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ASSESSMENT OF N_2 FIXATION BY *CASUARINA EQUISETIFOLIA* INOCULATED WITH *FRANKIA* ORS021001 USING ^{15}N METHODS

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Summary—*Casuarina equisetifolia* seedlings, uninoculated or inoculated with *Frankia* strain ORS021001 were grown for 4.5 months in pouches, then transplanted into 1 m^3 concrete containers forming 1 m^2 microplots. Trees were harvested 6.5 months later when they were 11 months old. N_2 fixation was measured using three methods of assessment: the direct isotopic method, the A-value method and the difference method. Estimations of N_2 fixation during the 6.5 months following transplantation were respectively 3.27, 2.31 and 3.07 g N_2 per tree. From these values it was calculated that about 40–60 kg N_2 would be fixed per hectare in a year at normal densities of 10,000 trees ha^{-1}. The results of this experiment confirm that *Frankia* strain ORS021001 can be confidently recommended to inoculate casuarinas in the field. Means to improve nodulation and subsequently N_2 fixation by casuarinas are discussed.

INTRODUCTION

(Dommergues, 1963). By comparing the soil and tree N content of a planted plot with the soil N content

MATERIALS AND METHODS

The experiment was conducted from July 1982 to May 1983 at the ORSTOM Bel Air Station in Dakar, Senegal, in twelve 1 m^3 concrete containers forming 1 m^2 microplots. The soil used was Bel Air soil, a typical sandy (93% sand), neutral (pH 7.0) soil, with C and N contents of 0.3 and 0.025% respectively (psamment; vernacular name: Dior). The soil was carefully homogenised, introduced into the concrete containers and finally fumigated with methyl bromide.

Seeds of *Casuarina equisetifolia* harvested in the vicinity of Dakar were sown in sterile Bel Air soil. When 1-month-old, the seedlings were planted into 5 × 25 cm polyethylene pouches filled with a mixture of vermiculite and sterile soil (1:5). The seedlings were inoculated by dipping their roots in a suspension of a 2-month-old culture of *Frankia* ORS021001 grown at 28°C in liquid QMOD medium (Lalonde and Calvert, 1979), the amount of *Frankia* inoculum added to each plant being equivalent to 3 μg protein.

Plants were raised in the pouches for 3.5 months and then transplanted into the microplots with four plants per microplot, all of which received PK as K_2HPO_4 at the rate of 17 g m^{-2}.

Treatments

At transplantation time three treatments with four replications each were used as follows:

Treatment 1. No inoculation; application of ^{15}N-labelled NH_4^+-N at the rate of 2 g N m^{-2} (i.e. 0.5 g N $plant^{-1}$) as a solution of $(^{15}NH_4)_2SO_4$ containing 10.5 atom% ^{15}N excess.

Treatment 2. No inoculation; application of ^{15}N-labelled NH_4^+-N at the rate of 10 g N m^{-2} (i.e. 2.5 g N $plant^{-1}$) as a solution of $(^{15}NH_4)_2SO_4$ containing 1.9 atom% ^{15}N excess.

Treatment 3. Inoculation with *Frankia* ORS021001; application of ^{15}N-labelled fertilizer as in treatment 1.

Throughout their growth the plants were carefully irrigated. When the plants were 8 months old, they exhibited symptoms of an undefined nutrient deficiency which was eliminated following application of 11 of Hewitt (1966) N-free nutrient solution to each microplot every 2 weeks. Plants were harvested 6.5 months after transplantation to the microplots when they were 11 months old. Three out of the 16 plants in treatment 1 and three out of 16 plants in treatment 2 were found to bear nodules. These con-

e of inoculation with *Frankia* ORS021001 on nodule weight, height, dry weight and N content of 11-month-old *Casuarina equisetifolia*[1]

	Dry weight (g $tree^{-1}$)				N content (%)			
Height (cm)	Cladodes	Branchlets (dia ⩽4 mm)	Stems and branches (dia >4 mm)	Total	Cladodes	Branchlets (dia ⩽4 mm)	Stems and branches (dia >4 mm)	N total (g $tree^{-1}$)
170 ± 22[a]	151 ± 32[a]	43 ± 13[a]	100 ± 27[a]	295 ± 70[a]	0.86 ± 0.05[a]	0.36 ± 0.02[a]	0.30 ± 0.01[a]	1.77 ± 0.45[a]
192 ± 20[ab]	204 ± 58[ab]	66 ± 18[b]	140 ± 34[ab]	409 ± 105[ab]	1.00 ± 0.09[a]	0.39 ± 0.03[a]	0.29 ± 0.04[a]	2.71 ± 0.75[a]
216 ± 21[b]	260 ± 88[b]	76 ± 20[b]	188 ± 58[b]	525 ± 162[b]	1.30 ± 0.14[b]	0.54 ± 0.05[b]	0.40 ± 0.09[b]	4.78 ± 1.90[b]

0.05).

plication of ^{15}N-labelled fertilizer, 0.5 g N $tree^{-1}$; Treatment 2: uninoculated trees with application of ^{15}N-labelled fertilizer, 2.5 g N $tree^{-1}$; Treatment 3: inoculated fertilizer, 0.5 g N $tree^{-1}$. For each experiment, numbers in column with same letter do not differ significantly, $P = 0.05$ (Duncan, 1955).

emission spectrometry. For each individual tree N and ^{15}N values were calculated taking into account the weight, N and ^{15}N contents of the different fractions of the tree (cladodes, branchlets, stems and branches).

As already indicated in the Introduction N_2 fixation was assessed using three methods: the direct

spectively:

$$fn = \frac{en}{efn} \quad \text{and} \quad fo = \frac{eo}{efo}$$

en and *eo* being the atom% ^{15}N excess in N_2-fixing and non-N_2-fixing plants respectively and *efn* and *efo* the atom% ^{15}N excess in the fertilizer applied on N_2-fixing

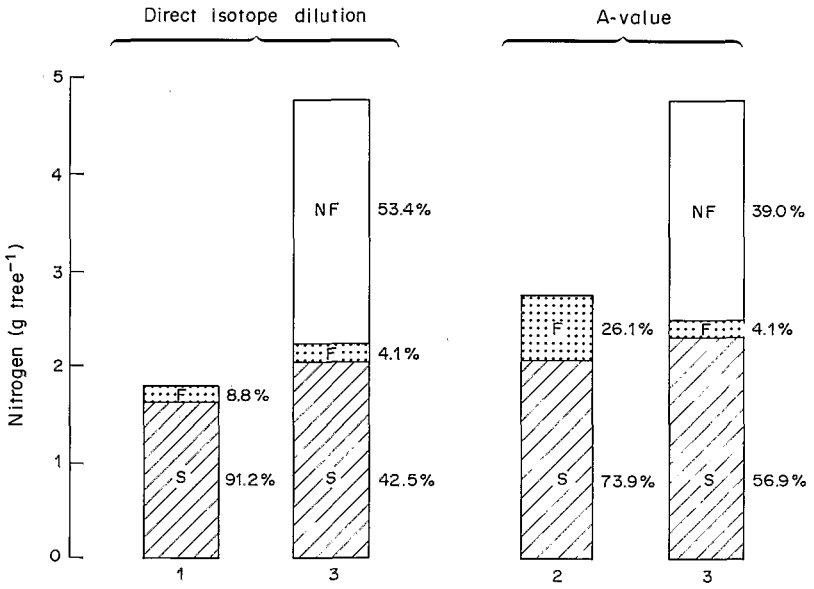

Fig. 1. Contribution of soil (S), fertilizer (F) and N_2 fixation (NF) to the N nutrition of *Casuarina equisetifolia* as assessed using direct isotope (left) and A-value (right) methods. Results expressed as g of N tree^{-1} or as percentage of total N content of the trees. Numbers 1, 2 and 3 refer to the treatments as defined in the text.

Nitrogen uptake from soil and fertilizer

The percentage of plant N derived from soil by N_2-fixing plants was 42.5 or 56.9% according to the method of assessment used (Fig. 1). The percentage of plant N derived from fertilizer was 4.1–8.8% (lower application of fertilizer) and 26.1% (higher application of fertilizer). The contribution of N fertilizer to plant nutrition was small probably because the percentage of utilization of N fertilizer remained low (27.12–31.26%), a situation comparable to that reported for soybean and millet in other Senegalese soils (F. Ganry, unpublished data).

DISCUSSION

N_2 fixation by Casuarina equisetifolia

throughout the experiment (Witty, 1983). In addition it would be desirable (1) to study more extensively the influence of plantation density on N_2 fixation taking into account the fact that in semi-arid conditions many plantations do not contain more than 2000 trees ha^{-1} (Andeke-Lengui and Dommergues, 1983; Ataia, 1983); (2) to follow up the N_2-fixing activity of aging plantations, this activity supposedly increasing up to 5–10 yr of age, then decreasing with the progressive accumulation of N in the forest litter.

Variation in the N_2-fixing potential of individual trees

This variation could not be attributed to the endophyte, since trees were inoculated with a pure strain of *Frankia* and contamination by external strains was unlikely. Nor could the variation be due to soil heterogeneity, because the soil was carefully

application of N fertilizer at a rate of 2.5 g tree^{-1}, considered a high rate by silvicultural standards, induced a much lower, and non-significant, yield increase ($\times 1.53$). Thus inoculation appears to be a more efficient way to improve the growth of casuarinas than N fertilization whenever the soil is devoid of *Frankia*, a situation which is most often encountered in tropical countries where casuarinas have not yet been introduced. In addition, the result of the experiment reported here together with unpublished experiments using non-sterile soils devoid of *Frankia* confirm that strain ORS021001 can be confidently recommended to inoculate casuarinas in the field.

Preliminary work in field-simulating conditions has shown that by applying a heavy inoculum (equivalent to 30 μg *Frankia* proteins per plant) instead of a sparing inoculum (3 μg *Frankia* proteins), nodulation was more regular and the nodule dry weight expressed per 9-month-old tree nearly doubled (27 ± 5 g nodule tree^{-1}). Since we have been able to improve the culture of *Frankia* strains specific to casuarinas, we can now envisage massive inoculation of these trees, thus hopefully inducing a homogeneous and more abundant nodulation, which is the prerequisite to high N_2 fixation.

Improving the nodulating ability of the host-plant

We have already discussed the origin of the large variation in the N_2-fixing capacity of the casuarina tree. This variation can be partly related to the nodulating ability of the host-plant genotype. Attempting to explore this variability would probably be a fruitful approach to increasing N_2 fixation of casuarina stands.

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