INCO-DC: International Cooperation With Developing Countries (1998 – 2002)

Contract number: ICA4-CT-2002-10017

Second Annual Report: 1/12/2003 – 30/11/2004

Title: Utilisation of wastewater for fuel and fodder production and environmental and social benefits in semi-arid, peri-urban zones of sub-Saharan Africa.

Project homepage: www.bioman.ceh.ac.uk/ubenefit.htm

Key words: peri-urban, wastewater recycling, irrigation, fodder, fuelwood, microsymbionts

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INCO-DC:International cooperation with Developing Countries (1998-2002)

Utilisation of wastewater for fuel and fodder production and environmental and social benefits in semi-arid, peri-urban zones of sub-Saharan Africa.

Second Annual Report: 1/12/2003 – 30/11/2004

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Compiled by J. Wilson

Summary report

A major constraint to progress on this project has been the delays in installation of fully-functioning water treatment and irrigation facilities in some countries. These delays arise from several causes:

- Difficulties in obtaining the necessary agreements for the construction of the facilities (partner 4)
- Difficulties in obtaining the necessary materials within budget and cashflow problems arising from the heavy startup costs for such facilities and the difficulties of managing this within the EU framework of disbursement of funds (partner 2, 3, 4, 5)

These delays inevitably create delays to other work packages and have affected the efficiency of conducting the whole study. A concomitant of this is that now that treatment plants are at different stages of development in the different countries, it is difficult for the SCP subcontractor to make the necessary site visits within their budget. These difficulties are being overcome, enabling field studies to commence.

Work package 1 Water treatment and irrigation

SCP subcontractor has provided comprehensive advice to all partners constructing treatment plants and made modifications to designs to meet local requirements as necessary. In Niger, construction of the treatment plant is completed and the irrigation system is being constructed. In Mali, the installation of the irrigation system, and wastewater evaluation is ongoing. Delays are greatest in Burkina Faso where difficulties have been experienced in obtaining permission for construction etc. Nonetheless, there has been progress and most of the technical difficulties have been surmounted, but the cash flow difficulties continue to create problems. Soil and water analyses have been conducted. In Mali, detailed soil descriptions have been produced of the irrigation site

Work package 2 Tree growth and management

Selection of tree species for the experimental trials was coordinated between partners at the first annual meeting and experimental protocols for the design of trials were produced. Progress was made in nursery screening trials and plants are in production for outplanting. Studies are interlinked with those of work package 4.

Work package 3 Tree water use and soil water status

Equipment for measuring tree water use and soil water status was shipped to Niger and was installed during a 2 week intensive training period in May 2004. Unfortunately, a key item of equipment was damaged during the final stages of the training period and had to be returned to the US for repair, creating a delay of several months. This delayed the measurement of tree water use until late 2004 and data will be provided in the next report.

Work package 4 Microsymbionts and N fixation

Working in controlled glasshouse conditions, Partner 1 has conducted the experiment delayed due to illness last year. The results highlight the importance of good

mycorrhizal infection for tree growth, the difference in effectiveness of isolates and the presence of tree species x isolate interactions. In Mali, studies in non-sterilised soils highlighted the role of inoculation in increasing the amount of mycorrhizal infection on nursery plants. In Burkina Faso, results demonstrated variations in response of tree species to different treatments. In Niger, nursery experiments with waste water and symbionts, highlighted the beneficial effects of waste water on plant growth. Inoculants were also often effective, but to a lesser extent overall. However, preliminary molecular studies by Partner 6 indicate that inoculant rhizobia had been replaced by wild types. This emphasises the difficulties of running and interpreting experiments conducted in nursery conditions.
Partner 6 has refined molecular methods, and tested different DNA extraction procedures. A procedure using guanidine thiocyanate was found to be both a safe and highly effective extraction procedure. Sequencing of selected rhizobia strains, to enable the development of specific probes is in progress.

Work package 5 Economics and quality of produce

Led by Niger, partners have collaborated in the refinement of the questionnaires. Three questionnaires have been produced, targeted at wood suppliers, commercial wood-cutters and domestic wood-cutters. A review of fuelwood production in Niger has been produced.

Work package 6 Soil and plant nutrition

Nutrient contents of irrigation water and soil nutrient status at the irrigation sites are being determined. The main work in this package cannot commence until the irrigation trials have been established.

Work package 7 Planting stock quality

This work package is focussed on determining the ideal morphology of planting stock destined for irrigated sites. Partner 1 made a presentation about attributes of planting stock quality at the first annual meeting. Activities on this package will commence once the irrigation sites are up and running.

Work package 8 Pest monitoring and management

Personnel have been allocated to this task and preliminary studies conducted. The main activities will commence when the irrigation trials are underway.

Scientific annual report

Work package 1 Water treatment and irrigation

SCP subcontractor has provided comprehensive advice to all partners constructing treatment plants and made modifications to designs to meet local requirements as necessary. In Niger, construction of the treatment plant is completed and the irrigation system is being constructed. In Mali, the installation of the irrigation system, and wastewater evaluation is ongoing. Delays are greatest in Burkina Faso where difficulties have been experienced in obtaining permission for construction etc. Nonetheless, there has been progress and most of the technical difficulties have been surmounted, but the cash flow difficulties continue to create problems. Soil and water analyses have been conducted and plant material is being prepared for the field studies.

A detailed description of the Minimana irrigation site in Mali has been prepared. The soils are loamy-sand on the surface horizons and increasingly clayey at depth with a presence of ferruginous concretions. For these reasons these soils are classified as "ferrugineux tropicaux lessivés" (CPCS, 1967) or ultisols (Soil taxonomy, 1998). Although there is variation in the soil profile, the C content is low in all profiles, including the surface layers.. Beyond 100 cm depth; the C content is much less than that of the surface horizons. Similarly, low amounts of N were recorded in all three profiles where it is lower than 0.8 g kg^{-1} The distribution of N in the profiles is similar to that of C. The C/N ratios are low and present very few variation in the first two horizons. They vary from 2.90 to 6.22 in the deepest horizons. The total P content varies between 200 and 260 mg kg^{-1}. The deepest horizons are less rich in phosphorus than those on the surface. This difference varies approximately from 3% to 15%. The low pH values of the profiles indicate an acid soil in all the horizons. The pHs of the major horizons are higher than those of the horizons which precede them. For the pH (H2O), this difference varies from 0.3 to 0.9 unit pH, which is considerable. The pH (H2O) is not much higher than the pH KCl, which is logical because there is very little exchangeable aluminium in these soils.The microbial biomass is low; it varies from 89.07 to 121.27 mg kg^{-1} and it is higher in the horizons of surface than in those of depths.

The amounts of carbon and nitrogen are low as is typical in the highly weathered soils from tropical regions of Africa. This situation is in relation with the high rate of mineralisation of the organic matter especially at high temperatures and in rainy season when the soil moisture is sufficient (Scholes and Al, 1994; Pieri, 1989). This is confirmed by the low C/N ratio indicating mineralized soils with low organic matter along the profile. This remark is comforted by the low microbial biomass (an average of about 100 mg kg^{-1} both in surfaces and deeper horizons). In the deepest layers, the organic matter probably results from various iron-orgnanic matter complexes which are then drained by water through the pores create the activities of the soil fauna (termites and worms) and other interstices of the soil.

Phosphorus contents are low, but could be corrected by fertilizer addition. In these circumstances, mycorrhizal inoculation, which enhances uptake of immobile nutrients such as P, will be especially important.

Work package 2 Tree growth and management

Selection of tree species for the experimental trials was coordinated between partners at the first annual meeting and experimental protocols for the design of trials were produced. Progress was made in nursery screening trials and plants are in production for outplanting. Studies are interlinked with those of work package 4.

In Mali, 4 months after planting, *Leucaena leucocephala* was the tallest, followed by *Gliricidia sepium* and *Acacia seyal.* In terms of diameter, *Gliricidia sepium* was the best species followed by *Leucaena leucocephala* and *Acacia seyal.* At a later evaluation, *A. angustissima* has also performed well.

In Burkina Faso, *Gliricidia sepium*, *Leucaena spp.* and *Afzelia africana* displayed superior height growth compared with the other species under test. However, *G. sepium* and *A. africana* appeared to be the most vigorous and leafy at this stage. In general, acacias and *Pterocarpus* sp. had the slowest growth and it was suggested that these species might benefit from more management care (inoculation for example) to accelerate their juvenile growth.

In Niger, the use of waste water, frequently improved plant growth, although it was noted that it often reduced the extent of nodulation on root systems.

Work package 3 Tree water use and soil water status

Equipment for measuring tree water use and soil water status was shipped to Niger and was installed during a 2 week intensive training period in May 2004. Unfortunately, a key item of equipment was damaged during the final stages of the training period and had to be returned to the US for repair, creating a delay of several months. This delayed the measurement of tree water use until late 2004 and data will be provided in the next report.

Work package 4 Microsymbionts and N fixation

Working in controlled glasshouse conditions, results of Partner 1 highlight the importance of good mycorrhizal infection for tree growth, the difference in effectiveness of isolates and the presence of tree species x isolate interactions. The most effective inoculants increased tree growth by 2 – 3 fold over the period of the study. In Mali, studies in non-sterilised soils highlighted the role of inoculation in increasing the amount of mycorrhizal infection on nursery plants. In Burkina Faso, four moths after inoculation, significant differences were revealed for all the parameters. On average, acacias performed poorly with similar growth whereas *Gliricidia* showed the best performance. Applying either Rhizobium or endomycorrhizas improved plant growth while double inoculated plants performed poorly ($p<.001$). Some species like *Gliricidia*, *Leucaena diversifolia* and *L.* hybrid, responded better to mycorrhizal inoculation alone or to inoculation with Rhizobium alone. Growth of *L. leucocephala* and acacias was better without inoculation. However the response to the double inoculation is more important than that of the single inoculation with either Rhizobium or mycorrhizas. Inoculants had not been pre-tested and differences in response may indicate incompatibilities or ineffectiveness.

In Niger, nursery experiments with wastewater and symbionts, highlighted the beneficial effects of waste water on plant growth. Inoculants were also often effective, but to a lesser extent overall than use of waste water. However, preliminary molecular studies by Partner 6 indicate that inoculant rhizobia had been replaced by wild types. This emphasises the difficulties of running and interpreting experiments conducted in nursery conditions.

Partner 6 has refined molecular methods, and tested different DNA extraction procedures. A procedure using guanidine thiocyanate was found to be both a safe and highly effective extraction procedure. Sequencing of selected rhizobia strains, to enable the development of specific probes is in progress.

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Work package 8 Pest monitoring and management

Personnel have been allocated to this task and preliminary studies conducted. The main activities will commence when the irrigation trials are underway.

Management annual report

Organisation of the collaboration
Management of the collaboration is through annual coordination meetings and also by regular email communication, with all participants and especially with work package leaders. A major change in this year is that Dr Julia Wilson of CEH replaced Mr J Douglas Deans as project coordinator due to Mr Deans' retiral. To facilitate the handover, Dr Wilson accompanied Mr Deans to the annual meeting in 2004 and also participated in the field training which was conducted in Niger. She has already collaborated with IRD Senegal on other projects and will visit INERA in Burkina Faso in 2005 for the first time.

All members were represented at the coordination meeting in Mali in 2004 (Figure 1). The field site at Minimana was visited and all workpackages were extensively discussed. The harmonisation of approaches by different partners was a major source of discussion. CEH has provided additional support in terms of advising on suitable experimental designs for field trials.

Figure 1**Group photo of the second meeting participants in the second annual meeting visiting the experimental site of Minimana**

Exchanges

During this second year, Ousmane Sacko (Université of Bamako, Mali, Partner 3) and Alzouma Mayaki Zoubeirou (Université Abdou Moumouni, Niger, Partner 5) spent respectively eight and six months in laboratory of Partner 6 in Dakar.

Problems
As will be seen from the partners' reports, the setting up of the water treatment plants has been the main factor delaying progress. In some countries there have been difficulties in obtaining the necessary permissions, especially when the people who gave provisional permission during the project preparation phase were no longer in post

when the project was approved by the EU. All partners have experienced cash flow difficulties in purchasing expensive components, which often needed to be imported. Once each partner has received their advance payment at the commencement of the contract, it has not been easy for partners to predict the amount or timing of subsequent payments, and it would be helpful if the formula used in calculating this could be provided by the EU together with a clearer timeframe. In order to help one partner, and prevent further delays to progress, CEH has advanced 10000 euro in anticipation of further receipts from the EU.

Most, but not all, bank transfers have proceeded more smoothly than last year, though partners have been seriously hampered by late payments, but problems remain with the mechanics of some transfers, especially to Mali. All partners, except Partners 1 and 6, report that work has been delayed due to financial problems.

Overall, the difficulties of buying capital items, often requiring import, have been exacerbated by uncertain cash flow from Brussels

Partner 1 Centre for Ecology and Hydrology, UK
CENTRE FOR ECOLOGY AND HYDROLOGY
(NATURAL ENVIRONMENT RESEARCH COUNCIL)

K Ingleby, RC Munro, AL Decorde and J Wilson

Mr JD Deans retired in October 2004 and Dr Julia Wilson took over from him as project coordinator.

1. Summary of Progress

- A screening experiment has been conducted in the glasshouse. Isolates of 7 AM fungi were grown with 4 different tree species.
- Mycorrhizal inoculation improved the growth of all 4 tree species; *Senna siamea*, *Leucaena leucocephala* and *Khaya senegalensis* showed a greater response to inoculation than *Gliricidia sepium*. Levels of mycorrhizal infection were lower for *Leucaena leucocephala* than the other 3 species.
- All 4 tree species grew best when inoculated with the AM isolates *Glomus mosseae* and/or *Glomus fasciculatum*; these 2 isolates also formed the highest levels of mycorrhizal infection.
- Growth of all 4 tree species was poorest for the uninoculated trees and those inoculated with AM isolate *Gigaspora albida* 1b, which formed very few mycorrhizas.
- Mycorrhizal infection was positively correlated with all parameters of tree growth for all 4 tree species.
- The irrigation experiment has been set up and irrigation treatments are being applied.
- AM cultures are being maintained in the glasshouse.
- Chemical analysis of wastewaters was conducted for partners
- Experimental design protocols were produced for field trials
- Training was provided in the installation and use of sapflow equipment in Niger

2. Activities

2.1 Workpackage 1 Water treatment and irrigation

The experimental sites in Niger and Mali were visited with local partners and progress and problems were discussed. Issues in connection with the site in Burkina Faso have been discussed with the local partner. Chemical analysis of wastewaters was conducted for partners (Table 1).

Table 1 Results of wastewater analysis

	SampDesc	Be ug/l	Al(27) ug/l	V ug/l	Cr ug/l	Mn ug/l	Co ug/l	Ni(60) ug/l	Cu ug/l	Zn ug/l	As ug/l	Se ug/l	Cd ug/l
Burkina Faso	eb43503,PX	<0.060	537	1.96	7.72	77.7	1.08	42.1	2100	2290	0.874	<0.600	0.97
Burkina Faso	eb43504,P138	<0.060	502	2.29	6.83	75.6	1.06	46	2390	2610	0.984	0.876	0.736
Burkina Faso	eb43505,P14	0.99	24800	70.2	63.6	369	11.4	47.8	2040	2220	2.08	1.21	0.728
Burkina Faso	eb43506,Fin Campus	0.172	4080	19.4	13.7	322	5.01	51.9	2480	2990	1.97	<0.600	0.906
Mali	eb43501,eau filtre - 1	0.239	3570	6.42	6.85	17.2	1.37	8.89	32.2	127	0.594	0.066	4.36
Mali	eb43502,eau filtre - 2	0.204	1830	5.22	4.39	16.8	1.14	7.97	33	133	0.579	0.122	4.4
Mali	eb43507,Minimana (drain)	0.376	6800	16.3	13.8	104	2.43	8.43	15.6	99	1.28	<0.600	0.41
Mali	eb43508,Minimana (non-filtered)	0.162	2000	6.3	3.15	25.7	0.62	3.04	47	62.3	0.71	<0.600	0.148
Niger	eb43509, crude - 30/4 - 14h	<0.060	382	1.67	<0.800	74.6	0.43	4.29	10.7	65.2	0.63	<0.600	0.116
Niger	eb43510, tap - 30/4 - 14h	0.043	235	0.377	0.154	37.2	0.178	1.05	1.2	35.5	0.301	0.054	0.048
Niger	eb43511, purified - 30/4 - 14h	<0.060	97.5	0.984	<0.800	90.3	0.84	20.2	2.34	25.1	0.786	<0.600	<0.040
Niger	eb43512, crude - 1/5 - 16h	<0.060	129	1.15	<0.800	79.7	0.268	3.44	8.65	92.2	0.528	<0.600	0.076
Niger	eb43513, tap - 1/5 - 16h	<0.003	11.3	0.426	0.146	6.68	0.027	0.782	0.822	9.12	0.306	<0.030	0.092
Niger	eb43514, purified - 1/5 - 16h	<0.060	449	2.58	1.4	60	1.18	3.06	4.16	42.8	0.844	<0.600	<0.040
Niger	eb43515, crude - 2/5 - 16h	<0.060	218	1.45	<0.800	81.7	0.328	3.44	7.71	33.5	0.51	<0.600	0.072
Niger	eb43516, tap - 2/5 - 16h	0.031	300	0.363	0.256	18.5	0.092	1.12	0.83	9.45	0.286	0.112	0.017
Niger	eb43517, purified - 2/5 - 16h	<0.060	101	1.15	<0.800	351	1.23	2.14	3.64	21.3	0.792	<0.600	0.04
Niger	eb43518, crude - 3/5 - 16h	<0.060	534	3.19	2.13	66.8	0.958	5.11	22	82.2	0.778	1.05	0.146
Niger	eb43519, tap - 3/5 - 16h	<0.003	76.5	0.469	0.234	12.9	0.073	0.767	1.12	6.58	0.287	0.106	0.007
Niger	eb43520, purified - 3/5 - 16h	<0.060	30	0.61	<0.800	9.65	0.434	1.73	3.9	<20.0	0.622	<0.600	<0.040

2.2 Workpackage 2 Tree growth and management

CEH has participated in the selection of species for the different trials and has produced protocols for experimental designs (Annex 1).

2.3 Workpackage 3 Tree water use and soil water status

All necessary equipment was shipped to Niger and two weeks' hands-on training was provided to partner 5 in the operation of equipment for measuring sapflow and soil water.

Work package 4 Microsymbionts and N-fixation

2. 4 Glasshouse screening experiment

The overall objective was to test AM inoculants for effectiveness with 4 tree species (2 N-fixing species and 2 non N-fixing species. The experiment is in two phases: in phase 1, growth of tree species with different mycorrhizal inoculants is tested (with rhizobium when appropriate); in phase 2, the growth of these tree/AM fungal combinations is being tested in the presence/absence of wastewater irrigation.

2. 4. 1. Treatments (32)

Trees (4)

Gliricidia sepium Dakar 6/03, ILG50 (ex. Mali)
Leucaena leucocephala Dakar 6/03 (T2C Odonto)
Senna siamea CNSF 1154 (Bobo Prov.)
Khaya senegalensis CNSF 1156 (Mondon Prov.)

AM inoculants (8)

Glomus aggregatum IR14
Glomus aggregatum ISRA
Glomus fasciculatum ISRA
Glomus mosseae ISRA
Glomus etunicatum 1 (BEG 176)
Gigaspora albida 1b (BEG 172)
Gigaspora albida 2 (BEG 173)
Control

Replication (10)

2. 4. 2. Inoculation (Figure 2)

Seeds of all 4 tree species were chipped and pre-germinated in Petri dishes. Seedlings were transferred to 150cc pots containing sterilised loam/grit-sand/coir (mixed 1:1:1) and 20g of the appropriate inoculum. Control pots contained 20g of autoclaved inoculum. Pots were arranged in 32 trays (1 inoculant/tree species per tray) with tray positions on the bench rotated each week. After 1 week, seedlings of *Leucaena* and *Gliricidia* (the two nodulating legumes in this study) were inoculated with 2 ml of appropriate Rhizobium culture (isolates LdK4 and GlirY3616 respectively).

Figure 2 Trays of germinated tree species

2. 4. 3. Experiment set up (Figure 3)

After 4 weeks, 10 seedlings of each treatment (4 trees x 8 fungi) were transferred to 1500cc pots containing sterilised loam/grit-sand mixed 1:2. The 4 tree species were arranged in a split plot design with fungal treatments randomised within each plot. Measurements of stem diameter were made every 2 weeks.

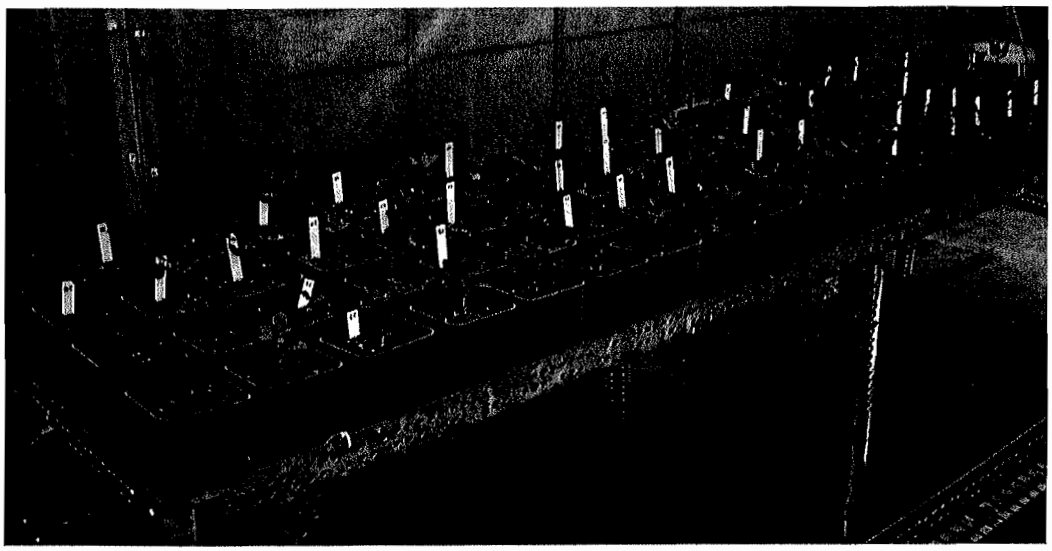


Figure 3 After potting on, seedlings were arranged in a split-plot design in the glasshouse

2. 4. 4. Harvest and assessment

After 24 weeks, shoots of those plants not selected for inclusion in the Phase 2 irrigation experiment were removed for assessments of shoot biomass. Two soil cores (20cc volume) were removed from each pot for assessment of mycorrhizal infection on the roots. Roots were extracted from the cores, stained in Trypan Blue and assessed using the gridline intersect method. During the staining process however, it was apparent that an alternative method of assessment was needed for roots of *Senna siamea*. Even after prolonged treatment in KOH and bleaching solution, epidermal cells of *S. siamea* remained black and internal mycorrhizal structures could only be observed by squashing roots under a cover slip and examining under a high power microscope (Figure 4). In order to assess infection of *S. siamea* roots, the roots were cut into 1mm fragments and 20 fragments randomly removed from each sample. These were squashed open and assessed either as mycorrhizal or non-mycorrhizal. As the gridline intersect method was therefore not appropriate for the assessment of infection in *S. siamea*, levels of infection cannot be directly compared with those found for the other 3 tree species, and, because total length of roots in the soil cores was not measured, root concentrations could not be calculated for this species.

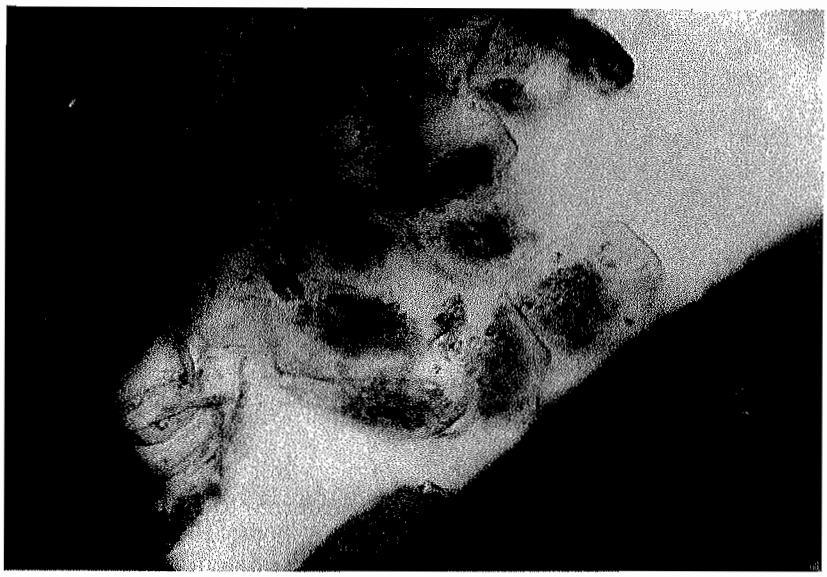


Figure 4 Mycorrhizal structures in roots of *Senna siamea*, showing dark colouration of epidermal cells

2. 4. 5. Results

The harvest at 24 weeks showed that mycorrhizal inoculation significantly improved growth of all 4 tree species. Growth benefits were most pronounced for *Leucaena leucocephala* (Figure 5, Table 2), *Khaya senegalensis* (Figure 6, Table 3) and *Senna siamea* (Figure 7, Table 4) and least pronounced for *Gliricidia sepium* (Figure 8, Table 5), indicating that this species was least responsive to mycorrhizal inoculation. Overall levels of mycorrhizal infection were high in *S. siamea* (65.7%), *K. senegalensis* (61.2%) and *G. sepium* 61.2%) but significantly lower in *L. leucocephala* (51.2%) indicating this species was less mycotrophic.

Generally, all 4 tree species grew best when inoculated with the *Glomus mosseae* and *Glomus fasciculatum* isolates, and these 2 isolates also formed the highest levels of infection (79.6 and 76.9% respectively). However, several significant tree species x AM isolate interactions were seen. Growth of *L. leucocephala* and *K. senegalensis* was best when inoculated with the *G. mosseae* isolate, whereas growth of *S. siamea* and *G. sepium* was best when inoculated with the *G. fasciculatum* isolate. Both *Gigaspora albida* 2 and *Glomus aggregatum* ISRA isolates were most effective and formed high levels of mycorrhizal infection with *S. siamea*, and least effective with *L. leucocephala*.

Growth of all 4 tree species was poorest for the uninoculated trees and those inoculated with the *Gigaspora albida* 1b isolate. After 24 weeks, uninoculated trees remained non-mycorrhizal while those inoculated with isolate *Gigaspora albida* 1b had formed very few mycorrhizas (4.4%). It was no surprise therefore that mycorrhizal infection was positively correlated with all parameters of tree growth for all 4 tree species (Table 6).

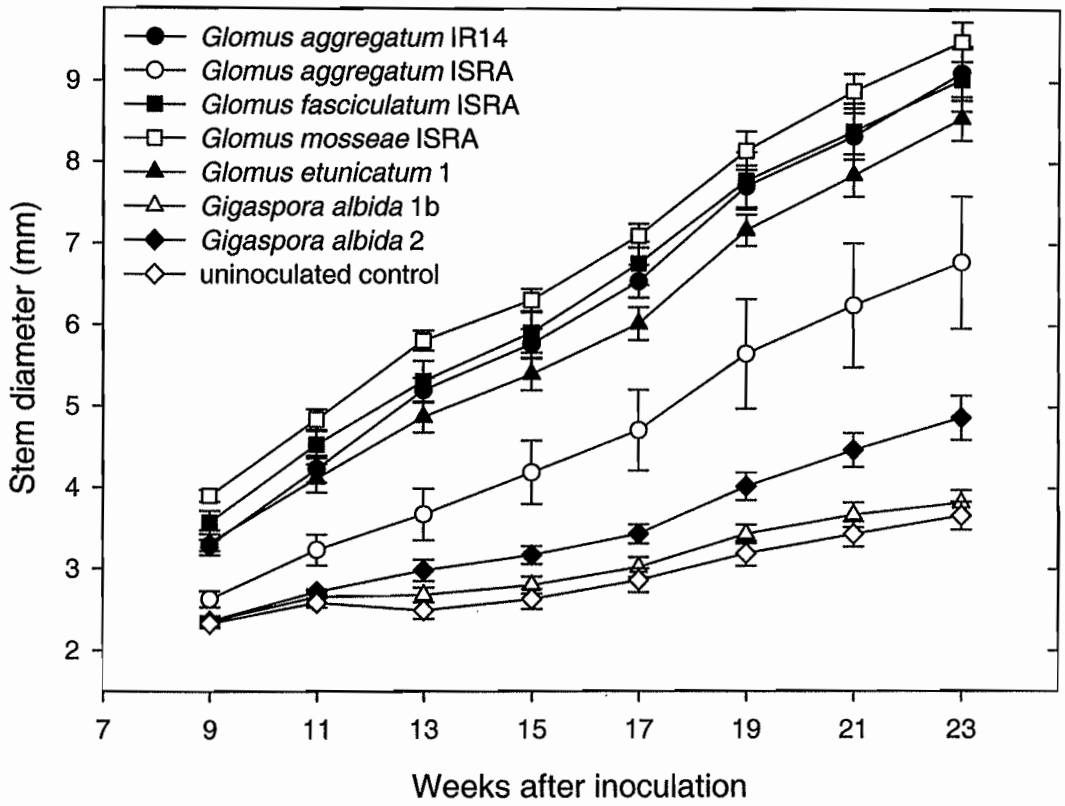


Figure 5 Growth of *Leucaena leucocephala* seedlings after inoculation with 7 different fungi (Bars indicate ± SE)

Table 2 Shoot and root growth and mycorrhizal infection of inoculated and uninoculated *Leucaena leucocephala* seedlings after 24 weeks

	Inoculation treatment								*P* value
	G.agg. IR14	G.agg. ISRA	G.fas. ISRA	G.mos. ISRA	G.etu. 1	Gi.alb. 1b	Gi.alb 2	Cont.	
Stem diameter (mm)	9.11 a[1]	6.78 b	9.03 a	9.50 a	8.55 a	3.82 d	4.87 c	3.66 d	<0.001
Stem Dry Wt. (mg)	1052 a	501 b	959 a	-	-	26 c	79 c	-	<0.001
Leaf Dry Wt. (mg)	560 a	308 b	403 ab	-	-	38 d	139 c	-	<0.001
Myc. infection (%)	59 c	46 d	67 b	75 a	64 bc	0.6 e	46 d	0 e	<0.001
Root conc. (cm/40cc)	242 a	126 c	191 b	252 a	121 c	18 e	47 d	42 d	<0.001

[1] Values within a row with the same letter are not significantly different from each other (LSD and Fisher's F test)

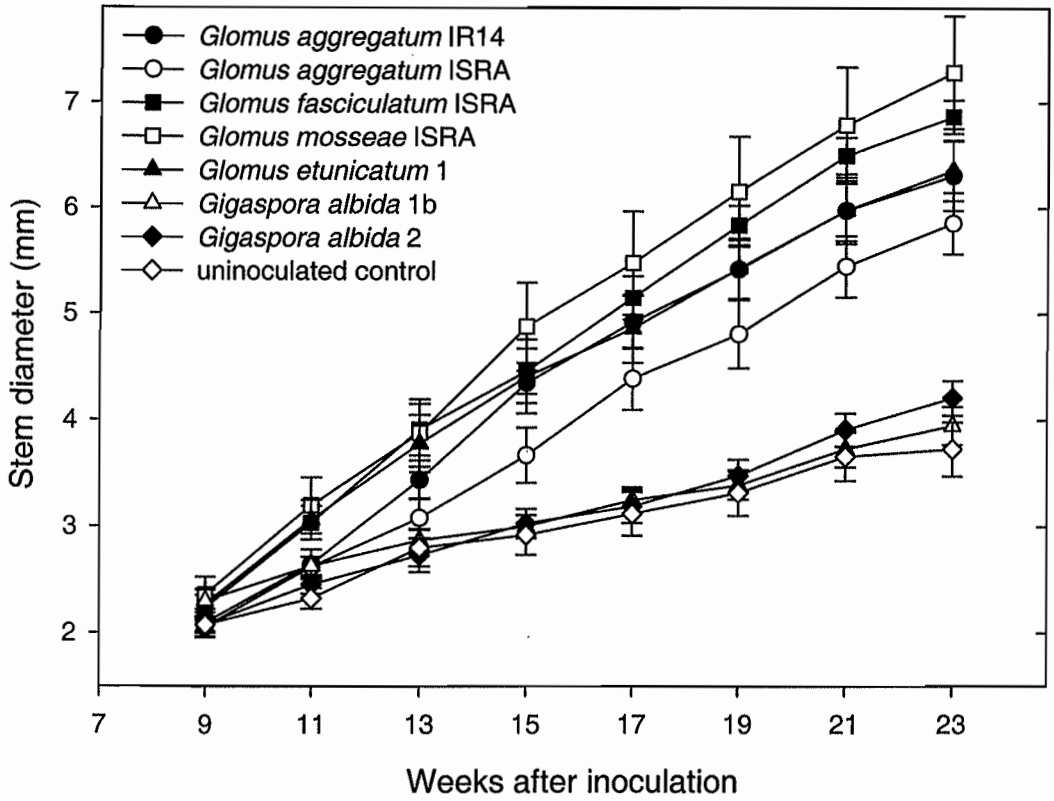


Figure 6 Growth of *Khaya senegalensis* seedlings after inoculation with 7 different fungi (Bars indicate ± SE)

Table 3 Shoot and root growth and mycorrhizal infection of inoculated and uninoculated *Khaya senegalensis* seedlings after 24 weeks

	Inoculation treatment								*P* value
	G.agg. IR14	G.agg. ISRA	G.fas. ISRA	G.mos. ISRA	G.etu. 1	Gi.alb. 1b	Gi.alb 2	Cont.	
Stem diameter (mm)	6.31 bc[1]	5.86 c	6.87 ab	7.29 a	6.36 abc	3.95 d	4.21 d	3.73 d	<0.001
Stem Dry Wt. (mg)	725 a	593 a	853 a	-	-	192 b	198 b	-	<0.001
Leaf Dry Wt. (mg)	1089 a	995 a	1278 a	-	-	573 b	602 b	-	<0.001
Myc. infection (%)	87 a	79 b	80 b	79 b	57 c	1.7 e	45 d	0 f	<0.001
Root conc. (cm/40cc)	662 a	518 ab	578 ab	510 ab	564 ab	226 c	484 b	114 c	<0.001

[1] Values within a row with the same letter are not significantly different from each other (LSD and Fisher's F test)

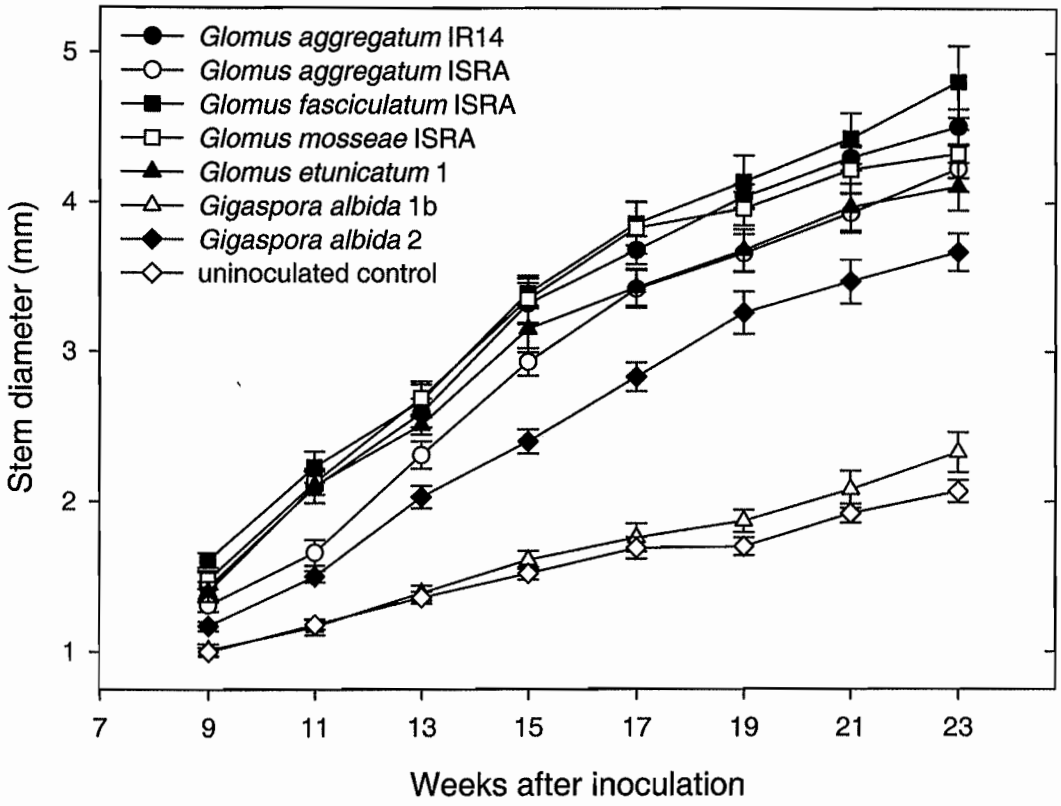


Figure 7 Growth of *Senna siamea* seedlings after inoculation with 7 different fungi (Bars indicate ± SE)

Table 4 Shoot growth and mycorrhizal infection of inoculated and uninoculated *Senna siamea* seedlings after 24 weeks

	Inoculation treatment								*P* value
	G.agg. IR14	**G.agg. ISRA**	**G.fas. ISRA**	**G.mos. ISRA**	**G.etu. 1**	**Gi.alb. 1b**	**Gi.alb 2**	**Cont.**	
Stem diameter (mm)	4.51 ab[1]	4.23 b	5.11 a	4.33 b	4.11 bc	2.33 d	3.67 c	2.07 d	<0.001
Stem Dry Wt. (mg)	558 a	332 b	507 ab	-	-	49 c	276 b	-	<0.001
Leaf Dry Wt. (mg)	1651 b	1670 b	2261 a	-	-	320 c	1419 b	-	<0.001
Myc. infection (%)	71 b	72 b	82 a	79 ab	60 c	12 d	84 a	0 e	<0.001

[1] Values within a row with the same letter are not significantly different from each other (LSD and Fisher's F test)

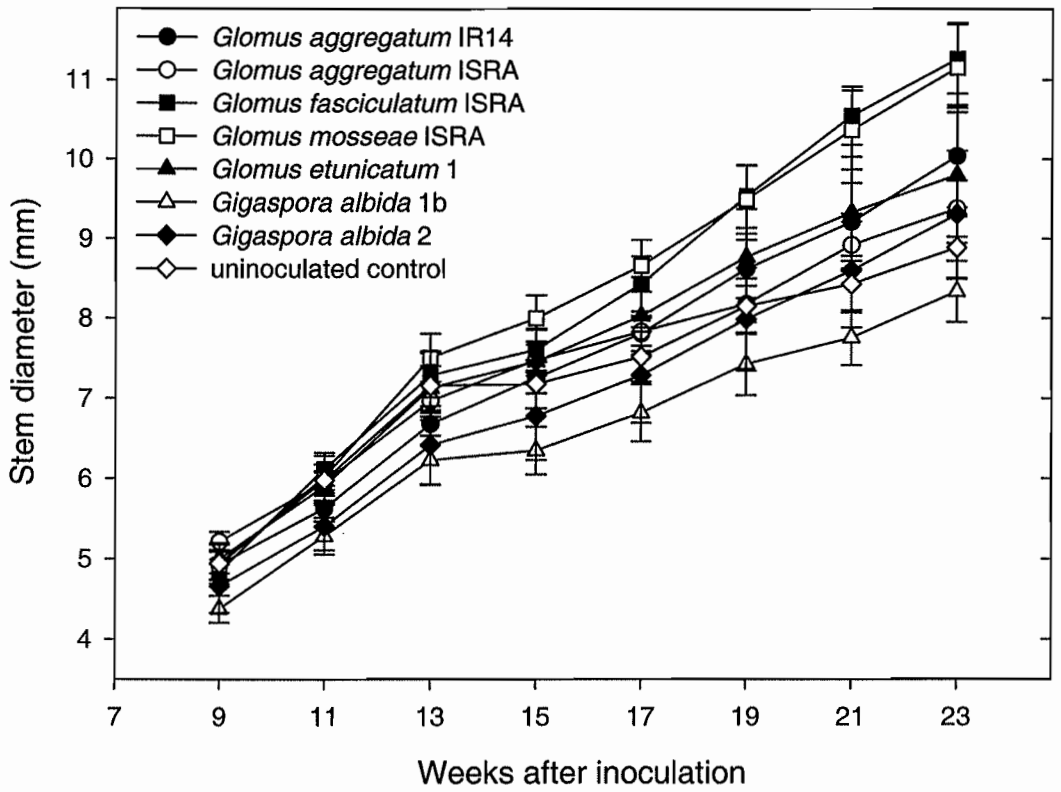


Figure 8 Growth of *Gliricidia sepium* seedlings after inoculation with 7 different fungi (Bars indicate ± SE)

Table 5 Shoot and root growth and mycorrhizal infection of inoculated and uninoculated *Gliricidia sepium* seedlings after 24 weeks

	Inoculation treatment								*P* value
	G.agg. IR14	G.agg. ISRA	G.fas. ISRA	G.mos. ISRA	G.etu. 1	Gi.alb. 1b	Gi.alb 2	Cont.	
Stem diameter (mm)	10.0 abc[1]	9.4 bc	11.3 a	11.2 ab	9.8 abc	8.3 c	9.3 c	8.9 c	<0.001
Stem Dry Wt. (mg)	257 b	179 b	445 a	486 a	234 b	132 b	237 b	142 b	<0.001
Leaf Dry Wt. (mg)	393 b	202 bcd	628 a	605 a	266 bcd	133 cd	306 bc	111 cd	<0.001
Myc. infection (%)	69 c	61 d	79 b	85 a	74 bc	3.1 e	58 d	0 f	<0.001
Root conc. (cm/40cc)	151 bc	66 e	191 a	182 ab	104 d	20 f	125 cd	31 f	<0.001

[1] Values within a row with the same letter are not significantly different from each other (LSD and Fisher's F test)

Table 6 Correlation coefficients (r) between mycorrhizal infection and parameters of tree growth for the 4 tree species

	S. siamea	*L. leucocephala*	*K. senegalensis*	*G. sepium*
Stem diameter	0.724***	0.734***	0.715***	0.389***
Stem dry wt.	0.661***	0.729***	0.652***	0.474***
Leaf dry wt.	0.809***	0.740***	0.594***	0.523***
Root conc.	-	0.818***	0.706***	0.818***

*** significant at $P<0.001$
n=80 for stem diameter and root concentration and n=50 for dry weight measurements

The results of the screening experiment show a large response to mycorrhizal inoculation with differences between the 4 tree species and between the 7 AM isolates tested. For logistical reasons (i.e. materials and glasshouse space needed) it was necessary to restrict the irrigation phase of the experiment to just 3 tree species and 3 inoculation treatments. For the tree species, the 3 most responsive and varied in terms of N nutrition were selected; *Leucaena leucocephala* (N-fixing legume), *Senna siamea* (non-nodulating legume) and *Khaya senegalensis* (non-legume). For the AM inoculants, the most effective inoculant (*Glomus mosseae*) and the uninoculated control were selected along with *Glomus etunicatum* 1, an isolate for which molecular markers are available and that has been used in several other inoculation experiments.

2.5. Irrigation experiment

The overall objective of the experiment was to test selected tree species x AM fungal combinations watered with filtered glasshouse water or with a solution designed to simulate the wastewater recycled in Mali.

2.5. 1. Treatments

Trees (3)
Leucaena leucocephala Dakar 6/03 (T2C Odonto)
Senna siamea CNSF 1154 (Bobo Prov.)
Khaya senegalensis CNSF 1156 (Mondon Prov.)

AM inoculants (3)
Glomus mosseae ISRA
Glomus etunicatum 1 (BEG 176)
Uninoculated control

Irrigation (2)
Watered as normal or with solution simulating wastewater recycled in Mali: It was decided to use Ingestad's nutrient solution modified to provide increased levels of N (132 mg/l - **x2**) Zn (1.46 mg/l – **x100**) and Cu (0.157 mg/l – **x10**). These concentrations may be increased once the trees begin growing faster in May 2005.

Replication (4)

2.5.2 Experiment set up (Figure 9)

Twenty four weeks after inoculation, 8 plants of each of 9 treatments (3 tree species x 3 AM inoculants) were potted into 32 litre tubs containing sterilised loam/grit-sand mixed 1:5. Four plants were then allocated to each irrigation treatment. The new treatments were laid out in 4 randomised blocks with tree species arranged in a split plot design and fungal inoculants and irrigation treatments allocated to sub plots. Application of the irrigation treatment began on 7th January 2005, with each plant receiving 250 ml of nutrient solution or water per week. Monthly measurements of stem diameter are being continued.

Figure 9 Plants of *Leucaena leucocephala, Senna siamea* and *Khaya senegalensis*, transferred to larger pots (32 l) for the irrigation phase of the study.

4. Progress against activities defined in technical annex (WP4)

- Glasshouse experiment to test effectiveness of mycorrhizal inoculants with 4 tree species completed.
- Glasshouse experiment to test the effect of wastewater irrigation on effective tree species x AM inoculants set up.
- Isolates of AM fungi are being maintained in pot cultures in the glasshouse.

5. Forward look WP4

- Glasshouse experiment; continued measurements to monitor effect of irrigation treatment on tree growth.
- Assessments of irrigation treatment effects on mycorrhizal populations and soil microbial activity, including application of molecular techniques.
- Maintenance of pot cultures of AM fungi.

Partner 2: Institut d'Economie Rurale, Bamako, Mali

Work package 1: Water treatment and Irrigation

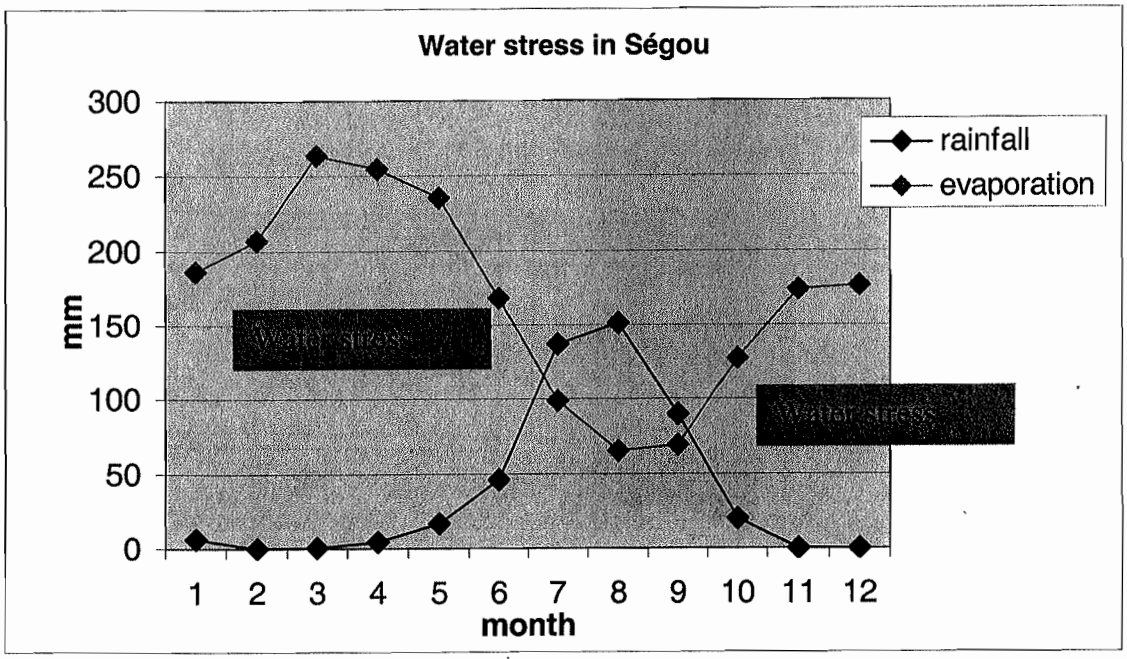
The objective of this work package is to treat and evaluate waste water from rice irrigation from Siribala irrigated perimeter for fodder and fuelwood production.

An experimental site (4 ha) has been chosen near a village called Siribala situated at 30 km from Niono. The site is situated at 14°4 latitude north, 6°03 longitude west and at an altitude of 274.3 m. The drainage canal which will be used to irrigate experimental site is called the drain of Minimana.
Experimental plots (1 ha) have been delimited and surrounded with wire netting.
Using the data collected in Mali, SCP has produced a report on the project which includes:
1. Potentialities and constraints linked to climate and its incidence on the project (climatic data and water deficits).
2. Potentialities and constraints of the experimental site (Topography and soil studies).
3. Description of wastewater treatment and evaluation project.

1. Potentialities and constraints linked to climate and its incidence on the project (Climatic data and water stress).

Using climatic data for the Ségou region, we can say that the climate is Sudan-Sahelian type with the annual rainfall between 450 and 600 mm. The high rainfalls occur in July and August. The mean annual temperature during 22 years (1980-2002) is 28.6°C. The highest temperature recorded is 40.8°C and the lowest is about 17.4°C in January. The mean daily evapotranspiration is estimated to 5.6 mm per day. The water stress (Figure 10) in the region is very important during all the year except the rainy season. A balance between rainfall and evaporation is reached only during the rainy season from July to September.

Figure 10 Annual variation in the occurrence of water stress in the Segou region

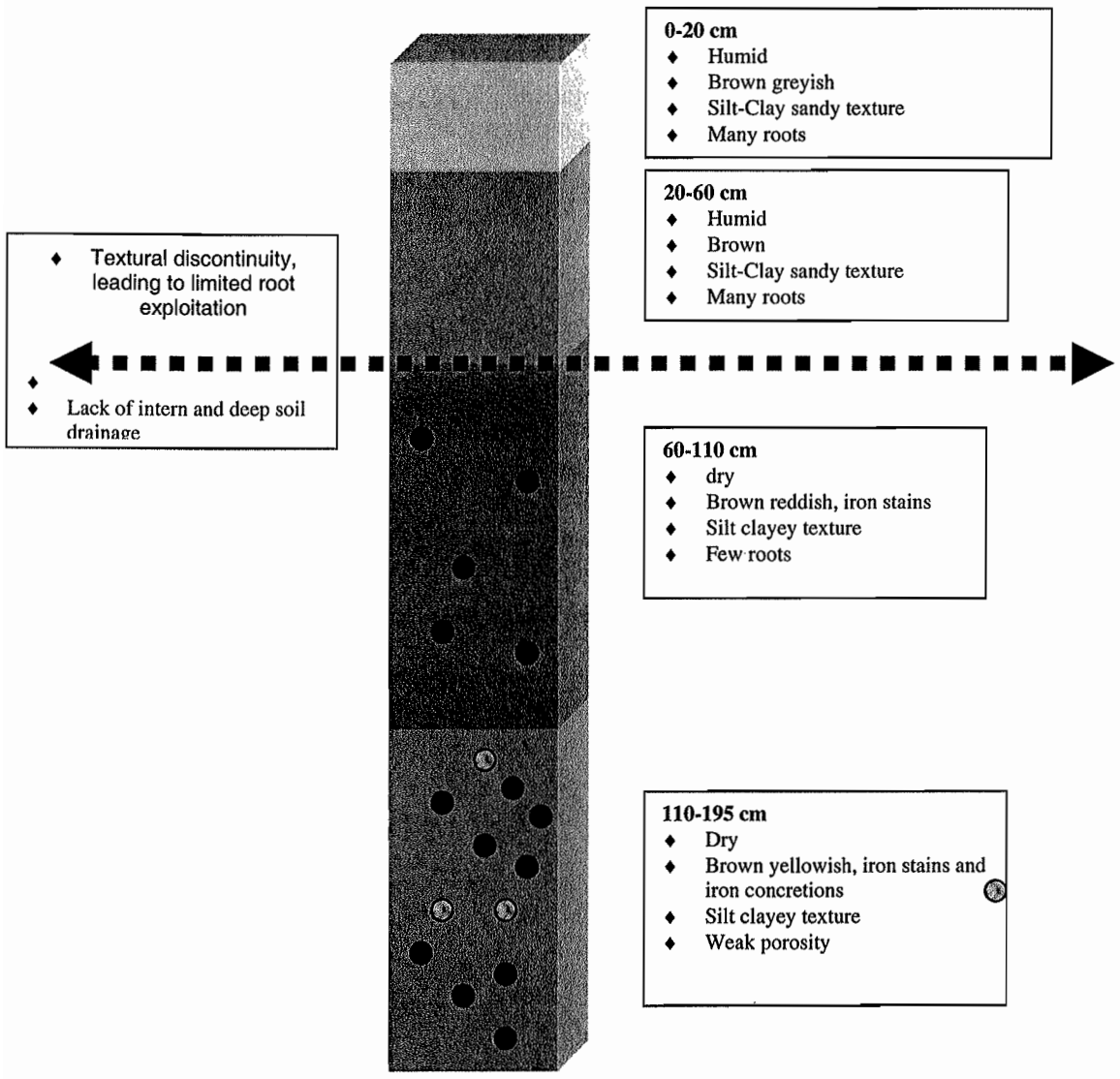


2. Potentialities and constraints of the experimental site (Topography and soil studies).
The following site maps have been produced by SCP: Morpho-pedological map, global management plan map, irrigation plan map and the map of the artificial basin. These maps are contained in the SCP report produced in November 2003.

2.1. Soil studies

Three types of soil have been identified in the experimental site. Arenosols classified as 'ferrugineux tropicaux' are the dominant soil type. The description of the profile of soil chosen for the setup of the experiments is presented in Figure 11. Soil samples have been collected from all the profile layers for chemical analyses and this for the three types of soils.

Figure 11 Soil profile at the experimental site



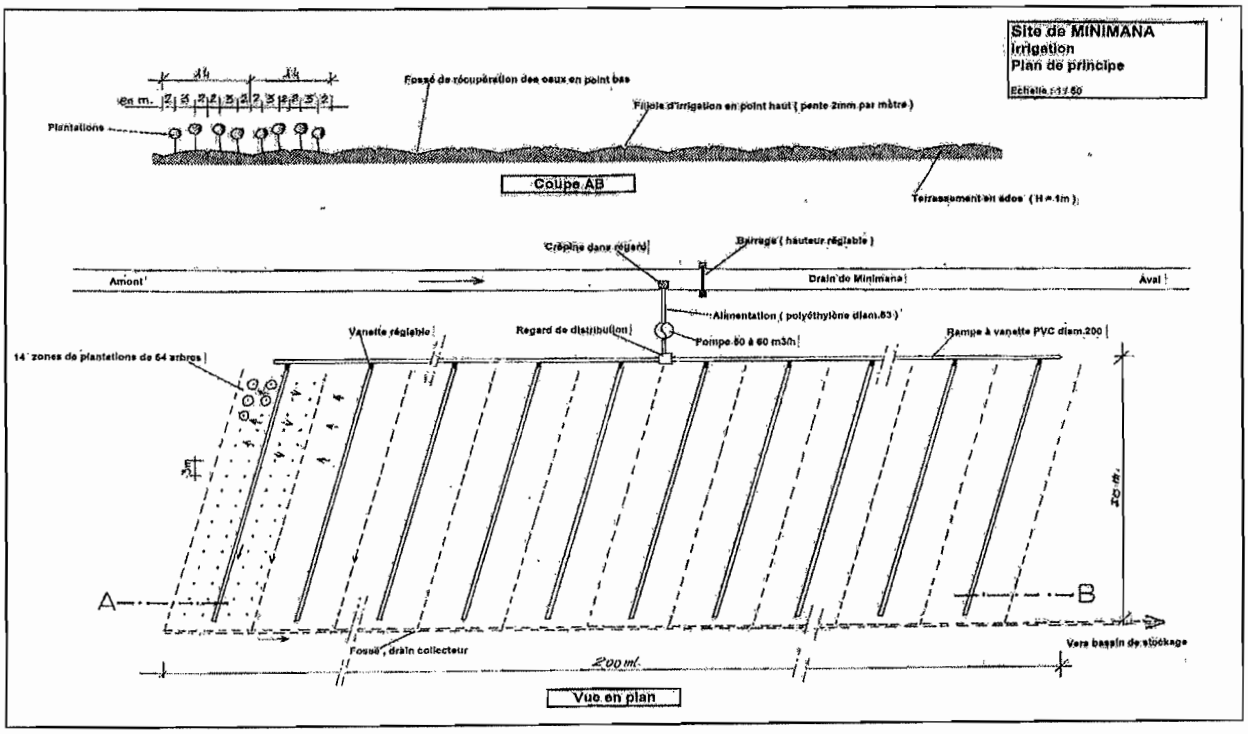
3. Description of wastewater treatment and valorisation project.

Using the conceptual irrigation plan produced by SCP, the Mali team have installed the irrigation system in the field. This irrigation system involves the following things: site management (earthworks, house, guard room, dam building, fencing the experimental site), and equipment (water pump engine and irrigation materials).

3.1. Experimental site management

Figure 12 shows the plan of the irrigation system. This irrigation system including experimental plots has been surrounded using iron fences (Figure 13, on the right). Earthworks, construction of the collector drain, and excavation of the dyke of the artificial basin have been completed. Trenching has been done on the secondary embankments, and the emptying drain from the artificial basin to the drain of Minimana has been excavated. Preparation of the planting holes in the experimental plots has started. Activities such as building dam and proper management of the artificial basin still have to be done.

Figure 12 Plan of the irrigation site (SCP report, November 2003)



A house and a guard room have been built for the caretaker and for safe keeping of all the equipment and small materials of the project.

3.2. Irrigation equipments

All the equipment (thermal water pump engine and accessories, adjustable floodgates, PVC tubes…) needed for the irrigation system to work properly has been bought and is available at the experimental site. PVC tubes (Figure 13) have been placed on the primary embankment, and adjustable floodgates have been placed on the secondary embankments. Trenches have been dug out on the last ones in order to facilitate drainage of water to plants. Figure 14 shows the water pump in the Minimana drain.

Figure 13 PVC tube (200 m in length) placed on the primary embankment. An adjustable floodgate is shown on the secondary embankment

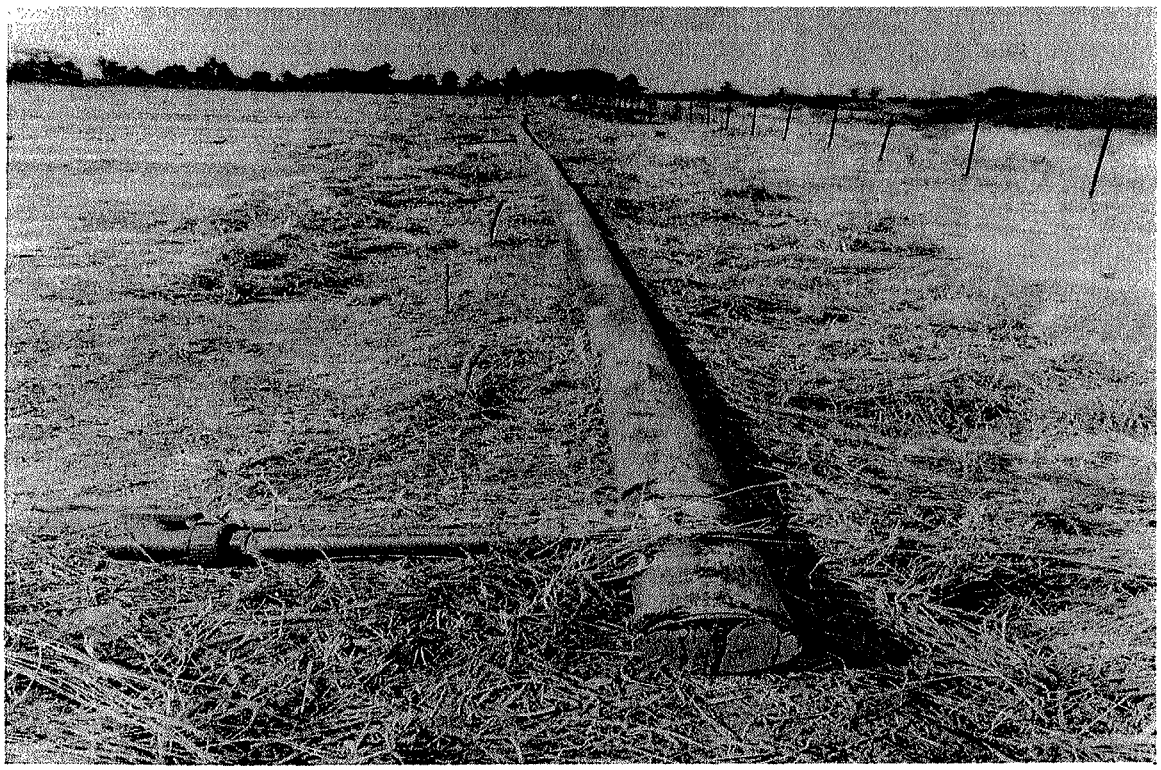
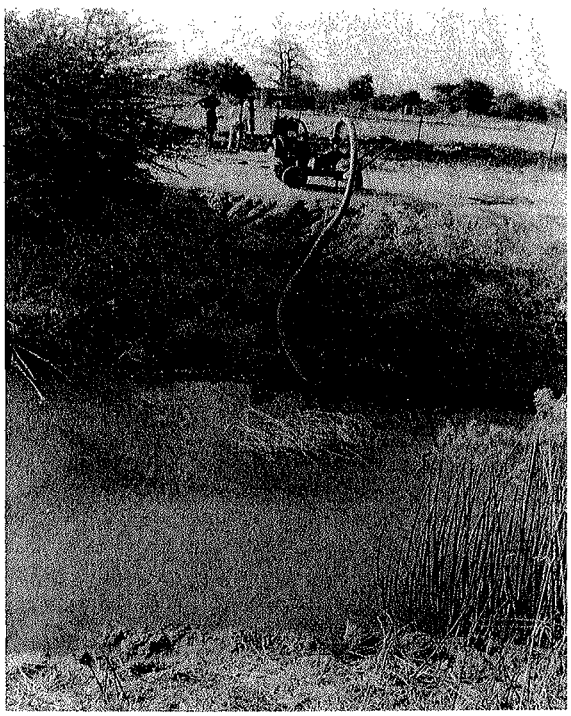


Figure 14 Water pump engine and the filter in the drain at Minimana



Work package: 2 Tree growth and Management

1. Screening experiment in nursery:
Nursery works started in July in the nursery of the Forestry Resources Program. In Mali, 11 tree species are incorporated in the screening trial. Australian tree seeds have been purchased from the AgroForester Tropical Seeds Co. in USA.

1.1. First screening trial
Results were reported last year

1.2. Second screening trial

1.2.1. Materials and Methods

Tree species
Tree species used in the screening experiment are: *Acacia crassicarpa*, *Acacia mangium*, *Acacia auriculiformis*, *Leucaena leucocephala*, *Gliricidia sepium*, *Calliandra calothyrsus*, *Acacia angustissima*, *Acacia Senegal*, *Acacia seyal*, *Pterocarpus lucens* and *Khaya senegalensis*.

The experimental design
The experimental design is a Complete Randomized Block Design, with the eleven afore-mentioned tree species and four inoculation treatments: 1) + Mycorrhizas, 2) + Rhizobium, 3) +Mycorrhizas + Rhizobium and 4) the control treatment (-M-R).
There are 5 blocks, and each species x treatment combimation is represented by a plot of 30 plants in each block. This experiment therefore required 6600 seedlings to be produced.

Mycorrhizal Inoculation
Mycorrhizal inoculum was provided by Partner 4 from INERA Burkina Faso. The potting compost used, was that usually used in the nursery of the Forestry Resources Programme (1/3 field soil +1/3 pure sand +1/3 compost). The soil was not sterilized. During the inoculation with mycorrhizas, efforts were taken to avoid cross contamination (allowing sufficient space between treatments, and ensuring cleanliness of hands and all tools during the inoculation process. For inoculation, seeds were soaked overnight in inoculum solution, and before sowing seeds, inoculum was poured in the planting hole using a garden syringe. The second inoculation was done at the seedling stage using the syringe.

Data Collection: Seeds were sown on 01/11/ 2003. Germination observations started one day later. The first measurement of growth parameters started on 22/12/2003. Since then parameters such as seedling diameter, seedling heights, number of branches and percentage of survival have been measured each month.

Data Analyses: Data have been analysed using MINITAB statistical software.

1.2.2. Results and Discussion
The percentage of germination of all the 11 species is available. Apart from some species like *Calliandra calothyrsus, Pterocarpus lucens* and *Khaya senegalensis,* the percentage of germination of the tree species is rather good (Table 7). Tree species such as *Gliricidia sepium, Acacia angustissima, Acacia seyal* have the best percentage of germination (100%) followed by *Acacia auriculiformis, Leucaena leucocephala, Acacia mangium* with more than 90%. This group is followed by species like *Acacia crassicarpa* and *Acacia senegal.*

Table 7 Percentage of germination of tree species (4 weeks after sowing seeds). Mean of all treatments.

Species	Means
Acacia angustissima	100 **A**
acacia auriculiformis	96 **A**
Acacia crassicarpa	81 **AB**
Acacia mangium	93 **A**
Acacia senegal	59 **B**
Acacia seyal	100 **A**
Calliandra calothyrsus	15 **D**
Gliricidia sepium	100 **A**
Khaya. senegalensis	32 **C**
Leuceana leucocephala	94 **B**
Pterocarpus lucens	22 **C**
P =	**0.000**

NB: Means followed by different letters are significantly different at P = 0.05

After 2 months of germination, we can see from Table 8 that the survival rate is good for most of the species except *Pterocarpus lucens*. Highly significant differences have been found between tree specie s concerning the growth in height and diameter. *Acacia seyal* has the best height followed by *Leucaena leucocephala* and *Gliricidia sepium*. But concerning the growth in diameter, it appears that Gliricidia is the best species followed by *Leucaena leucocephala* and *Acacia seyal*.

Table 8 Tree growth 2 months after germination. Mean of all treatments

Species	Height (cm)	Diameter (cm)	Survival rate (%)
Acacia angustissima	4.40 **C**	0.11 **E**	81 **A**
Acacia auriculiformis	4.66 **C**	0.10 **E**	85 **A**
Acacia crassicarpa	2.80 **D**	0.10 **E**	72 **B**
Acacia mangium	5.53 **C**	0.10 **E**	90 **A**
Acacia senegal	4.45 **C**	0.15 **D**	52 **C**
Acacia seyal	17.08 **A**	0.20 **C**	99 **A**
Gliricidia sepium	13.65 **B**	0.44 **A**	100 **A**
Leuceana leucocephala	13.82 **B**	0.27 **B**	94 **A**
Pterocarpus lucens	3.42 **C**	0.10 **E**	28 **D**
P =	**0.000**	**0.000**	**0.000**

NB: Means followed by different letters are significantly different at P = 0.05

After 4 months of germination, Table 9 shows that the survival rate remained good which means that good survival of seedlings exist. Highly significant differences have been found between tree species concerning the growth in height and diameter (Figure 15). At this stage *Leucaena leucocephala* has the best height followed by *Gliricidia sepium* and *Acacia seyal.* Concerning the growth in diameter, *Gliricidia sepium* remains the best species followed by *Leucaena leucocephala* and *Acacia seyal.*

Table 9 Tree growth 4 months after germination. Mean of all treatments.

Species	Height(cm)	Diameters(cm)	Survival rate (%)
Acacia. angustissima	14.0 **D**	0.306 **C**	81 **A**
Acacia. auriculiformis	9.90 **D**	0.144 **D**	85 **A**
Acacia. crassicarpa	7.10 **D**	0.191 **D**	71 **B**
Acacia mangium	15.5 **D**	0.323 **C**	90 **A**
Acacia senegal	13.4 **D**	0.247 **C**	52 **C**
Acacia seyal	34.3 **C**	0.395 **C**	99 **A**
Gliricidia sepium	47.7 **B**	1.010 **A**	100 **A**
Leucaena leucocephala	68.9 **A**	0.749 **B**	94 **A**
Pterocarpus lucens	8.5 **D**	0.278 **C**	28 **D**
P =	**0.000**	**0.000**	**0.000**

NB: Means followed by different letters are significantly different at P = 0.05

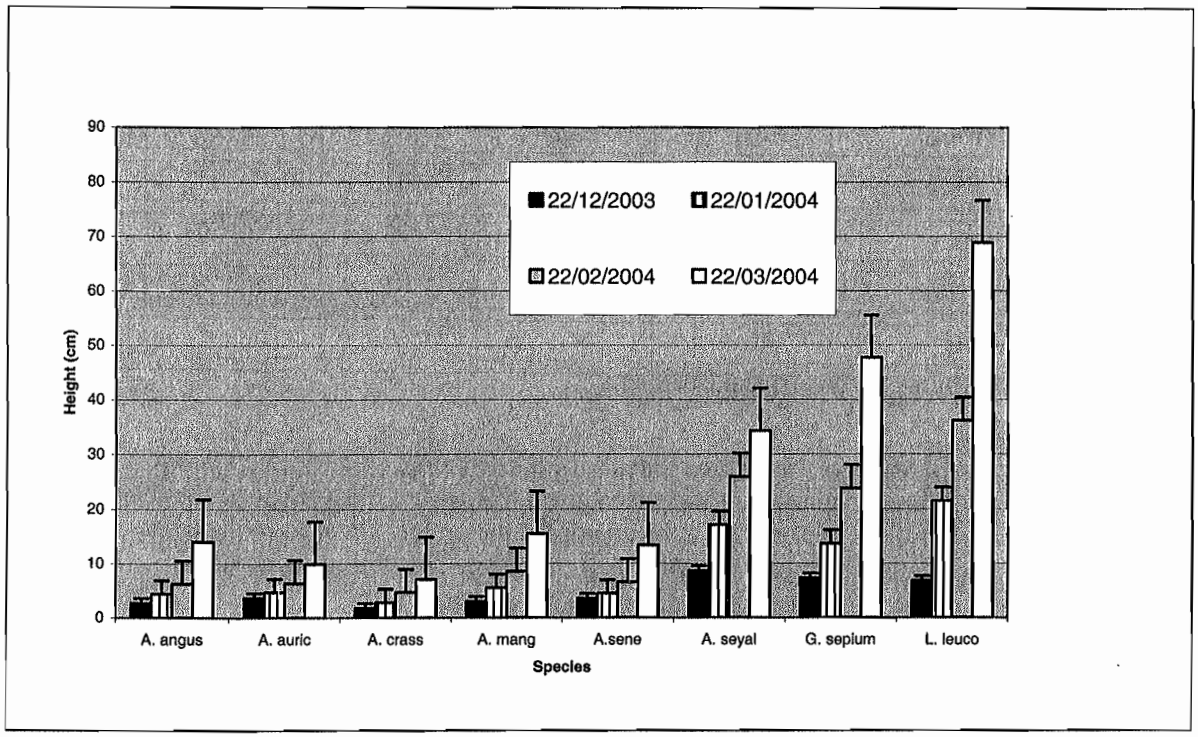


Figure 15 Tree height growth 4 months after germination, mean of all treatments.

NB: Bars represent standard errors of the means at 5% level.

At this stage of experimentation, the results show that the best performing species are: *Gliricidia sepium, Leucaena leucocephala* and *Acacia seyal*. At a later stage, the growth (diameter and height) of *Acacia angustissima* was significantly higher than the other species. This is why these three tree species have been chosen for field trials during the coordination meeting in Bamako.

Mycorrhizal and rhizobial treatments had some positive effects on tree growth but they were not significantly different.

Table 10 shows preliminary results on the percentage of mycorrhizal infection according to species and treatments. Since the soil was not sterilised, the control plants become infected with 'wild symbionts'. Plants from mycorrhizal and mycorrhizal + rhizobium (R+M) treatments have the highest percentage of infection. This suggests that the symbionts used for the inoculation have either infected roots of the plants more quickly, or are more suitable than the wild types occurring in the nursery.

Table 10 Percentage of mycorrhizal infection according to species and treatments

Treatments **Tree species**	**- R - M**	**+R**	**+M**	**+ R + M**
Gliricidia sepium	50.77	25	85.11	58.33
Acacia senegal	41.26	50	65.88	65.31
Acacia seyal	18.75	28.57	72.06	71. 78
Acacia mangium	0	31.80	61.42	53.65
Acacia crassicarpa	0	52.23	40.97	55.32
Acacia angustissima	35.33	48.48	93.05	88.33
Acacia auriculiformis	0	25.89	64.13	27.77
Leuceana leucocephala	37.10	27.77	64.44	51.78
Pterocarpus lucens	-	55.55	73.55	57.14
Calliandra calothyrsus	-0	-	81.78	28.57
Khaya senegalensis	40.47	-	63.37	-

NB: + R + M = Rhizobium + Mycorrhizal treatment

2. Field experiments:

During the meeting in Bamako, it was agreed that all the participating countries will do the same experiments in different countries. It was also agreed that two of the tree species will be same in all countries. For these reasons, the coordinator for the project Dr; Julia Wilson has worked out two experiments which will be done in all countries. The objectives and the experimental designs of the two experiments are presented in Annex 1.

2.1. Experiment 1.

Objective: To compare the growth of tree species in different countries under irrigated field conditions in Burkina Faso, Niger and Mali.

Nursery works have already started in the experimental field in Minimana. Seeds of the following tree species were sown in plastic pots on 10/12/2004: *Gliricidia sepium*, *Leucaena leucocephala, Acacia angustissima* and *Khaya senegalensis*.

Mycorrhizal inoculum was send to us by Partner 4 from INERA Burkina Faso. At the meeting in Bamako it was agreed to use the mixed inoculum that Partner 2 obtained from AgroForester. So partner 2 sent this inoculum to Partner 4 for mass production. This mixed inoculum is used by all the countries. The biotic ingredients of this inoculum are: Endomycorrhizal (VAM) spores; minimum 40 spores/cc of blended *Glomus brasilianum*, *Glomus clarum*, *Glomus deserticola*, *Glomus intraradices*, *Glomus monosporus*, *Glomus mosseae* and *Gigaspora margarita*.

An unsterilized soil mix (1/3 field soil +1/3 pure sand +1/3 compost) was used to fill plastic pots. In order to avoid cross contamination during the inoculation, treatments were kept

separately and pots were raised off the ground (Figure 16). Seeds have been soaked overnight in inoculum solution. Before sowing seeds, inoculum was poured in the planting hole using a garden syringe.

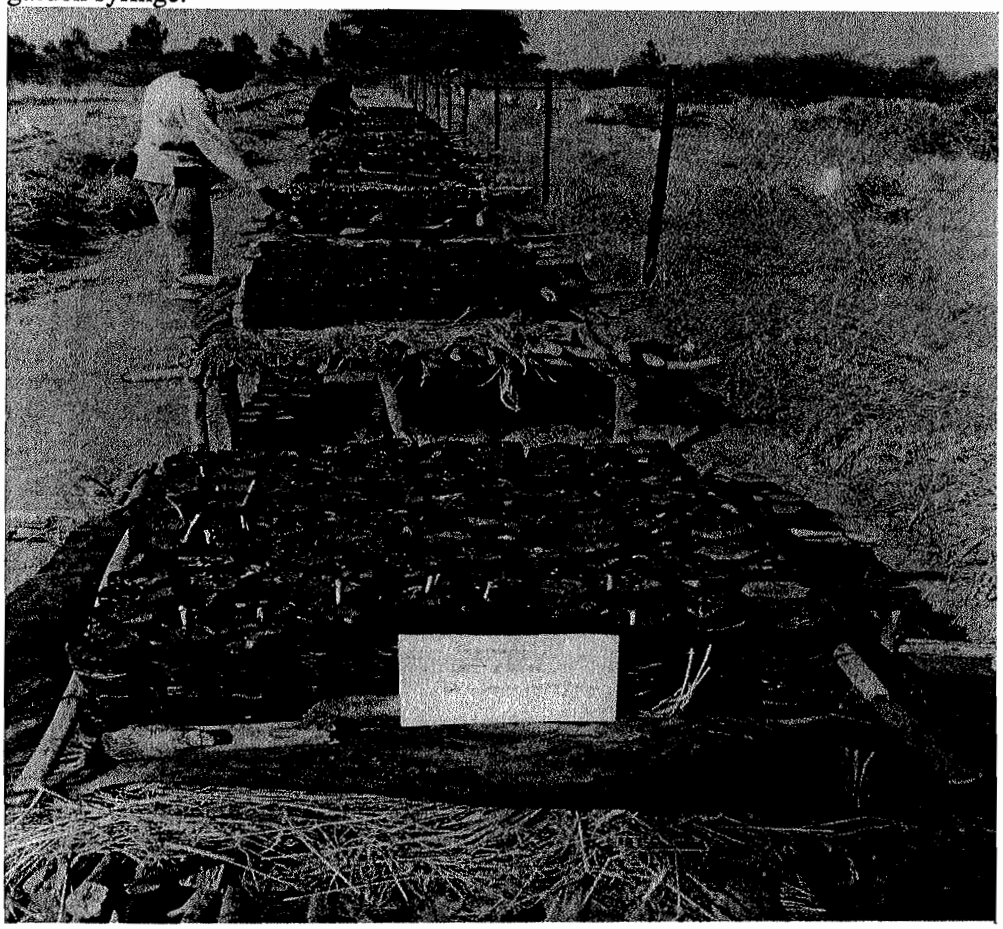


Figure 16 Preparation **of plants for the Minimana outplanting: treatments are kept separate and pots are raised off the ground to avoid cross contamination**

Germination: Seed germination is not yet finished for all the species. Growth parameters will be measured once germination is completed.

2.2. Experiment 2

Objective: to do a quick screening of the species already studied in the nursery to check their performance under field conditions

Seeds of the ten following tree species have been sown on 10/12/2004: *Acacia crassicarpa*, *Acacia mangium*, *Acacia auriculiformis*, *Leucaena leucocephala*, *Gliricidia sepium*, *Calliandra calothyrsus*, *Acacia angustissima*, *Acacia senegal*, *Pterocarpus lucens* and *Khaya senegalensis*. Concerning the seeds of *Acacia seyal*, there is a problem of availability due to the strong attack by insects.
Experiment 2a will test the exotic species and experiment 2 b will test indigenous species.
The designs are the same for both experiment 2a and 2b.

The same soil substrate used in experiment 1 has been used in this experiment. The inoculum and method of inoculation are the same in the two experiments.

Germination has also started in this experiment but it is not yet complete. Figure 17 shows the germination of seedlings in the second experiment.

Figure 17 Germination stage of seeds in the nursery at Minimana site

Planting
Planting holes have been prepared on the experimental plots for the 2 experiments.
We are planning to plant the seedlings out May-June 2005.

Work package 3: Tree Water–use and soil water status.
Soil samples have been taken from the experimental site and the dynamics of water use according to species will be assessed after tree planting.

Work package 4: Microsymbionts and N-fixation: We have received 1 kg of mixed inoculum from Partner 4 (Burkina Faso). This inoculum has been used in the first and second experiments.

Work package 5: Socio economic surveys have been done by Partner 3. Preliminary results have been presented during the coordination meeting in Bamako.

Work package 6: Soil and Plant nutrition
Soil chemical analyses have been done in Burkina Faso (Partner 4) and the results are already available.

Characterisation of the Minimana site

1-Generalities on the area
The site of Minimana, with a surface of 4 ha belongs to the north Sudanian climatic zone with an annual rainfall varying from 550 to 600 mm. Geomorphologicaly, the area of Kala (Minimana is located in upper Kala) belongs to the Central Delta of Niger more precisely to the dead Delta which is a fossil plain of the Niger river. This fossil plain which no longer receives flood water from the Niger River when in spate is nowadays become again a wetland thanks to the agricultural hydro installations installed during the colonial period. The majority of the population of the "Office du Niger" live in a production system called "Colonat": The managed fields are rented to peasants who also pay a royalty (for irrigation) to the managing authorities of the hydraulic network. This network is composed of primary channels which bring water,

secondary canals which are the distributors and tertiary channels which are the sprinklers. Drains are installed at the low sides of the fields to evacuate waste water of the sugar cane plantations and the rice fields. This waste water is used only in a partial way by peasants in the non arranged zones.

2. The site of Minimana

The experimental site of Minimana is in the commune of Siribala; about 30 km of Niono. It covers a surface of 4 ha near by the drain called "drain of Minimana" on right bank. The field is rectangular, with a length of 400 m skirting the drain which will be used as water intake for the primary pipe from which the plots will be sprinkled. Its width is 100 m directed North-South.

3. Methodological approach of the study of the ground

Nine forest tracks originating at the drain were opened over a 100 m length with an azimuth of 190°. The distance between forest tracks are 50 m. Transverse and orthogonally a forest track of 400 m crossed the 9 forest tracks in their medium. The layout thus produces a square grid of 50 m / 50 m of the 4 ha. Thus 16 plots of 2500 m^2 were delimited. Surveys with the drill to 1.20 m depth were carried out at the centre of each pilot. The materials resulting from the survey holes were examined for: moisture content, colour, texture, the presence of coarse elements, of gray tasks, ochres or rusts are taken as parameters of discrimination. The objective of the survey was to chart the edaphic diversity of the field. Once this diversity was identified, pedological pit of 2m depth were opened in each soil unit.

4. Results of soil study:

The site belongs to a great geomorphological entity, the dead delta of the Niger River which is a fossil plain with weak slope. The alluvial material is mainly fine and clayey.

4.1. Surveys with the drill.

Fifteen of the 16 probed plots contained at least one ant-hill with an average height of 1.75 m and 6 to 8 m of diameter. These biological disturbances were taken into account at the installation period. Indeed the subjacent materials re-installed on the surface obstruct much the development of the seedlings. The herbaceous cover is not homogeneous on the soil. One meets there stripped area with encrusting on the soil surface. This phenomenon is accentuated in the Western half of the 4 ha.

The materials resulting from the survey holes revealed three distinct cartographic units. The criteria of moistening front and the appearance of the spots indicating the dynamics of water in the profile are those having had a significant level of discrimination to differentiate the soil units. The examination of the surveys gave three units

Unit 1: The material is dry starting from 60 cm of depth and becomes compact; the soil reveals the first spots of oxidation of iron. They are the elementary plots identified as n° 1; 3; 6 and 9 (see Figure 18)

Unit 2: The material is wet from 0 to 120 cm depth; the spots of oxidation appear in the soil starting at 40 cm. The concerned plots are: N° 2, 4, 8, 10, 14, 15, 16

Unit 3: This material is wet from 0 to 120 cm depth. The spots of oxidation start at 25 cm. The plots which are on this unit are: N° 5, 7, 11, 12 and 13.

4.2 Description of the pedological profiles

On each identified cartographic unit, a pedological pit of 2 m depth was dug. The notations P1, P2, P3 correspond respectively to the profile of units 1, 2 and 3.

P1:

Horizon 1: 0-20 cm. The material is wet; it has a brown-gray colour without iron spots of oxidation. Its texture is loamy-clay-sand (LCS) with coarse sand. This organo-mineral horizon is flaky and reveals many impacts of fine roots.
Horizon 2: 20-60 cm. The material is wet. It has a brownish color neither without iron oxidation spots nor of reduction. Its texture is LCS but with a small proportion of sand compared to horizon 1. It is not very plastic, rather dirtying that sticking. The impacts of roots are numerous with presence of big and fine roots. This layer of the profile (0 to 60 cm) is porous.
Horizon 3: 60-110 cm. The material on this depth is almost dry and compact with a shining reddish–brown colour. Its texture is loamy-clay (LC). It is plastic and sticking. Concerning its structure, it produces oblique plates. One meets in this layer some roots of medium size and many reddish brown iron oxidation spots.
Horizon 4: 110-195 cm It is dry and compact, the material is shining brown–yellowish and reveals spots of oxidation and reduction of iron but with a predominance of the Fe +++ spots. This horizon does not contain any roots. The material pours out in oblique plates. It has a clayey-loam texture (CL) coating with ferruginous concretions towards the bottom of the pit. The layer of the profile from 60 to 195 cm is a material with very low porosity.

This is a ferruginous soil with a drainage classified from moderate to normal.

P2: This profile is wet at all depths (0-200 cm)

Horizon 1: 0-20 cm. It is the horizon where the organic matter and the mineral matter are closely built-in. The material has a loamy-clay-sand (LCS) texture with coarse sand, but without any ferruginous spot. Its colour is brown -yellowish with matte appearance. It is not very plastic and not sticking. The impacts of roots are numerous with the presence of fine roots.

Horizon 2: 20-40 cm. This material has a yellowish orange colour with matte appearance. Its texture is loamy-clay (LC), it is plastic and sticking and does not reveal ferruginous spots, the roots are very numerous, and they are fine or medium size.

Horizon 3: 40-120 cm. This layer of the soil has clayey-loam texture (CL); the colour of this material is yellow orange with matte appearance. The profile shows ferruginous spots of oxidation and reduction and very few iron concretions of a gravel size (diameter varying from 0.2 to 2 cm); some impacts of roots are perceptible.

Horizon 4: 120-200 cm. Only the dynamics of water distinguishes this horizon from that overlying. Indeed the grey spots are more numerous here i.e. that the indices of hydromorphia of the soil are more clear in the subjacent layers. Horizons n°3 and 4 have a very porous material.
The soil is hydromorphic with gley at the same time oxidized and reduced.

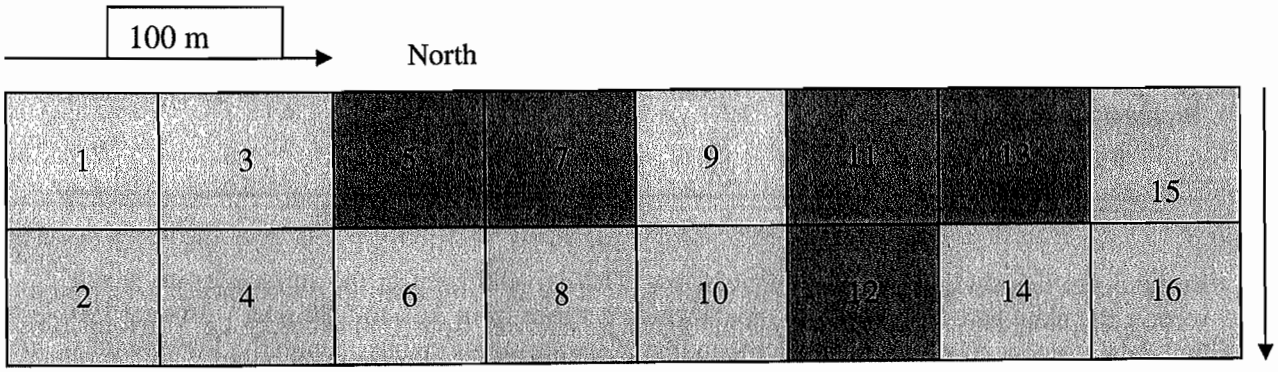
P3
Horizon 1: 0-25 cm. The material is wet; it has a LCS texture and a yellow brown-gray colour. It is not very plastic, does not reveal any ferruginous spots but some specks related to the roots, which are numerous in this horizon. Sand met here has contrary to that of the two previous profiles a medium size.

Horizon 2: 25 to 55 cm. The material has a LA texture and a yellowish brown colour with matte appearance. It is wet, plastic and sticking. This horizon contains many impacts of roots of fine and medium sizes. One observes there also spots of oxidation and reduction with a prevalence of ferric iron.

Horizon 3: 55 to 120 cm. Like the two preceding overlying horizons, the material of this horizon is wet. It has a CL fine texture and a matte brown yellowish colour. The indices of hydromorphia are very clear; the spots of oxydo- reduction cover all the walls of the profile in this section. The grey overrides oxidation. The material is plastic and sticking and one observes many roots generally of a medium size.

Horizon 4: 120-185 cm. The material is dry and very compact; it has a CL texture and a matte yellowish brown colour. The material is plastic and sticking. The spots of oxydo reduction are numerous, the impacts of some roots suggest that the dry and compact character of this material is seasonal and temporary. This profile is porous in the two horizons of surface and not very porous to far from porous in the subjacent horizons.
The drainage in this soil is bad; it's a hydromorphic soil with reduced gley.

Side drain of Minimana (right bank)



Caption

P1
Pedological pit 1

P2
Pedological pit 2 (P2)

P3
Pedological pit 3

Length of the arrow: 100 m

Figure 18 Distribution of soil units at Minimana

4.3 Chemical characteristics of the profiles

4.3.1 Organic matter, phosphorus, pH and microbial biomass

The actual values come from 3 soil profiles; they are presented in **Table 11.**

Table 11 Soils C, N, P content and pH in various profiles in Minimana

Profiles	Depth	N	C	C/N	P(total)	pH	pH
		($g\ kg^{-1}$)			($mg\ kg^{-1}$)	(H20)	(KCl)
Profile n°1 (P1)	0 - 20	0.61	4.52	7.38	259.17	5.2	5.0
	20 - 60	0.34	2.31	6.87	248.76	4.3	3.7
	60 - 110	0.24	1.64	6.73	231.23	4.9	4.2
	110 - 195	0.15	0.44	2.90	226.48	5.5	5.1
Profile n°2 (P2)	0 - 20	0.43	3.30	7.64	226.48	4.6	4.3
	20 - 40	0.34	1.77	5.25	213.65	4.6	3.7
	40 - 120	0.24	1.03	4.23	204.97	4.9	3.8
	120 - 200	0.15	0.52	3.42	205.89	5.0	4.4
Profile n°3 (P3)	0 - 25	0.71	5.80	8.16	212.20	4.1	3.8
	25 - 55	0.34	2.87	8.43	206.00	4.1	3.5
	55 - 120	0.25	1.78	7.21	202.95	4.5	3.6
	120 - 185	0.20	1.24	6.22	204.97	5.0	4.4

In the 3 profiles studied, the C content is low. That is valid even in the surface layers which receive the main part of the organic inputs where it varies from 3.30 to 5.80 g kg^{-1} Beyond 100 cm; the C content is two times smaller than that of the surface horizons. Similarly, low amounts of N were recorded in all three profiles where it is lower than 0.8 g kg^{-1} The distribution of N in the profiles is similar to that of C.

The C/N ratios are low and present very few variations in the first two horizons. They vary from 2.90 to 6.22 in the deepest horizons. The total P content varies between 200 and 260 mg kg^{-1}. The deepest horizons are less rich in phosphorus than those on the surface. This difference varies approximately from 3% to 15%.

The low pH values of the profiles indicate an acid soil in all the horizons. The pHs of the major horizons are higher than those of the horizons which precede them. For the pH (H2O), this difference varies from 0.3 to 0.9 unit pH, which is considerable.
The pH (H2O) is not much higher than the pH KCl, which is logical because there is very little exchangeable aluminium in these soils (< 0.001 cmol kg^{-1} according to Ndiaye (1987)

The microbial biomass is low; it varies from 89.07 to 121.27 mg kg^{-1} and it is higher in the horizons of surface than in those of depths (Figure 19).

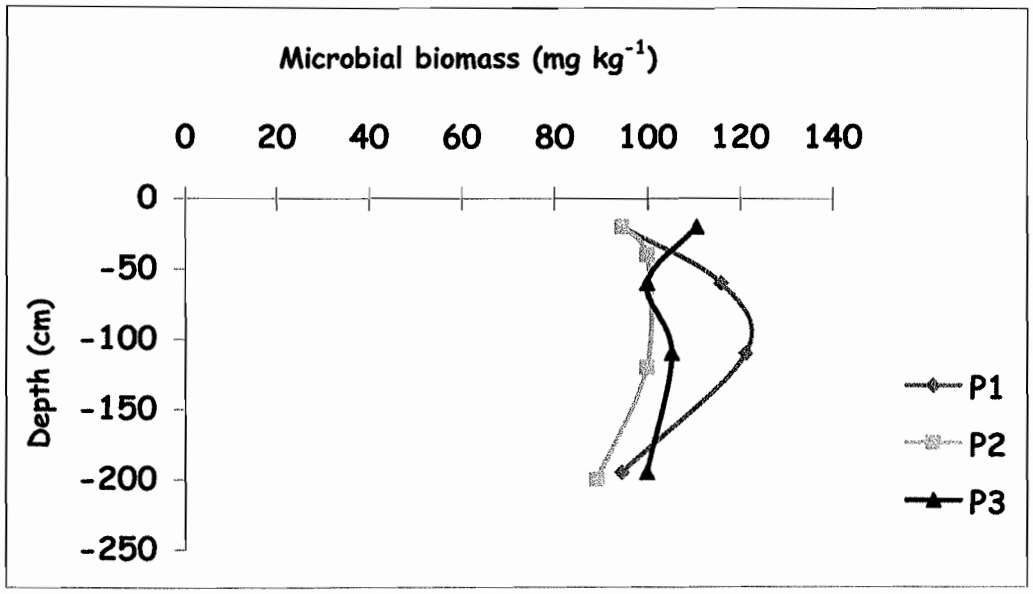


Figure 19 Variation in microbial biomass with depth at Minimana

4.3.2 Exchangeable cations

4.3.2.1. Calcium

On the 3 profiles, the exchangeable Ca content is variable at the surface horizons are not distinguished in a clear way in the 2 horizons of surface (Figure 20). However the content at depth was at least double that at the surface.

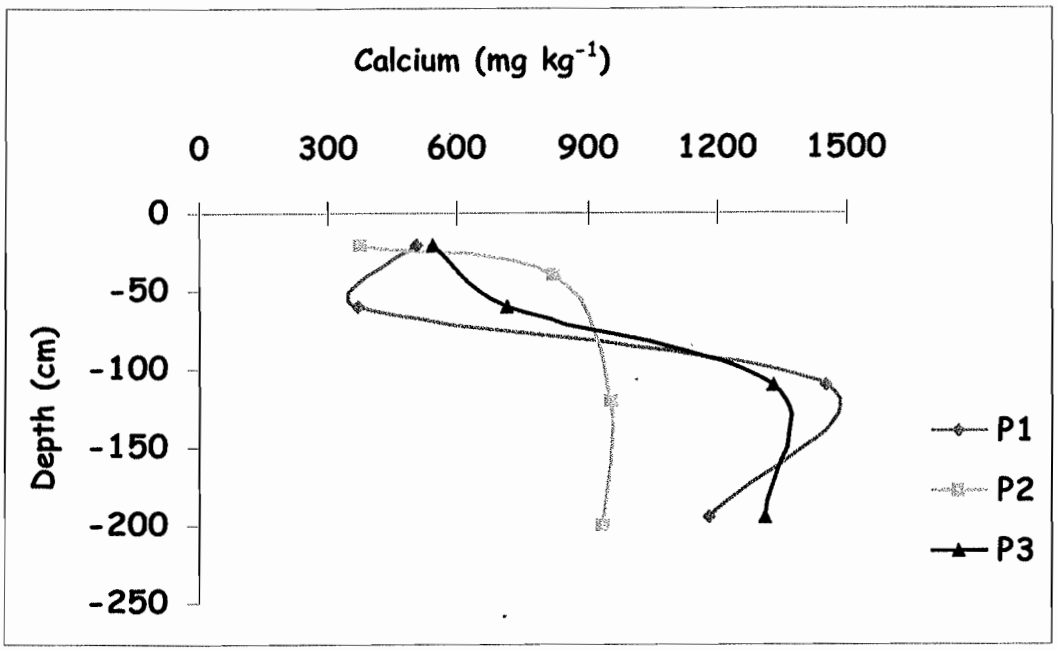


Figure 20 Variation of calcium content according to soil depth in 3 profiles in Minimana

4.3.2.2 Magnesium:

The magnesium content (Figure 21) varies between 70 and 400 mg kg $^{-1}$. Like calcium, the magnesium content at the surface is far lower than that at depth.

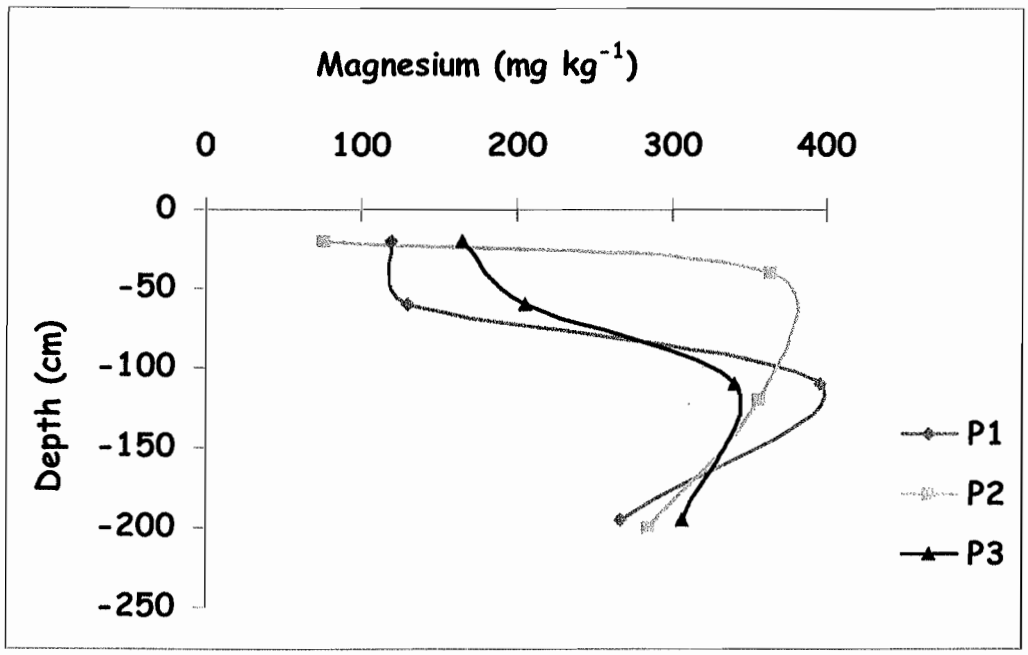


Figure 21 Variation of magnesium content according to soil depth in 3 profiles in Minimana

4.3.2.3 Potassium:

The exchangeable K contents are low at all depths (< 20 mg kg $^{-1}$), they vary between 6 and 16 mg and present a rather heterogeneous distribution in the surface layers of the profiles contrary to those at depth.

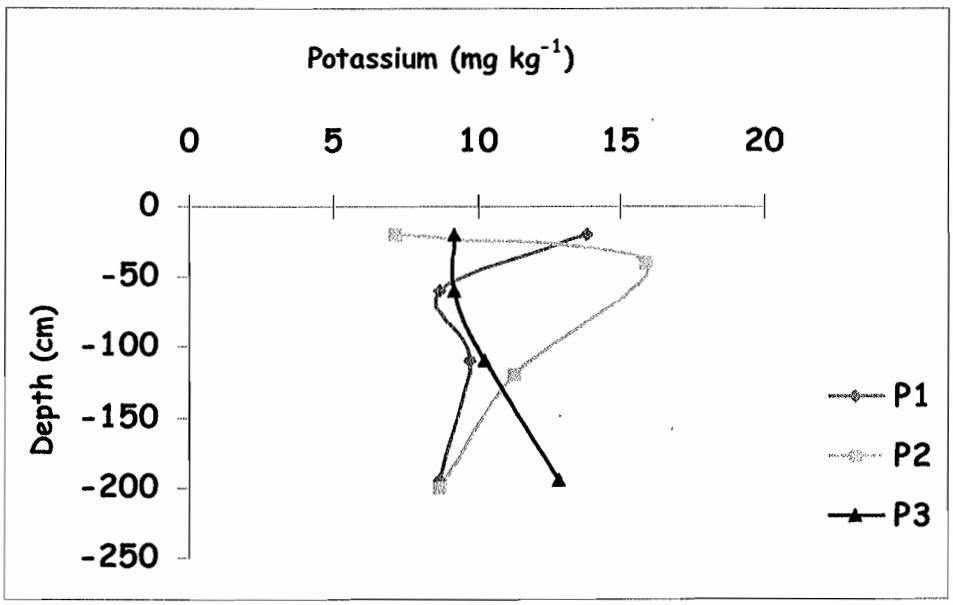


Figure 22 Variation of potassium content according to soil depth in 3 profiles in Minimana

4.3.2.4 Sodium:

The exchangeable sodium contents (Figure 23) vary between 155 and 700 mg kg $^{-1}$. Unlike calcium and magnesium, the amounts of sodium in the 2 surface horizons of higher than those at depth.

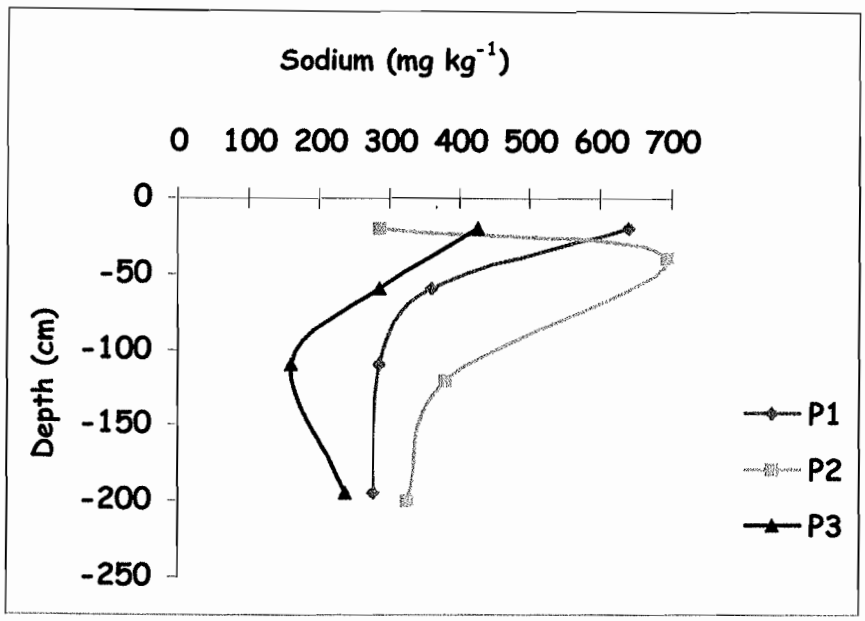


Figure 23 Variation in sodium content with soil depth in 3 profiles in Minimana

5- Discussion:

Examination of the profiles shows that the textures of these soils are loamy-sand on the surface horizons and increasingly clayey at depth with a presence of more or less tender ferruginous concretions. For these reasons these soils are classified as "ferrugineux tropicaux lessivés" (CPCS, 1967) or ultisols (Soil taxonomy, 1998).

The amounts of carbon and nitrogen are low as is typical in the highly weathered soils from tropical regions of Africa. This situation is in relation with the high rate of mineralisation of the organic matter especially at high temperatures and in rainy season when the soil moisture is sufficient (Scholes and Al, 1994; Pieri, 1989). This is confirmed by the low C/N ratio indicating mineralized soils with low organic matter along the profile. This remark is comforted by the low microbial biomass (an average of about 100 mg kg^{-1} both in surfaces and deeper horizons). In the deepest layers, the organic matter probably results from various iron-orgnanic matter complexes which are then drained by water through the pores create the activities of the soil fauna (termites and worms) and other interstices of the soil.

The low phosphorus contents are characteristic of the soils in Mali and have been previously reported by Poulain (1975). However with soil cultivation, fertilizer additions can correct this deficiency in the top-soil layers. That is useful because there is an interdependence between nitrogen, phosphorus and pH.

The relatively low contents of Ca and Mg on the soil surface and averages in depths and the low amount of K could be explained by the migration of Ca and and Mg to the bottom of the profile and furthermore by the nature of clays which are not only of kaolinitic nature but there are also some illite and smectite (Condom, 2000) which are a little richer in these elements. If the amounts of calcium are deficient in the top-soils layers, those of magnesium are adequate. On average, balance between the cations (Ca/Mg = 3) is optimal along all the profiles; it is the same as the Mg/K ratio = 16 for the horizons of surface. On the other hand the Mg/K ratio value > 25 indicates a very strong imbalance between this elements in the deeper layers. On average, the Na content is high especially on the surface than on the sub-soil layers although this element is very mobile. This presence of Na in the horizons of surface could be due to the contributions by water of irrigation. The accumulation of sodium involves a degradation of the conditions of soil

physics by dispersion of clays, loss of permeability, degradation of the structure (Duchaufour, 1997) but in our case the contents are much lower than the weakly saline (1150 mg k^{-1}) and saline (2300 mg k^{-1}) classifications.

6 Conclusions:

These soils are suited for agriculture in general but especially arboriculture in particular because of its depth. The chemical analyses also show that these soils have convenient properties for trees in this area.

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Problems

Our activities are really in progress but we are really in short of money, because we did not receive a payment from the EU in response to the first year's cost statement. We are really anxious about the future of our activities since we don't have means to deal with the project staff salaries, manual labours, security of our infrastructure and materials.

Visitors and presentations:

In addition to the visit of all partners during the annual meeting, the following visits, presentations and trainings have taken place.

Field Visits: Siribala's Town house representatives, women association and breeders association have visited the experimental site of Minimana.

The Deputy Director of IER, the World Bank representative in Mali and the Head of Sotuba Research Station has visited the screening trial in the nursery of Forestry research Programme.

The project and the results of the screening experiment have been presented in Niono during the Meeting between the Scientific Committee of IER and Research Result Users Committee.

Three students have done their field training on the project: Bourema Coulibaly and Mariam Coulibaly for BSc degree from IPR (High School from Mali); Lassina Niaré for Master degree from the University of Bamako (FAST). Another PhD student Penda Sissoko is planning to carry out her research in Siribala on the socio-economic aspect of the project.

Partner 3: University of Mali, UMALI. DB.LMB

Summary

Liaison with partners: ***Good collaboration has been established between IER, AAVNU and FAST for the implementation of field experimentation at Minimana irrigqtion site.***

Water, Soil and Plant Chemical Analyses. Analyses were performed in Mali by different laboratories. Soil chemical analysis were performed by the lab of IPR/IFRA at Katibougou. Water quality control is being conducted with the collaboration of « Laboratoire de la Qualité des Eaux » de la Direction Nationale de l'Hydraulique, Ministère des Mines et de l'Energie à Bamako.

Plant growth and management.. Two fields experimental design were set up at Minimana in order to compare selected tree species' growth and their management under irrigated conditions. Our main interest is **microsymbionts and N-fixing trees** studies using inoculant technology.

Pest monitoring and management. Nematodes and termite occurence is being investigated in the soil of site of Minimana before tree planting. Entomological studies (attacks by insects) are being undertaken in nursery and field under irrigated conditions.

Economics. Socio-economic studies on utilisation of wastewater for fuelwood and fodder production were performed following discussions between Mali and Niger.

Formal Training: ***Mr Fallaye KANTE officially registered for Ph. D degree at ISFRA in Mali, has returned to Mali, after a period of formal training at IRD in Senegal with Partner 6. He is implementing the main field experiments in irrigated conditions at Minimana.***

General objectives

To use microsymbionts (N-fixing bacteria and mycorrhizas) to fix nitrogen and sequester nutrients, hence reducing the needs for fertilizer inputs in the irrigated plantations.

Technical specific objectives

Tree growth and management (wp 2)

The first stage of the project is to screen nitrogen fixing trees in nursery conditions before carrying out experiments under irrigated field conditions.

In the second stage, under irrigated conditions we have:

- to compare the growth of selected tree species in nursery and under irrigated field conditions in Mali
- to do a quick screening of the trees already produced to make sure that the species which have been selected under irrigated field conditions are the best species

- To continue with the socioeconomics studies (wp 5)
- To analyse soil, water and plants for their nutrient contents and bacterial occurrence (wp 6)
- To investigate pests on the target tree species (wp 8)
- To train DC researchers in modern molecular microbiological methods

Results

Work Package 2: Tree growth and management.

2.1. Tree growth and management at Sotuba

Last year, studies of tree growth in nursery conditions at Sotuba station, showed that three species: *Gliricidia sepium, Leucaena leucocephala* and *Acacia angustissima* were the best species in terms of their growth. Hence, these species were selected for the field experiment under irrigated conditions at Minimana.

2.2. Tree growth and management under irrigated conditions

At the close of the project year, two field experiments were set up at Minimana in order to compare the growth of selected trees under irrigated field conditions in Mali. Experiment 1, outlined below, is the Mali component of the dispersed field experiment which was agreed at the 2004 annual meeting, described in the protocol in Annex 1.

Experiment 1. The Objective is to compare the growth of tree species in different countries under irrigated field conditions at Minimana (Mali).

The four tree species which were agreed at the meeting in Mali were used in this experiment (Table 12). *Gliricidia sepium, Leucaena leucocephala* and *Acacia angustissima* are leguminous and *Khaya senegalensis* is a non legume tree.

Table 12 Tree species and inoculant strains used in Experiments 1* and 2 at Minimana (Mali).

Tree species	Rhizobia/Mycorrhizas strains
*Leucaena leucocephala**	LdK4
*Gliricidia sepium**	GsK4
*Acacia angustissima**	13c
*Khaya senegalensis**	Mixed inoculum of mycorrhizas
Acacia crassicarpa, Acacia mangium, Acacia auriculiformis	13c + 11c
Calliandra calothyrsus	Kwn35
Acacia senegal	CIRADF 300 ; CIRADF301 ; CIRADF 302
Pterocarpus lucens	13c

Plant production in nursery at Minimana site

Production of those tree species in plastic bags (12 cm x 30cm x 15cm) is undertaken in nursery conditions. Experimental design is Complete Randomized Block. Five blocks were used and four treatments were applied: Control, Rhizobia (R), Mycorrhizas (M) and R+M Due to the size limitations of the field site, our field studies will focus on two treatments (+R+M) and control. About 150 plants of each species per treatment were produced in the nursery. To limit contamination with soil microbial strains in nursery all pots were placed on plastic sheeting.

Inoculation stage

Seeds of leguminous tree species were sown and inoculated with both specific rhizobial and mycorrhizal cultures. *Khaya senegalensis* was inoculated only with mycorrhizas.

Depending on seed size, two to three seeds were sown in the same hole for the largest seeds and three seeds were sown for the smallest seeds. For *Khaya senegalensis* four seeds were sown to ensure good germination. Two to four weeks after sowing (depending on the rate of germination) seedlings or young plants were inoculated (Figure 24). Each plant was inoculated at the root collar with 10ml of the appropriate rhizobial strain.

Figure 24 Applying inoculants to nursery plants for Experiment 1.

Experiments at Minimana

Experiment 1:

Date of sowing and inoculation of seeds: 10/12/ 2004

Date of inoculation of young plants : 25/12/2004

Experiment 2 :

Date of sowing and inoculation of seeds : 28/12/ 2004

Date of inoculation of young plants 2 6/01/2005

Experiment 2.

The objective is to conduct a quick screening of the trees that have already been produced, and compare their performance with the species used in experiment 1.

Two experiments: 2a and 2 b using exotic and indigenous species respectively will be tested under irrigated conditions. At this time all species were sown in plastic bags at Minimana.

Work Package 4: Microsymbionts and N-Fixation.

Experiments 1 and 2 listed above were used to fix nitrogen, sequester nutrients to determine the microsymbionts inoculum potential of irrigated soils.

In all experiments, selected leguminous trees are at seedling stage and inoculation has been done. Assessment of mycorrhizal and rhizobial infection before planting will done in experiment 1 and growth parameters will be measured after planting as indicated in Annex 1.

Work Package 5 : Economics. At this second step, data collection and processing cover two levels:

-description and analysis of wood and forage subsectors at the level of city like NIONO

-description and analysis of wood and forage availability and consumption at a sample of households in side the city.

Work Package 6 : Water, Soil and Plant chemical analysis.

Water samples were taken in three time on February 2004, May (2004) and December 2004. Water samples for February and May were sent to CEH for analysis.

For samples collected in December 2004, nutrients contents and bacteria occurrence are analysed with the collaboration of Laboratoire de la Qualité des Eaux (LQE) de la Direction Nationale de l'Hydraulique at Bamako (Table 13, Table 14). Main results are presented in Table 14. Soil chemical content are analysed by the laboratory of IPR/IFRA at Katibougou (Mali).

Table 13 Equipment used for chemical and nutrient analysis of irrigation water at Minimana. Lab of LQE (Bamako, Mali)

Assessments	Equipment used for water analysis
Nitrites	Spectrophotomètre- Dr/2010-HACH
Nitrates	
Fer	
Azote Ammoniacal	
Phosphates	
Chlorures	
Sulfates	
Conductivité	Conductimètre wtw. LF 521
pH	MicropHmetre 2001 Crison
Dureté , Ca^{++} , Mg^{++}	Agitateur magnétique, Titreur digital
Sodium, Potassium Na^{+}, K^{+}	Dataloging Flame Spectrophotometer
DCO	COD reactor model : 45600, Spectrophotomètre-Dr/2010-HACH
DBO5	DBOmetre : wtw. Oxytop^{R}Box
-----bacteriological analysis-----	Rame filtrante

Table 14 Results of analysis of samples originating from irrigation water at Minimana on 28/12/2004.

Analyses physico-chimiques			
Water samples	nutrients	Results	Comments
1*	Nitrate NO_3^-	0.40 mg/l	*Could be used without negative effect*
2#		1.00 mg/l	
3~		0.00 mg/l	
1-	Nitrite NO_2-	0.002 mg/l	
2-		0.000 mg/l	---//--
3-		0.001 mg/l	
1-	Fer Fe^{2+}	0.458 mg/l	
2-		0.25 mg/l	---//--
3-		0.124 mg/l	
1-	Azote ammoniacal NH_4^+	0.35 mg/l	
2-		0.53 mg/l	---//--
3-		0.48 mg/l	
1	Phosphates PO^{3-}_4	0.22 mg/l	
2-		0.47 mg/l	---//--
3-		0.28 mg/l	
1-	DCO	0 mg/l	
2-		0 mg/l	---//--
3-		1.00 mg/l	
1-	Demande Biochimique en	0 mg/l	

2-	Oxygène DBO5	0 mg/l	---//--
3-		0.25 mg/l	
1-	Sodium Na^+	3.43 mg/l	
2-		3.81 mg/l	---//--
3-		4.61 mg/l	
1-	Potassium K^+	1.23 mg/l	
2-		2.55 mg/l	---//--
3-		4.02 mg/l	
1-	Conductivité	54 µS/cm	
2-		51 µS/cm	---//--
3-		199 µS/cm	
1-	pH	6.8	
2-		5.64	
3-		5.83	
1-	Sulfates	0 mg/l	
2-		0 mg/l	---//--
3-		0.25 mg/l	
1-	Calcium Ca^{2+}	3.4 mg/l	
2-		3.76 mg/l	---//--
3-		3.1 4 mg/l	
1-	Magnesium Mg^{2+}	1.84 mg/l	
2-		1.60 mg/l	---//--
3-		1.01 mg/l	
1-	Chlorure Cl^-	1.0 mg/l	
2-		1.4 mg/l	---//--
3-		1.3 mg/l	
	Bacteriological analysis		
1-	*Total Coliforms /100ml*	1100 + 14 00CNI	*Mauvaise qualité bactériologique*
2-		400 + 1600 CNI	
3-		1400+500CNI	
1-	*Coliformes fécaux /100ml*	600	---//--
2-		1100	
3-		100	

* Water used for plant irrigation: experiment 1 and 2 (new channel of Minimana)
# Water from rice field (old channel)
~ Water from old channel with Typhae

Work Package 8: Pest monitoring and Management: Nematodes, termites occurency were investigated in the soil of site of Minimana before tree planting. Entomology studies (attacks by insects) are undertaken in nursery and filed under irrigated conditions.

Formal Training: Mr Fallaye KANTE registered for Ph. D degree at FAST/ISFRA University of Bamako in Mali is now following up the field experiments set up at Minimana.

Problems

Our colleague B. DIOP, from the Department of Chemistry at FAST who is charged for Water, Plants and Soil nutrients contents analysis is not available to carry out those studies in his laboratory We will do those analysis with the collaboration of another laboratory.

We are faced with money availability. The money did not arrive at time. This caused us many problems with our staff and administration officers.

Partner 4: INERA, Burkina Faso

Wp 1. Water treatment and irrigation

Experimental site acquisition

At the beginning of the second year of the project (January 2004), a consultation was held between INERA and its local partners, particularly the University of Ouagadougou via LPCE laboratory, in order to formalise the concession of the experimental site for UBENEFIT project in the area of the University. This process was concluded by formal authorisation of the University through an official letter sent to INERA allowing the implementation of the activities of the project on a site commonly agreed. On the official authorisation of the University, it is stated that:

1. The experimental site remains the property of the University of Ouagadougou re-presented by the 'Laboratoire de Physique et de Chimie de l'Environnement' (hereafter LPCE) directed by Dr. Koulidiati Jean.

2. LPCE authorizes INERA (our team) to install a wastewater treatment system and to conduct the research activities of UBENEFIT project during the lifespan of the project.

3. The two collaborators (INERA and the University) can continue their collaboration on the experimental site of UBENEFIT project after the end of the project.

Complementary characterisation of the experimental site

After obtaining the site, soil profiles description was undertaken by INERA following the recommendations of the SPC team. The data was sent to SPC to assist in the design of the wastewater purification system. Samples of wastewater were also sent to CEH for heavy metal analyses.

Installation of the wastewater purification system

The construction of the station for the wastewater treatment as such has been delayed due to technical and administrative difficulties. Technically it has been laborious to select the suitable wastewater facilities to be installed. Finally, a plan was agreed (Figure 25) and sent the University administration for its approval. At the administrative level, the procedures were exceptionally long: two competitive bids for wastewater treatment systems were not conclusive. Thus a special authorization was required from the administration to select one company among the companies that applied though some modifications on his offer were necessary for the appropriate wastewater treatment system at a reasonable cost. At the moment, the administrative procedures are being finalized to allow the installation of the wastewater treatment facilities as soon as possible.

Wp 2. Tree growth and management

Preliminary Experiments

One of the recommendations of the meeting of Niamey (launching of UBENEFIT project) was that developing country partners should conduct preliminary irrigation trials in order to screen the potential species and select candidate species for the main

experiments using the treated wastewater. Thus INERA carried out irrigation and inoculation experiments with 20 local and introduced species. One experiment dealt with the response to irrigation of the species while a second evaluated the combined effect of both irrigation and inoculation. In both studies, seeds were pre-treated following the recommendations of the suppliers. After germination, the seedlings were transplanted in pots containing 2 kg of sand and abundantly watered using well water. An excess of water was applied daily, and pots were allowed to drain. Growth and nodulation data were analysed using GLM ($P < .05$) procedures followed by the comparison of the means using Duncan or Scheffe tests with SAS Software package (SAS institut, inc.). The main results of these experiments were presented at the annual meeting of the project held at Bamako (Mali) in 2004.

Experiment 1 Potential growth in irrigated conditions of species for fodder and wood production

The experiment had 20 species considered as 20 treatments and each treatment was composed of 20 plants. The design was a complete randomised block and the measurements (height and number of leaves) were done monthly. Table 15 presents the height and the number of leaves after three months of irrigation. The four species comprising *Gliricidia sepium*, *Leucaena spp.* and *Afzelia africana* displayed a similar height growth that was significantly superior to the growth of the rest the species ($P < .001$). However, *G. sepium* and *A. africana* appeared to be the most vigorous probably due to their higher number of leaves at this stage ($p < .001$; Table 15). In general, acacias and *Pterocarpus* sp. had the slowest growth and therefore these species might benefit from more management care (inoculation for example) to accelerate their juvenile growth.

Figure 25 DESIGN OF THE STORAGE, TREATMENT AND IRRIGATION SYSTEM USING THE WASTEWATER

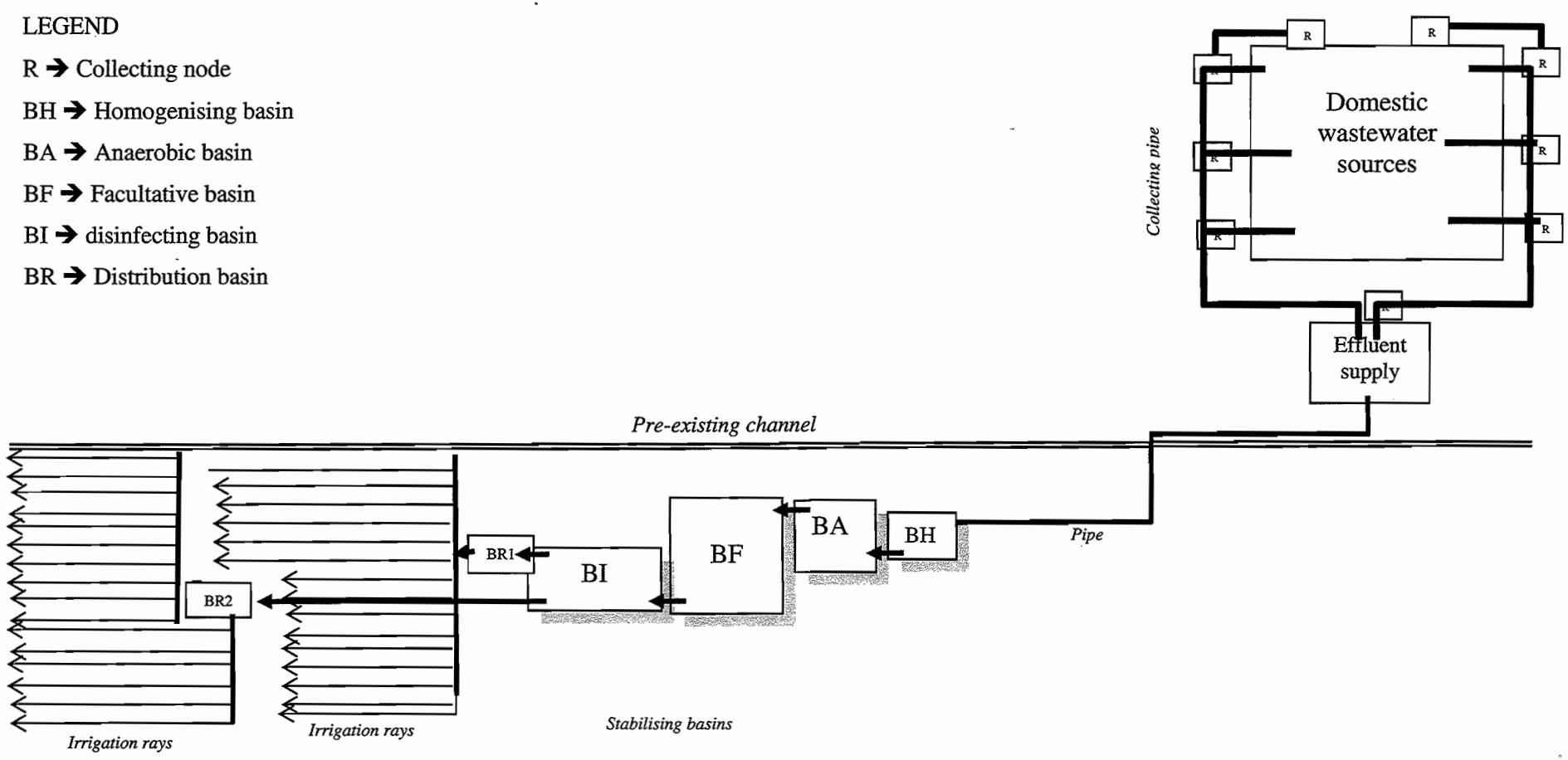


Table 15 Growth of woody species irrigated with well water

No.	Species	n	Height (mean ±SE) (cm)	No. of leaves /plant
1	*Senna siamea*	20	6.3 ±0.2 d-g	6.6 ±0.3 bc
2	*Gliricidia sepium*	20	12.5 ±0.6 ab	7.9 ±0.6 ab
3	*Leucaena leucocephala*	20	13.6 ±0.7 a	6.1 ±0.5 b-e
4	*leucaena sp. (local)*	20	11.9 ±0.4 abc	6.2 ±0.5 b-e
5	*Acacia angustissima*	20	5.9 ±0.3 efg	3.4 ±0.2 d-g
6	*Albizia lebbeck*	20	8.9 ±0.4 c-f	2.8 ±0.2 g
7	*Leucaena hybrid (L x L)*	20	11.9 ± 0.5 abc	6.4 ±0.6 bcd
8	*Leucaena diversifolia*	20	9.1 ±0.5 b-e	3.8 ±0.4 c-g
9	*Ziziphus mauritiana*	20	4.7 ±0.3 g	6.4 ±0.5 bcd
10	*Pterocarpus erinaceus*	20	3.8 ±0.3 g	3.1 ±0.2 e-f
11	*Acacia mangium*	20	5.6 ±0.3 fg	2.3 ±0.2 g
12	*Antada africana*	20	9.7 ±0.4 bcd	2.1 ±0.1 g
13	*Calliandra calothyrsus*	17	6.6 ±0.3 d-g	5.1 ±0.3 b-g
14	*Pterocarpus lucens*	20	4.1 ±0.2 g	2.9 ±0.3 fg
15	*Acacia auriculiformis*	20	5.3 ±0.2 g	4.2 ±0.3 c-g
16	*Acacia crassicarpa*	20	6.1 ±0.3 efg	4.9 ±0.4 b-g
17	*Afzelia africana*	20	13.5 ±0.8 a	10.8 ±0.9 a
18	*Khaya senegalensis*	15	4.7 ±0.7 g	2.9 ±0.2 efg
19	*Kigelia africana*	20	3.2 ±0.2 g	6.5 ±0.4 bcd
20	*Eucalptus camaldulensis*	20	3.9 ±0.2 g	6.2 ±0.2 b-e

Experiment 2 Response of local fodder species to irrigation and mycorrhizal inoculation

This experiment was conducted with material which was locally available (arbuscular mycorrhizal inoculum, fuel and fodder species). At this time, Rhizobial inoculants and exotic species were still to come from IRD and Agroforester company. The treatments were the combinations of two factors with the species factor composed of six species and the inoculation factor with two treatments (inoculated and non inoculated with arbuscular mycorrhizas). The mycorrhizal inoculant was *Glomus intraradices* strain IR14, provided by IRD/Dakar. The inoculum consisted of soil containing spores, hyphae and infected roots from pot cultures of *Sorghum bicolor.* Approximately 10 g was applied to each inoculated seedling.

Ten plants were allocated to each treatment and four months after planting, height, diameter, biomass and nodulation were determined (Table 16). Significant differences were revealed among species for both the height and the diameter parameters ($p < .001$) with Prosopis, Khaya and Afzelia performing better compared to the rest. In turn, Pterocarpus displaying the lowest values for the two growth parameters. In general, mycorrhization exerted a beneficial effect on diameter and biomass production of some species (Khaya), unfavorable effects in other cases (Prosopis, Senna, Pterocarpus), and neutral impacts for Afzelia, which is a typical ectomycorrhizal species. However,

the growth performance is not entirely representative of inoculation effects due to the fact that roots of some individuals got through the pots and penetrated in the soil underneath. Such behaviour is typical of local species, many of which displayed a long tap root that usually pierced the pots.

Table 16 Reponses of local fodder species to mycorrhizal inoculation

Species	Myco	N	Height (cm)	Diameter (mm)	Shoot (g)	Root (g)	Nodules Number	Nodules DW (mg)
Afzelia	-	10	20± 2.2	4.1± 0.3	1± 0.1	0.6± 0.2		
Afzelia	+	10	18.7± 2.2	4.7± 0.2	1.1± 0.2	0.7± 0.1		
Khaya	-	10	16.2± 0.6	4.3± 0.1	0.7± 0.1	0.3± 0		
Khaya	+	10	14.2± 0.7	5.1± 0.4	1± 0.2	0.4± 0.1		
Prosopis	-	10	31.6± 2.3	2.5± 0.2	1± 0.1	0.3± .0	8.7± 1.8	58± 9
Prosopis	+	10	29.8± 4.8	2± 0.2	0.4± 0.1	0.1± .0	2.7± 0.9	30± 13
Senna	-	10	17.8± 1.5	2.8± 0.3	1± 0.3	0.2± .0		
Senna	+	10	12.4± 1	2.2± 0.1	0.4± 0.1	0.1± .0		
Ziziphus	-	10	14.8± 2.3	2.5± 0.3	0.4± 0.1	0.3± .0		
Ziziphus	+	10	15.6± 1.6	2.6± 0.3	0.3± 0	0.3± .0		
P. lucens	-	10	12.3± 1.7	2.9± 0.2	0.2± 0	0.1± .0	0.9± 0.5	3± 1.8
P. lucens	+	10	8.4± 0.7	2.7± 0.2	0.1± 0	0.1± .0	0.4± 0.4	3± 2.8
ANOVA		DI						
Species (S)		5	***	***	***	***		
Myco (M)		1	ns	ns	*	Ns		
S*M		5	ns	*	*	Ns		

Experiment 3: Response of leguminous fuel and fodder species to double inoculation in irrigated conditions

This experiment was conducted with 10 local and exotic N-fixing species with a potential for producing either fuel or fodder or both (**Figure 26** and **Table 17**). To each species was applied the following treatments:

1) Control = non inoculated plants;
2) Plants inoculated with endomycorrhizas alone
3) Plant inoculated with endomycorrhizas and Rhizobium
4) Plant inoculated with appropriate Rhizobium (according to the recommendations of IRD)

Each experimental treatment was composed of 10 plants.

Acacia species were inoculated with two rhizobial strains, applied separately (11c and 13c), provided by IRD Dakar. Preliminary data analysis showed that both strains had similar effects on plant growth, and data presented here are means of the two strains. Other species were also inoculated with strains from IRD as follows: Gliricidia – strain GSK4, Calliandra – strain KWN35, Leucaena – strain LDK4. The mycorrhizal inoculant used was the same as in experiment 2.

After four months of experimentation, eight species still had all the experimental treatments represented. Thus the statistical analysis (ANOVA) was conducted using only these species. The two remaining species were left out of the analysis due to their low survival rates (**Table 17**). Significant differences were revealed for all the parameters. On average, acacias performed poorly with similar growth whereas Gliricidia showed the best performance. Applying either Rhizobium or endomycorrhizas improved plant growth while double inoculated plants performed poorly ($p<.001$). Some species like *Gliricidia*, *Leucaena diversifolia* and *L.* hybrid, responded better to mycorrhizal inoculation alone or to inoculation with Rhizobium alone. Growth of *L. leucocephala* and acacias was better without inoculation. However the response to the double inoculation is more important than that of the single inoculation with either Rhizobium or mycorrhizas. Inoculants had not been pre-tested and differences in

Figure 26 Growth of N-fixing exotic species inoculated or not with Rhizobium and arbuscular mycorrhizas (MVA) 1 *Acacia auriculiformis*; 2 *A. crassicarpa*, 3 *Gliricidia sepium* ; 4 *Albizia lebbeck* ; 5 *Leucaena*. local 6 *Leucaena diversifolia*; 7 L. hybrid 'LxL) ; 8 *L. leucocephala*

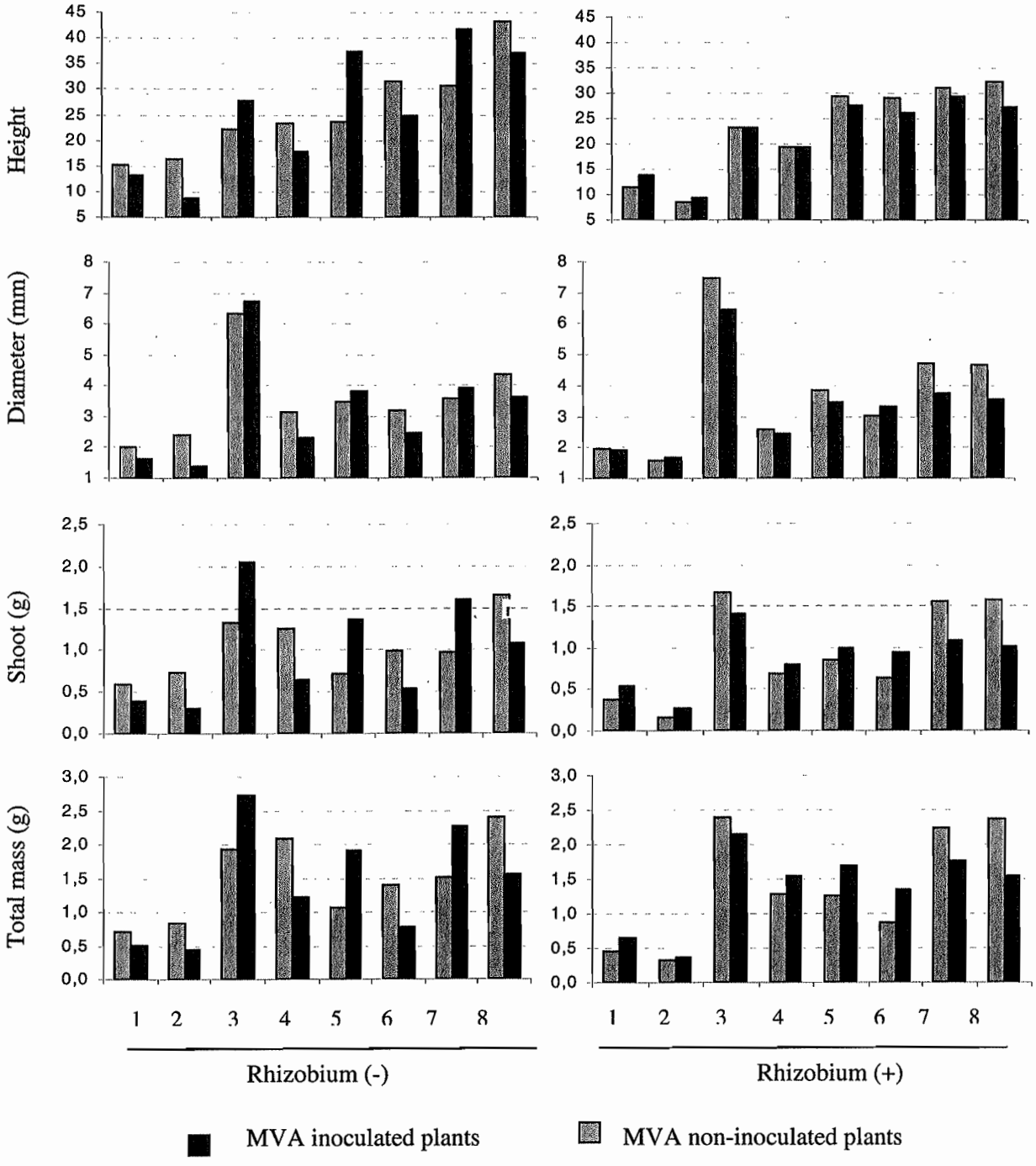


Table 17 Growth characteristics of *Calliandra* and *A. angustissima* Inoculated with Rhizobium and arbuscular mycorrhizas

Species	Myco	Rhiz	N	Height (cm)	Diameter (mm)	Shoot (g)	Root (g)	Nodules	
								Number	DW (mg)
Calliandra	0	1	3	8.9 ± 0.5	2.1 ± 0.2	0.1 ± 0.0	0.1 ± 0.0	3.0 ± 3.0	0.5 ± 0.5
Calliandra	1	1	11	9.8 ± 1.2	2.3 ± 0.2	0.4 ± 0.1	0.2 ± 0.0	4.5 ± 1.6	6.0 ± 2.1
A. angustissima	0	0	10	26.9 ± 2.4	2.9 ± 0.2	0.6 ± 0.1	0.2 ± 0.0	8.6 ± 2.4	14.6 ± 3.8
A. angustissima	1	0	10	18.2 ± 1.7	2.1 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	3.7 ± 1.7	12.6 ± 4.7

Wp 4. Microsymbionts and N-fixation

After the meeting of Bamako as well as for the previous year, INERA was in charge of producing the mycorrhizal inoculant to be used for the experiments of all the partners. It was suggested during the meeting of Bamako to use mixed inoculants, which compared to a single strain inoculant, has better potential to be adapted to flooded sites. IER (Mali) having such mixture of inoculants, it was commonly decided by partners from south to use the inoculant available in Mali. Therefore IER provided the inoculant that was multiplied by INERA for all the partners.

Wp 5. Economics and produce

INERA is contributing to the elaboration of questionnaire for the socio-economic studies in collaboration with the team of Niger in charge of this task.

Wp 8. Pest monitoring and management

A phytopathologist has been associated to our team to monitor the sanitary status of the plants irrigated with the treated wastewater. The implementation of this activity will start when the water treatment facilities will be installed.

Other activities

- INERA team participated (2 participants) to the second annual meeting of UBENEFIT project held in Bamako from the 3 au 8 May 2004
- INERA team advertised UBENEFIT project through its participation to forums organized locally in Ouagadougou like:

1. FRSIT (Forum National de la Recherche Scientifique et de l'Innovation Technologique) from 29 May to 5 June 2004. At this occasion an interview was given by the technician of the project in local well-known journal.
2. Forum on" Assainissement communautaire,hygiène et systèmes d'eau pour la lutte contre la pauvreté" of CREPA from 6 to 10 December 2004

Perspectives

- INERA's team is working hard to the complete installation of the wastewater purification system needed to continue the rest of the activities of the project;
- Nursery activities will start soon for the main trials according to what was commonly agreed;
- A film is in preparation to serve for future advertising activities following the success of our participation to forums as mentioned above (FRSIT and CREPA forum);
- Our team is pleased to be the organiser of the next annual meeting of the UBENEFIT project to be held in 2005 in Ouagadougou in Burkina Faso.

Partner 5: Universite Abdou Moumouni

INTRODUCTION

During this second year of the UBENEFIT Project the construction of the station for water purification has finished. Plants of different species were also grown in nursery in order to study the effect of inoculation (with rhizobia, mycorrhiza, or rhizobia + mycorrhiza) and type of water irrigation (tap or waste) on plant growth. Thereafter, these plants were transferred to field conditions. A literature review on fuel wood production in Niger was done in order to develop some guidelines for the socio economics questionnaire. This questionnaire elaborated by Mrs Germaine Ibro was improved at the second coordination meeting held in Bamako. However some of the planned activities during this second year were not realized because of the delay in receiving the second payment from the EEC.

I. WATER IRRIGATION AND TREATMENT

The construction of the station for water purification, which was began at the end of the first year of the project, has now finished. The prefabricated tarpaulin, necessary to keep purified water in the settling basin is installed. However although it has been completed, this station cannot be used because of the lack of irrigation equipment.

In order to compare the composition of waste and tap water in nutrients, samples were taken for analysis. In addition sample of waste water will also be analyzed for heavy metals at CEH.

II. TREE GROWTH, MANAGEMENT, AND MICROSYMBIONTS

2.1. Material and method

2.1.1. Plant inoculation and irrigation

Plants of local species (*Acacia nilotica, A. raddiana, A. seyal, Bauhinia rufescens, Leucaena leucocephala and Piliostigma reticulatum*) and Australian species (*A. anguistissima, A. auriculiformis, A. crassicarpa, A. mangium, Calliandra calothyrsus, Gliricidia sepium*) where grown in nursery from October 2003 to March 2004. The effect of two factors on plant growth were studied (See report of the first year for details) :

- type of water for irrigation : waste water or tap water.
- type of inoculation : rhizobia, mycorhiza, rhizobia + mycorhiza, control.

The experiment design was a split plot with the type of water in the main plot.

2.1.2. Plant measurements

During plant growth, the following measurements were done at weekly interval using ten individual plants per specie and per treatment : diameter of stem (at the base of the plant), plant height, number of leaves of the plant, number of ramifications of the plant. On March 10, 2004, corresponding to 4 months after planting, twelve (12) plants per species and per treatment were harvested. Each individual harvested plant was separated into different organs: shoot, roots and nodules. Plant organs were dried then weighed.

The remaining plants from the nursery were planted in the field according to five plants per species and per treatment for sap flow measurements.

2.1.3. Microsymbionts and N-fixation

Samples of roots and nodules were collected from each individual harvested plant for following determination :
- the level of mycorrhization in roots;
- the PCR/RFLP characterization in nodules;
- ELISA test in inoculated plants with specific rhizobia.
(see partner 6 report, page 84)

2.2. Results and discussion

2.2.1. Effect of type of water on plant growth

At early stage of plant growth (80 days after planting), the type of water for irrigation influenced significantly (P=0.05) the diameter of the stem, plant height (Table 18) and number of leaves (Table 19) for all species except *A. nilotica*, *A. seyal* and *Bauhinia rufescens*. Therefore plant watered with waste water had better development than those watered with tap water. For the three previous species, the effect of type of water was not significant on plant growth.

Table 18 Diameter of the stem (cm) and plant height (cm) for different tree species as influenced by type of water for irrigation and type of inoculation, 80 days after planting.

	Diameter of the stem (cm)						Plant height (cm)					
	Type of water		Type of inoculation				Type of water		Type of inoculation			
Genotypes	Waste	Tap	C	-M+R	+M-R	+R+M	Waste	Tap	C	-M+R	+M-R	+R+M
A. crassicarpa	2,137a	1,870b[1]	2,265a	1,915bc	1,700c	2,135ab[1]	13,25a	11,66b	15,53a	10,50c	10,60c	13,20b
A. mangium	2,542a	2,350b	2,530ab	2,305bc	2,205c	2,745a	16,70a	14,66b	16,12a	16,00a	13,57b	17,02a
A. auriculiformis	2,745a	2,417b	2,565	2,500	2,650	2,61	18,26	17,44	17,88	18,23	19,02	16,88
Leucaena leucocephala	4,945a	4,415b	4,370b	5,140a	4,035b	5,175a	21,21a	18,36b	17,73b	23,18a	15,80b	22,45a
Gliricidia sepium	6,47a	5,900b	6,190	5,955	6,090	6,51	15,86	15,66	16,77a	16,95a	14,50b	14,82b
A. angustissima	1,834a	1,449b	1,734a	1,348b	1,782a	1,702a	9,12a	7,20b	8,48a	6,37b	8,31a	9,48a
A. nilotica	5,105	5,152[2]	4,915b	4,875b	5,235ab	5,490a	26,99a	22,91b	24,27	24,50	25,38	25,65
A. seyal	3,872	4,025	4,015	3,955	3,915	3,91	31,85a	28,44b	27,38b	29,75b	28,53b	34,92a
A. raddiana	3,942a	3,292b	4,020a	3,590b	3,600b	3,260b	26,68a	18,74b	26,60a	23,18a	22,65a	18,40b
Piliostigma reticulatum	2,723a	2,450b	2,800a	2,400b	2,370b	2,775a	12,91a	10,69b	13,38a	9,98b	9,90b	13,95a
Bauhinia rufescens	3,230	3,300	3,220	3,170	3,275	3,395	19,30b	21,71a	20,83	18,80	20,05	22,35

[1] : For each specie data of the type of water or inoculation which has the same letter are not significantly different at 0,05 probability level.

[2] : not significant

C=control, M=mycorrhiza, R=rhizobia

For *B. rufescens*, waste water decreased the plant height and number of leaves at early stage of growth compared to tap water (Table 18). At later stage of plant growth (4 months after planting) the effect of the type of water varied according to the species and the plant organs (Table 19; Table 20; Table 21). Therefore waste water decreased significantly root dry weight for *A. auriculiformis, A. nilotica, P. reticulatum and Bauhinia rufescens* (Table 21). For the remaining species, the effect of the type of water for irrigation was not significant. On the other hand, the irrigation with waste water decreased dramatically the nodules dry weight for all species except *B. rufescens* and

P. reticulatum which do not nodulate. The effect of type of water on shoot dry weight varied according to the species. Waste water decreased significantly the shoot dry weight for *A. nilotica* and *A. seyal*, but increased shoot dry weight for *A. mangium, L. leucocephala, G. sepium* and *A. raddiana*. For the remaining species, there was no significant difference between the shoot dry weight of plant watered with tap or waste water.

The increase of plant development by waste water should be attributed to its high nutrient content compared to tap water. Therefore waste water should have more nutrients such as nitrogen resulting from mineralization of organic matter coming from domestic waster of the university campus. The presence of nitrogen in the irrigation water may have caused the reduced nodulation of the plants.

Table 19 **Number of plant leaves of different tree species as influenced by type of water for irrigation and type of inoculation, 80 days after planting.**

	Number of leaves					
	Type of water		Type of inoculation			
Genotypes	Waste	Tap	C	-M+R	+M-R	+R+M
A. crassicarpa	10,25	9,87	10,15	9,95	9,34	10,80
A. mangium	11,88a	9,88b	10,15b	10,55b	10,70b	12,10a
A. auriculiformis	16,34a	11,80b	12,83b	13,10b	16,35a	14,00ab
Leucaena leucocephala	14,98	13,38	14,15	14,30	13,75	14,50
Gliricidia sepium	8,80	7,72	8,30	8,55	8,00	8,20
A. angustissima	11,00	9,72	10,24b	8,67b	9,27b	13,25a
A. nilotica	87,68a	82,50b	88,60a	82,80b	84,20b	84,75b
A. seyal	37,00	37,47	34,55b	37,60ab	36,94ab	39,85a
A. raddiana	43,55a	41,50b	43,65	42,75	42,15	41,55
Piliostigma reticulatum	9,22a	8,05b	10,30a	7,40b	7,9b	8,95ab
Bauhinia rufescens	23,35b	25,90a	25,60a	24,75a	22,60b	25,55a

Table 20 Diameter of the stem (cm) and plant height (cm) for different tree species as influenced by type of water for irrigation and type of inoculation, 4 months after planting.

	Diameter of the stem (cm)						Plant height (cm)					
	Type of water		Type of inoculation				Type of water		Type of inoculation			
Genotypes	Waste	Tap	C	-M+R	+M-R	+R+M	Waste	Tap	C	-M+R	+M-R	+R+M
A. crassicarpa	3,940	3,799	4,304a	3,863a	3,196b	4,115a	20,62	19,88	24,42a	18,44c	16,90c	21,26b
A. mangium	4,205	4,244	4,125	4,196	4,083	4,496	28,20	28,88	28,33	29,21	26,88	29,73
A. auriculiformis	4,010	3,929	3,763b	3,804b	4,154a	4,158a	30,65b	33,23a	30,90	31,58	32,73	32,54
Leucaena leucocephala	6,440a	5,990b	5,833b	6,467a	5,792b	6,767a	29,17	26,65	25,38	28,19	29,04	29,02
Gliricidia sepium	8,730a	8,160b	8,030b	8,100b	8,480ab	9,160a	26,34	26,28	26,50	27,21	26,67	24,88
A. angustissima	3,340a	2,720b	3,170	2,950	2,620	3,390	21,27a	16,08b	17,33b	16,25b	17,54b	23,58a
A. nilotica	6,565	6,662	6,487bc	6,158c	6,817ab	6,992a	27,62	27,99	26,33	27,38	28,98	28,54
A. seyal	4,156b	4,427a	3,983b	4,279ab	4,408a	4,496a	36,24	35,61	31,83b	36,75a	36,42a	38,71a
A. raddiana	4,433	4,208	4,550a	4,012b	4,583a	4,137ab	31,14	30,94	32,71	29,04	31,62	30,77
Piliostigma reticulatum	4,263	4,396	4,229	4,225	4,288	4,575	35,60b	40,60	37,98	38,62	37,85	37,96
Bauhinia rufescens	5,060	4,690	4,340	4,490	4,820	5,840	16,93a	14,94b	16,29	15,19	15,00	17,25

2.2.2. Effect of type of inoculation on plant growth

For most of the species, the inoculation affected the plant growth even at early (Table 18,Table 19) or later stage (Table 20, Table 21). Moreover, the combined inoculation with rhizobia and mycorhiza was more effective in increasing plant development than single inoculation with rhizobia or mycorhiza. Therefore the double inoculation with rhizobia and mycorhiza provided more nutrients to the plants for their growth. Hence, the irrigation with waste water increased plant growth better than with tap water. However this effect was higher at the early stage of plant growth. At later stage, the difference of plant growth between the two types of water was lower. This can be attributed to limited condition of plant growth in the pot.

Table 21 Nodules and root and shoot dry weight of different tree species as influenced by type of water for irrigation and type of inoculation, 4 months after planting.

	Roots dry weight (g/plant)						Nodules dry weight (g/plant)						Shoots dry weight (g/plant)					
	Type of water		Type of inoculation				Type of water		Type of inoculation				Type of water		Type of inoculation			
Genotypes	Wast	Tap	C	-M+R	+M-R	+R+M	Wast	Tap	C	-M+R	+M-R	+R+M	Wast	Tap	C	-M+R	+M-R	+R+M
A. crassicarpa	0,702	0,775	0,942a	0,546b	0,525b	0,942a	84,0	119,0	150,0a	47,0b	39,0b	169,0a	2,41	2,15	3,07a	1,85b	1,48b	2,72a
A. mangium	0,646	0,752	0,842	0,675	0,604	0,675	28,2b	133,4a	15,9c	113,5b	25,1c	168,8a	3,35a	2,80b	3,01b	3,10ab	2,54b	3,66a
A. auriculiformis	0,746b	1,023a	0,848	0,779	0,896	1,008	74,0b	152,0a	136,0	122,0	78,0	116,0	3,41	3,20	3,09ab	2,83	3,68a	3,63a
Leucaena leucocephala	2,730	2,610	2,17c	2,860ab	2,44bc	3,220a	96,0	130,0	103,0	93,0	133,0	122,0	3,49a	2,99b	2,91b	3,48ab	2,86b	3,72a
Gliricidia sepium	1,885	1,842	1,717	1,671	1,875	2,192	146,0	216,0	201,0	220,0	164,0	139,0	3,325a	2,90b	3,03	3,10	3,22	3,10
A. angustissima	1,079	1,117	1,442a	0,750c	0,850bc	1,350ab	62,0b	238,0a	166,0	107,0	153,0	174,0	1,59	1,40	1,78a	0,99b	1,10b	2,12a
A. nilotica	1,073b	1,302a	1,054bc	1,004c	1,267ab	1,425a	9,7b	39,0a	25,9	16,7	14,8	39,9	3,92b	4,45a	3,39b	3,66b	4,23ab	4,93a
A. seyal	2,388	2,533	1,817b	2,438a	2,625a	2,962a	35,6b	120,9b	51,1b	114,6a	74,8ab	72,4ab	2,25b	2,79a	1,95b	2,51b	2,44b	3,17a
A. raddiana	1,235a	0,881	1,517a	0,937bc	1,117b	0,662c	35,6b	146,0a	77,5	71,7	124,0	90,1	3,09a	2,48b	3,06	2,58	3,04	2,47
Piliostigma reticulatum	1,35b	2,940a	1,86b	1,63b	1,20b	2,690a							1,59	1,66	1,64	1,58	1,40	1,88
Bauhinia rufescens	1,019b	1,671a	1,325	1,304	1,267	1,483							2,83	3,03	2,77	2,81	3,02	3,12

III. ECONOMICS AND QUALITY OF PRODUCE

Two types of activities were carried out for this work package during this second year of the project : i) a literature review on fuel wood production in Niger and ii) the finalization of the questionnaire.

3.1. Literature review on fuel wood production in Niger

3.1.1. Situation of fuel wood production in Niger

The area of Niger country is about 1,267,000 km². About 4/5 of this area is desert and many socio economics activities are limited by climatic constraints. Niger is dependant on other countries for its energies such as gas, electricity and fuel. Therefore wood is the main source of energy for more than 90% of households. Thereafter the quantity of required wood each year still very important contributing to destroy the forest. Moreover the forest resources are reduced by several factors : the satisfaction of the demand for fuel wood, the repetitive droughts and the use of lands for agriculture. Furthermore, the policy of reduction of the deforestation is very weak according to the situation.

Previous investigations of fuel wood utilization indicated that the consumption of wood varied according to several factors such as the zone (rural or urban) and the size of the family. Moreover a study conducted in 1989 classed the consumption of fuel wood in 3 categories according to the type of cities:
- big urban cities : 0.6 kg of fuel wood/person/day
- middle towns : 0.7 kg of fuel wood/person/day
- rural zone : 0.8 kg of fuel wood/person/day

Therefore the need of fuel wood was estimated in 1991 at about 1.8 million tons for rural populations and 512,784 tons for population of big cities and middle towns. This quantity represent respectively 78% and 22% of the total need of the country which is estimated at about 2,312,784 tons. In addition, the need for fuel wood has increased at the same rate of increase of the population (3.2% per year).

Another study conducted by the World Bank indicated a global need of 293 kg (1.23 ster) of fuel wood/person/year.

3.1.2. Evolution of forest policy in Niger

Four (4) periods can be distinguished in the evolution of Niger in policy forestry:

- ***From 1935 to 1960*** :

During this period a law was adopted on July 7, 1935 which fixed the forest system in West Africa. There was a classification of forest and the creation of parks with very limited authorization to cut wood. The main objective of this law was to conserve natural resources. There was no problem of fuel wood but species used for timber wood began to disappear around big cities such as Niamey, Zinder and Maradi. Fast growing species began to be planted around these cities.

- ***From 1960 to 1984*** :

There were very severe droughts during this period and the promotion of species such as *Eucalyptus* and *Dalbergia* for timber wood.

- ***From 1984 to 1992***:

The main facts of this period were:
- the emergence of fuel wood problems;
- "The Act of Maradi" which decided the plantation of community forests;
- The importance of agroforestry;
- the creation of forest cooperatives and the first laying out of natural forests by rural communities.

- ***From 1992 to 1999***:

This period was characterized by :
- aggravation of fuel wood problems mainly around big cities;
- the conception of the domestic energy strategy which promote the sustainable use of natural forest resources in order to assure the satisfaction of population in fuel wood.

3.1.3. Supply of urban centres in wood

Before 1984, there was no veritable preoccupation in the fuel wood supply for Niger country. The need of population was satisfy from dead wood. The supply of big centres was only assured through wood commercialization. In order to solve the increasing problem of fuel wood, Niger developed the creation of forest cooperatives and the domestic energy strategy. From 1984 to 1992, there was an increase of the urbanization and its corollary, commercialization in fuel wood. For example, the quantity of wood for energy wood was estimated at about 133,000 tons per year in 1990 for Niamey while it was about 110,000 tons in 1983.

Two ways of wood supply were distinguished :
- the auto supply in rural areas and secondary towns generally by women and children. The dead wood and wood from fields were mainly removed for this supply. This type of supply was the major system of wood supply of the country and represented about 2.4 millions of tons of wood in 2000. Its impact on natural forest resources is weak because it is using a crop residue.
- the commercial system of wood supply in urban zones which include several actors such as the woodcutters, the local wood sellers, the wholesaler, the dealer and the households.

3.1.4. Wood exploitation

The exploitation of forest resources is anarchic because of the lack of control of the whole system : location of wood exploitation, quantity of wood which is cut, forestry developer etc. The wood cutter has only to pay a small forest tax which allowed them to go any where to cut wood. Therefore the exploitation of forest resources was essentially guided by economic income. The main consequence of this system was the highest exploitation of forest from the peri urban zones.

Wood is transported to towns mainly by cars (about 66 to 88%), animals (camels, donkeys and carters : 10 to 31%). The contribution of the pedestrian and others way of transport is very low. There is an increase in the transport of wood by cars because of the quantity of wood which can be transported and the generated income of this activity.

The main persons involved in the exploitation and commercialization of wood are :
- **the wood cutter** : there are farmers who cut the dead wood or living trees for their own use in the forests or in the fallows. The wood is thereafter sold locally to wholesalers or transported and sold in the neighbouring towns. There is a second

category of wood cutter who work for the wholesalers. They are native people or come from the town to practice this activity.

- the local wood sellers : they sell wood in the main axis of the roads. They buy the wood from the wood cutter and sell it to the passengers or the people who transport it by cars.

- the wholesaler : there are two categories according to the mean of transport : small or big car. The wholesaler buys the wood in the markets of the main road axis or collect it from the forest. They supply the need of fuel wood for the big cities such as Niamey.

- the dealers : they sell wood in details to urban households. Some of them are women.

- the households : they buy the wood from the dealer, the wholesaler or sometimes from rural markets.

3.2. The socio –economics questionnaire

The questionnaire elaborated by Mrs Germaine Ibro was presented and discussed at the second coordination meeting held in Bamako from 4 to 7 May, 2004. The outcome of discussion with the other partners was to concentrate the socio economics studies on wood for fuel and aerial fodder. The literature review on wood for fuel in Niger indicated that there are five (5) groups of people involved in the wood commercialization : the woodcutters, the local wood sellers, the wholesaler, the dealer and the households. Three questionnaires were developed to explore the actual system of production of fuel food in the tree countries, Burkina Faso, Mali and Niger :
- Questionnaire 1 : to wood supplier (APPENDIX II). This questionnaire will be applied at the different entry points of wood to the town and require the attendance of the investigators during the whole day (24 hours)
- Questionnaire 2 : to wood cutter of the peri-urban zones (APPENDIX II): this questionnaire will be appied to some wood cutters from different zones of wood supply mentioned by in the suppliers.
- Questionnaire 3 : to wood cutter of the household (APPENDIX III). This questionnaire will be applied in some quarters of the town, mainly to women who use generally wood for cooking.

Another questionnaire related to the commercialization of fuel wood will also be elaborated. In addition the cost of production in nursery of the species for wood will be estimated.

Questionnaires for fodder production will also be elaborated similarly to those of fuel wood.

IV. Tree water use and soil water status

The activities of this work package began by the training of Niger team in meteorological, sap flow and soil moisture measurements by the CEH team (J. Douglas Deans and J. Wilson). This training was carried out in April, 2004.

In addition, plants from the nursery were planted in the field on May 22, 2004. 5 plants per species and per treatment (per type of water for irrigation and type of inoculation) were planted in randomized blocks. Within block, the distance of planting was about 1 m between trees of the same line and 1.5 m between two consecutive tree lines. The

distance between two consecutive blocks was about 2.5 m. Photographs taken on January, 13, 2005 illustrate the stages of development of the species (Figure 27).

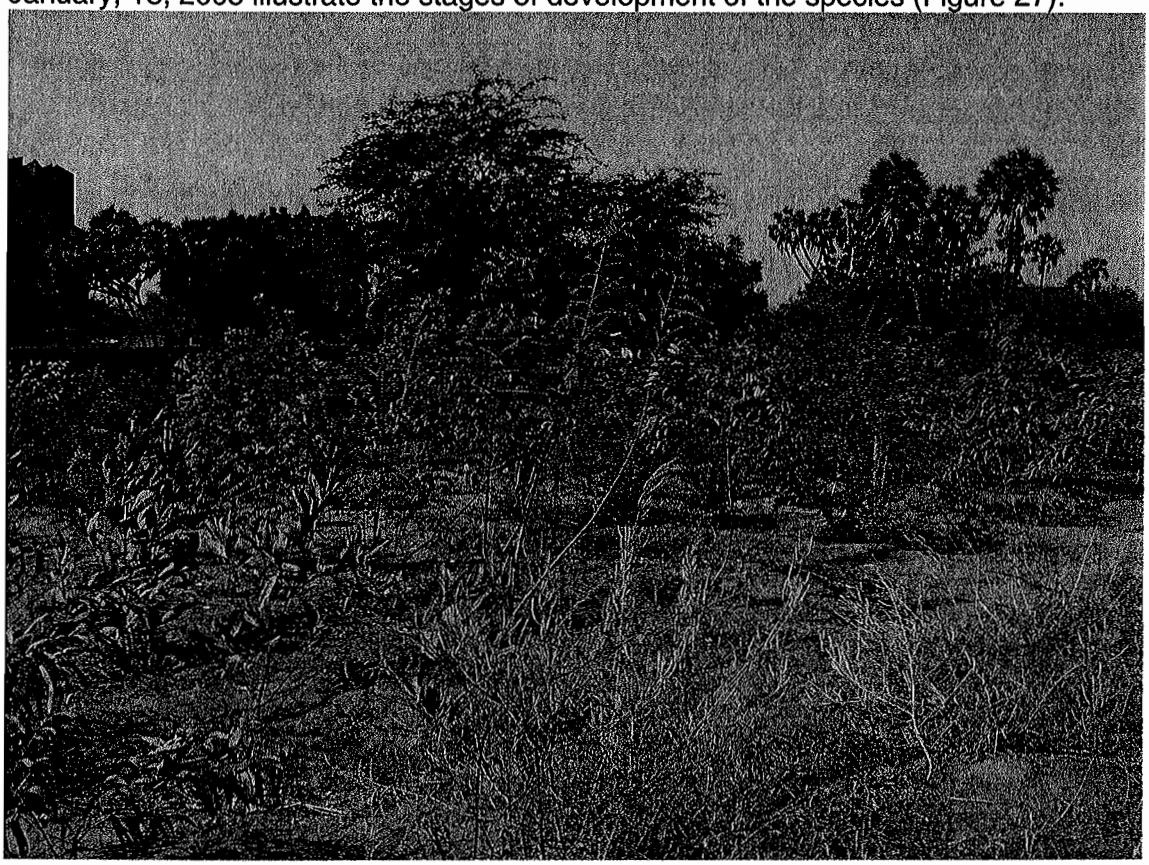


Figure 27 General view of study plots in Niger, January 2005

V. PARTICIPATION IN THE SECOND COORDINATION MEETING

Two members of Niger team participated in the second coordination meeting which was held in Bamako (Mali) from 4 to 7 May, 2004. At this meeting, some of the results of plant growth in nursery were presented.

VII. PLAN OF ACTIVITIES

The socio economics investigation, the sap flow measurements, the tree growth and management experiment will be the main activities of the third year of the project

3.1. Socio economic investigations

In order to assess the socio economics of fuel wood supply for Niamey city, the questionnaire will be used to make investigation. Six (6) axis of wood supply will be studied :

- Torodi to Niamey;
- Ouallam to Niamey;
- Dosso to Niamey;
- Filingué to Niamey;
- Tillabéry to Niamey;
- Say to Niamey.

3.2. Sap flow measurements

The variation of sap flow according to the species and the whether conditions and soil moisture will also be studied. This study will be conducted for the 11 species.
Sap flow of one plant of each specie is studied during 9 days + 1 day without power. Data of sap flow, whether and soil moisture are daily collected. Plants identified for this study were previously watered with waste water during one week. The irrigation with the waste water continued also during the study.

3.3. Tree growth and management

In order to compare the production of species for fuel wood and fodder production, two experiments will be conducted :

Experiment 1 :
- *Objective:* To compare the growth of tree species in different countries under irrigated field conditions in Burkina Faso, Niger and Mali.
- *Species :* Gliricidia sepium, Leuceana leucocephala, A. crassicarpa + 2 species
- *Experimental design :* complete randomized block with one factor (tree species). There will be 5 blocks, each containing one plot of each species. Each plot will contain 16 trees arranged 4 x 4. Only the central 4 trees will be measured. The distance will be about 1 m between trees within a row, and 2 m between the rows.
- *inoculation :* All trees should be inoculated in the nursery with mycorrhizas, and rhizobia where appropriate.
- *measurements :* Soon after planting, the height and root collar diameter of the 4 central plants will be measured. These measurements will be repeat every 1 – 2 months. These trees will be coppiced when they are big enough.
- *data analysis :* The data will be analysed by one-way ANOVA using a randomised block design. Analysis will be done on the plot means.

Experiment 2 :
- *Objective:* to do a quick screening of the produced trees in order to make sure that the selected species for experiment 1 are the best species.
- *Experimental design :* Experiment 2a will test the exotic species and experiment 2 b will test indigenous species.
The designs are the same for both experiment 2a and 2b.
These experiments will use a very simple design, with one tree per species per block, randomised within the block. There will be 20 blocks. Therefore 20 trees of each species will be used. This experiment uses the trees that have been grown in the nursery.
The space will be of about 1 m between trees within block and 1.5 m between blocks.

VI. PROBLEMS AND DIFFICULTIES

The prefabricated tarpaulin (membrane) necessary to keep purified water in the settling basin, was ordered in France. The duration of the transport from France to Lomé (Togo) and to Niamey (Niger) was very long. This has delayed the completion of the construction of the station for waste water purification.

The station of purification, however finished, cannot be used because of the lack of irrigation equipment: pumps and pipes for localized irrigation which must be ordered from another country. This irrigation equipment was not bought because the delay in the second payment by the EEC. The lack of money has also slowed the activities of the second year. We didn't get another payment from the EEC since the first advance which we received on April, 2003. Therefore the planned second experiment in the technical annex of the project has also been delayed. Furthermore, the activities of the socio economic work package were also delayed because of lack of money.

APPENDIX I

DESCRIPTION DU SYSTEME ACTUEL DE PRODUCTION DU BOIS DE CHAUFFE

Enquête production et commercialisation

(Questionnaire aux bûcherons)

(Nous entendons par bûcherons ceux qui coupent le bois de chauffe pour la vente).

Zone : Date:

Nom de l'enquêteur :

Nom du répondeur :

Profession du répondeur :

Identification

					Réponses
Genre (**SEX**)	1 = masculin 2 = féminin				
Age (**AGE**)					
Situation familiale (**MSTATUS**)	1=marrié(e) 2=divorcé(e) 3=veuf (ve) 4=célibataire				
Niveau d'éducation du Répondant (**EDUC**)	0= aucune 1= étude primaire 2= étude secondaire 3= étude supérieure 4= coranique 5= autres				
Taille de famille (**TAILLE**)	< 15 ans < 15 à 50 ans > 60 ans	**H**	**F**	**E**	
Activité principale (**PACT**)	1=agriculture 2=commerce 3= élevage 4= autres (à préciser)				
Activité secondaire	1=agriculture 2=commerce 3= élevage 4= autres (à préciser)				

1. Depuis quand exercez-vous cette activité ? .....

2. D'où provient le bois que vous coupez ? .....

2.1 = plantation
2.2 = forêts naturelles

A. Exploitation des forêts naturelles :

3. Comment est organisée la coupe du bois dans les forêts naturelles ?

3.1 de manière collective
3.2 de manière individuelle

4. Quels type de bois êtes vous autorisé à couper ?

4.1 Bois mort
4.2 Bois vif
4.3 Les deux

5. quelles sont les mesures de gestion adoptées pour une bonne régénération de la forêt ?

5.1 Coupe sélective (selon le diamètre)
5.2 Protection des jeunes plantes
5.3 Coupe par zone
5.4 Plantation
5.5 Autre à préciser.

6. Quelles sont les différentes périodes de coupes et les raisons?.....

Période de coupe	Raisons

7. Combien de stère coupez-vous par mois ? et par période ? .....

Période de coupe	**Espèces coupée**	**Fréquence de coupe par mois**	**Quantité coupée (stère)**

8. S'il existe une différence entre les périodes de coupe, donnez les raisons : .....
.....

9. Qui se charge de la coupe dans la famille ? .....

9.1 Les hommes
9.2 Les femmes
9.3 Les enfants
9.4 Les hommes et les femmes

10. Qui s'occupe de la commercialisation du bois ?.....

10.1 Les hommes
10.2 Les femmes
10.3 Les enfants
10.4 Les hommes et les femmes

(précisez le rôle de chaque intervenant)

11. Où se fait la vente du bois ? .....
11.1 au village
11.2 en ville

12. Décrivez le processus de la vente sur place .....

13. Comment transportez-vous le bois du lieu de coupe au lieu de vente ?
131 = charrette
132 = dos d'âne
133 = dos de chameau
134 = autre à préciser

14. Quel coût cela engendre t-il (par quantité transportée et par voyage ?).....

15. A quelle distance se situe le lieu de prélèvement du bois par rapport au village.....

16. Quelles sont les charges mensuelles auxquelles vous faites face ?

Rubrique de dépense	Montant mensuel

17. Combien y a t-il d'exploitants de bois au village
hommes _____ femmes _____

18. Quel pourcentage représente-ils par rapport à la population du village ?

19. Combien de temps passez –vous sur cette activité ?

combien de mois dans l'année ?
combien de jours par mois ?

20. Est ce que vous utilisez la main d'œuvre salariée ? oui ____ non ____

Si oui, quel est le mode de rémunération ? .....

21. A quelle distance se situe votre zone de coupe par rapport au centre urbain le plus proche ?

22. Existe t-il un marché rural de bois dans votre village ? oui ----- non ------

23. De quel type de marché s'agit –il ? .....
231. contrôlé
232. non contrôlé

24. Si non comment transportez-vous le bois jusqu'au centre urbain ?
241 charrette
242 dos d'âne
243 dos de chameau
244 autre à précisez.

25. Combien vous coûte un voyage (selon le moyen de transport).....

26. A combien vendez – vous un voyage en centre urbain (selon le moyen de transport).....

27. Combien de jours vous restez en ville pour vendre le chargement ?.....

28. Quelles sont les espèces de bois que vous coupez ? .....

29. Quelles sont les espèces de bois préférées par les consommateurs

1 .....
2 .....
3 .....

30. Est-ce que vous parvenez à satisfaire les préférences des consommateurs ?
oui non
si non pourquoi ?

31. Quelles sont les principales contraintes rencontrées dans l'exercice de cette activité ?

311 insuffisance de bois
312 insuffisance de main d'œuvre
313 taxes insupportables
314 mévente
315 charge importantes
316 autres à préciser

Justifiez chaque fois votre réponse

32. Quelle part ce votre revenu total représente les revenus tirés de la vente du bois ?.....
.....
33. Quelle utilisation faite-vous de revenu tiré du bois.

34. Selon vous est-ce que les besoins en bois sont satisfaits pour la ville ? .....

35. Si non quelle solution proposez-vous pour améliorer l'offre en bois de chauffe ?
.....

APPENDIX II

DESCRIPTION DU SYSTEME ACTUEL DE PRODUCTION DU BOIS DE CHAUFFE

(Questionnaire aux approvisionneurs en bois de chauffe)

Nom de l'enquêteur : ..... Date : .....

Nom : .....

Sexe : .....

Poste de Contrôle : .....

1.Identification

					Réponses
Genre **(SEX)**	1 = masculin 2 = féminin				
Age **(AGE)**					
Situation familiale **(MSTATUS)**	1=marrié(e) 2=divorcé(e) 3=veuf(ve) 4=célibataire				
Niveau d'éducation du répondant **(EDUC)**	0= aucune 1= étude primaire 2= étude secondaire 3= étude supérieure 4= coranique 5= autres				
Taille de famille **(TAILLE)**	< 15 ans < 15 à 50 ans > 60 ans	**H**	**F**	**E**	
Activité principale **(PACT)**	1=agriculture 2=commerce 3= élevage 4= autres (à préciser)				
Activité secondaire	1=agriculture 2=commerce 3= élevage 4= autres (à préciser)				

2. Depuis quand exercez-vous cette activité ? .....

3.Quelles sont vos zones d'approvisionnement et leur distance du centre ville ?

Zones d'approvisionnement	**Espèces achetées**	**Distance**

4. Comment est organisé la collecte de bois?.....

44 Achat auprès du bûcheron du village
45 bûcherons contractuels

5. Comment transportez-vous le bois des zones rurales en zones urbaines ?.....

51 Automobile 53 charrette 55 pied
52 âne 54 pirogue 56 vélo
57 autre à préciser

6. Quelle est l'évolution du prix du bois -énergie par période ?

Période	Volume acheté	Prix d'achat	Prix de vente

7. Existe t –il d'autres formes de taxes cachées ?.....
.....

8. quelles sont les coûts liés à la commercialisation du bois ?

Poste de dépense	Montant mensuel

9. Quelles sont les différentes périodes d'approvisionnement du bois ?..... .....
.....

Période	Volume acheté	Volume vendu	observation

10. Combien de voyages faites vous par mois selon les périodes ?

Périodes	Nombre de voyage /mois

11. Le moyen de transport vous appartient-il ?.....

12. Quels sont les coûts liés à son entretien ? .....

13. Quelles sont les espèces les mieux appréciées pour le bois de chauffe ?.....

14. Quelles sont vos principales contraintes ?.....

15. Le système actuel de production de bois de chauffe permet –il de satisfaire tous les besoins en bois de chauffe ? .....

16. Si non que proposez-vous ?.....

17. Que pensez –vous de la mise en valeur d'une plantation personnelle en vue de l'exploitation du bois de chauffe pour la vente ?.....

18. A qui revendez vous le bois ? .....

171 aux détaillants 172 aux détaillant -grossistes
173 aux consommateurs

19. Quelles sont les modalités de paiements ? (précisez les catégories de clients)
.....

191 vente à crédit
192 vente au comptant

20. Quelle part de vos revenus représentent les revenus tirés de la vente du bois (%)?.....

21. Quelle utilisation faite vous des revenus tirés de la vente du bois ?

APPENDIX III

DESCRIPTION DU SYSTEME ACTUEL DE PRODUCTION DU BOIS DE CHAUFFE

Questionnaire ménage
(Adressé à la mère de famille)

1. Identification

					Réponses
Genre (**SEX**)	1 = masculin 2 = féminin				
Age (**AGE**)					
Situation familiale (**MSTATUS**)	1=marrié(e) 2=divorcé(e) 3=veuf(ve) 4=célibataire				
Niveau d'éducation du répondant (**EDUC**)	0= aucune 1= étude primaire 2= étude secondaire 3= étude supérieure 4= coranique 5= autres				
Taille de famille (**TAILLE**)	< 15 ans < 15 à 50 ans > 60 ans	**H**	**F**	**E**	
Activité principale (**PACT**)	1=agriculture 2=commerce 3= élevage 4= autres (à préciser)				
Activité secondaire	1=agriculture 2=commerce 3= élevage 4= autres (à préciser)				

1. Quelles sont les sources d'énergies que vous utilisez pour la cuisine ?

   10combustible ligneux
   11gaz butane
   12 pétrole
   13électricité
2. Quelles sont vos sources d'approvisionnement en bois ?.....

   2.1 achat auprès de détaillant
   2.2 achat auprès de transporteurs
   2.3 achat auprès des bûcherons
   2.4 plantation
   2.5 collecte
3. Comment faite-vous votre approvisionnement en bois de chauffe ?.....
   31 par cuisine
   32 par jour
   33 par semaine
   34 par mois

4. A combien évaluez –vos dépenses en bois par cuisine ?

5. Combien de fois faites - vous la cuisine par jour ? .....

6. A combien s'élèvent vos dépenses hebdomadaires pour l'achat du bois énergie ?

7.Quelles espèces de bois préférez-vous utiliser ?

Espèces	Raisons de la préférence

8. Est-ce que vous trouvez à tout moment de l'année l'espèce préférée ?..... .....
Oui.... .....non.....
Si non pourquoi ? .....

9. Quels types d'équipement utilisez-vous ? .....

a. foyer à 3 pieds traditionnel
b. foyers améliorés
c. fourneau à charbon

10. Quelles sont les contraintes que vous rencontrez pour votre approvisionnement en bois énergie ?

.....

Partner 6 :IRD Senegal, Sub-Contractor : SCP France

Workpackage number 1 (Water treatment and irrigation) (SCP)

The aim was to design for the third experimental site (Ouagadougou) the most suitable and low cost domestic wastewater purification system, appropriate to local conditions in peri-urban environment in Burkina Faso and adapted to WHO standards.

The chosen one-hectare experimental site for UBENEFIT Project is located on the Ouagadougou University campus, on the North of E.I.E.R. (Ecole Inter-Etats d'Ingénieurs de l'Equipement Rural) fields.

Topography is plain with few reliefs, slope is very slight. Soil is rather homogeneous, comprising sandy or silty clays (10 to 45% of sand and 55 to 90% of silty clay). With this soil structure, the use of a membrane for lagooning basins is not mandatory to ensure water-tightness.

Effluent is composed of domestic wastewater with a high level of suspended matter, coming from the Faculty campus, restaurant and some offices. The BDO mean value used for calculations is 430 mg/l.

The lagooning method of water treatment is appropriate for the climate (high temperatures). A water deficit occurs throughout the year,apart from July and August.

In agreement with INERA and EIER partners, the wastewater treatment system proposed comprises:

- pre-treatment by screening/grit removal in a concrete sewer lift station, equipped with one electrical pump,
- a pipe will lead the effluent to the lagooning basins (about 400 linear meters)
- primary treatment with anaerobic lagooning (one 150 m^3 basin, 3 m depth basin, wastewater retention time is 2 days),
- secondary treatment with facultative aerobic lagooning (one 800 m^3 basin, 1,5 m depth basin, wastewater retention time is 9 days),
- tertiary treatment with maturation lagooning (one 500 m^3 basin, 1 m depth basin, wastewater retention time is 5 days),
- a concrete storage basin (reservoir) (one 100 m^3 basin, 1 m depth, storage is 1 day).

All basins will be fed by gravity. A homogenisation basin will be implemented before the anaerobic lagooning basin, in order to reduce effluent speed (see Figure 28).

Thus, and as requested by the Burkina Faso Authorities, the quality of treated effluent will correspond to A-type constraints (according to WHO standards): number of intestine nematode eggs<1 per litre and thermotolerant pathogens < 104 / l.

Irrigation will be gravity-fed irrigation with 50 m long lines.

Tree water supply is estimated 50 m^3/ha/day (as average daily evaporation is about 5 mm), and because of losses due to gravity-fed irrigation (estimated 50%), water requirement is finally estimated 100 m^3/ ha/day.

The experimental site is divided into two 5000 m^2 areas, each divided into four sub-areas of 5 irrigation lines. Each sub-group could be irrigated in an independent manner.

Mechanical earthworks are needed to build 1 m high, 50 m long and 5 m large earth boards between the lines, that would each be planted of two rows of trees. Irrigation lines would be about 20 x 40 cm, with a slope of 2 mm/m, with 5 m space between them.

Soil drainage will be important to follow in the project, as well as salts concentration in the root zone due to water accumulation. Salinity will have to be controlled regularly.

SCHEMA DE PRINCIPE GENERAL DU SYSTEME DE COLLECTE, DE TRAITEMENT ET D'IRRIGATION

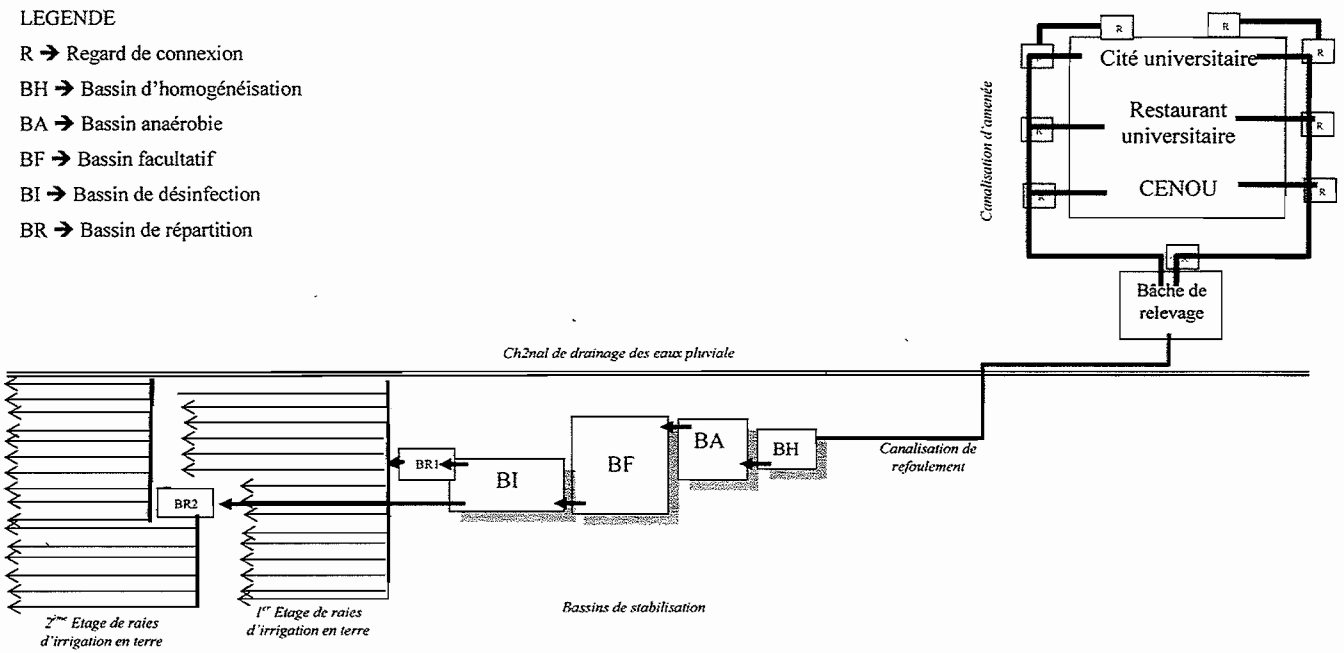


Figure 28 General scheme of wastewater treatment and irrigation Sources : CNRST/INERA – EIER – BET(ECODI) - 2004

R connection hole BH : homogenization basin BA : anaeroby basin BF : facultative aeroby basin
BI : maturation basin (disinfection basin) BR : distribution basin

Bâche de relevage : Sewer lift station Canalisation de refoulement : delivery pipe Chenal de drainage des eaux pluviales : rainwater drainage channel Cité universitaire : campus

Gravity-fed irrigation with two stages of lines

Conclusion - Progress of the planned tasks

The major problem encountered is the delays that have occurred in all the three projects. The implementation of wastewater treatment plants and irrigation systems have been delayed for different reasons (late supply of irrigation materials in Niger, late project planning and implementation in Burkina Faso because of delays in the selection of the experimental site...). Trees will be planted in experimental sites in spring 2005 (months 28 or 29) instead of month 14 as initially planned in the Technical Annex.

In spring/summer 2005, work planned is: seedling production, priming of wastewaters treatment plants, preliminary tests for irrigation, experimental site soil preparation and tree plantation.

1) Niger

Building of wastewater treatment plant is finished. Irrigation system implementation is in progress. Priming could soon occur (spring 2005).

2) Mali

The quality of initial effluent and final drainage waters has not yet been analysed. Implementation of irrigation – wastewater valorization project is on-going.

3) Burkina Faso

SCP helped for the conception of the project and for writing the terms of reference of the project, in tight partnership with EIER/INERA. Consultation with enterprises for the construction of the wastewater purification station has been launched by EIER / INERA partners. Implementation is in progress.

Work package 2 (Tree growth and Management)

In the first annual report, it was indicated that an irrigated field trial was set up in Bel Air Research station on November 2003 with the six common species of woody legumes tested under nursery conditions in Mali, Burkina Faso and Niger: *Acacia mangium*, *Acacia auriculiformis*, *Acacia crassicarpa*, *Gliricidia sepium* and *Leucaena leucocephala*. We continued to follow up this field trial in order to have vigorous trees well nodulated on which it will be possible to harvest nodules. An estimation of the symbiotic nitrogen-fixation could be done also in order to have some data which could be compared with data obtained in Mali, Burkina and Niger where the plants will have been irrigated with waste-water. One part of the nodules harvested in the three south countries will be, as soon as possible, analyzed by using ELISA technique in order to see if the strains LDK 4, GsK4 and the mixture Aust 13 C / Aust 11 C are able to compete for nodule occupancy of, respectively, *Leucaena leucocephala*, *Gliricidia sepium* and the several species of Australian acacias.

Plants of five tree species (*Acacia mangium*, *Acacia auriculiformis*, *Acacia crassicarpa*, *Leucaena leucocephala* and *Acacias senegal*) chosen for Mali, Burkina Faso and Niger to follow the next activities, were inoculated with the corresponding strains (respectively, Aust 13 C, Aust 11 C, Aust 13 C, LdK4, CIRAD 300, 301, 302) in nursery conditions in order to have a important number of nodules. These nodules will be used for molecular tests in the laboratory (DNA extraction and hybridization).

Work package 4 (Microsymbionts and Nitrogen-Fixation)

Development of molecular techniques

The probes described in the first annual report (IgS1, IgS2, IgS3 and IgS4) were tested on nodules formed by target strains. Results shown that the denaturation treatment (enzyme and high temperature) applied to crushed nodules was not strong enough. In order to improve protein denaturation and DNA yield, different treatments involving Guanidine Thiocyanate, a strong protein detergent (Chomczynski *et al.*, 1997, *Biotechniques* 22:550-553), were tested on cowpea nodules in the first time. The data were compared to those obtained by CTAB/PVPP (Hexadecyltrimethylammonium Bromide/Polyvinylpolypyrrolidon) extraction method associated to Phenol:Chloroform:Isoamyl Alcohol purification, actually used in laboratory.

To facilitate comparison between treatments, rehydrated and surface sterilised nodules were crushed together in TES / sucrose buffer (20 mM Tris-HCl pH 8.0; 50 mM EDTA di-sodium pH 8.0, 50 mM NaCl; 8 % p v^{-1} sucrose) (Rex, 2000, *Focus* 22:26-27) with 100 µl per nodule. Each treatment was performed in three replicates. Material lysis was done with Lysozyme (4 mg/µl) at 37°C for 15 min. Decreasing concentrations of Guanidine Thiocyanate and different incubation times at 65°C (30 min, 15 min, 10 min and 5 min) were tested. The best DNA yield was obtained with Guanidine Thiocyanate 0.0005 M and 15 min incubation at 65°C (Table 22). 2.7 fold DNA yield increasing was obtained: 3.15 µg of total DNA per gram of dry matter in comparison with 1.17 µg yielded by CTAB/PVPP treatment.

The proposed method is a safe procedure (without Phenol:Chloroform:Isoamyl Alcohol), shorter (1 h 20 min per nodule for GES protocol in comparison with 4 hours for CTAB/PVPP protocol) and 60 times more economic for products (5 FCFA per nodule spent by GES protocol in comparison with 300 FCFA by CTAB / PVPP protocol) than the extraction procedure with CTAB / PVPP. The isolated DNA is ready for PCR-RFLP without any additional purification

(Figure 29). It allows an easy transfer of DNA extraction technique to developing country laboratories.

Table 22 Comparison of treatment effects on DNA yield obtained on cowpea nodules

Treatment	Nodule dry weight (g)[a]	Protein purity A260 / A280[b]	DNA quantity (mg g^{-1} dry matter)[c]
CTAB / PVPP		1.63	1.17
GES (6 M)		1.71	1.07
GES (5 M)		1.58	1.24
GES (1 M)		1.7	1.7
GES (0.1 M)	0.0036	1,78	1.74
GES (0.005 M)		1.68	2.21
GES (0.001 M)		1.75	1.6
GES (0.0005 M)		**1.75**	**3.15**
GES (0.0001 M)		1.66	2.15
GES (0.00005 M)	0.0036	1.58	2.34
Without GES	0.0036	2.55	0.61

[a]The mean weight of one dry nodule determined as a ratio between the weight of all of nodules in crushed mixture and nodule number.

[b]Results are given as the mean of three replicates.

[c]Results are given as the mean of three replicates.

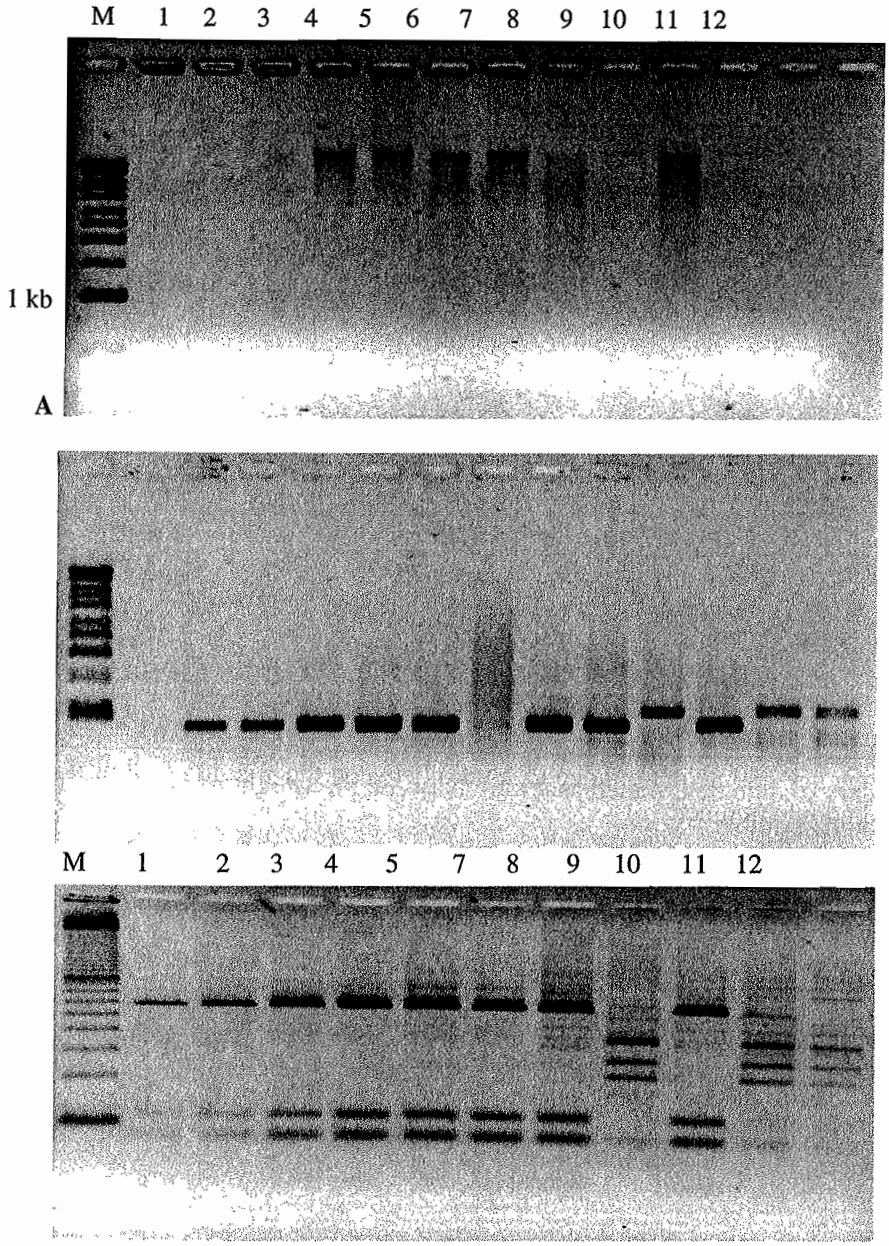


Figure 29 Gel electrophoresis of the total DNA isolated by the GES method (A), amplified 16S-23S rDNA IGS products (B) and digested with *Msp*I endonuclease (C).

Lane M, size marker (Pharmacia Biotech), 1 kb (A) and (B); 100 pb (C), lanes 1 to 12, different cowpea nodules

The method was tested on nodules collected from the woody legumes grown in nursery. The results were compared with those obtained by CTAB/PVPP protocol (Table 23). For each tree species, a mixture of six crushed nodules was divided into six samples to allow three replicates per treatment (GES protocol or CTAB/PVPP protocol).

Table 23 DNA yield obtained on woody tree nodules by GES protocol

Tree species	Nodule dry weight (g)[a]	Protein purity A260/A280[b]		DNA quantity (mg g^{-1} dry matter)[c]	
		GES	CTAB/PVPP	GES	CTAB/PVPP
Acacia mangium	0.0025	1.5	1.67	1.67	0.39
A. auriculiformis	0.005	1.3	1.55	2.06	0.28
A. crassicarpa	0.005	1.6	1.63	0.76	0.20
A. senegal	0.005	1.4	1.53	0.83	0.21
Leucaena leucocephala	0.005	1.7	1.45	0.87	0.26
Gliricidia sepium	0.0025	1.4	nd	0.93	nd

[a]The mean dry weight of one nodule determined as a ratio between the weight of all the crushed nodules in a mixture and nodule number.

[b]Results are given as the mean of three replicates for both DNA extraction protocols with GES or with CTAB/PVPP.

[c]Results are given as the mean of three replicates for both DNA extraction protocols with GES or with CTAB/PVPP.

nd, not determined

The results show that irrespective of the nodule origin, the GES protocol provides a 3 (example of *Leucaena* nodules) to 7 (*A. auricoliformis*) times improvement in the amount of recovered DNA yield of all of tree species in comparison to data obtained by CTAB/PVPP protocol, with similar $A_{260/280}$ ratios (indicating DNA purity) being acceptable for PCR-RFLP and hybridization studies.

DNA probes design for inoculants

DNA of selected rhizobia strains (Aust 11 C, Aust 13 C, GsK4, LdK4) corresponding to each tree host plants that will be used in inoculation trials were amplified and purified for sequencing of 16S-23S rDNA IGS region (Figure 30). The three strains CIRAD 300, 301 and 302, used for *Acacia senegal* inoculation, were partially sequenced for 16S-23S rDNA. A complete sequencing will be finished later. The obtained sequences will be used for development of the strain-specific DNA probes.

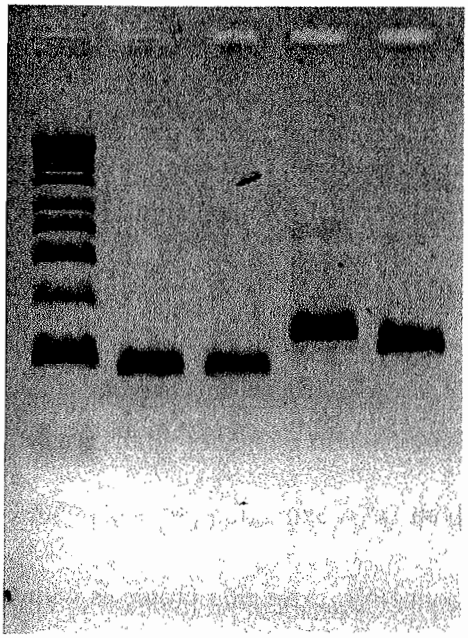


Figure 30 Amplified and purified 16S-23S rDNA IGS samples.

Line M, 1 kb size marker (Pharmacia Biotech); 1, 2, 3 and 4, PCR products of strains, respectively, Aust 11 C, Aust 13 C, GsK4 and LdK4

Molecular analysis of nodules formed in nursery

Nodules obtained by partner 5 (Univ. A. Moum, Niger) in nursery trials on plants inoculated with selected strains in conditions of normal or wastewater irrigations were analysed by PCR-RFLP technique in collaboration with IRD (partner 6), in Laboratoire Commun de Microbiologie, in Dakar. Analysis of 81 nodules showed that the inoculated strains did not persist and that the diversity of "wild type" strains in the nodules was depending on water conditions.

Training of DC researchers

During this second year, Ousmane Sacko (Université of Bamako, Mali, Partner 3) and Alzouma Mayaki Zoubeirou (Université Abdou Moumouni, Niger, Partner 5) spent respectively eight and six months in laboratory of Partner 6 in Dakar.

Annex 1.

Work Package 2 – Tree growth and management

Protocols for field experiments

NB. These experiments should all be done on irrigated plots. SCP have provided you with information about the amounts of water which should be supplied.

Experiment 1.
Objective: To compare the growth of tree species in different countries under irrigated field conditions in Burkina Faso, Niger and Mali.

Two of the tree species will be the same in all countries. Partners will also plant at least two more species. The species which were agreed at the meeting in Mali were as follows:

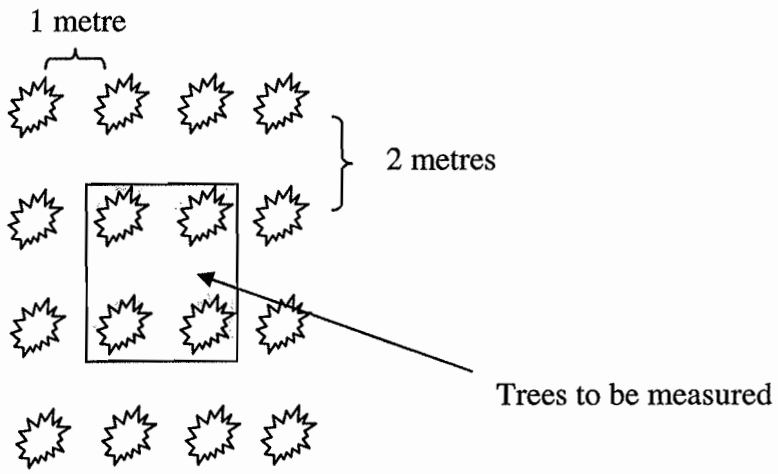
Species	Burkina-Faso	Niger*	Mali
Gliricidia sepium	✓	✓	✓
Leucaena hybrid	✓	✓	✓
Acacia angustissima	✓		✓
Acacia crassicarpa		✓	
Khaya senegalensis			✓
Afzelia africana	✓		

* Niger will select another tree species

We will use a randomised block design, with one factor (tree species).

There will be 5 blocks, each containing one plot of each species. Each plot will contain 16 trees arranged 4 x 4. Only the central 4 trees will be measured. There will be 1 m between trees within a row, and 2 metres between the rows.

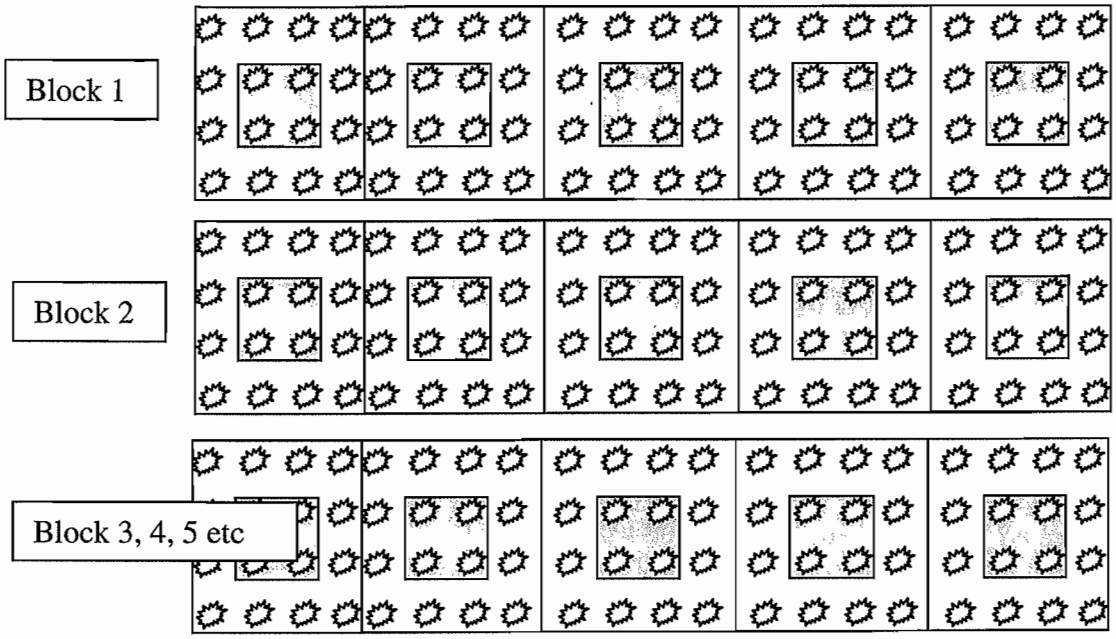
Thus a single plot of trees will look as follows



All trees should be inoculated in the nursery with mycorrhizas, and rhizobia where appropriate.

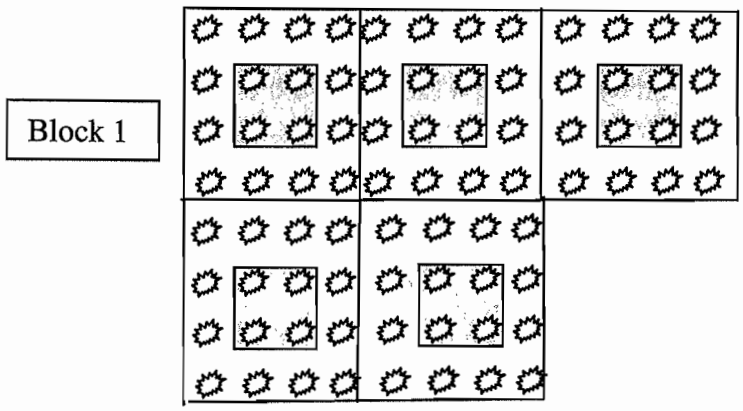
We use experimental blocks to increase the precision of treatment comparisons. Plots which are physically close to each other or have similar soil properties would be expected to give similar yields. Thus in a field experiment, where soil fertility or moisture are not uniform, blocks would be placed so that all plots within a block would be on soil of similar type.

The shape of the block can be varied according to the land available, but you must maintain the 4 x 4 arrangement of each plot within the block, and the plots must be together.
For example, if there are 5 species



In this arrangement, each block will take 21 m x 8 m

OR



The arrangement of the plots must be randomised in each block

For example, with 5 tree species

Block no.	Tree species				
1	4	2	1	5	3
2	5	1	4	3	2
3	1	3	5	2	4
4	5	4	3	2	1
5	3	1	5	2	4

This experiment will use 80 trees of each species. All of these need to be inoculated with mycorrhizas (and rhizobium, where appropriate). Because some seedlings will not grow well in the nursery, produce about 120 plants of each species in the nursery and select the 80 for planting which are closest to the mean size. Keep the spare plants in the nursery as they may be needed to replace plants which die.
You need to start preparing the inoculum and sowing the trees now, so that they are ready for planting as soon as your sites are ready.

If tree species use different mycorrhizas and rhiizobium, take precautions about cross contamination in the nursery and when planting in the field. Take advice from Didier and Marc as to how to avoid this. Assess mycorrhizal and rhizobial infection before planting.
Soon after planting, measure the height and root collar diameter of the 4 central plants. Repeat these measurements every 1 – 2 months. the data as soon as it has been collected to see what is happening.

We plan to coppice these trees when they are big enough. The age of the trees at coppicing will depend on their growth rates, but it is expected that coppicing will be done when the trees are about 15 months old.

Produce a plan of your experimental layout and make sure that all personnel who collect data for you understand the layout of the site, so that trees are not muddled up.

Data collection and analysis
I attach an example of a data collection sheet. Please check it. Have I left anything out?

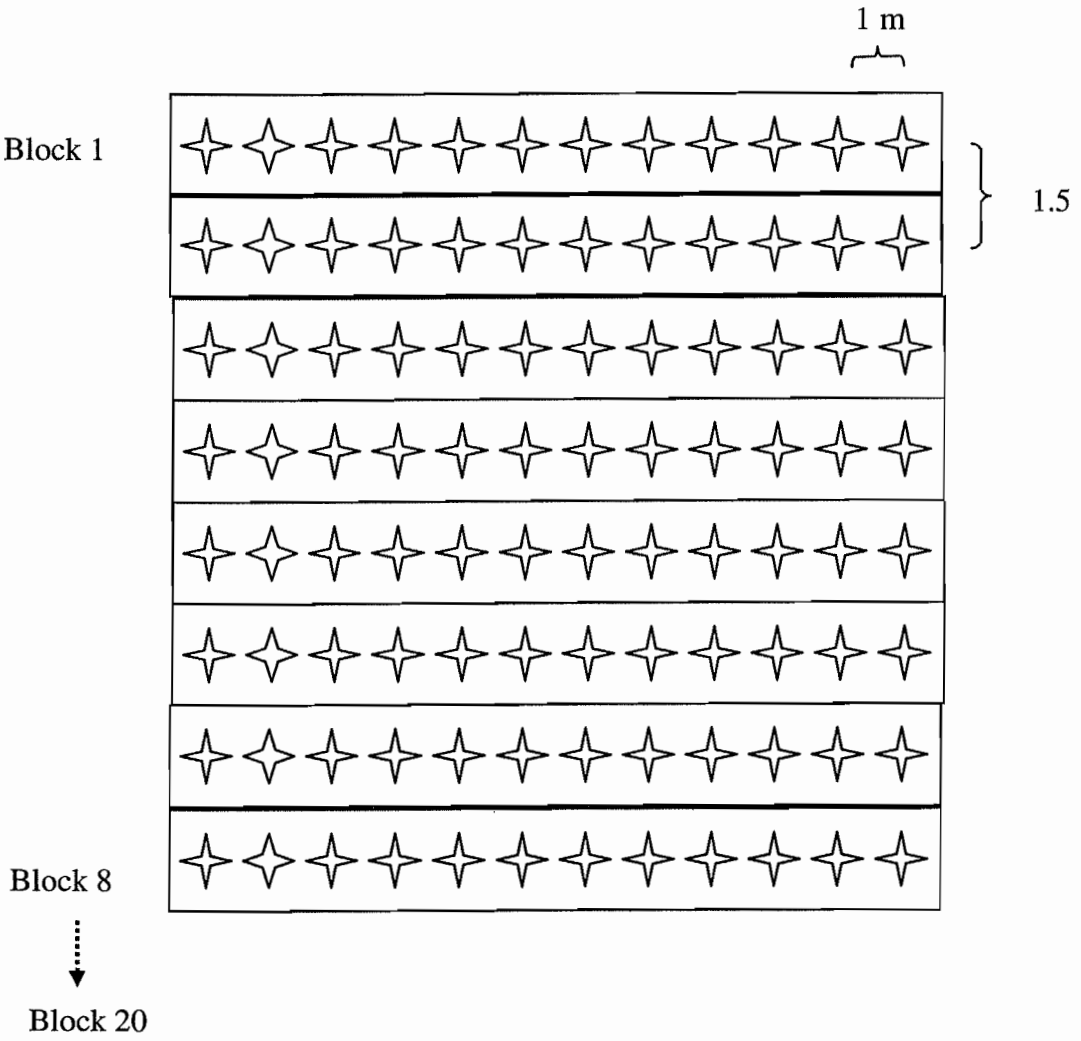
The data will be analysed by one-way ANOVA using a randomised block design. Analysis will be done on the plot means.

Experiment 2
Objective: to do a quick screening of the trees that you have already produced, just to make sure that the species that you have selected for experiment 1 are the best species

Experiment 2a will test the exotic species and experiment 2 b will test indigenous species.

The designs are the same for both experiment 2a and 2b.

These experiments will use a very simple design, with one tree per species per block, randomised within the block. There will be 20 blocks. Therefore you need 20 trees of each species. This experiment uses the trees that you have already grown in the nursery. Try to take all the trees from the +M+R treatment, and use those with the sizes which are closest to the mean size for the treatment. Measure once a month (height and root collar diameter)
Use a spacing of 1 m between trees within block and 1.5 m between blocks.



The example above uses 12 species. You can use more.

This is an example of a few randomised blocks. As in experiment 1, the blocks do not have to be this shape. Choose what fits best with your land.

Block no.	Tree species number											
1	11	2	6	10	8	3	5	7	9	1	4	12
2	8	5	7	1	12	2	10	3	9	6	11	4
3	1	10	3	7	2	8	11	12	4	6	5	9
4	9	10	2	11	5	4	8	1	3	12	7	6
....20	2	7	12	4	10	1	9	6	5	11	3	8

Data analysis. This is a simple randomised block design, with one factor –tree species

Contract number: ICA4-CT-2002-10017 Year 2 2003-2004

Data sheet
For annual report

(to be completed by the co-ordinator at 12-monthly intervals from start of contract. Figures to be up-dated cumulatively throughout project lifetime)

1. *Dissemination activities*	Totals (cumulative)	
Number of communications in conferences (published)		
Numbers of communications in other media (internet, video)		
Number of publications in refereed journals (published)		
Number of articles/books (published)		
Number of other publications	1	
2. *Training*		
Number of PhDs	2	
Number of MScs		
Number of visiting scientists		
Number of exchanges of scientists (stays longer than 3 months)	4	
3. *Achieved results*		
Number of patent applications		
Number of patents granted		
Number of companies created		
Number of new prototypes/products developed		
Nubmer of new tests/methods developed		
Number of new norms/standards developed		
Number of new softwares/codes developed		
Number of production processes		
4. *Industrial aspects*		
Industrial contacts	Yes	No ✓
Financial contribution by industry	Yes	No ✓
Industrial partners:- Large	Yes	No ✓
SME[1]	Yes ✓	No
5. *Comments*		
Other achievements (use separate page if necessary)		

[1] Less than 500 employees