

Response of field-grown *Casuarina equisetifolia* to inoculation with *Frankia* strain ORS 021001 entrapped in alginate beads

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Abstract

A large scale field experiment (ca 1 ha) was carried out in Senegal, to evaluate the response of *Casuarina equisetifolia* to inoculation with *Frankia* strain ORS 021001 entrapped in alginate beads. Biomasses (expressed as dry weight or total nitrogen) of assimilatory branchlets, wood and roots, and nodules were measured in uninoculated and inoculated trees, randomly sampled 1, 2 and 3 years after transplantation in the field. When biomasses were expressed as dry weight, increases due to inoculation were similar at the three sampling dates, 45, 36 and 40%, respectively. When biomasses were expressed as total nitrogen, the response to inoculation with time was much higher in the 2nd year than in the 1st and 3rd year. N₂ fixation, estimated using the difference method reached 2.48, 12.25 and 13.44 g N₂ fixed annually per tree. Correspondingly, nodule dry weights, expressed in g per tree, were 2.5, 12.18 and 22.75 at the end of the 1st, 2nd and 3rd year, respectively. In spite of the positive response of field-grown *Casuarina equisetifolia* to inoculation, the decrease of N₂ fixation observed in the third year was probably due to unfavorable climatic conditions coupled with insect attacks at the beginning of the third year.

Introduction

In the last few years, trials carried out under controlled conditions, in greenhouses and on microplots have demonstrated that various species of Casuarinaceae can become more productive if young plants are inoculated with a *Frankia* strain (Gauthier *et al.*, 1985; Reddell and Bowen, 1985).

Until recent years, no field trials were initiated to confirm the success of the trial practices if used under practical forestry conditions. For this reason, experiments were organized in Australia and Zimbabwe by P. Reddell and his group (Reddell *et al.*, 1988) and at the same time by the authors in Senegal.

This report presents the results of a trial, established in 1984, in the interdune zone north of Dakar, Senegal.

Material and methods

Preparation of plants in the greenhouse

Casuarina equisetifolia seeds collected at Mbao, near Dakar, were sown on 16 April 1984 at Bel Air where the soil (0.4% C, 0.026% N) was first sterilized with methyl bromide. On 15 May 1984 the young plants were planted in polyethylene bags filled with the sterilized soil. At the same time half the plants (300 plants) were inoculated with a suspension which was obtained as follows: 15 g of air-dried alginate beads, containing *Frankia*, were rehydrated in phosphate buffer for a few hours and then crushed in 1.5 liters of distilled water.

The air-dried alginate beads were prepared by entrapping hyphae and sporangia of *Frankia* strain ORS 021001 (Diem *et al.*, 1982) in an alginate



Table 1. Characteristics of the soil in the eight plots at Notto, Senegal

	F1	F2	F3	F4	T1	T2	T3	T4
Clay (%)	1.9	2.5	1.7	1.8	1.3	2.1	1.5	2.3
Fine silt (%)	0.5	0.5	1.2	1.3	0.8	1.3	1.0	1.3
Fine sand (%)	33.0	34.8	34.7	27.2	31.4	35.0	28.7	33.4
Nitrogen (%)	0.047	0.071	0.065	0.055	0.057	0.121	0.065	0.057
Avail P ppm	25	13	13	13	13	61	21	36
Total P ppm	170	180	200	170	170	320	160	210
pH	5.7	5.1	5.7	5.2	6.0	5.9	6.0	5.3

F1, F2, F3, F4 = plots planted with inoculated *Casuarina equisetifolia*.

T1, T2, T3, T4 = plots planted with uninoculated *Casuarina equisetifolia*.

solution according to the method used for microbial immobilization (Diem *et al.*, 1988). The beads were then air-dried and stored for *ca* 2 years before being used. While in the greenhouse, the plantlets received 100 ml of Hoagland solution (1/4 th dilution) once a week.

Transplanting to the field

On 23 July 1984, when the greenhouse plants were about 30 cm high, they were transplanted to the permanent plantation site at Notto, 80 km north of Dakar and 5 km from the coast. Soil characteristics are shown in Table 1. The plantation was established after rain had wetted the soil to a depth of 30–40 cm. Throughout the first year of the experiment each plant was given 200 liters of water. The plants were divided over 8 plots, 416 m² each (4 controls and 4 inoculated plots), 84 plants per plot, which amounts to 2.500 trees ha⁻¹. The plots were surrounded by a row of *Eucalyptus camaldulensis* to reduce contamination and later to make an estimate of nitrogen fixation using the natural abundance technique.

To avoid contamination, plots with each of the two treatments were laid out on both sides of an axis that followed the fertility gradient line. The result was that comparisons between plots needed to be calculated in pairs, the F plots were inoculated, the T plots were the controls: couple F1-T1, couple F2-T2, couple F3-T3 and couple F4-T4.

Fertilization and rainfall

In the first year 5 g K₂HPO₄ were applied to each

tree. In the second and the third years, the amounts were increased to 10 g and 15 g respectively.

Annual rainfall: 215 mm in 1985, 375 mm in 1986 and 463 mm in 1987. Except at the first year of planting out, the trees were not given supplemental irrigation.

Sampling

For three consecutive years (July 1985–July 1986 and July 1987), five randomly selected trees were pulled out from specially planted rows in each of the 8 plots. After being measured and weighted, the trees were divided into four parts: assimilatory branchlets, branches, roots and nodules. These parts were dried and then ground to a powder. Total nitrogen was determined on subsamples of the ground powders.

Results and discussion

Emphasis could be placed on the fact that, except for a few trees in control plots T4 and T2, no control plot trees were nodulated, which shows that the soil had no, or at least very few, *Frankia* strains infective for *Casuarina*. During sampling, all nodulated trees found in the control plots were discarded. There were very few: one in 1985, two in 1986 and two in 1987.

Table 2 shows that, except for height, the response of *Casuarina equisetifolia* to inoculation was significant ($P \leq 0.05$) to very significant ($P \leq 0.01$). The beneficial effect of inoculation related to the biomass of the different fractions of the trees, whether it was expressed in terms of dry weight or total N. The shoot/root ratio calculated

Table 2. Effects of inoculation with *Frankia* strain ORS 021001 on the height and the biomass, expressed as dry weight or total nitrogen, of the assimilatory branchlets, branches (including trunks, and roots) and the nodules of *Casuarina equisetifolia* 1, 2 and 3 years after being transplanted in the field (Notto, Senegal)

Inoculation	Height (cm)	Dry weight (g tree ⁻¹)				Total N (g tree ⁻¹)				Shoot/root expressed on	
		bran.	a.b.	roots	nod.	bran.	a.b.	roots	nod.	Weight basis	Total N basis
July 1985 (1yr. after transplantation)											
0	151	163	89	266	0	0.63	1.46	1.24	0	0.95	1.69
+	182	324	119	309	2.55	1.99	2.17	1.60	0.04	1.42	2.54
	*	**	**	*		**	**	*		*	**
July 1986 (2yrs after transplantation)											
0	291	890	628	507	0	5.64	9.03	2.52	0	2.99	5.82
+	313	1349	871	626	12.18	9.39	16.24	3.21	0.20	3.48	7.52
	NS	**	**	**		**	**	**		*	*
July 1987 (3yrs after transplantation)											
0	500	1451	1000	840	0	7.32	15.57	4.74	0	2.91	4.85
+	575	2220	1356	1031	22.75	12.20	23.35	5.12	0.40	3.39	6.47
	NS	**	**	**		**	**	NS		**	*

Significance level: * ($P \leq 0.05$); ** ($P \leq 0.01$); NS = not significant.

a.b. = assimilatory branchlets.

bran. = branches and trunks.

nod. = nodules.

0 = uninoculated plants. + = inoculated plants.

on dry weight or total N basis was favorably affected at the three sampling times.

Assuming that N_2 fixation can be measured by the difference between the total N content of inoculated trees (that fix N_2) and the total N content of uninoculated trees (that do not), N_2 fixed by the end of the first year (July 1985) can be estimated at an average $5.80 - 3.32 = 2.48$ g N_2 per tree. By the end of the second year (July 1986) the average N_2 fixed per tree was $29.44 - 17.19 = 12.25$ g N_2 . By the end of the third year (July 1987) the average N_2 fixed was $41.07 - 27.63 = 13.44$ g N_2 .

Annual N_2 fixed rose from a low 2.48 g per tree in the first year, by 9.38 g in the second year and decreased sharply to 1.19 g in the third year. Corre-

lation matrices obtained at each sampling time showed high coefficients of correlation (*ca* 0.90) between nodules and tree biomass and also between percentage of N in assimilatory branchlets and tree biomass. By contrast, the coefficient of correlation between percentage of N in branches and biomass was much lower (Table 3).

Conclusion

The present paper reports for the first time the successful use of entrapped *Frankia* in alginate beads as an inoculant for field grown *Casuarina* and that *Frankia* beads seem to be still effective

Table 3. Correlations between nodule biomass expressed in terms of dry weight (NODW) or total N (NODN), and percentage of N in the assimilatory branchlets (E) or branches (F) expressed in terms of arc sin on the one hand and biomass of aerial parts of the trees (assimilatory branchlets + branches) expressed in terms of dry weight (DW) or total N (TOTN) on the other hand

Year	Coefficient of correlation							
	NODW/DW	NODW/TOTN	NODN/DW	NODN/TOTN	E/DW	E/TOTN	F/DW	F/TOTN
1	0.94	0.95	0.93	0.95	0.85	0.85	0.03	0.97
2	0.93	0.90	0.92	0.89	0.86	0.92	0.67	0.72
3	0.92	0.91	0.92	0.91	0.92	0.93	0.27	0.37

after a two year storage at room temperature. Instead of using an inoculant made of a *Frankia* culture merely entrapped in alginate beads, a procedure followed in the experiment reported here, Diem *et al.* (1985) (quoted by Diem *et al.*, 1988) and Burleigh *et al.* (1988) proposed to improve this type of inoculant by allowing *Frankia* to grow within the alginate beads, a procedure which also appeared to be most successful.

Inoculating *Casuarina equisetifolia* with *Frankia* significantly improved tree biomass, expressed as dry weight for total N, and the shoot/root ratio, expressed as dry weight or total N.

The positive response of *Casuarina equisetifolia* to inoculation at Notto can be explained by the fact that the soil, like most soils in Western Africa, is devoid of *Frankia* or contains a very low number of native *Frankia*. The nodulated trees found in the control (uninoculated) were probably infected through contamination in the nursery. However, the hypothesis that *Frankia* preexisted in the soil cannot be entirely rejected. Further studies are planned to verify whether the nodulated trees in the control plot were infected by unknown native strains or by ORS 021001.

Height was not significantly increased by inoculation, a result showing that, when dealing with trees, the use of height as the sole criterion for assessing the effect of inoculation may not be sufficient.

The response to inoculation in the Notto trial was less spectacular than that reported in the experiment carried out recently in Zimbabwe by Reddell *et al.* (1988) which is the only other field trial reported, to date, using liquid cultures of *Frankia* as inoculant for Casuarinaceae.

The reason for this dissimilarity is probably because at Notto drought limited N₂ fixation and plant growth, whereas in Zimbabwe the environmental conditions were much more favorable.

N₂ fixation assessed by the difference method, a method which we found to probably overestimate the actual N₂ fixation in soils similar to that of Notto (Sougoufara *et al.*, 1989b) was much higher in the second year than in the first and third years. These differences can be attributed either to soil chemical status (the presence of combined N possibly inhibiting N₂ fixation during the first year and nutrient deficiencies being possibly involved in the third year) and/or insect attacks from borers (co-

leopters of bostrychidae). In absolute value, the amount of N₂ fixed was in the range of that observed in field-simulating conditions, that is 3 g per tree in 6.6 months (Gauthier *et al.*, 1985) with *Casuarina equisetifolia* of the same origin. However it was much lower than that obtained with a selected clone of *Casuarina equisetifolia* which fixed as much as 40–70 g of N₂ in 2 years (Sougoufara *et al.*, 1989b).

As was observed in other experiments, carried out in containers (Sougoufara *et al.*, 1989a), inoculation with *Frankia* significantly increased the shoot/root ratio, the relative increases for ratios calculated from total N being 50, 29 and 33% at the end of 1st, 2nd and 3rd year respectively.

Correlations between the different parameters were examined in order to find out whether some of them could be used to predict the effect of inoculation on the relative increase of tree biomass expressed as dry weight or total N. From Table 3 it is clear that there was a significant positive correlation between tree biomass and nodule biomass, a correlation that we have already observed in all other trials, with *Casuarina equisetifolia* (Sougoufara *et al.*, 1989a). As suggested by recent trials, nodulation is not necessarily correlated with N₂ fixation per se. This absence of correlation is illustrated here by the fact that N₂ fixation sharply decrease during the 3rd year in spite of the doubling of the nodule weight during the same year.

The fact that tree biomass was correlated with the N% in the assimilatory branchlets—and not that of the branches—is of interest because a non destructive N analysis of the assimilatory branchlets might be used as an indicator of the response of trees to inoculation.

From this experiment, which concerned ca 0.5 ha (surface of inoculated plots), and Reddell's experiment in Zimbabwe, it can be inferred that the technology of inoculation with cultures of *Frankia* or *Frankia* entrapped in alginate beads is realistic and feasible on a large scale in the tropics.

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