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Nitrogen fixation of field-inoculated *Leucaena leucocephala* (Lam.) de Wit estimated by the ^{15}N and the difference methods

N. SANGINGA[1], K. MULONGOY and A. AYANABA[2]
International Institute of Tropical Agriculture, PMB 5320, Ibadan, Nigeria. Present addresses: [1]IAEA Agricultural Laboratories, Joint FAO/IAEA Programme, A-2444 Seibersdorf, Austria and [2]Del Monte Corporation, Box 36, San Leandro, CA 94557, USA

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Abstract

The amount of nitrogen fixed by *Leucaena leucocephala* (Lam.) de Wit was assessed on an Alfisol at the International Institute of Tropical Agriculture located in southwestern Nigeria. Estimated by the difference method, nitrogen fixation of leucaena inoculated with Rhizobium strain IRc 1045 was 133 kg ha^{-1} in six months. Inoculation with Rhizobium strain IRc 1050 gave a lower nitrogen fixation of 76 kg ha^{-1}. Fertilization with 40 and 80 kg N ha^{-1} inhibited nitrogen fixation by 43–76% and 49–71%, respectively. Estimates with the ^{15}N dilution method gave nitrogen fixation of 134 kg ha^{-1} in six months when leucaena was inoculated with Rhizobium strain IRc 1045 and 98 kg ha^{-1} for leucaena inoculated with Rhizobium strain IRc 1050. This nitrogen fixation represented 34–39% of the plant nitrogen. Inoculated leucaena derived 5–6% of its nitrogen from applied fertilizer and 56–54% from soil.

Introduction

Leucaena leucocephala (Lam.) de Wit can yield prunings containing over 500 kg N ha^{-1} annually (Guevarra, 1976 cited by Kang *et al.*, 1981). Hogberg and Kvarnstrom (1982) used the acetylene reduction assay and found annual fixations of 110 ± 30 kg N ha^{-1} in Tanzania. Sanginga *et al.* (1986) took advantage of leucaena's requirement for specific rhizobia to estimate nitrogen fixation by the difference method with the uninoculated and unnodulated leucaena plants as the non-nitrogen-fixing control. They found that in six months, leucanea fixed 224–274 kg N ha^{-1}, equivalent to about 56% of the plant nitrogen. Acetylene reduction assays and the difference method provide only indirect measures of nitrogen fixed, and cannot distinguish between nitrogen derived from atmosphere, soil and fertilizer (Hardarson *et al.*, 1984).

The present investigation was undertaken to compare nitrogen fixation of field-inoculated leucaena evaluated by the difference and the ^{15}N dilution methods, and to determine the proportion of nitrogen fixed as compared to nitrogen derived from soil and fertilizer.

Materials and methods

The experiment was carried out at the International Institute of Tropical Agriculture (IITA) located in southwestern Nigeria. The soil is an Alfisol of the Egbeda series. Soil properties, determined on samples taken at a depth of 0–15 cm, were: pH (H_2O), 6.1; organic C, 0.85% total N, 0.07%; CEC, 5.4 meq 100 g^{-1}; and extractable P (Bray-1), 6.5 ppm. The number of leucaena rhizobia was 360 per g of soil.

The experimental plot was ploughed and single superphosphate at 80 kg P ha^{-1} and muriate of potash at 50 kg K ha^{-1} were broadcast before planting. The field design was a split plot with 4 replications.

Main treatments consisted of uninoculated leucaena plants and plants inoculated with Rhizobium strains IRc 1045 or IRc 1050 isolated earlier in southwestern Nigeria at Fashola and Ibadan, respectively (Sanginga *et al.*, 1985; 1987). Subtreatments were 0, 40 and 80 kg N ha^{-1} as ammonium sulfate applied at one week after plant emergence. Forty kilograms N ha^{-1} of ammonium sulfate with 10% atom ^{15}N excess were applied to microplots located in the center of the 40 kg N ha^{-1} subtreatments. Main plots measured 8 × 9 m and subplots 8 × 3 m, with a row spacing of 50 cm and a planting distance of 20 cm within rows. Microplots measured 1.50 × 4 m.

Leucaena seeds were scarified with concentrated sulfuric acid for 30 minutes and rinsed several times in sterile water. Four seeds per hill were sown immediately after they had been coated with peat inoculants of Rhizobium strains IRc 1045 or IRc 1050 following the procedure described by Vincent (1970). Rhizobia numbers averaged 1 × 10^7 cells per seed. Seedlings were thinned to two per hill a week after emergence. At 24 weeks after planting (WAP), nodule numbers and dry weights and shoot dry weight and nitrogen content were determined on 10 plants harvested at random in 2 m sections within the second row of each plot. Plants were also dug up in the two middle rows of the ^{15}N microplots. Plant materials were dried in an oven at 65°C for 48 hours. Soil and plant nitrogen were determined with a Technicon Autoanalyser after micro-Kjeldahl digestion.

Nitrogen-15 analysis was carried out by mass spectrometry at Boyce Thompson Institute, Cornell University, Ithaca, New York, USA. Nitrogen derived from soil and fertilizer was calculated from ^{15}N data following the method of Fried and Middelboe (1977). For the difference method, uninoculated leucaena plants were used as reference.

Results and discussion

Inoculation of leucaena with rhizobia increased nodule mass (Fig. 1), shoot dry matter (Fig. 2) and nitrogen content (Fig. 3) in the no-fertilizer-nitrogen treatment. Adequate nodulation improves establishment and growth of leucaena (Ahmad and Ng, 1981; Diatloff, 1973; Sanginga *et al.*, 1986). Fertilization with 40 and 80 kg N ha^{-1} depressed

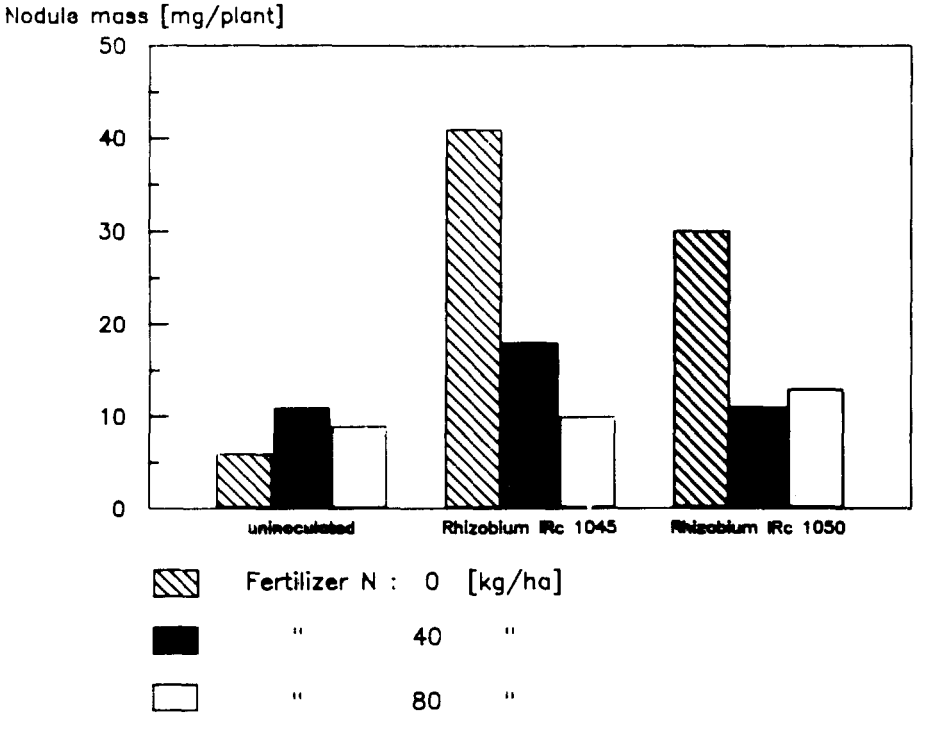

Fig. 1. Effect of inoculation and N fertilizer on nodule mass by *Leucaena leucocephala*.

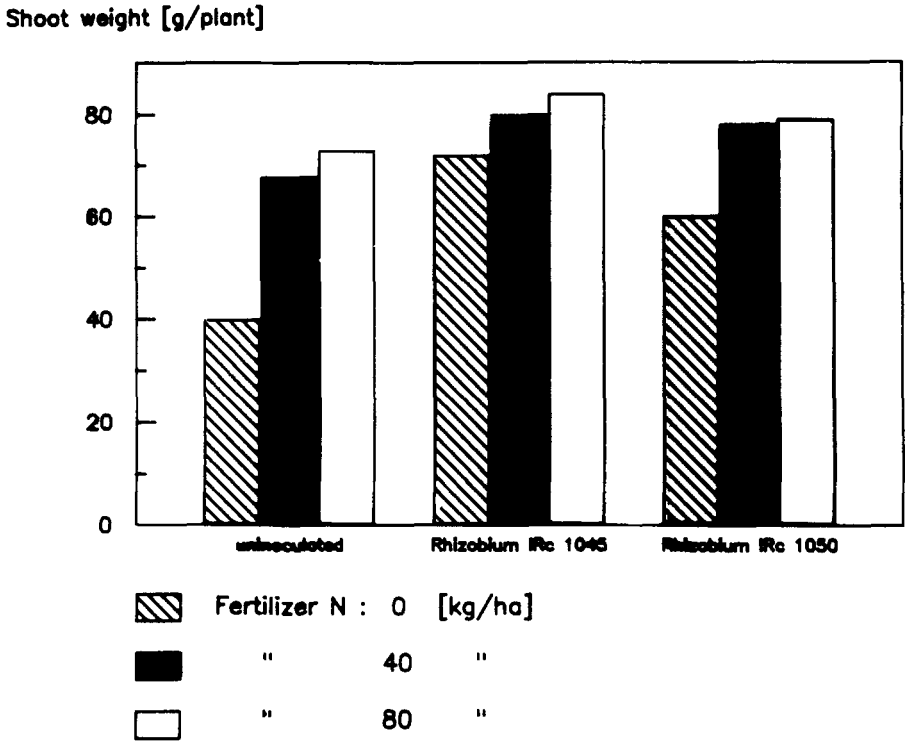

Fig. 2. Effect of inoculation and N-fertilization on shoot dry matter by *Leucaena leucocephala.*

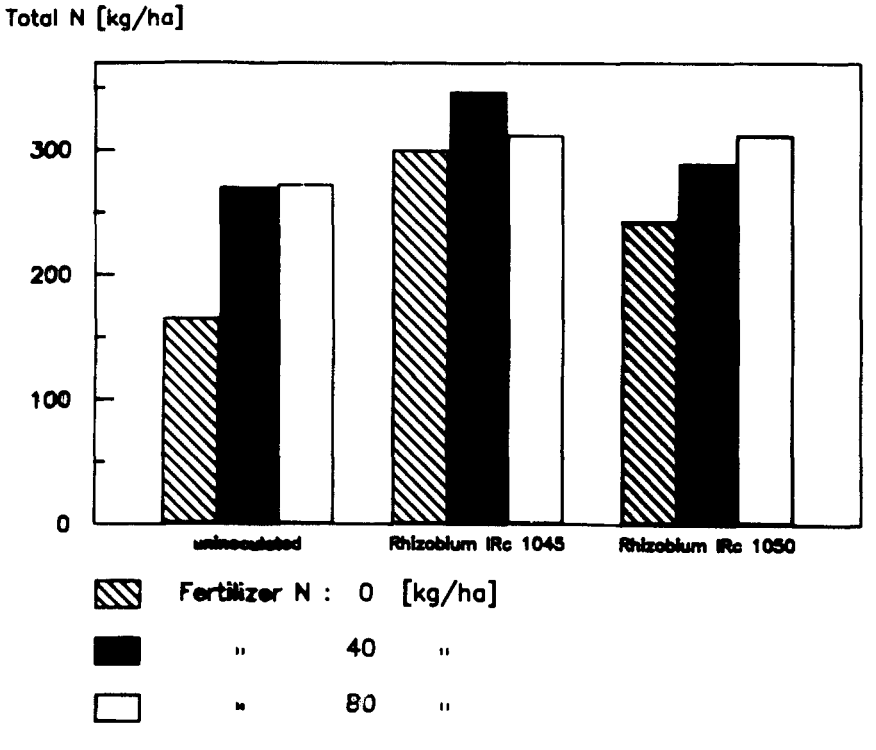

Fig. 3. Effect of inoculation and N fertilizer on total N of *Leucaena leucocephala.*

nodulation in inoculated plants (Fig. 1), but it increased leucaena shoot dry weight and nitrogen content in inoculated plant to the same levels as did inoculation with rhizobia (Figs. 2 and 3).

The amount of N_2 fixed calculated by the difference method are shown in Fig. 4. The symbiosis with Rhizobium strain IRc 1045 fixed 133 kg N ha^{-1} in six months, while the symbiosis with IRc 1050 fixed 76 kg N ha^{-1} during the same period when no fertilizer N was applied. In this study, uninoculated plants, which were used as reference plant, nodulated. This could lead to underestimation of the amounts of N_2 fixed using either the difference or the isotope dilution method. However, although nodules on uninoculated plants were as numerous as on inoculated ones, they were inefficient. This was indicated by their low specific weight and low activity per g of nodules. In addition nitrogen contents and shoot dry weights of uninoculated plants that received no nitrogen were the same as in an experiment where uninoculated plants had no nodules (Sanginga *et al.*, 1986).

The problem of the reference crop has been discussed by many authors (Chalk, 1985; Fried *et al.*, 1983; Rennie and Rennie, 1983; Wagner and Zapata, 1982) and still there are a lot of controversies. This is even more complicated for nitrogen fixing tree such as leucaena where no non-fixing isolines are yet not available. The only alternative is therefore the use of the uninoculated control as the reference crop. However, cross-contamination may occur and could lead to errors due to fixation by nodules developed on the control plants. Several workers (Rennie and Rennie, 1983) have used a nodulated legume with an ineffective Rhizobium, as a reference crop. When N_2 fixed in the reference crop is negligible and/or high in the fixing crop, this should normally result in only small deviations from the true values of N_2 fixed, and in many cases of this nature the error could be therefore neglected. In the present case, the late nodules that developed on our uninoculated treatments as a result of rhizobia contamination had little effects on estimates of N_2 fixed. This has been observed by other investigators (Danso *et al.*, unpublished) and confirmed in many of our ongoing research on N_2 fixation by nitrogen fixing trees.

Estimates with the ^{15}N dilution technique gave nitrogen fixation of 134 kg and 98 kg ha^{-1} in six months for leucaena in symbiosis with Rhizobium

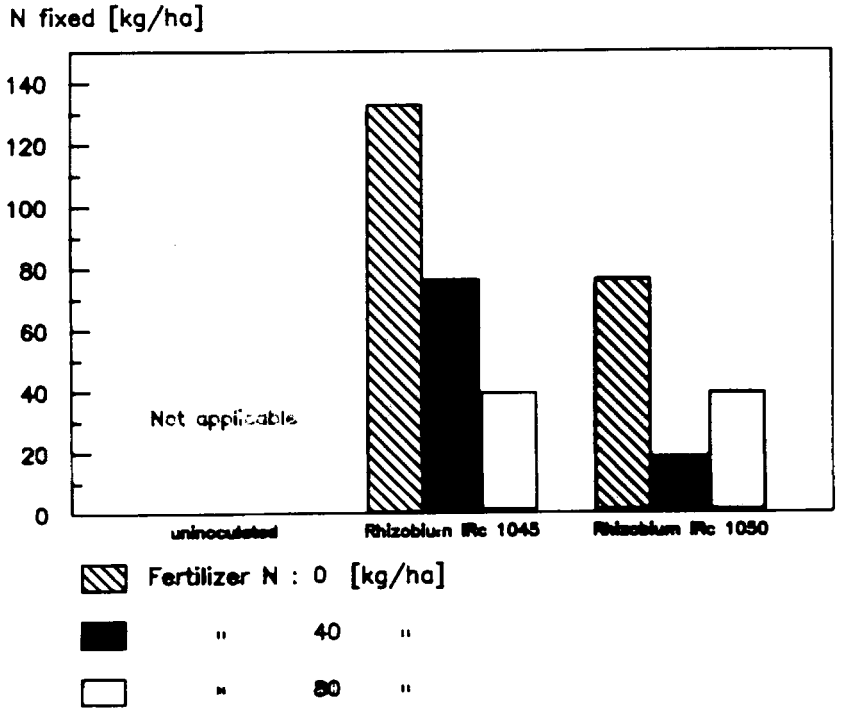

Fig. 4. Effect of inoculation and N fertilizer on N fixed by *Leucaena leucocephala.*

Table 1. Amounts and proportions of nitrogen fixed by *Leucaena leucocephala* inoculated with Rhizobium strain IRc 1045 or IRc 1050 estimated by the difference method and the ^{15}N dilution technique at 2 weeks after planting

Rhizobium strains	^{15}N dilution method			
	N fixed ($kg\,ha^{-1}$)	N fixed (%)	Ndfs[b] (%)	Ndff[b] (%)
IRc 1045	134	39	50	5
IRc 1050	98	34	54	6
LSD (5%)	10	4	1	3

[a] Percentage of total plant N.
[b] Ndfs = Nitrogen derived from soil; Ndff = Nitrogen derived from fertilizer.

IRc 1045 and 1050, respectively (Table 1). The amounts of N_2 fixed by this method represented 34–39% of the total plant N. Inoculated leucaena derived 5–6% of its N from applied fertilizer and 50–54% from soil.

Ammonium sulfate depressed nitrogen fixation. At $40\,kg\,N\,ha^{-1}$, the rate used for ^{15}N application, nitrogen fixation was reduced by 43–76% (Fig. 4). However, the isotope dilution method gave similar proportion (%) and amount of N_2 fixed to that of the difference method calculated at $0\,kg\,N\,ha^{-1}$. Although it is well established that large amounts of applied N reduce nodulation and nitrogen fixation the good agreement between the two methods on N_2 fixed by leucaena inoculated by Rhizobium IRc 1045 is difficult to explain. Understanding the conditions in which this occurs may facilitate the justification of the choice of the method to measure N_2 fixation.

Data in Table 1 show a low % of N derived from fertilizer by leucaena. This represented only a utilization of $2\,kg\,N\,ha^{-1}$ from the $40\,kg\,N\,ha^{-1}$ applied. Leaching, runoff and probably N immobilization are possible cause of these losses. In contrast to N fertilizer leucaena derived between 59 and 54% of its nitrogen from the soil. One attribute of leucaena in alley cropping is its deep rooting systems which allow to recycle N and other nutrients from deep soil layer to the soil surface. This N usually taken late, in deep soil layer where N from different sources accumulate, could be responsible of the inhibition of N_2 fixation observed in this experiment.

The most important conclusion to be drawn from the data presented is that inoculation with specific and effective rhizobia increases the early growth of leucaena through biological nitrogen fixation. The amount of nitrogen fixed was influenced by the strain of Rhizobium used for inoculation. Studies are however needed to validate these observations in various ecologies and to assess the influence of inoculation on later stages of leucaena growth. The amount of nitrogen fixed by inoculated plants was high and justifies the use of Rhizobium and leucaena symbiosis as a source of nitrogen in cropping systems. The difference method was less accurate than the ^{15}N dilution technique, but it indicated that ^{15}N fertilizer application at the rate of $40\,kg\,N\,ha^{-1}$ also underestimated nitrogen fixation because this rate of nitrogen fertilizer reduced nodulation and nitrogen fixation (Fig. 4).

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References

Ahmad N and Ng F S P 1981 Growth of *Leucaena leucocephala* in relation to soil pH, nutrient levels and *Rhizobium* concentration. Leucaeana Research Report, 2, 5–10.

Chalk P M 1985 Review. Estimation of N_2 fixation by isotope dilution: An appraisal of techniques involving ^{15}N enrichment and their application. Soil Biol Biochem. 17, 389–410.

Diatloff A 1973 Leucaena needs inoculation. Q. Agric. J. 99, 642–644.

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

Fried M, Danso S K A and Zapata F 1983 The methodology of measurement of N_2 fixation by non-legumes as inferred from field experiments with legumes. Can. J. Microbiol. 29, 1053–1062.

Hardarson G, Zapata F and Danso S K A 1984 Field evaluation of symbiotic nitrogen fixation by rhizobial strains using ^{15}N methodology. Plant and Soil 82, 369–375.

Hogberg P and Kvarnstrom M 1982 Nitrogen fixation by woody legume *Leucaena leucocephala* in Tanzania. Plant and Soil 66, 21–28.

Kang B T, Sipkens L, Wilson G F and Nangju D 1981 *Leucaena leucocephala* (Lam.) de Wit prunings as nitrogen source for maize (*Zea mays* L.). Fert. Res. 2, 279–287.

Rennie R J and Rennie A D 1983 Techniques for quantifying N_2 fixation in association with non-legumes under field and greenhouse conditions. Can. J. Microbiol. 29, 1022–1035.

Sanginga N, Mulongoy K and Ayanaba A 1986 Effect of inoculation and mineral nutrients on nodulation and growth of *Leucaena leucocephala* (Lam.) de Wit. *In* Biological Nitrogen Fixation in Africa. Eds. H Ssali and S O Keya. pp 419–427. The Nairobi Rhizobium Microbiological Resources Center, Nairobi, Kenya.

Sanginga N, Mulongoy K and Ayanaba A 1986 Inoculation of *Leucaena leucocephala* (Lam.) de Wit with Rhizobium and its nitrogen contribution to a subsequent maize crop. Biol. Agric. Hortic. 3, 341–347.

Sanginga M, Mulongoy K and Ayanaba A 1987 Evaluation of indigenous strains of*Rhizobium* for *Leucaena leucocephala* (Lam.) de Wit in Nigeria conditions. *In* Les Arbres Fixateurs d'Azote et L'Amélioration Biologique de la Fertilité du Sol. Colloques et Séminaires. pp 416–436. ORSTOM, Paris.

Talbott H, Kenwort W J and Legg J D 1982 Field comparison of the ^{15}N and difference methods for measuring nitrogen fixation. Agron. J. 74, 799–804.

Vincent J M 1970 A Manual for the Practical Study of Root-Nodule Bacteria. IBP Handbook No. 15. Oxford, Blackwell Scientific Publications.

Wagner G H and Zapata F 1982 Field evaluation of reference crops in the study of nitrogen fixation by legumes using isotope techniques. Agron. J. 74, 607–612.