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ESTIMATING N_2 FIXATION AND N DERIVED FROM SOIL BY *CASUARINA EQUISETIFOLIA* USING LABELLED ^{15}N FERTILIZER: SOME PROBLEMS AND SOLUTIONS

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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 N_2 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 N_2 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 N_2 per tree during 1 yr. N_2 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 N_2 fixed per tree in Bel Air soil (0.025–0.030% N) during 1 yr. The amount of N_2 fixed was much higher in larger containers. In the case of 1 m^3 containers with Camberene soil, the amount of N_2 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 N_2 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 N_2-fixing trees—woody legumes or actinorhizal plants—as producers of wood, fuel-wood and biomass or as soil improvers. Suspected N_2-fixing trees have however often been introduced into different ecosystems, without first verifying that they fix significant amounts of N_2 (Dommergues, 1987). It is essential to quantify N_2 fixation in different species and provenances before promoting their extensive use. This knowledge would assist in designing better management practices for improving the N_2 fixed in various systems.

Some practical problems are bound to arise in the initial attempts to evaluate the N_2-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 N_2 fixation in *Casuarina equisetifolia* and to compare the effect of the following factors on the plant biomass and N_2 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 N_2 fixation measurements and also to enhance the N_2-fixing potential of *C. equisetifolia*, chosen as a model of fast-growing N_2-fixing trees.

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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 m^3 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 N_2-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

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 N_2 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 ^{15}N 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 N_2 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 N_2 fixation.

In experiments 1 and 2, P and K were added to the soil as 5 g K_2HPO_4 before planting. In experiment 3, P and K were added to the soil as 20 g K_2HPO_4 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 N_2 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 ^{15}N. Isotopic ^{15}N 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 ^{15}N values for the whole plant were calculated by first weighting the atom % ^{15}N 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 N_2 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)
Experiment 1 (clones S, α, β)						
1	0.232	9.19	0			
2	1.400	1.89	0			
3	0.232	9.19	+			
Experiment 2 (clone β)						
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	
Experiment 3 (clone β)						
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

[1]In all cases, ^{15}N urea applied at planting. In addition, in experiment 3, treatment 3, 5.590 g of non-labelled urea was applied 12 months after planting.
[2]0: no inoculation; +: inoculation with *Frankia* strain ORS 021001 (Diem *et al.*, 1983).
[3]C: 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}) Dry weight	Total N	Atom % ^{15}N excess	NDFS (g plant^{-1})	Nodule dry weight (g plant^{-1})	Estimates of N_2 fixation (g plant^{-1}) ID	AV	TND
Treatment 1 (uninoculated plants, low rate of urea)								
S	204c	1.51c	0.211a	1.48c				
α	295b	2.18b	0.139a	2.14b				
β	417a	3.31a	0.167a	3.25a				
Treatment 2 (uninoculated plants, high rate of urea)								
S	379c	2.25c	0.285a	1.91c				
α	421b	2.75b	0.156b	2.52b				
β	534a	3.35a	0.222ab	2.95a				
Treatment 3 (inoculated plants, same rate of urea as in treatment 1)								
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 % ^{15}N excess, NDFS, nodule dry weight and N_2 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 N_2-fixing, i.e. inoculated (NDFFinoc.) and non-N_2-fixing i.e. uninoculated (NDFFuninoc.) plants, this correction factor being: + (NDFFuninoc − NDFFinoc.).

Estimation of N derived from soil

For the non-N_2-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 N_2-fixing plant, NDFS was the difference between total N uptake and N derived from fertilizer plus N_2 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 N_2 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 N_2 fixation and, except for the TND estimate, clone S derived more N from N_2 fixation than clone α.

For clones α and β amounts of N_2 fixed were significantly higher by the TND than the isotopic methods (ID and AV) (Table 2).

The mean estimates of N_2 fixed for the three methods were: ID = 2.55, AV = 2.70 and TND = 3.90 g N_2 fixed plant^{-1}. The statistical analysis (not presented here) showed that the ID and AV methods assessed statistically similar N_2 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 S	α	β	Mean (treatment 1 vs 2)
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: Inoc.[1]	Soil type[2]	Volume[1]	Plant biomass (g plant^{-1}): Dry weight	Total N	Atom % ^{15}N excess	NDFS (g plant^{-1})	Nodule dry weight (g plant^{-1})	Estimates of N_2 fixation[3] (g plant^{-1}): 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 % ^{15}N excess, NDFS, nodule dry weight, N_2 fixed measured by the AV and the TND methods.
Significance level: $^{**}P < 0.01$; $^{*}P < 0.05$; NS = not significant.
†Including nodules.
[1]0: no inoculation; +: inoculation with *Frankia* strain ORS 021001.
[2]C: Camberene soil; B: Bel Air Soil.
[3]Estimates of N_2 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 N_2 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 % ^{15}N excess was not significantly affected by soil type, but it was significantly affected by volume of containers, the mean atom % ^{15}N 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 N_2 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 N_2 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 ^{15}N in the different plant parts. The atom % ^{15}N 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 % ^{15}N 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 % ^{15}N 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)

	Experiment 1		Experiment 2	
Plant part	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 N_2 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 N_2 fixation. Nodule dry weight and N_2 fixed measured by the TND method increased significantly ($P < 0.01$) during the second year.

Estimates of N_2 fixed based on the either AV or TND method were similar, with a doubling of the amount of N_2 fixed in the second year (Table 6).

DISCUSSION

Methodology

Comparison of the methods of estimation of N_2 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 N_2 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 N_2-fixing than in non-nodulated soybeans. Such a phenomenon in our study could have resulted in the higher TND estimates of N_2-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 m^3) (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 % ^{15}N excess, which, by definition, is inversely related to the % of N derived from N_2 fixation, was always much higher in roots than in aerial parts. The atom % ^{15}N 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 ^{15}N 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

	Factors studied		Plant biomass (g plant^{-1})				Nodule dry weight (g plant^{-1})	Estimates of N_2 fixation[2] (g plant^{-1})	
Treat. No.	Inoc.[1]	Age (months)	Dry weight	Total N	Atom % ^{15}N excess	NDFS (g plant^{-1})		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 % ^{15}N excess, NDFS, nodule dry weight and N_2 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 m^3.
[1]0: no inoculation; +: inoculation with *Frankia* strain ORS 021001.
[2]See 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 N_2-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 ^{15}N labelling of the soil declines with time (Witty, 1983), and with the roots formed before most other organs, their tissue should have a higher ^{15}N content than the rest of the plant.

Similar differences in the atom % ^{15}N 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 N_2 fixed it is mandatory to sample and analyse separately the different parts of the plant before deriving a weighted ^{15}N average for the whole plant.

In addition, the distribution of ^{15}N in the uninoculated plant declined with age (Table 6), as a result of the decrease of ^{15}N 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 % ^{15}N excess, were high probably because of the variability of this characteristic in the different plant parts. Because of the high variability of atom % ^{15}N excess, the estimations of N_2 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 % ^{15}N excess, one should increase the amount of ^{15}N 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 ^{15}N enrichment in soil (Witty, 1983). Consequently the decrease in the atom % ^{15}N 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 N_2 fixation. The solution to this problem has already been given by different authors who recommend the incorporation of ^{15}N labelled organic matter, the addition of various carbon sources to immobilize the N before planting, or the use of slow-release ^{15}N fertilizer formulations (Witty and Ritz, 1984; Boddey, 1987).

Because of the fast tree growth and high rate of N_2 fixation, labelled ^{15}N was markedly diluted in the inoculated plants, resulting in low atom % ^{15}N excess values, likely to be drastically affected by otherwise small absolute errors. To avoid such problems, it may be necessary to use higher ^{15}N enrichments or higher amounts of labelled ^{15}N 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 N_2 fixed (AV, TND) and the amount of NDFS. This was also true for the % of N_2 fixed (results not presented). It is unlikely that the low biomass and N_2 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 N_2 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 N_2-fixing potential of a tree, i.e. its N_2 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 N_2 fixed by *C. equisetifolia*. This result suggests that *C. equisetifolia*, like most N_2-fixing plants, probably prefer utilizing combined N than fixing their own N_2 from the atmosphere (Allos and Bartholomew, 1955; McAuliffe *et al.*, 1958; Harper, 1974; Hinson, 1975).

Increasing the N_2-fixing potential of C. equisetifolia

The data presented here strengthen the concept that an increase in N_2 fixation can be achieved through two approaches:

(i) screening for host plants with potential for high N_2 fixation; and
(ii) inoculating the host with a highly effective N_2-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 N_2 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 N_2 in association with *Frankia*.

Variations in the ability of legumes to fix N_2 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 N_2 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 N_2 fixation capabilities in their early stages.

Inoculation with effective strains of Frankia. If clone selection appears to be a promising approach to improve N_2 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.

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