

ESTIMATING N₂ FIXATION AND N DERIVED FROM SOIL BY *CASUARINA EQUISETIFOLIA* USING LABELLED ¹⁵N FERTILIZER: SOME PROBLEMS AND SOLUTIONS

B. SOUGOUFARA

Ministère de la protection de la Nature, B.P. 1386, Dakar, Sénégal

S. K. A. DANSO

FAO/IAEA Division, P.O. Box 100, 1-1400 Vienna, Austria

H. G. DIEM and Y. R. DOMMERGUES*

BSSFT (ORSTOM/CTFT/CNRS), 45 bis Avenue de la Belle Gabrielle, 94736 Nogent-sur-Marne,
Cedex, France

(Accepted 15 January 1990)

Summary—Three experiments were carried out in Senegal to assess the effect of four factors, clone, soil type, volume of containers and plant age, on N₂ fixation by *Casuarina equisetifolia* using isotope dilution (ID), A value (AV) and total N difference (TND) methods. Three clones grown in 15 l. containers differed significantly in their ability to fix N₂ and also to absorb soil N, the best clone (β) fixing 3.27 (ID) and 3.85 (AV) and the less active 2.51 (ID) and 2.73 (AV) g N₂ per tree during 1 yr. N₂ fixation by clone β was markedly affected by soil N: it decreased from 7.60 (AV) in Camberene soil (0.010% N) down to 4.64 (AV) g N₂ fixed per tree in Bel Air soil (0.025–0.030% N) during 1 yr. The amount of N₂ fixed was much higher in larger containers. In the case of 1 m³ containers with Camberene soil, the amount of N₂ fixed (AV) per tree (clone β) was 40.44 g during the first year and 84.43 g during the 2 first years of growth. In experiments 1 and 2, ID and AV estimations were significantly less than ID or AV estimations, but, in experiment 3, AV and TND estimations were similar. Problems raised by the estimation of N₂ fixation are discussed: Inoculation with *Frankia* strain ORS 021001 always markedly increased plant biomass expressed as dry weight and total N.

INTRODUCTION

Attention is focused worldwide on the exploitation of N₂-fixing trees—woody legumes or actinorhizal plants—as producers of wood, fuel-wood and biomass or as soil improvers. Suspected N₂-fixing trees have however often been introduced into different ecosystems, without first verifying that they fix significant amounts of N₂ (Dommergues, 1987). It is essential to quantify N₂ fixation in different species and provenances before promoting their extensive use. This knowledge would assist in designing better management practices for improving the N₂ fixed in various systems.

Some practical problems are bound to arise in the initial attempts to evaluate the N₂-fixing potentials of trees, for which solutions will have to be sought. Our aim was to identify some of these potential problems and to propose possible solutions.

Three methods, isotope dilution (ID) and A value (AV) (both isotopic methods), and the total N difference method (TND) were used to evaluate N₂ fixation in *Casuarina equisetifolia* and to compare the effect of the following factors on the plant biomass and N₂ fixation: tree clone, soil type, size of containers for growing trees and plant age. The last part of the paper is devoted to discussions on ways to improve N₂ fixation measurements and also to enhance the

N₂-fixing potential of *C. equisetifolia*, chosen as a model of fast-growing N₂-fixing trees.

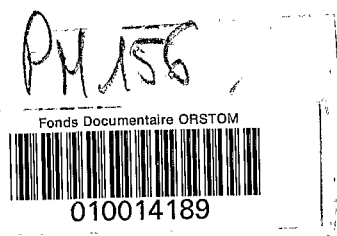
MATERIALS AND METHODS

Soil and plant material

The experiments were made in 1985 and 1986 at the ORSTOM Bel Air station in Dakar Senegal, using two types of soil: Camberene soil, a dune soil (97% sand), poor in nutrients, slightly acidic (pH 6.0), with 0.10 and 0.010% C and N respectively; and Bel Air soil, a sandy (93% sand), neutral (pH 7.0) soil, with C and N contents of 0.30 and 0.025–0.030% respectively. Each soil type was carefully homogenized, and fumigated with methylbromide. Three types of containers were used: 15 and 50 l. plastic containers which were fitted into holes dug out in the soil of the experimental field at 1.5 × 1.5 m intervals, and concrete 1 m³ containers (vol: 1000 l.) that were also fitted into the soil.

Cuttings of three clones of *C. equisetifolia* which had been shown (Sougoufara *et al.*, 1989) to have different N₂-fixing potentials were used. These were: clone S (a relatively-poor nodulator); clone α (an intermediate nodulator); and clone β (a very good nodulator). The explants were placed in sterile Camberene soil in a growth chamber. After 1 month the rooted plantlets were transferred in polyethylene bags into the nursery. Two months later, the 3-month-old cuttings were placed in the

*To whom all correspondence should be addressed.



containers (two plants per container), and inoculated at the base with a 3-week-old liquid culture of *Frankia* strain ORS021001 (equivalent to 20 µg protein plant⁻¹). Later they were thinned to 1 plant per container.

Treatments

In experiment 1 the three treatments indicated in Table 1 were applied to clones S, α, β, with 6 replications. In this experiment the two isotope methods were used to estimate N₂ fixation: the isotope dilution method (ID) with treatment 1 (uninoculated plants) as the reference crop (Fried and Middelboe, 1977), and the AV method with a higher ¹⁵N rate applied (treatment 2) to the uninoculated reference crop (Fried and Broeshart, 1975; Danso, 1986). Trees were grown in 15 l. containers containing Camberene soil. Other experimental details are given in Table 1.

In experiment 2 only clone β was used to study the effects of inoculation (no inoculation and inoculation), soil type (Camberene and Bel Air), and volume of container (15 vs 50 l.) on N₂ fixation (using only the AV and TND methods). Each treatment was replicated three times. Other experimental details are given in Table 1.

In experiment 3, as in experiment 2, clone β of *C. equisetifolia* was used as plant material. Two factors at two levels each were studied: inoculation (no inoculation and inoculation), and plant age (12 and 24 months). Treatments were replicated five or six times. Trees were grown in 1000 l. containers containing Camberene soil. Other experimental details are given in Table 1. As in experiment 2, only the AV and TND methods were used to evaluate N₂ fixation.

In experiments 1 and 2, P and K were added to the soil as 5 g K₂HPO₄ before planting. In experiment 3, P and K were added to the soil as 20 g K₂HPO₄ before planting.

In the three experiments, plants were carefully irrigated throughout growth and, in addition, received 50 ml (15 and 50 l. containers) or 200 ml

(1000 l. containers) of N-free Hewitt (1966) solution every week. Plants were harvested at 9 months after transplanting [experiments 1, 2 and 3 (treatments 1 and 2)] or 21 months after transplanting [experiment 3 (treatments 3 and 4)], when they were 12- or 24-months-old, respectively.

Estimation of N₂ fixation

In experiments 1 and 2, the following plant parts were sampled separately:

- (i) photosynthetic branchlets [terminology recommended by Flores (1978); Johnson (1982); Torrey and Berg (1988), the term cladode previously used being inappropriate];
- (ii) branchlets, branches and trunk;
- (iii) roots; and
- (iv) nodules.

In experiment 3, because the trees were much larger than in experiments 1 and 2, the branchlets (dia < 4 mm) were further separated from branches and trunk (dia > 4 mm) so that there were five parts.

Each plant part was dried to constant weight at 70°C and ground into 100 µm powder. Subsamples of each powdered plant part were analysed for total N and ¹⁵N. Isotopic ¹⁵N analyses were carried out at Seibersdorf Laboratory (IAEA) using Dumas' method and a VG 602 mass spectrometer (Fiedler and Proksch, 1975). For each individual tree, N and ¹⁵N values for the whole plant were calculated by first weighting the atom % ¹⁵N excess of the different plant parts (including nodules in inoculated plants) (Fried *et al.*, 1983).

In experiment 1, because the amount of urea applied in treatments 1 and 3 was the same, each estimate of N₂ fixation by the TND was simply based on the difference between the average total N content of each inoculated plant and that of the uninoculated control (Bergersen, 1980). In experiment 1 (treatment 2 vs treatment 3) and in experiments 2 and 3, the principle of TND calculation was similar, but a

Table 1. Treatments in experiments 1, 2 and 3

Treatment No.	Application of ¹⁵ N urea (g N plant ⁻¹) ¹	Atom % ¹⁵ N excess in urea	Factors studied			
			Inoc. ²	Soil type ³	Volume of containers (l)	Age at harvest (months)
<i>Experiment 1 (clones S, α, β)</i>						
1	0.232	9.19	0			
2	1.400	1.89	0			
3	0.232	9.19	+			
<i>Experiment 2 (clone β)</i>						
1	1.400	1.89	0	C	15	
2	0.232	9.19	+	C	15	
3	1.400	1.89	0	C	50	
4	0.232	9.19	+	C	50	
5	1.400	1.89	0	B	15	
6	0.232	9.19	+	B	15	
7	1.400	1.89	0	B	50	
8	0.232	9.19	+	B	50	
<i>Experiment 3 (clone β)</i>						
1	5.590	1.89	0			12
2	0.930	9.19	+			12
3	5.590	1.89	0			24
4	0.930	9.19	+			24

¹In all cases, ¹⁵N urea applied at planting. In addition, in experiment 3, treatment 3, 5.590 g of non-labelled urea was applied 12 months after planting.

²0: no inoculation; +: inoculation with *Frankia* strain ORS 021001 (Diem *et al.*, 1983).

³C: Camberene soil; B: Bel Air soil.

Table 2. Experiment 1, comparison of the following parameters in three 12-month-old uninoculated (treatment 1) and inoculated (treatment 3) clones (S, α , β) of *C. equisetifolia*

Clones	Plant biomass (g plant ⁻¹)		Atom % ¹⁵ N excess	NDFS (g plant ⁻¹)	Nodule dry weight (g plant ⁻¹)	Estimates of N ₂ fixation (g plant ⁻¹)		
	Dry weight	Total N				ID	AV	TND
<i>Treatment 1 (uninoculated plants, low rate of urea)</i>								
S	204c	1.51c	0.211a	1.48c				
α	295b	2.18b	0.139a	2.14b				
β	417a	3.31a	0.167a	3.25a				
<i>Treatment 2 (uninoculated plants, high rate of urea)</i>								
S	379c	2.25c	0.285a	1.91c				
α	421b	2.75b	0.156b	2.52b				
β	534a	3.35a	0.222ab	2.95a				
<i>Treatment 3 (inoculated plants, same rate of urea as in treatment 1)</i>								
S	417c	4.54c	0.095a	1.99c	11.5c	2.51ab	2.73b	3.03b
α	534b	5.62b	0.092a	3.69b	17.3b	1.87c	1.54c	3.45b
β	943a	8.51a	0.103a	5.14a	27.5a	3.27a	3.85a	5.20a

Soil used: Camberene; volume of containers in which plants were grown: 15 l.

Within each treatment, data in each column followed by the same letter are not significantly different according to Newman-Keuls' test ($P < 0.05$).

Plant biomass expressed as dry weight or total N, atom % ¹⁵N excess, NDFS, nodule dry weight and N₂ fixed measured by the ID, the AV and the TND methods.

correction factor was applied taking into account the amount of N derived from fertilizer in N₂-fixing, i.e. inoculated (NDFFinoc.) and non-N₂-fixing i.e. uninoculated (NDFFuninoc.) plants, this correction factor being: + (NDFFuninoc. - NDFFinoc.).

Estimation of N derived from soil

For the non-N₂-fixing plant, N derived from soil (NDFS) was estimated as the difference in total N uptake in each plant and N derived from fertilizer. For the N₂-fixing plant, NDFS was the difference between total N uptake and N derived from fertilizer plus N₂ fixed.

RESULTS

No control was contaminated by *Frankia*.

Experiment 1

Plant biomass. The biomass of uninoculated and inoculated clones differed significantly in terms of dry weight or total N (Table 2). For dry weight and total N they ranked $S < \alpha < \beta$. The biomass increases resulting from inoculation were significant ($P < 0.05$); the mean dry weight of uninoculated plants (treatment 1) was 305 compared to 762 g plant⁻¹ in the inoculated ones (treatment 3) with corresponding values for total N being 2.33 and 6.23 g plant⁻¹, respectively.

Nodule weight and N₂ fixation. The ranking of the clones based on nodule biomass expressed in dry weight or total N was similar to that based on plant

dry weight or total N. Clone β was superior in N₂ fixation and, except for the TND estimate, clone S derived more N from N₂ fixation than clone α .

For clones α and β amounts of N₂ fixed were significantly higher by the TND than the isotopic methods (ID and AV) (Table 2).

The mean estimates of N₂ fixed for the three methods were: ID = 2.55, AV = 2.70 and TND = 3.90 g N₂ fixed plant⁻¹. The statistical analysis (not presented here) showed that the ID and AV methods assessed statistically similar N₂ fixed, but that the TND gave significantly higher estimations ($P < 0.05$).

Table 3 shows that the A values of the soil did not differ significantly ($P < 0.05$) with the rate of fertilizer applied. In contrast, the AVs of soil differed significantly with the clones.

N derived from soil (g plant⁻¹). The amount of NDFS differed significantly ($P < 0.05$) between the clones, their ranking based on this trait being similar to that based on the biomass.

Experiment 2

Plant biomass. For each treatment, means are given in Table 4. Interpretation of the data organized factorially is as follows.

—Soil type significantly ($P < 0.01$) affected plant biomass expressed as total N (g plant⁻¹), the means being 8.93 and 8.36 for the Camberene and Bel Air soils, respectively.

—Volume of container affected significantly ($P < 0.01$) plant dry weight and total N. The

Table 3. Experiment 1, A values of soil in treatments 1 (low rate of urea) and 2 (high rate of urea), uninoculated plants

Treatment	Application of ¹⁵ N urea (g N plant ⁻¹)	Clones			Mean (treatment 1 vs 2)
		S	α	β	
1	0.232	11.7	21.3	14.3	15.8a
2	1.400	8.8	16.4	11.1	12.1a
Mean (clones)		10.2B	18.9A	12.7B	

Treatments 1 and 2, whose means are followed by the same small letter, do not differ significantly ($P < 0.05$).

Clones whose means are followed by the same capital letter, do not differ significantly ($P < 0.05$).

Table 4. Experiment 2, effect of inoculation (Inoc.), soil type and volume of containers on the following parameters of 12-month-old uninoculated and inoculated *C. equisetifolia*, clone β

Treat. No.	Factors studied			Plant biomass (g plant ⁻¹)		Atom % ¹⁵ N excess	NDFS (g plant ⁻¹)	Nodule dry weight (g plant ⁻¹)	Estimates of N ₂ fixation ³ (g plant ⁻¹)	
	Inoc. ¹	Soil type ²	Volume ¹	Dry weight	Total N				AV	TND
1	0	C	15	453	3.14	0.214	2.78			
2	+	C	15	953	8.51†	0.097	4.57	18.3	3.85	5.64
3	0	C	50	958	4.86	0.203	4.34			
4	+	C	50	2386	19.20†	0.070	7.66	32.0	11.36	14.67
5	0	B	15	522	3.45	0.191	3.11			
6	+	B	15	882	6.95†	0.105	4.49	15.0	2.38	3.80
7	0	B	50	1020	5.12	0.121	4.79			
8	+	B	50	2250	17.92†	0.062	10.90	25.3	6.90	13.02
—Factor inoculation				**	**	**	**			
—Factor soil type				NS	**	NS	*	NS	*	**
—Factor volume				**	**	*	**	**	**	**
—Interactions										
Inoc. × soil			**	**	NS					
Inoc. × volume				**	**	NS	*			
Soil × volume				NS	NS	NS	NS	NS	NS	NS
Inoc. × soil × volume				NS	NS	NS	NS			

Plant biomass expressed as dry weight or total N, atom % ¹⁵N excess, NDFS, nodule dry weight, N₂ fixed measured by the AV and the TND methods.

Significance level: ***P* < 0.01; **P* < 0.05; NS = not significant.

†Including nodules.

¹0: no inoculation; +: inoculation with *Frankia* strain ORS 021001.

²C: Camberene soil; B: Bel Air Soil.

³Estimates of N₂ fixation using AV and TND methods.

mean dry weights were 702 and 1653 g plant⁻¹ for 15 and 50 l. containers, respectively, with corresponding total N values of 5.51 and 11.77. —Inoculation significantly (*P* < 0.01) affected plant dry weight and total N, the mean dry weights of uninoculated and inoculated plants being 738 and 1617 g respectively. The mean total N contents were 4.14 and 13.14 for uninoculated and inoculated plants, respectively (Table 4).

Nodulation and N₂ fixation. Treatment means are given in Table 4. Interpretation of the data organized factorially is as follows.

- Nodule dry weight (g plant⁻¹) was not affected by soil type, but was significantly (*P* < 0.01) higher in larger than in smaller containers, the mean nodule dry weights being 16.7 and 28.7 g plant⁻¹ in the 15 and 50 l. containers, respectively.
- Atom % ¹⁵N excess was not significantly affected by soil type, but it was significantly affected by volume of containers, the mean atom % ¹⁵N excess values being 0.152 and 0.114 in the 15 and 50 l. containers, respectively.
- Nitrogen fixed using the AV method was significantly (*P* < 0.05) affected by soil type, with means of 7.60 and 4.64 g plant⁻¹ for the Camberene and Bel Air soils respectively. Nitrogen fixed using the AV method was significantly (*P* < 0.01) higher in the larger than in the smaller containers, with means of 3.15 and 9.13 g plant⁻¹ for 15 and 50 l. containers, respectively.
- Soil type significantly (*P* < 0.01) affected N₂ fixed as measured with TND method, TND means being 10.24 and 8.54 g plant⁻¹ for the Camberene and Bel Air soils, respectively. TND was also significantly (*P* < 0.01) higher in the

larger containers than in the smaller ones, with means of 4.82 and 13.96 g plant⁻¹ in 15 and 50 l. containers, respectively.

- Similar to the observation in experiment 1, AV values were significantly (*P* < 0.01) smaller than TND values, the mean values for N₂ fixed (calculated in a factorial design including data from both methods) being 6.12 when using AV and 9.28 g plant⁻¹ when using TND.

N derived from soil. Treatment means are given in Table 4. Interpretation of the data organized factorially is as follows.

- The effect of soil type on N derived from soil (NDFS) was significant (*P* < 0.05), with means of 4.83 and 5.82 g plant⁻¹ for the Camberene and Bel Air soils, respectively.
- The effect of the volume of containers was significant (*P* < 0.01), NDFS means being 3.73 and 6.92 g plant⁻¹ for the 15 and 50 l. containers, respectively.
- The effect of inoculation was significant (*P* < 0.01), the means NDFS being 3.67 and 6.66 g plant⁻¹ for uninoculated and inoculated plants, respectively (Table 4).

Distribution of ¹⁵N in the different plant parts. The atom % ¹⁵N excess differed within the different plant parts with the following ranking, observed both in experiments 1 and 2: photosynthetic branchlets < branches < roots. The ranking based on total N was the opposite: photosynthetic branchlets > branches > roots. The atom % ¹⁵N excess in the nodules was similar (*P* < 0.05) to that of the photosynthetic branchlets (Table 5).

Experiment 3

In treatments 1 and 2 there were 6 replications. In treatments 3 and 4 there were 5 replications because

Table 5. Mean atom % ¹⁵N excess and mean total N content (g plant⁻¹) in the different plant parts of *C. equisetifolia*, experiments 1 (means of clones S, α and β) and 2 (means of treatments 2, 4, 6 and 8)

Plant part	Experiment 1		Experiment 2	
	Atom % ¹⁵ N excess	Total N	Atom % ¹⁵ N excess	Total N
Photosynthetic branchlets	0.046c	2.50a	0.033c	5.83a
Branches	0.089b	1.95b	0.084b	3.54b
Roots	0.197a	1.51c	0.174a	3.51b
Nodules	0.059c	0.26d	0.040c	0.34c

Data in each column followed by the same letter are not significantly different according to Newman-Keuls' test ($P < 0.05$).

one tree in treatment 4 gave a negative value of N₂ fixed and one tree in treatment 3 was eliminated to get a balanced design for treatments 3 and 4. Means of data for the four treatments are given in Table 6.

Biomass of plants. The biomass of 24-month-old plants, as dry weight and total N, was significantly higher ($P < 0.01$) than that of 12-month-old ones, and the effect of inoculation was highly appreciable. Except in treatment 1, the coefficient of variation was low (2–7%) (Table 6).

Nodulation and N₂ fixation. Nodule dry weight and N₂ fixed measured by the TND method increased significantly ($P < 0.01$) during the second year.

Estimates of N₂ fixed based on the either AV or TND method were similar, with a doubling of the amount of N₂ fixed in the second year (Table 6).

DISCUSSION

Methodology

Comparison of the methods of estimation of N₂ fixation. As already indicated, estimations based on ID and AV methods did not differ significantly in any clone.

In experiments 1 and 2 (Tables 2 and 4) estimations of N₂ fixed based on the total N difference method were always significantly higher than those obtained from the isotopic methods. Ruschel *et al.* (1979) reported that soil N uptake was higher in N₂-fixing than in non-nodulated soybeans. Such a phenomenon in our study could have resulted in the higher TND estimates of N₂-fixed than by the isotopic methods,

assuming that the inoculated plants absorbed more N from soil (NDFS) than uninoculated ones. In addition, there were large variations in the ability of the different clones to utilize soil N (NDFS), even in the absence of inoculation (Table 2, treatments 1 and 2), so that the discrepancies between isotopic and TND methods varied with the clones tested.

In experiment 3, in which the plants were grown in large concrete containers (1 m³) (Table 6), there was good agreement between evaluations based on AV and TND methods, probably because the relative contribution of soil N to the plant total N was similar in uninoculated and inoculated treatments, a hypothesis validated by the fact that NDFS of uninoculated and inoculated plants did not differ significantly.

The AV of the soil in experiment 1, treatments 1 and 2, (Table 3) did not differ significantly, which indicates that increasing the amount of fertilizer did not affect the AV, a conclusion in conformity with the AV concept (Fried and Dean, 1982). The fact that AV varied with the three uninoculated clones indicates that this value reflects the variability in the N nutrition pattern of the plants.

Sampling of the different plant fractions. Table 5 (experiments 1 and 2) shows that the atom % ¹⁵N excess, which, by definition, is inversely related to the % of N derived from N₂ fixation, was always much higher in roots than in aerial parts. The atom % ¹⁵N excess of nodules ranged from 0.050 to 0.064 or from 0.031 to 0.057 in experiments 1 or 2, respectively, and close to those of the aerial parts. The mechanisms responsible for the distribution of ¹⁵N in the different plant parts are probably complex. The

Table 6. Experiment 3, effect of inoculation (Inoc.) and plant age on the following characteristics of uninoculated and inoculated *C. equisetifolia*, clone β

Treat. No.	Factors studied		Plant biomass (g plant ⁻¹)		Atom % ¹⁵ N excess	NDFS (g plant ⁻¹)	Nodule dry weight (g plant ⁻¹)	Estimates of N ₂ fixation ² (g plant ⁻¹)	
	Inoc. ¹	Age (months)	Dry weight	Total N				AV	TND
1	0	12	3256 (37)	18.8 (35)	0.201 (70)	17.2 (39)			
2	+	12	5548 (7)	62.3 (7)	0.046 (68)	21.5 (63)	78 (10)	40.4 (43)	45.0 (9)
3	0	24	12070 (7)	76.6 (6)	0.013 (54)	75.3 (4)			
4	+	24	23851 (2)	161.1 (4)	0.005 (114)	76.7 (47)	220 (1)	84.4 (47)	84.8 (7)
—Factor Inoc.		**	**	*	NS				
—Factor age			**	**	**	**	**	*	**
—Interaction									
Inoc. × age		**	**	*	*				

Plant biomass expressed as dry weight and total N, atom % ¹⁵N excess, NDFS, nodule dry weight and N₂ fixed measured by the (AV) and the TND methods. Coefficients of variation (%) between brackets.

Significance level: ** $P < 0.01$; * $P < 0.05$.

Soil used: Camberene soil; volume of containers where plants were grown: 1 m³.

¹0: no inoculation; +: inoculation with *Frankia* strain ORS 021001.

²See Table 1; in treatments 3 and 4 calculations were based on 5 out of 6 replications (see text).

following hypotheses can be proposed to explain this distribution:

- (1) the root may not have direct access to the N as it is fixed, as suggested by Pate *et al.* (1979) with a large amount of N derived from N₂-fixation translocated to the aerial parts of the plant where it may be needed for the rapid growth of the assimilatory organs;
- (2) because the ¹⁵N labelling of the soil declines with time (Witty, 1983), and with the roots formed before most other organs, their tissue should have a higher ¹⁵N content than the rest of the plant.

Similar differences in the atom % ¹⁵N excess in the different parts of plants have been reported by several authors [e.g. Montange *et al.* (1981); Fried *et al.* (1983); Rennie and Rennie (1983)]. Consequently to obtain an accurate estimation of the amount of N₂ fixed it is mandatory to sample and analyse separately the different parts of the plant before deriving a weighted ¹⁵N average for the whole plant.

In addition, the distribution of ¹⁵N in the uninoculated plant declined with age (Table 6), as a result of the decrease of ¹⁵N in the soil (Fried *et al.*, 1983).

Variability of the data. In our experiments, greatest care was taken not only to obtain a well homogenized soil, but also to ensure the use of homogenous plant material, cuttings were used instead of seedlings. The consequence was that, in experiment 3 (Table 6), with the exception of treatment 1, coefficients of variation (CV) related to dry weight, total N and nodule weight were much lower than those observed in a previous experiment on *C. equisetifolia* (Gauthier *et al.*, 1985).

CV of atom % ¹⁵N excess, were high probably because of the variability of this characteristic in the different plant parts. Because of the high variability of atom % ¹⁵N excess, the estimations of N₂ fixation based on AV were less precise (i.e. had a higher CV) than the estimations obtained from the TND method, a result in contradiction with a number of reports quoted by Boddey (1987). To reduce the CV of atom % ¹⁵N excess, one should increase the amount of ¹⁵N added to the soil in the case of fast-growing trees, like *C. equisetifolia*, which absorb large amounts of N. In addition, one should attempt to improve the subsampling technique (which does not seem to be involved in the case of nodules, since the biomass is low) and also to increase the number of replications.

Labelling of the soil for fast-growing trees. For ease of application the labelling of the soil was achieved by incorporating the labelled fertilizer into the soil at planting time, which unavoidably leads to a decline of ¹⁵N enrichment in soil (Witty, 1983). Consequently the decrease in the atom % ¹⁵N excess of plant tissues, such as the one observed in Table 6 (e.g. treatment 1 vs treatment 3) cannot be attributed only to the incorporation of N derived from N₂ fixation. The solution to this problem has already been given by different authors who recommend the incorporation of ¹⁵N labelled organic matter, the addition of various carbon sources to immobilize the N before planting, or the use of slow-release ¹⁵N fertilizer formulations (Witty and Ritz, 1984; Boddey, 1987).

Because of the fast tree growth and high rate of N₂ fixation, labelled ¹⁵N was markedly diluted in the

inoculated plants, resulting in low atom % ¹⁵N excess values, likely to be drastically affected by otherwise small absolute errors. To avoid such problems, it may be necessary to use higher ¹⁵N enrichments or higher amounts of labelled ¹⁵N added to the soil (ca 1/20 of the expected total N in tree at harvest) than normally used in annual grain legumes.

Effect of the volume of containers in which trees were grown. Table 4 (experiment 2) shows that the larger the container, the higher was the plant biomass (dry weight and total N), the amount of N₂ fixed (AV, TND) and the amount of NDFS. This was also true for the % of N₂ fixed (results not presented). It is unlikely that the low biomass and N₂ fixation values in the small containers were largely attributable to differences in nutrient status since the amount of fertilizer that was applied to 15 and 50 l. containers was the same, or to water limitation since the plants were profusely irrigated. Furthermore, within containers of the same volume, inoculation increased not only N₂ fixed, but also NDFS, indicating that there was more N in the soil than taken up in the soil planted with uninoculated trees as an example. It seems that root confinement in small containers, in some unclear fashion, might tend to underestimate the N₂-fixing potential of a tree, i.e. its N₂ fixation in the absence of any limiting factor, and this would have to be considered when experiments have to be carried out in closed systems, and in pots, in the field or greenhouse.

Effect of soil type. Although the total N content of Camberene and Bel Air soils was low, compared to that of most arable soils, the N content of Bel Air soil was still high enough to significantly reduce N₂ fixed by *C. equisetifolia*. This result suggests that *C. equisetifolia*, like most N₂-fixing plants, probably prefer utilizing combined N than fixing their own N₂ from the atmosphere (Allos and Bartholomew, 1955; McAuliffe *et al.*, 1958; Harper, 1974; Hinson, 1975).

Increasing the N₂-fixing potential of C. equisetifolia

The data presented here strengthen the concept that an increase in N₂ fixation can be achieved through two approaches:

- (i) screening for host plants with potential for high N₂ fixation; and
- (ii) inoculating the host with a highly effective N₂-fixing *Frankia* strain.

Clone selection. By comparing the characteristics of the three clones that were tested, differences in ranking were obtained, based on the characteristics examined.

Based on plant biomass, nodule or N derived from soil, the ranking for clones was: $S < \alpha < \beta$. For the amount of N₂ fixed, the ranking was: $\alpha < S < \beta$. This suggests that, at least for clones S and α , plant biomass, or the ability to use soil N are not overriding factors in the ability of these *C. equisetifolia* clones to fix N₂ in association with *Frankia*.

Variations in the ability of legumes to fix N₂ is well known [e.g. Hardarson *et al.* (1984)]. However, with the exception of the recent publication of Phillips *et al.* (1986) on *Cicer arietinum* and *Vigna unguiculata*, few papers have reported variations in their capacities to assimilate N from soil.

Exploiting this double variability is a most promising approach to improve N₂ fixation and growth, especially in the case of trees. The problem of the choice of the criterion to use for plant selection has been addressed in another paper, which concluded that evaluation of nodule biomass of trees grown in the absence of limiting factors is probably a simple and reasonably reliable one (Sougoufara *et al.*, 1989).

Considering only clones α and β , previous studies (Sougoufara *et al.*, 1987) using younger plants (7-month), have already shown the superiority of clone β over clone α . This similarity in the ranking of the clones in the 1987 study and in the present one where plants were 12- and 24-month-old suggests that it is probably possible to screen the host plants for N₂ fixation capabilities in their early stages.

Inoculation with effective strains of Frankia. If clone selection appears to be a promising approach to improve N₂ fixation, one should keep in mind that inoculation with *Frankia* is an absolute requirement in soils devoid of the compatible *Frankia* strains, a situation most often encountered in West Africa. In all three experiments, inoculation with the effective *Frankia* strain ORS 021001 dramatically increased the yield of *C. equisetifolia*, expressed as dry weight and total N.

Acknowledgements—Financial support for the study has been provided by FAO/IAEA division Vienna, the French Ministry of Research through a CIRAD grant, ORSTOM, the International Foundation for Science and Ministère de la Protection de la Nature du Sénégal. We are grateful to Marc Ducouso for performing statistical analyses on the data and Jean Bakhoum for technical assistance.

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ISSN 0038-0717

Soil Biology & Biochemistry



ISSS-AISS-IBG

ISSN 0038-0717

Vol. 22 No. 5

1990

Executive Editor

J. S. WAID



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