
L. leucocephala	4.5	4.0	4.25
Uninoculated plots	2.0	2.5	2.25
S. siamea	1.5	2.5	2.00
S. spectabilis			
Control	0	0	0
Total	8.0	9.0	8.5

LSD 5% (1) 0.8129
(2) 0.6637

(1) For comparing plant species
(2) For comparing sampling depths
- average value of 80 plots

RESULTS AND DISCUSSION

The ability of root-nodule bacteria to fix atmospheric nitrogen in symbiosis with compatible host legumes has been extensively studied but little attention has been given to the competitive ability and persistence of introduced *Rhizobium* strains which is the basis of their selection as inoculants for use in perennial legume cultivation (Danso et al., 1992). At the time of introduction of the inoculants, the number of homologous rhizobia able to nodulate *L. leucocephala* were 360 cells/g of soil on the experimental field while after the ten-year fallow period their population had risen to 8.5 x 10^4 cells/g of soil refers (Table 1). The substantial increase in population and dominance of the introduced *Rhizobium* strains in the rhizosphere can be attributed to: (i) a non-specific rhizosphere effect indicating that plant roots provide a more favourable environment than soil for rhizobia and other microorganism, and (ii) nodule senescence which encourages rhizobia release and thus increase the population of the appropriate *Rhizobium* symbiont in the rhizosphere and seed inoculation strategy adopted for this study. This submission aligned correctly with previous observation of earlier investigators (Bushby, 1984; Sanginga et al., 1990, 1994).

Soil collected beneath *L. leucocephala* plants on the field contained higher numbers of the inoculant than those collected under *S. siamea* and *S. spectabilis*, generally inoculated plots contained more rhizobia than the uninoculated plots after the fallow period (Table 1). The increase in the population of the inoculant was significantly influenced by the plant species.

After the ten-year fallow period, 100% of nodules formed on *L. leucocephala* were proved to be those of the introduced rhizobial strains IRC 1045 and IRC 1050 because the isolated nodules were resistant to 500 μg/ml of streptomycin. The indigenous strains were not resistant to this concentration of streptomycin, which corroborate earlier discovery (Sanginga, 1985). Precisely, *L. leucocephala* (Lam) de Wit proved to be highly specific for IRC 1045 and IRC 1050 in the sub-humid soil in Nigeria.

The nodulation that took place in the uninoculated nitrogen-fertilized soil samples occurred because soil water and rain water enhanced motility of rhizobial cells from *L. leucocephala* plots into *S. siamea* and *S. spectabilis* plots, the introduced rhizobial strains were not discovered in the adjacent control field (Table 2). Nodulation therefore has developed as an ecologically convenient mechanism in which the occurrences of one symbiont frequently accounts for the presence of the other (Woomer et al., 1988). Comparison of nodule fresh weights from the tree legumes revealed that the nodule fresh weights that developed from soil collected from beneath non-N2 fixing plant species were higher probably due to their high soil water absorbing capabilities which complemented their relative soil fertility refers (Table 3).

Table 2. Average nodule number and biomass performance of *L. leucocephala* at 6 weeks after plantng in the greenhouse with soil samples from experimental plots after 10 years of fallow.

	← 0 – 15 cm* →		← 0 – 15 cm* →		
Plant species	Nodule number	Nodule fresh weight (g)	Nodule number	Nodule fresh weight (g)	% Nodulation
Inoculated N-fertilized					
L. Leucocephala	76	1.13	79	1.11	36.9
Uninoculated N-fertilized					
S. siamea	76	1.38	70	1.31	36.4
S. spectabilis	54	0.82	70	1.18	26.2
Adjacent plot					
Control	0	0	0	0	0

* average value of 80 plots

Table 3: Means of agronomic parameters of *L. leucocephala* obtained from the greenhouse experiment at 6 weeks after planting.

Plant Species	Root fresh weight (g)	Root dry weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)	Mineral nitrogen (ppm)
L. Leucocephala	2.72	1.41	4.52	2.91	29.34
S. siamea	4.21	1.60	7.90	3.41	29.82
S. spectabilis	3.24	1.15	5.47	2.39	27.98
Control	4.13	1.18	6.09	2.41	19.0
LSD 5%	1.18	0.39	1.95	0.85	5.40

*Each value is an average of 80 plots

The comparison of the agronomic parameters deduced from the greenhouse experiment revealed a significant difference between the control field and experimental field in terms of latent mineral nitrogen level, this has significantly influenced the dry weights of both the shoots and roots of *L. leucocephala* harvested 6 weeks after planting in the greenhouse. Soil samples from beneath the reference trees afford *L. leucocephala* the opportunity to accumulate more water which explains for high freshweights detected from plant materials obtained from soil samples from beneath the reference trees (Table 3). The shoot and root dry weights of *L. leucocephala* plant materials obtained from soils from beneath *S. siamea* and *L. leucocephala* were found not to be significantly varied thus corroborating the fact that the *L. leucocephala* relatively has exceptional capacity to produce biomass, forage, wood and to improve soil fertility as reported by Kang et al. (1981). This suggests that higher rhizobia population from *L. leucocephala* soils did not translate to high dry matter accumulation in *Leucaena* when compared with the biomass production in *S. siamea.*

The implications of the results obtained in this study were that *Rhizobium* strains IRC 1045 and IRC 1050 introduced with *L. leucocephala* seeds were more competitive in the soil environment than the native rhizobia and were detectable in the soil in high numbers as well as being able to effectively nodulate *L. leucocephala* 10 years after their introduction which agrees with the report of Sanginga et al. (1994). Persistence of *B. japonicum* in a field soil for 2–year fallow period which sustained soybean yields after fallow without further inoculant or fertilizer nitrogen input has been reported by Ranga Rao et al. (1982).

We conclude that the inoculation of legume seeds with reasonably large population of effective and persistent rhizobial strains constituted an evident advantage over inorganic nitrogen fertilizer which has to be applied

frequently for consistent high yields. *Rhizobium* inoculants can be said to be a potent alternative to chemical nitrogen fertilizer as well as a renewable resource which is capable of sustaining food production with little or no purchased inputs. If properly harnessed, this technology would afford developing countries opportunity to avoid use of chemical nitrogen fertilizer which has been incriminated in most aquatic and terrestrial pollution.

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