Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

Report

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

Contract number: ICA4-CT-2002-10017

Fifth Annual Report: 1/12/2006 – 30/11/2007

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**

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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.

Fifth Annual Report: 1/12/2006 – 30/11/2007

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Compiled by J. Wilson

Summary Report

Since the beginning of the contract, the results achieved are as follows:

Work package 1 Water treatment and irrigation

Sites for waste water treatment plants and irrigation systems were identified and the systems have been constructed in Burkina Faso, Mali and Niger. Progress was slower than planned for a variety of reasons, including delays to obtaining permits for construction, delays associated with importation and shipping of components and delays in purchase of expensive items due to cash flow problems. Staff have been trained in how to use and maintain the systems. Some modifications and refinements and repairs have been necessary, but systems are functioning in each country. The irrigation sites were characterised in advance of tree planting, and soil and water analyses are being conducted regularly.

Work package 2 Tree growth and management

Tree species with potential for use in irrigated conditions in each country were identified and experimental designs for these trials were produced. Some species are being used in common in all three countries. Nursery screening trials were conducted and plants were then out planted to the irrigation sites. Trees have grown quickly under the irrigated conditions and many species are performing well. Few problems have been detected. Species which were selected for their performance in the nursery are not necessarily the best performers in the field plots. In this year, biomass production has been determined and the effects of coppicing at 2 different heights on regrowth have been assessed.

Work package 3 Tree water use and soil water status

Staff in all three countries with irrigation systems have received training in the use of sap flow, soil water and associated measuring equipment. In Mali, considerable variation between tree species in soil water use has been noted, together with differential effects according to the inoculation history of the plants. *Acacia angustissima* appears to have particularly high water use and is easily water-stressed, whereas *A. mangium* appears to be more robust in its performance. Even with irrigation, tree water use is declining by the late morning, indicating stomatal closure. In Burkina Faso, *L. hybrid* showed the highest transpiration rate (1.09 L cm^{-2} day^{-1}), followed by *L. leucocephala* (0.93 L cm^{-2} day^{-1}), *G. sepium* (0.93 L cm^{-2} day^{-1}) and *A. angustissima* (0.61 L cm^{-2} day^{-1}). Gliricidia did not show morning stomatal closure, whereas Acacia and the Leucaenas did. Stomatal closure was especially marked with *Acacia angustissima*, confirming the results previously obtained in Mali. The long term use of physiological equipment under tropical conditions has proved difficult as the equipment is not particularly robust.

Work package 4 Microsymbionts and N fixation

Working in controlled glasshouse conditions, using sterilised soil media, the UK partner has identified considerable variation in effectiveness of different mycorrhizal strains on different tree species. After the initial screening phase, selected tree species were taken on to the second phase of the study in which plant response to simulated irrigation water is being measured. Uninoculated plants grew very poorly irrespective of whether they are receiving the simulated irrigation water or not. The response of inoculated plants to irrigation varied with inoculant and tree species.Initially, nursery

and field studies in Mali, Niger and Burkina Faso gave much less clear results. However, assessments during this final year of the project have demonstrated positive effects of inoculation on tree growth in Mali and Niger.
These observations are reinforced by molecular studies, using strain-specific probes for nodule analysis, which have been successfully tested against the inoculants, studies on samples collected from the field experiments in Mali suggest that the inoculant strains are absent – other types are present. This suggests that either the original inoculation was unsuccessful, or that the inoculants have been out-competed by indigenous strains.

Work package 5 Economics and quality of produce
Questionnaires have been developed by the partner in Niger, in collaboration with other partners. All countries have now completed their surveys, which have generated a considerable amount of useful information about fuel wood and fodder supplies. For Ouagadougou (population 960000 in 2000), it is estimated that 225,004 tons of fuel wood and 6708 tons of charcoal per year are transported to the city. The average price of firewood was approximately 21 F CFA per kg, and charcoal was 60 – 110 F CFA per kg. Sellers can achieve a substantial income from sales. The large quantities of fuel imported into Ouagadougou highlight the pressure on fuel resources. This is further indicated by the observations in Niger, where wood cutters cut an average of 27 steres per month, and each village can have 40 – 80 woodcutters. In Mali, annual wood fuel consumption averages about 0.5 ton per capita, and collection of a cart load of wood can involve a journey of 30 km and 3 days. Increasing numbers of grazing cattle are creating conflicts between different land uses. Assessments of fuel wood quality and palatability to animals have been made in Mali.

Work package 6 Soil and plant nutrition
Nutrient contents of irrigation water and soil nutrient status are being monitored at each site. In Mali, studies showed that pesticide levels were not significant, but that there was sometimes a build up of ammonium and turbidity in water flowing out of the plantation. In Burkina Faso microbiological analyses showed that the water treatment was successful in reducing levels of bacteria. Analyses have continued in all countries and no problems have been detected.

Work package 7 Planting stock quality
Studies have been conducted in Burkina Faso and Mali.

Using various parameters of planting stock quality (shoot: root ratio, sturdiness quotient, Dickson's Quality Index), considerable variations in quality have been identified, between species, production methods and between partners testing the same species. In Burkina Faso, a previous pot experiment was planted out. Previous effects of inoculation, substrate and pot size were no longer evident, however there was considerable variation between species in growth. At the time of planting, there were considerable differences in shoot: root ratios between species.

Work package 8 Pest monitoring and management

Studies in Mali have highlighted attack by termites on Leucaena and Calliandra, causing death of experimental trees, and the susceptibility of Acacia angustissima to prolonged flooding.

Scientific Annual Report

Work package 1 Water treatment and irrigation
Water treatment and irrigation facilities have mostly run well, with occasional problems. However the facility in Burkina Faso (Partner 4) has required some modifications to be made this year as there were some underlying problems with the initial design of the system which made it unreliable. Water quality has continued to be monitored..
Deliverables 1.1, 1.2, 1.3 and 1.4 achieved.
Ongoing: Partners intend to continue using these irrigation systems and to extend their studies beyond the life of this project.

Work package 2 Tree growth and management
Work this year has concentrated on completing evaluations, and in holding field visits.
Assessments in **Mali** confirm those reported last year, in experiment 1, *Leucaena leucocephala* is the tallest species, having achieved 7 m in 2 years, double the height of *Acacia angustissima. Khaya senegalensis* has performed unexpectedly well under irrigation. Coppicing studies commenced this year, and coppicing height had no significant effect on regrowth. *Leucaena leucocephala* regrew fastest after coppicing. In Experiment 2, *Leucaena leucocephala* yielded the most dry wood from coppicing whereas *Acacia mangium* yielded the most fodder. In **Burkina Faso**, *Leucaena* hybrid was the best performing species, and *Afzelia africana* was the slowest growing. In **Niger**, assessments of fodder and fuelwood production indicated that *Acacia crassicarpa* yielded the most fodder, and that yield was positively influenced by symbiont inoculation.
Deliverables 2.1, 2.2, 2.3, 2.4 achieved,
Ongoing 2.5

Work package 3 Tree water use and soil water status
Studies this year have focused on the field site in **Burkina Faso**, where training was provided. Evapotranspiration was calculated. Some problems with ageing equipment were encountered. Leucaena hybrid showed the highest transpiration rates, and *A. angustissima* the lowest. As at previous sites, this latter species showed day time stomatal closure and appeared to be easily water-stressed.
Deliverable 3.1, 3.2, 3.3 achieved

Work package 4 Microsymbionts and N fixation
Assessments during this final year of the project Mali, Niger and Burkina Faso have demonstrated positive effects of double inoculation (with rhizobium and mycorrhizas) on tree growth in Mali and Niger. Averaged across species, fuelwood production by inoculated plants was 67% greater than uninoculated, and fodder production was 35% greater. Direct soil inoculum potential studies are incomplete, but the results from the inoculation studies indicate that inoculation in the nursery gets plants off to a good start and that indigenous inocula are either ineffective or deficient.

France has developed strain-specific probes for nodule analysis, which have been successfully tested against the inoculants, however results of analyses by FAST so far, on nodules collected from the field studies in Mali suggest that the inoculant strains are absent – other types are present. This suggests that either the original inoculation was unsuccessful, or that the inoculants have been out-competed by indigenous strains. No new effective rhizobial strains have been identified.
Deliverables: 4.1, 4.2, 4.3, 4.4 complete

Work package 5 Economics and quality of produce
In **Mali,** Prices and preferences for fuelwood were tested with women's groups. *Leucaena leucocephala* was the most preferred species, and *Acacia angustissima* was the least. With cattle breeders, animal preferences for fodder were also determined; oxen and donkeys exhibited much stronger preferences than sheep and goats.
Deliverable 5.1 5.2, 5.3, 5.4, 5.5 complete
5.6 – 5.8 in progress

Work package 6 Soil and plant nutrition
In **Mali** final soil and wood samples have been taken for chemical analyses, results are not yet available. Water samples indicate that quality conforms to WHO standards.
Deliverables 6.1, 6.2 6.5 completed
6.4 incomplete

Work package 7 Planting stock quality
Field studies were completed
Deliverable7.1, 7.2 completed

Work package 8 Pest monitoring and management
No significant problems with pests and diseases have been reported from studies in **Mali** and **Burkina Faso**.
Deliverables 8.1 -8.2 complete

Management Annual Report

Organisation of the collaboration

Management of the collaboration is through coordination meetings and also by regular email communication with all participants, and scientific visits and exchanges between partners.

The final meeting was planned to be hosted by partner 1 in Edinburgh in September 2007. However it had to be cancelled due to extreme problems in partners obtaining visas to attend. Not only was it necessary to obtain visas for the UK, but partners' travel advisers informed them that they also had to obtain visas for France, despite the fact that they were only intending to catch connecting flights and not enter the country. Thus they each had to obtain 2 visas. Some of the partner countries no longer have British Embassies, and some French embassies were temporarily closed and partners had to travel to neighbouring countries (Ghana, Senegal …) to make their applications in person, an expensive and time consuming process. The French Embassies demanded much documentation – birth and marriage certificates of all family members, proof of employment etc. These difficult processes proved too much and the final meeting was abandoned.

Partner 1 Centre for Ecology and Hydrology, UK
Julia Wilson, Kevin Ingleby, Robert C Munro

Summary of Progress

- WP3: training was provided to INERA in operation of sap flow and soil water equipment and in analysis of the data

- WP4: Results from the glasshouse experiment have been prepared for publication.

- Preparations were made for the final meeting, but difficulties with travel arrangements (visas) of several partners, prevented the meeting being held.

Activities

WP3: Tree water use and soil water status
Equipment was transferred from IER Mali to INERA in Burkina Faso early in 2007. Julia Wilson and Bob Munro from Partner 1 visited Burkina Faso in March 2007 to provide training in the operation of the equipment. Results of the study in Burkina are provided in the report prepared by Partner 4.

WP4: Glasshouse experiment
The glasshouse experiment was completed and a publication entitled Mycorrhizas in agroforestry: response of multi-purpose tree species and arbuscular mycorrhizal fungi to irrigation with simulated wastewater. Biology and Fertility of Soils J. Wilson, K. Ingleby and R.C. Munro has been prepared.

Partner 2: Institut d'Economie Rurale, Bamako, Mali
Mr. Daouda Sidibé, Dr Kalifa Traoré, Mr. Broulaye Koné

Work package 1: Water treatment and Irrigation
1. Objectives
The objective of this project is to treat and valorize the use of rice irrigation waste water from the Siribala irrigated perimeter for fodder and fuelwood production**.**
An experimental site (4 ha) was set up previously near a village called Siribala situated at 30 km from Niono. The site is situated at 14° 4' N, 6°03' W and at an altitude of 274.3 m**.** The drainage canal used to irrigate experimental site is called the drain of Minimana. The experimental site (1 ha) is delimited and surrounded with wire netting.
The information concerning this has been presented in previous reports.

Work package: 2 Tree growth and Management
Activities
During 2007, the main activities were related to tree growth and management and fuelwood and fodder quality assessment through survey. These issues have been approached through two experiments. The first experiment compared the growth of the tree species *Gliricidia sepium*, *Leucaena leucocephala*, *Acacia angustissima* and *Khaya senegalensis* inoculated with or without mycorrhizas and rhizobium under irrigated field conditions in Mali. The number of factors is two (tree species and inoculation treatments). Two levels of treatment have been done: the double inoculation with mycorrhizas and rhizobia (+R+M) and the control treatment. There are five randomised blocks, each containing one plot of each species. Each plot contains 16 trees arranged 4 x 4. Only the central 4 trees are measured. There is 1 m gap between trees within a row, and 2 m between the rows. The second experiment is related to a quick screening of 10 tree species (*Acacia crassicarpa*, *Acacia mangium*, *Acacia auriculiformis*, *Leucaena leucocephala*, *Gliricidia sepium*, *Calliandra calothyrsus*, *Acacia angustissima*, *Acacia senegal*, *Pterocarpus lucens* and *Khaya senegalensis)* to assess their performance relative to those species assessed in experiment 1.The experimental design is simple, with one tree per species per block, randomised within the block. In total we have used 30 blocks, therefore 30 trees of each species were used. This experiment used the trees that we had already grown in the nursery in Sotuba. Trees from the R+M treatment with the sizes closest to the mean size for the treatment were used.

Biophysical parameters such as height of trees, root collar diameter, diameter at 1.30m and the number of branches have been measured monthly.

On May 04 2007 a field visit was organized with the women's associations of the villages of Laminibougou (one association) and Siribala (two associations) and the association of cattle breeders of Siribala (one association). A survey was done and much information gathered. It enabled the local population to assess the fuelwood quality, and equivalent price (women), and fodder quality (cattle breeders). Several samples of firewood of known weight were prepared and shown to the women for appreciation. Fodder of each species was also cut and given to animals to assess palatability
.

Aerial biomass was determined after coppicing at two heights (25 cm and 50 cm from soil surface) in order to assess the regeneration performance. All the data have been analyzed using MINITAB statistical software and recently using STATISTIX 7.0, 2000 analytical software, USA.

Results achieved

Experiment 1

The results of analysis of variance (two-way ANOVA) on diameter at the base, diameter at breast height, height and number of branches concerning the four species are presented in Table 1. It appears that all these parameters vary significantly according to species. Furthermore, the double inoculation has a significant effect on them. *Khaya senegalensis* had the greatest diameter at the base but the smallest number of branches (only 6 against 70 for Leucaena). By contrast, *Leucaena leucocephala* grew better, it had the greatest diameter at the base, diameter at breast height and number of branches, demonstrating its superiority in fuelwood and fodder production compared to the other species.

Table 1: Growth parameters of various species with or without rhizobium and mycorrhizal inoculation in semi arid region in Siribala (Mali).

Species	**D_{00} (cm)**		**$D_{1.3}$ (cm)**		**Height (m)**		**Number of branches**	
	Means	**Groups**	**Means**	**Groups**	**Means**	**Groups**	**Means**	**Groups**
Gsep	6,33	B	3,28	C	4.97	B	19	C
Lleuc	7,32	B	5,10	A	6.98	A	70	A
Aang	4,23	C	2,32	D	3.97	C	28	B
Ksen	8,54	A	4,47	B	4.75	B	6	D
Mean	6.61		3.79		5.17		30.81	
P(0.05)	0.000		0.000		0.000		0.000	
Inoculation								
R+M	7.1	A	4.14	A	5.64	A	34	A
Control	6.1	B	3.43	B	4.69	B	28	B
Mean	6.6		3.79		5.16		31	
P(0.05)	0.000		0.000		0.000		0.01	

Figure 1 shows the difference in growth parameters according to species.

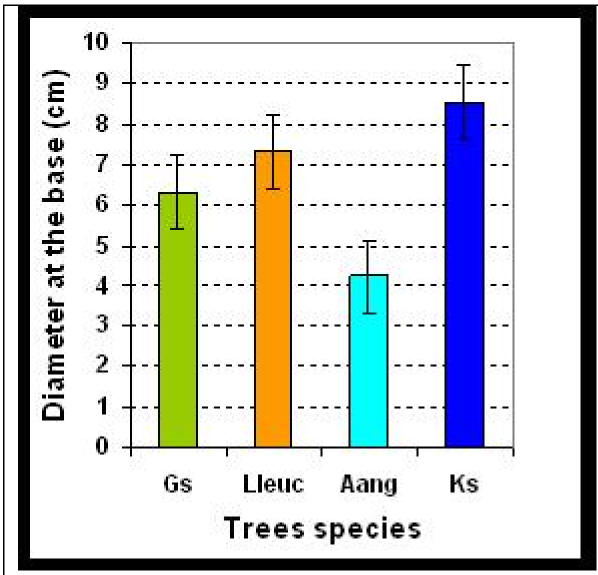

Fig1a. Mean tree diameter at the base

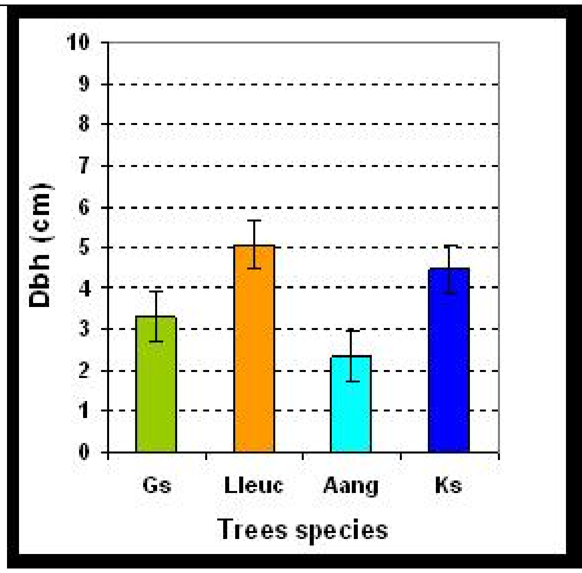

Fig1b. Mean tree diameter at breast height

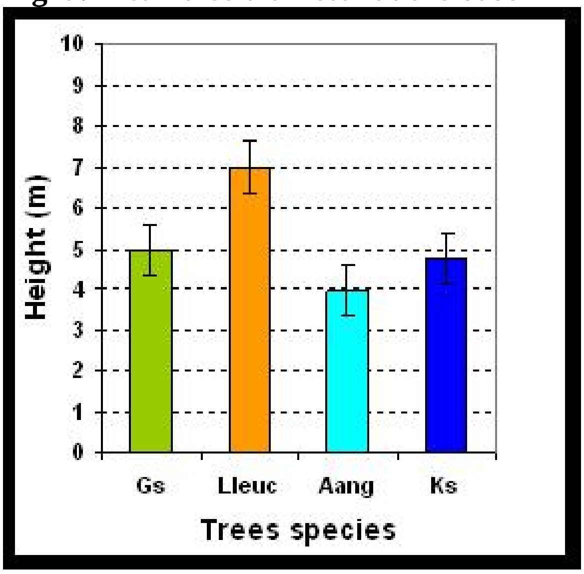

Fig1c. Mean tree height

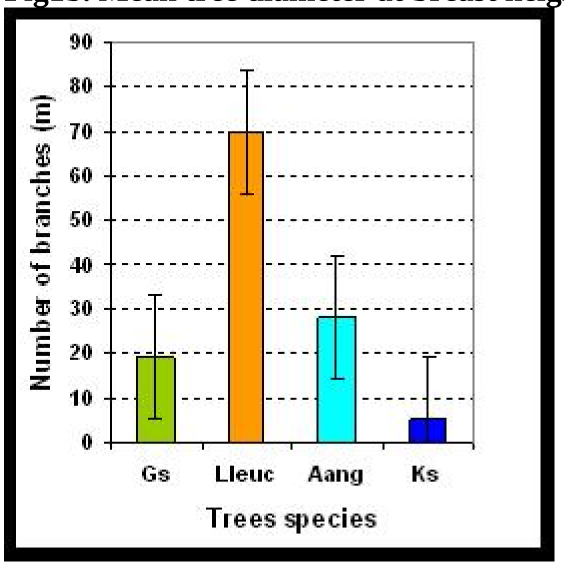

Fig1d. Mean number of branches

Figure 1: Growth parameters of 4 species in semi arid region in Siribala (Mali).

The height of *Leucaena leucocephala* is about 7 m only two years after planting. Its height is double that of *Acacia angustissima.*
The double inoculation has a significant effect on tree growth. For instance the mean number of branches of inoculated trees is about 22% greater than non-inoculated trees (Table 1). These positive effects are shown in Figure 2.

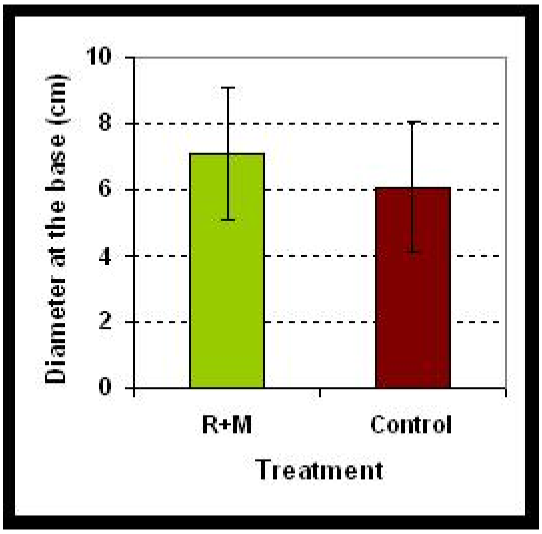

Fig 2a Diameter at the base

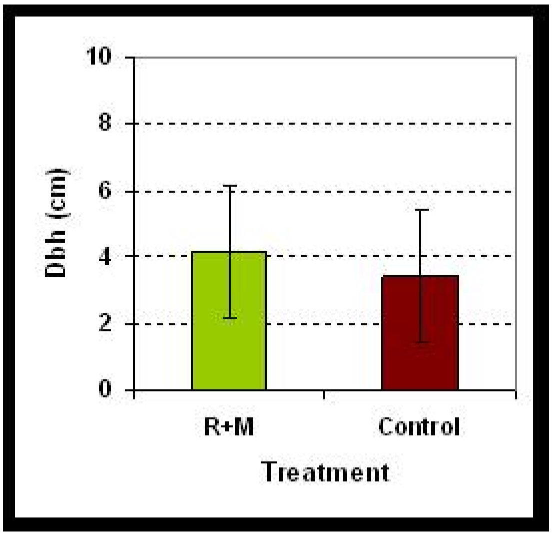

Fig 2b Diameter at breast height

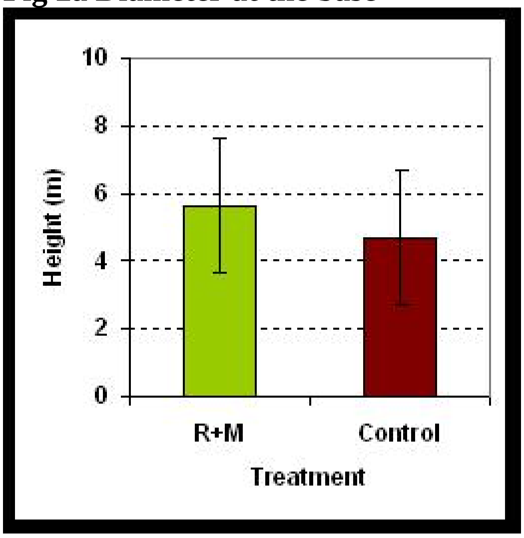

Fig 2c tree height

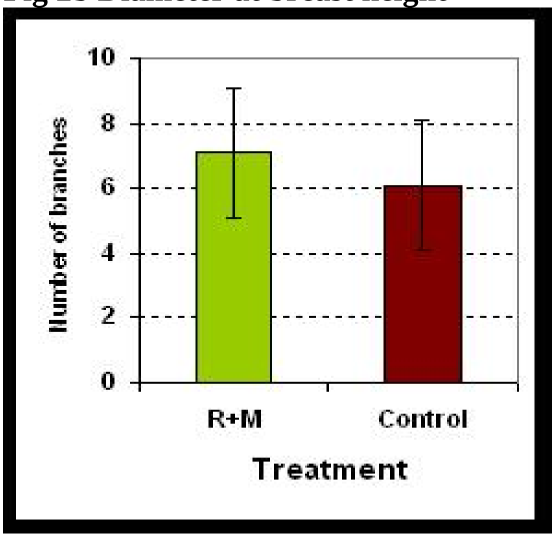

Fig 2d number of branches

Figure 2: Effect of inoculation on mean diameter at the base, mean diameter at breast height (Dbh), mean height and mean number of branches of 4 species inoculated or not with rhizobium and mycorrhiza in semi arid region in Siribala (Mali).

The results of analyses of variances concerning aerial biomass (fuelwood and fodder) are given in Table 2.

Table 2: **Fuelwood and fodder production (t ha-1) by various species inoculated with or with out rhizobium and mycorrhiza in semi arid region in Siribala (Mali).**

Species	Dry fuelwood production		Fresh fodder production		
	Means	Homogeneous groups	Species	Means	Homogeneous groups
Lleu Gsep Aang Ksen	36.100 25.600 17.300 14.200	A B C D	Gsep Lleu Aang Ksen	24.900 22.700 18.200 16.900	A AB B BC
M (tons) SE (tons) P (0.05)	23.300 2.089 0.0038		20.675 1.3466 0.047		

Figure 3 highlights the effects of inoculation. The inoculation with rhizobium and mycorrhiza (p = 0.0087 for fuelwood production and p = 0.0294 for fodder production) has a positive effect on plant production. It is 67% greater than the control (29.150 t ha-1 against 17.450 t ha-1) for fuelwood production and 35% for fodder production (23.750 t ha-1 against 17.600 t ha-1).

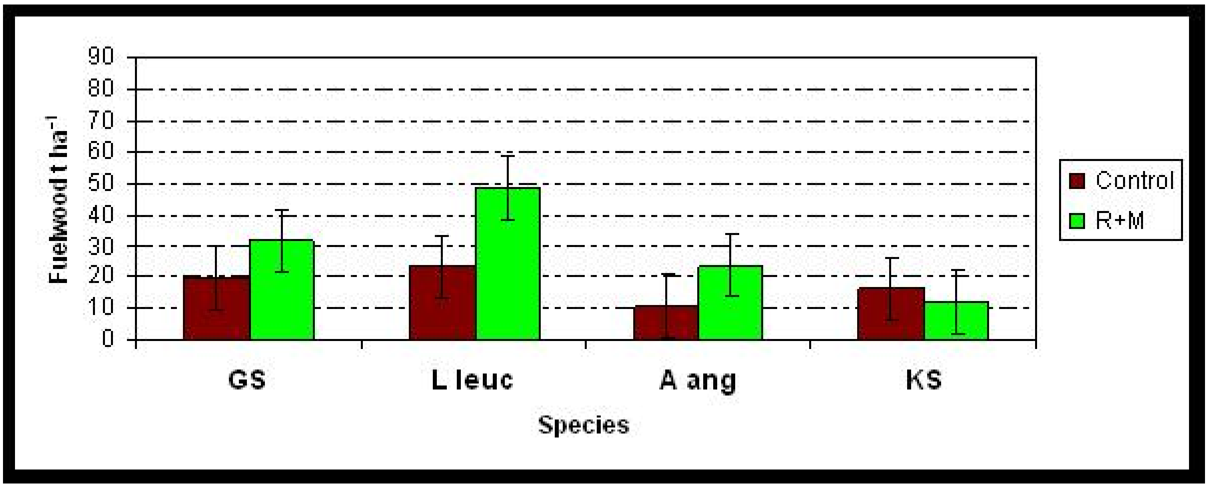

Figure 3: **Dry fuelwood production by 4 tree species with or without inoculation with rhizobium and mycorrhiza in semi arid region in Siribala (Mali).**

The production levels obtained are comparable to those reported by 'Trees for the future' (2006) which mentioned 60 t ha^{-1} 3 years after planting. Fodder production follows the same tendency (Figure 4).

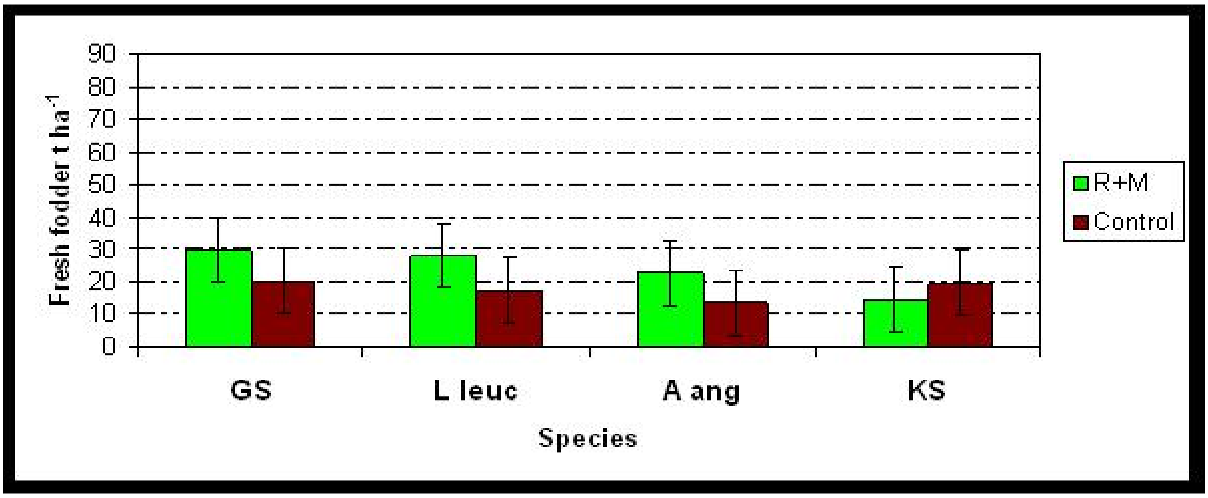

Figure 4: **Fresh fodder production by 4 species inoculated or not with rhizobium and mycorrhiza in semi arid region in Siribala (Mali).**

Comparing growth in the two studies shows that these species are very sensitive to competition. In the experiment 2 because of the number of species (10) and the small spacing between trees the production for the same species diminished considerably. For instance Khaya in experiment 2 produced only 0.5 t ha-1 of fuelwood when mixed and 14 t ha^{-1} in pure plantation. However, planting many species could be beneficial mainly because of the diversity gained in fodder and the management of disease risk or other environment constraints. When summed on a land area basis, the total fuelwood or fodder production are comparable to those of the more widely spaced pure plantation.

Coppicing: Two months after coppicing, regrowth as assessed to determine whether regeneration is better when the coppicing was done at 25 cm or 50 cm height above the ground. The results are reported inTable 3. It appears that the cutting height and inoculation don't have significant effects (NS) on the diameter at the base, the height and the number of sprouts of the tree species. However, the diameter at the base and the height of sprouts are greatest with *Leucaena leucocephala* which grew faster. For instance, the height of sprouts of Leucaena is 2.5 times that of *Khaya senegalensis* and its diameter at the base is 2 times higher than that of *Acacia angustissima*.

Table 3: Growth parameters of sprouts after coppicing at two heights (50 and 25 cm from soil surface) of four species inoculated with or with out rhizobium and mycorrhiza in semi arid region in Siribala (Mali).

Species	Diameter at the base (cm)		Height of sprouts (m)		Number of sprouts	
	Means	Groups	Means	Groups	Means	Groups
Gsep	1.39	AB	1.4	B	22	NS
Lleuc	1.67	A	2.19	A	20	NS
Aang	0.79	C	0.92	BC	14	NS
Ksen	1.08	B	0.85	C	14	NS
Mean	1.23		1.34		18	
P(0.05)	0,023		0,003		0,183	
Inoculation						

R+M Control	1.32 1.15	NS NS	1.41 1.27	NS NS	18 18	NS NS
Mean P(0.05)	1.23 0,690		1.34 0,168		18 0,804	
Cutting Height						
50 cm 25 cm	1.24 1.22	NS NS	1.41 1.27	NS NS	19 17	NS NS
Mean P(0.05)	1.23 0,863		1.34 0,297		18 0,452	

Lines affected by the same letter are not significantly different at p=0.05 level.

The good performance of Leucaena which is followed by *Gliricidia sepium* is highlighted in Figure 5.

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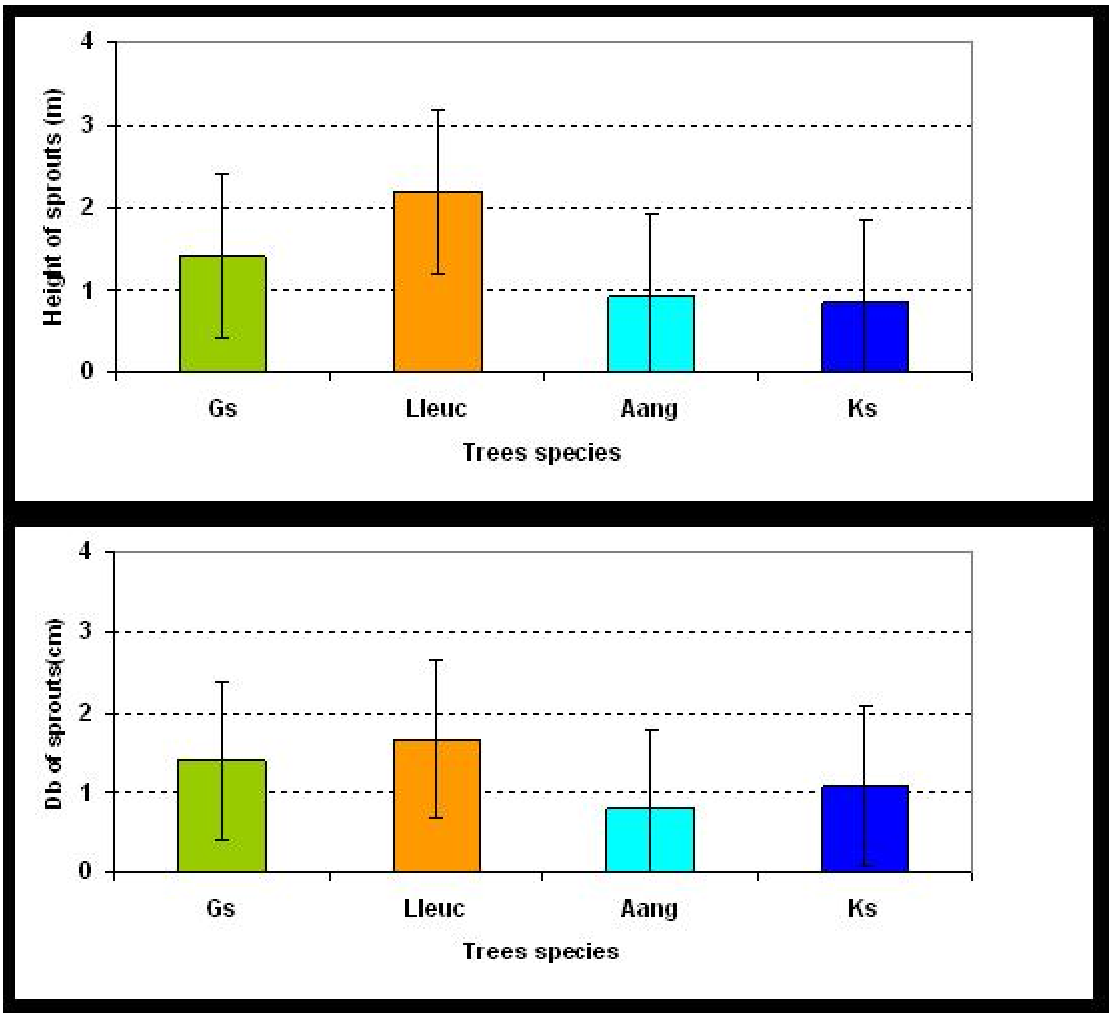

Figure 5: Height and diameter at the base of sprouts after coppicing at two heights (50 and 25 cm from soil surface) of four species in semi arid region in Siribala (Mali).

Experiment2

Coppicing height had no significant effect on dry fuelwood and fodder production (respectively P = 0.5799 and 0.9240 with a probability level of 5%). When cut at 25 cm fuelwood and fodder production were 2.9638 t and 4.1153 t respectively, compared with cutting at 50 cm, when they were 3.2491 t and 4.2010 t. However, dry fuelwood production differed significantly according to species (Table 4) and it ranged from 1 kg ha^{-1} to 7.6916t ha^{-1}. *Pterocarpus lucens* was the least productive (only 1kg ha^{-1} of dry fuelwood and 56 kg ha^{-1} of fodder) because it genetically grows very slowly but also because it doesn't tolerate the competition. *Leucaena leucocephala* and *Acacia mangium* have significantly the greater dry fuelwood production followed by *Gliricidia sepium*, *Acacia auriculiformis* and *Acacia angustissima*. There are 4 species (*Acacia crassicarpa*, *Acacia senegal*, *Calliandra calothyrsus* and *Khaya senegalensis*) in which the dry fuelwood means are not significantly different from one to another. One surprising and interesting thing is the production of *Khaya senegalensis* which is well known as a very slow growing species (Yengue and Callot, 2002). In our screening experiment where 10 species are mixed, the production of this species was found to be up to 5 t ha^{-1} and 7.7 t ha^{-1} respectively for fuelwood and fodder, after only 2 years.

With 14.333 t ha^{-1}, *Acacia mangium* has the greatest fodder production which is double that of its dry fuelwood. *Acacia auriculiformis* which produced less wood (3.7912 t ha^{-1}) produced most fodder (6.5667 t ha^{-1}).
To summarize, the fuelwood and fodder production are greater for *Acacia mangium*, *Gliricidia sepium*, *Leucaena leucocephala*, *Acacia auriculiformis*. But the best is ***Acacia mangium*** which produces much wood and much fodder.

Table 4: Fuelwood and fodder production (t ha^{-1}) of various species in semi arid region in Siribala (Mali).

Species	Dry fuelwood production		Fresh fodder production		
	Means	Homogeneous groups	Species	Means	Homogeneous groups
Lleu	7.6916	A	Aman	14.333	A
Aman	7.4603	AB	Aaur	6.5667	B
Gsep	5.5463	B	Gsep	6.5133	B
Aaur	3.7912	BC	Lleu	5.4333	B
Aang	2.3940	C	Acra	3.9000	BC
Acra	1.5714	CD	Asen	1.9000	C
Asen	1.3533	CD	Aang	1.7400	C
Ccal	0.7632	D	Ksen	0.7713	CD
Ksen	0.4925	D	Ccal	0.3673	CD
Pluc	1.00E-03	E	Pluc	0.0561	D
M (tons)	3.1065		4.1581		
SE (tons)	0.2567		0.4475		
P (0.05)	0.0000		0.0000		

Lleu (*Leucaena leucocephala*), Aman (*Acacia mangium*), Gsep (*Gliricidia sepium*), Aaur(*Acacia auriculiformis*), Aang (*Acacia angustissima*), Acra (*Acacia crassicarpa*), Asen (*Acacia senegal*), Ccal(*Calliandra calothyrsus*), Ksen (*Khaya senegalensis*), Pluc (*Pterocarpus lucens*), M(mean), SE (standard error), P (probability level of 5%).

In this experiment too, the coppicing was done at two heights (25 cm or 50 cm) after two months and the growth parameters have been measured to assess the regeneration capability of the tree species. The results are reported in Table 5.

Table 5: Growth parameters of sprouts two months after coppicing of 10 species in semi arid region in Siribala

Species	Diameter at the base (cm)		Height of sprouts (m)		Number of sprouts	
	Means	Groups	Means	Groups	Means	Groups
Aman	0,4525	CDE	0,6417	CD	13	NS
Ksen	0,9039	C	0,6917	CD	7	NS
Ccal	0,2856	CDE	0,3733	D	4	NS
Pluc	0,5194	CD	0,6600	CD	9	NS
Acra	0,8328	C	0,9972	C	17	NS
Aaur	0,3333	CDE	0,6056	CD	21	NS
Lleu	1,8739	A	2,2806	A	23	NS
Aang	0,8233	C	1,3083	BC	14	NS
Asen	0,8822	C	1,1306	C	32	NS
Gsep	1,5167	B	1,6583	B	24	NS
Mean	0.84		1.034		17	
P(0.05)	0.000		0.000		0,085	
Coppicing Height						
50 cm	0,84	NS	1,14	NS	19	NS
25 cm	0,85	NS	0,93	NS	14	NS
Mean	0.85		1.035		17	
P(0.05)	0,937		0,056		0,155	

After two months, the coppicing height had no significant effect on the diameter at the base, the height and the number of sprouts.
The diameter at the base and the height of the sprouts were both significantly higher for *Leucaena leucocephala* compared to the other species. It is followed by *Gliricidia sepium. Calliandra calothyrsus* has the smaller diameter at the base and sprout height. *Leucaena leucocephala* and *Gliricidia sepium* have the better aptitude of regeneration compared to the other species.

Field visit and farmers' opinions on species production and palatability

Fuelwood quality
This parameter was determined on the four species of the experiment 1 because they were well developed and were in pure plantation.

According to their cocking experiences, 70% of the women interviewed said that *Gliricidia sepium* is a good fuelwood and evaluated the price of 55 kg at 200 FCFA (less than ½ US dollar). 90% of the women found that *Leucaena leucocephala* is a good fuelwood and evaluated the price of 85 kg at 500FCFA (1 US dollar).
Khaya senegalensis has been identified as the most energetic fuelwood by 80% of the women and they evaluated the price of 85 kg at 500 FCFA (1 US dollar). This conclusion is without doubt linked to the knowledge they have on this local species rather than an objective assessment.
Only 40% of the women identified *Acacia angustissima* as a good fuelwood and evaluated the weight of 45 kg at 200 FCFA.
The classification of the species by the women can be summarized as follows:
Leucaena leucocephala > *Gliricidia sepium* > *Khaya senegalensis* > *Acacia angustissima.*

Palatability test
This test used the species from experiment 2 because of their number (10 species), and was conducted using fresh fodder.

Among the 10 species the **goats** have greatly eaten *Acacia angustissima, Leucaena leucocephala, Calliandra calothyrsus, Pterocarpus lucens and Acacia senegal. Acacia mangium and Gliricidia sepium have been less eaten when fresh.* This is not surprising because in this area it is known that animals like very much Gliricidia when it is dry or pretty dry. *Acacia crassicarpa* and *Acacia auriculiformis* were the only species not eaten by goats.
All the 10 species have been greatly eaten by **sheep**.
Only *Pterocarpus lucens* was eaten by **donkeys**, the other species were not eaten probably because fodders were fresh. **Oxen** ate only 3 species: *Leucaena leucocephala, Acacia angustissima* and *Acacia auriculiformis*

Conclusion:
Inoculation with rhizobium and myccorhiza had a positive effect on plant production. It is 67% greater than the control (29.150 t ha^{-1} against 17.450 t ha^{-1}) for fuelwood production and 35% for fodder production (23.750 t ha^{-1} and 17.600 t ha^{-1}).
Acacia mangium has the greater fodder production (14.333 t ha^{-1}) which is the double of its dry fuelwood (7.4603 t ha^{-1}). This species is well adapted to our irrigated condition in Siribala *Acacia auriculiformis* which produced less wood (3.7912 t ha^{-1}) produced much fodder (6.5667 t ha^{-1}). *Acacia angustissima* shows poorer performance in the field because of its high water demands and small production (2 t ha^{-1} of fuelwood and 1.7 t ha^{-1} of fodder).
Sheep and goats are the main animals which eat greatly the fodder coming from the tree species whereas donkeys ate only *Pterocarpus lucens*. They are followed by oxen which eat mainly *Leucaena leucocephala, Acacia angustissima* and *Acacia auriculiformis.*
For fuelwood quality, women prefered respectively *Leucaena leucocephala, Gliricidia sepium, Khaya senegalensis* and *Acacia angustissima.*

Work package 3. To assess and understand the adaptation factors of these species, climatic data, soil water status and water used (sapflow measurement) data have been collected.
The crop evapotranspiration (ET_0) has been calculated using the FAO software CROPWAT 5.7 because the major parts of the effects of various weather conditions are incorporated into its estimation. So, parameters such as temperature, relative humidity, solar radiation, wind speed, and location (altitude and latitude) have been measured. All these data are available and have been presented in the past report and final report.

Work package 4: Mycrosymbionts 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. Inoculation has been done in nursery at Siribala experimental site.
Mycorrhizal infection data of all the tree species from the different treatments are necessary for the interpretation of the growth results found in the field. For this reason, fine roots of all the species have been collected regularly and stored in a cool place to await assessment of mycorrhizal infection. Prior to coppicing activities

(March 2007), soil from the experimental site have been collected and an experiment will be set up in nursery in order to assess the inoculum potential of the field site. Data concerning root length, shoot dry weight and root dry weight are available. Soil samples are regularly collected for spore extraction. All these activities will be performed because the data that will be gathered are absolutely necessary for the interpretation of our results.

Work package 5: Socio economic surveys are the responsibility of Partner 3.

Work package 6: Soil and Plant nutrients,
Before the preparation of field sites, soil samples have been collected and their chemical analyses have been done. Another set of soil sample have been taken one year after tree planting. Soil samples have been taken just before the coppicing activities. Plant component of all the species used in the experiments have been collected and oven dried. Plant components were sampled just before coppicing trees. All these samples have been sent to our laboratory for analysis of their chemical content.

Work package 7: **Planting stock qualities**,
Planting stock qualities such as total plant dry weight shoot: root ratio, sturdiness quotient Dickson's quality index have been determined for all the species used in the experiments and the results are available.

Dissemination activities:

1. Stakeholders' day in Minimana experimental site. The site will be open to farmers, women associations, breeders associations, NGO's, Staff of 'Office du Niger', State development offices working in Niono, Marakala, Dougabougou, researchers from the IER centre in Niono;
2. The results of the project are disseminated to local population through broadcasting of the local radios of Siribala and Niono.
3. A team of Malian National TV will visit the site, and the images, the objectives, the results of the project will broadcast.
4. Scientific publications and posters will be produced.

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Summary

During the last year of the project several groups (women, rural population, scientist and NGO, community leaders) visited all experimentation set up in Minimana where irrigated plantations are located. Results obtained on main activities are summarized as defined in the different work packages.

Inoculation potential assessment on plant growth planned after coppicing was not performed because of inundation by rainfall during this period. Main investigations concerned rhizobia strains molecular characterization studies and strains isolation using previously collected nodules. Great diversity of indigenous strains was found

In the framework of training two scientists Fallaye KANTE (FAST) and Youssouf CISSE (IER) in Mali may achieve their thesis with the collaboration of LCM (Dakar) and ISFRA (Mali). This project allowed us to be supported by AUF and University of Bamako by contributing to train Fallaye KANTE (for 4 months at LCM) and Mohamed TRAORE for a DEA in biotechnology at FAST.

Scientific annual report

Objectives: For this period the main objectives were:

- To verify persistence of inoculants in tree leguminous nodules
- To train scientists in molecular biology,
- To disseminate results of these studies to the user communities (GOs, NGOs, farmers, peri-urban landowners etc.) via participatory workshops and visits to sites.

Meetings and visits

Local groups, rural population and NGO visited all experimentation where irrigated plantations were set up.

Activities

Workpackage 4: Microsymbiont & N-Fixation

Main investigations concerned molecular characterization studies and strains isolation using previous collected nodules.
At 21 and 24 months after planting, the effects of inoculation on plant growth and nodulation were not assessed: because of flooding, measurements were not performed.

At Minimana site (Siribala), rhizobia diversity studies were performed in the nursery and field using DNA extracted from nodules collected from plants in experiment 1 and 2. Nodules were collected from the three tree species: *Acacia angustissima*, *Gliricidia sepium* and *Leucaena leucocephala*.

DNA quality was checked using gel electrophoresis. PCR of rDNA IGS 16S-23S was performed in our lab at FAST. For molecular studies two restriction enzymes were used: *Hae* III and *Msp* I for RFLP. Main molecular studies were carried out at the laboratory of LCM (Partner 5) at Dakar using the protocol described by Krasova Wade *et al.,* 2005. For each experiment, relevant results are illustrated using types of genetic profiles presented in Table 6 - Table 17 and Figure 6 - Figure 11.

1. Nursery results concerning experiments 1 and 2 are as follows:

Experiment 1 For each tree leguminous species, 60 root nodules were used for strain molecular characterisation. Among them, 30 nodules were collected from trees inoculated with R+M and the other 30 nodules from control plants. Results given in Table 6 - Table 8 show a very low frequency of the strains which had been used as inoculants. *Leucaena* and *Gliricidia* nodules were dominated by single indigenous strains. Were inoculants less competitive than indigenous strains, or were inoculation processes unsuccessful?

Table 6 Profiles of rhizobial strains nodulating *Acacia angustissima* in Experiment 1 in the nursery

Treatments	Types of genetic profiles				
	Inoculant strains		Indigenous strains		
	11c	13c	A	B	C
Inoculation (R +M)	5	0	3	1	8
Control	0	0	4	0	0
Total number of profiles	5	0	7	1	8

Table 7 Profiles of rhizobial strains nodulating *Gliricidia sepium* in Experiment 1 in the nursery

Treatments	Types of genetic profiles	
	Inoculant strain	Indigenous strain
	GsK_4	D
Inoculation (R +M)	0	25
Control	0	22
Total number of profiles	0	47

Table 8 Profiles of rhizobial strains nodulating *Leucaena leucocephala* in Experiment 1 in the nursery

Treatments	Types of genetic profiles		
	Inoculant strain	Indigenous strains	
	LdK_4	E	F
Inoculation (R +M)	0	28	0
Control	1	22	1
Total number of profiles	1	50	1

Experiment 2

In this experiment, one treatment was applied (R+M). Thirty (30) nodules were used for strain molecular characterization, and results are presented in Table 9 - Table 11. As in experiment 1, the occurrence of inoculant strains was rare.

Table 9 Profiles of rhizobial strains nodulating *Acacia angustissima* in Experiment 2 in the nursery

Treatments	Types of genetic profiles										
	Inoculant strains		Indigenous strains								
	11c	13c	G	H	I	J	K	L	M	N	O
Inoculation (R +M)	1	0	2	1	3	2	5	1	2	2	1
Total number of profiles	20										

Table 10 Profiles of rhizobial strains nodulating *Gliricidia sepium* in Experiment 2 in the nursery

Treatments	Types of genetic profiles				
	Inoculant strain	Indigenous strains			
	GsK_4	D	P	Q	R
Inoculation (R +M)	0	10	5	5	1
Total number of profiles	21				

Table 11 Profiles of rhizobial strains nodulating *Leucaena leucocephala* in Experiment 2 in the nursery

Treatments	Types of genetic profiles	
	Inoculant strain	Indigenous strains
	LdK_4	R
Inoculation (R +M)	0	27
Total number of profiles	27	

In our experimental conditions, using irrigated wastewater for plant production in experiment 1 and 2.

- Rhizobial strains used as inoculants for *Gliricidia sepium, L. leucocephala and A. angustissima* rarely occurred in the harvested nodules. Strain 11c, with *A. angustissima* was the most persistent, but nodules were dominated by indigenous strains.
- In experiment 2, a considerable diversity of indigenous strains was found with Acacia and Gliricidia.

Experiment 3 in nursery Nodules were collected in order to follow the persistence of single inoculated rhizobia strains in the field. Nodule DNA was extracted for strain diversity investigation, as before**.**

2. In the field in experiment 1 and 2, fifteen (15) nodules per treatment and per tree species were used for DNA extraction and strain diversity investigation by PCR RFLP studies. The main results are shown below (Table 12 - Table 17, Figure 6 - Figure 11).

Table 12 Occurrence of IGS types of rDNA 16S-23S obtained using HaeIII and MspI in PCR-RFLP of *Acacia angustissima* **nodules collected from the field experiments**

Treatments	Type of IGS		
	Inoculant strains		Indigenous strains
	11c	13c	D
R + M	0	0	8
T control	0	0	7
Total	0	0	15

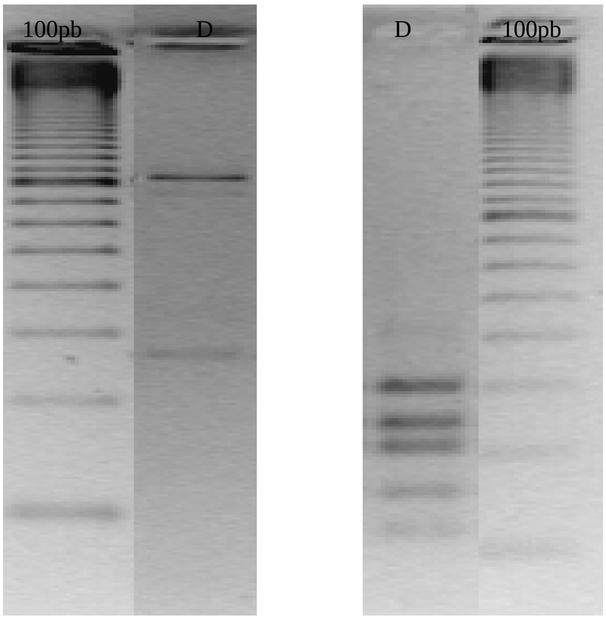


Figure 6 IGS profiles found using *Hae* III and *Msp* I in RFLP of rhizobia strains nodulating *A. angustissima* from field samples

Table 13 Occurrence of IGS types of rDNA 16S-23S obtained using HaeIII and MspI in PCR-RFLP of *Gliricidia sepium* nodules collected from the field experiments

Treatments	Type of IGS	
	Inoculant strain	Indigenous strains
	GsK_4	D
R + M	4	3
Control	2	3
Total	6	6

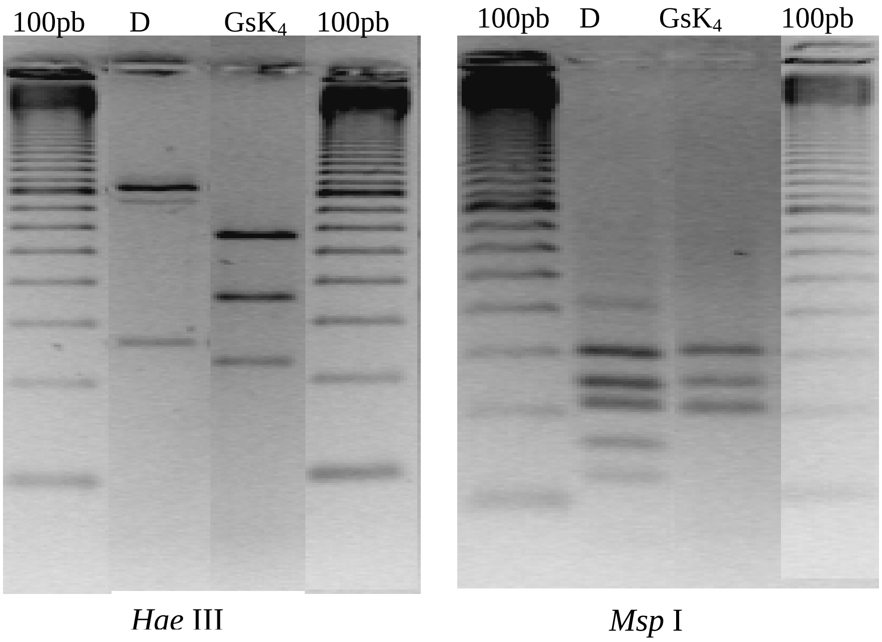
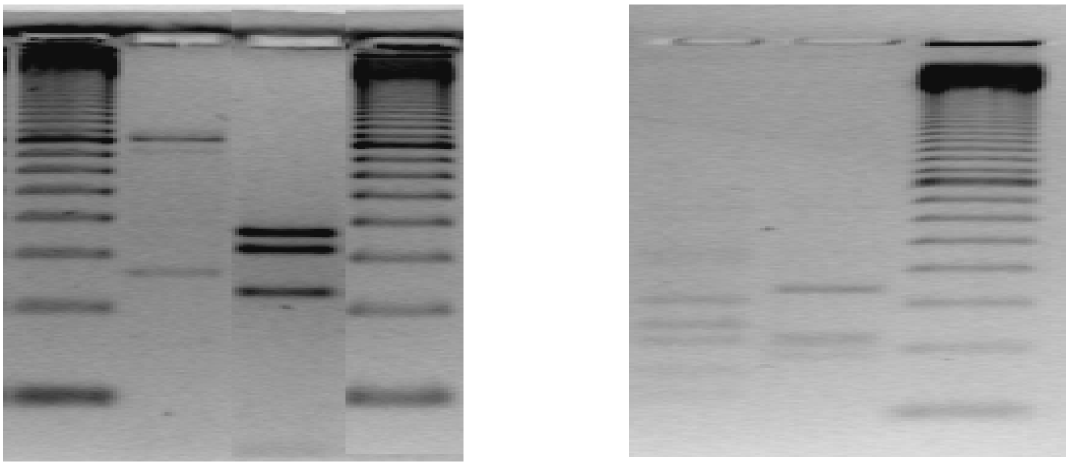


Figure 7 IGS profiles found using *Hae* III and *Msp* I in RFLP of rhizobia strains nodulating *G. sepium* from field samples

Table 14 Occurrence of IGS types of rDNA 16S-23S obtained using HaeIII and MspI in PCR-RFLP of *Leucaena leucocephala* nodules collected from the field experiments

Treatments	Type of IGS	
	Inoculant strain	Indigenous strains
	LdK_4	D
R + M	7	2
Control	9	0
Total	16	2



Hae III *Msp* I

Figure 8 IGS profiles found using *Hae* III and *Msp* I in RFLP of rhizobia strains nodulating *L. leucocephala* from field samples

Experiment 3 in field.

Table 15 Occurrence of IGS types of rDNA 16S-23S obtained using HaeIII and MspI in PCR-RFLP of *Acacia angustissima* nodules collected from experiment 3 in the field

Treatment	Type of IGS			
	Inoculant strains		Indigenous strains	
	11c	13c	T	D
M	0	0	4	3
R	0	0	2	9
Total	0	0	6	12

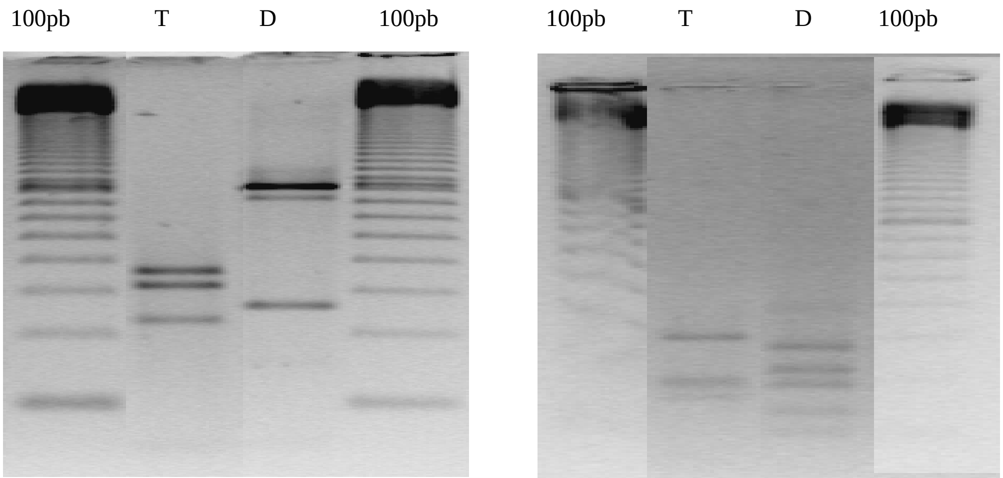


Figure 9 IGS profiles found using *Hae* III and *Msp* I in RFLP of rhizobia strains nodulating *A. angustissima* from field samples

Table 16 Occurrence of IGS types of rDNA 16S-23S obtained using HaeIII and MspI in PCR-RFLP of *Gliricidia sepium* nodules collected from experiment 3 in the field

Treatment	Type of IGS	
	Inoculant strain	Indigenous strain
	GsK_4	D
R	3	7
M	3	6
Total	6	13

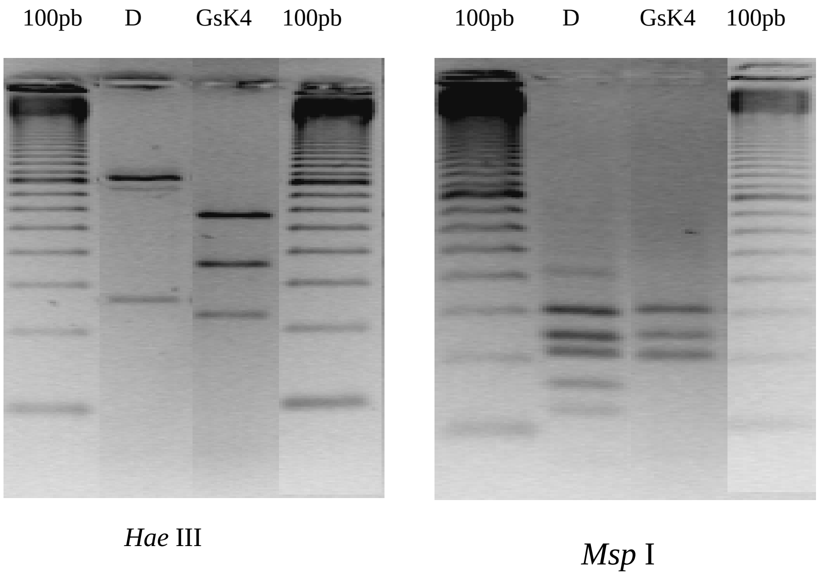
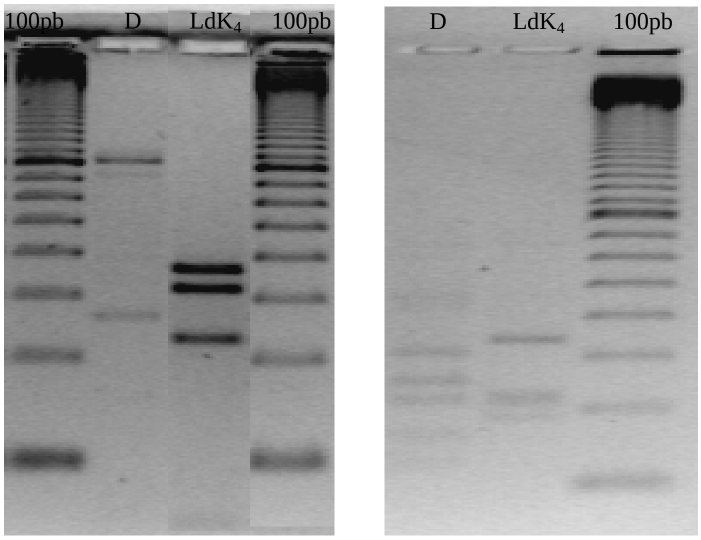


Figure 10 IGS profiles found using *Hae* III and *Msp* I in RFLP of rhizobia strains nodulating *Gliricidia sepium* from field samples

Table 17 Occurrence of IGS types of rDNA 16S-23S obtained using HaeIII and MspI in PCR-RFLP of *Leucaena leucocephala* nodules collected from experiment 3 in the field

Treatments	Type of IGS	
	Inoculant strains	Indigenous strains
	LdK_4	D
R	3	5
M	3	2
Total	6	7



Hae III *Msp* I

Figure 11 IGS profiles found using *Hae* III and *Msp* I in RFLP of rhizobia strains nodulating *Leucaena leucocephala* from field samples

In conclusion, In the nursery, the frequency of inoculant strains is very low, whilst in field trials, they were observed more frequently. Strains of rhizobia used as inoculants of *Gliricidia* and *Leucaena* are mainly present in investigated nodules in all experiments. Of the indigenous strains, type D was found in nodules of all three leguminous tree species. Also we found that numbers of indigenous strains were higher in treatment R+M than in control or single strain inoculation.

Strain isolation. Exp 1 and 3. From crushed nodules collected in Experiment 1 before coppicing from treatments (R+M) and Control, only five rhizobia strains were isolated from *A angustissima* and *G. sepium*, and 1 isolate of *L . leucocepahala* with R+M . We plan to undertake molecular characterizationof these strains by PCR RFLP.

Workpackage 5: economics

Questionnaires were used to collect data and information and main results on utilisation of wastewater for fuelwood and fodder production at different levels: villages, associations, households were developed in French.

Opinions of the population on proposed tree species and wastewater utilization are given below (Table 18 - Table 20). Origins of wood used for different purposes, constraints of wood supply and energy use are listed in Table 21 - **Error! Reference source not found.**.

Table 18 Evaluation or opinion on proposed tree species by local populations in the different villages

Exotic leguminous trees species	*Evaluation*
Acacia crassicarpa	***Unknown in Mali***
Acacia mangium	***Without success in irrigation conditions***
Acacia auriculiformis	***Well***
Leucaena spp.	***Well***
Gliricidia sepium	***Good***
Local and Known leguminous tree species in Mali	
Acacia senegal	**-**
Acacia seyal	***A. nilotica and tomentosa better in Mali than A. seyal***
Pterocarpus erinaceus	***Medium***
Pterocarpus santaniloides	**-**
Khaya senegalensis	***Well***
Eucalyptus	***Well***

Table 19 Opinions on wastewater « eaux de drainage » quality and quantity in 3 villages

Opinion		*Villages*			*Total*
		Boh	*Laminibougou*	*Minimana*	
Yes	*Number*	*38*	*30*	*11*	*79*
	% of total	*40.4%*	*31.9%*	*11.7%*	*84.0%*
No	*Number*	*7*	*8*		*15*
	% of total	*7.4%*	*8.5%*		*16.0%*
	Total	*45*	*38*	*11*	*94*
	% of total	*47.9%*	*40.4%*	*11.7%*	*100.0%*

Table 20 Activities using wastewater « eaux de drainage »

Villages		Uses of drainage water					Total
		Vehicles	Arboriculture	Watering of animals	Rice growing	Fish	
Boh	Number	21	4	35	11	3	74
	% of total	16.5%	3.1%	27.6%	8.7%	2.4%	58.3%
Laminibougou	Number	3	11	22			36
	% of total	2.4%	8.7%	17.3%			28.3%
Minimana	Number	2	4	7	4		17
	% of total	1.6%	3.1%	5.5%	3.1%		13.4%
	TOTAL	26	19	64	15	3	127
	% du total	20.5%	15.0%	50.4%	11.8%	2.4%	100.0%

Table 21 Origin of dry wood « bois sec »

Villages	Origin of dry wood			Total
	Village land	Other villages	No response	
Boh	42,3%	3,1%	4,1%	49,5%
Minimana	8,2%		3,1%	11,3%
Laminibougou	24,7%	1,0%	13,4%	39,2%
Total	75,3%	4,1%	20,6%	100,0%

Table 22 Origin of wood for construction

Villages	Origin of wood for construction				Total
	Village land	Other villages	Purchased	No response	
Boh	41,9%	8,6%		1,0%	51,4%
Minimana	9,5%			1,0%	10,5%
Laminibougou	33,3%	1,9%	2,9%		38,1%
Total	84,8%	10,5%	2,9%	1,9%	100,0%

Table 23 Origin of wood for other uses

	Provenance du bois de service				Total
Villages	Village land	Other villages	Purchased	No response	
Boh	28,3%	9,4%	1,9%	11,3%	50,9%
Minimana	8,5%		,9%	,9%	10,4%
Laminibougou	30,2%	1,9%	2,8%	3,8%	38,7%
Total	67,0%	11,3%	5,7%	16,0%	100,0%

Table 24 Constraints of wood supply

Constraints	Frequency	Percent (%)
Scarcity	80	37,6
Distance	67	31,5
Quantity	16	7,5
Quality	16	7,5
Insufficiency of equipment	5	2,3
High cost	9	4,2
Disappearance of the biodiversity	7	3,3
Increase in population	3	1,4
Purchase	6	2,8
Availability of men	1	,5
Repression by the forest agents	3	1,4
Total	213	100,0

Table 25 Annual consumption of wood energy per capita (tons)

Wood		
Rural area Aranged zones	Villages or cities surveyed	Consumption/ha/an/T
Cities	**Niono**	0.46
	Macina	0.65
	Diabaly	0.26
	Siribala	0.87
	Dougabougou	0.68
	Cities	0.53
		0.56
Charcoal	Niono	0.05
Craft Industry (Artisans)		1.080

Information on costs, margins and prices of existing production chains and systems have been obtained.

Several meetings were held in the frame work of liaison with end users, NGOs, womens' groups, which were documented by film and photographs.

In May 2007, tree coppicing was done without the collaboration of Youssouf Cisse from IER who is responsible for the economics workpackage.

Workpackage 6: Water, Soil and plant nutrition

Following previous analyses (see report of 2005-06), further analyses of water samples was conducted (Table 26).

Table 26: Results of irrigation water analysis site of Minimana on February and May2007.

Water contents	Results of irrigation water analysis ($mg.l^{-1}$)			
	***Minimana* 02/2007**	***Minimana* 05/2007**	***Ricefield* 05/2007**	***Typha* 05/2007**
Nitrites $N0_2^-$	0	0.004	0.006	0
Nitrates $N0_3^-$	2.1	-	-	-
N ammoniacal NH_4^+	0.43	0.56	0.4	0.37
$OrtPO_4^{3-}$	0.9	0.1	0.08	0.2
SO_4^-	4.8	-	-	-
pH	7.4	7.06	6.58	6.84
Fe^{2+}	0.85	-	-	-
Ca^{2+}	3.2	-	-	-
Mg^{2+}	1.9	-	-	-
Mn	0	0	0.014	0.001
Na^+	7.2	-	‘	-
K^+	0.7	-	-	-
Cl^-	2.5	-	-	-
Zn	0.08	0.04	0.014	0.12
Ni	-	0.01	0.005	0.046
Cu	0.05	0.08	0.02	0.06
Cr	-	-	-	-
Bicarbonate	18	-	-	-
Fluor	0.31	-	-	-
DBO5	0	-	-	-
DCO	0	98	80	25
Conductivité (µS/cm)	45	265	218	187
Turbidité	69	-	-	-
Couleur	143	38	75	
Alcalinité	14	-	-	-
Matières Solides Totales	61.42	-	-	-
Indice de Rayznar	11.98	-	-	-
Comments	No Good Quality	Colored water High DCO and Nickel concentration	Colored water DCO élevée	Colored water High DCO and Nickel concentration
		QPC conform	QPC conform	QPC conform

These analyses highlight water colour, high DCO and high concentration of Ni. Concentration of pesticide residues in water did not presented a potential risk. Although some values were high, overall concentrations conformed to WHO standards.

Workpackage 8: Pest Monitoring and Management

Before coppicing, under irrigation conditions, observations made during dry season showed the adverse effect of attacks by termites on two tree species. Those attacks led to the reduction of numbers of *Leucaena leucocephala* trees and poor survival of *Calliandra calothyrsus* plants in experiments 1 and 2.

Twenty four months after planting, in the dry season (May 2007), we also observed that all experiments were invaded by crickets or locusts identified as *Micratauria microtaurides*. But no significant damage was noted because the problem was overcome by rapid intervention of local population and authorities, project technician and technical service agencies.

After tree coppicing 27 months after planting (MAP), in September 2007, during the rainy season, the experimental site was flooded by water. All plots planted with *Acacia angustissima* were fully occupied by water causing the death by lack of oxygen in water of those tree species: film and CD are available to document this aspect.

In field, according to observations on plant health in experiment 1 *Gliricidia sepium*, *Khaya senegalensis* followed by *Leucaena leucocephala* were more resistant than the others to termites and pests in irrigated conditions. In Experiment 2, local tree species were well adapted. In all experiments *Acacia angustissima* and *Calliandra calothyrsus* seemed the most affected by termites.

Training

Fallaye KANTE at (FAST) and Youssouf CISSE (IER) registered for Ph. D degree at ISFRA in Mali are completing their theses.
This project allowed Fallaye KANTE to be supported by AUF for 4 months at Laboratoire Commun de Microbiologie de Dakar, for molecular characterization studies. Mohamed TRAORE (FAST) has joined this project in the framework of MSc in inoculant technology. He has been supported by University of Bamako.

Dissemination of the results:
*** Visits to experimental site at MINIMANA (Siribala)** were performed by
Delegation led by Dean of the department of Biology at FAST, University of Bamako
Staff of ONG AAVNU,
Association KAFO des femmes de Siribala
Visites des autorités municipales de Siribala
Association DEMBAGNOUMAN des femmes de Siribala et de Laminbougou
Association KAVURAL des Eleveurs peulhs

- **Publications and documentations**
  - 1 film
  - 1CD
  - 3 papers in prep

***Problems encountered*:**
Some data as plant sheet and soil samples content analysis are not available because of Dr Diafar CISSE from IPR/IFRA who is charged with this task, has abandoned his institute.

Partner 4: INERA

Mahamadi Dianda, Jules Bayala, Kadidia Sanon and Ouédraogo S. Jean

Work package 1. Water treatment and irrigation

Waste water purification system

During this final period of project activities, the functioning of the waste water treatment equipment was still irregular, despite the replacement of the pump which supplies it with waste water. However, updating the waste water equipment is essential for further dissemination and application of the system. Thus, the system was totally stopped to look for workable solutions with the assistance of the local waste-water treatment specialists, and the involvement of M Hervé Pinazze and Marie Deren (two French students) who were completing the fieldwork part of their engineer degree. A new device was designed that should be constructed in addition to the pre-existing equipment. This device is expected to properly prevent the flow of plastic materials carried in the waste water, while treating the fatty components that contribute to the malfunctioning of the system. This supplementary device is currently being completely installed. The students also investigated possibilities to optimise the flow of the treated effluent towards the irrigated plantation, by assessing sand particle sizes and composition suitable to be used for the disinfecting basin.

Work package 2: Tree growth and Management

MAIN EXPERIMENT

The main field experiment was established in March 2006 using *Acacia angustissima*, *Gliricidia sepium*, *Leucaena* hybrid LxL and *Afzelia africana*. Species were inoculated with *Rhizobium* and AM fungi and grown for three months in the nursery. The plantation was established with 2 m and 1 m spacing between and within lines. The layout was a Latin square. There were 16 plants (4 rows each of 4 plants) in each plot. The height, the diameter and the number of branches were measured in August 2007 on the inner 2 x 2 trees of each plot.

ASSESSMENT OF TREE GROWTH IN FIELD TRIALS

Out of the four species included in this trial, *Afzelia* was the slowest growing species with less than 0.4 m in height and 0.6 cm in diameter. The three other species (*A. angustissima, G. sepium* and *Leucaena* hybrid) irrigated with wastewater displayed similar growth performance (Table 27). For the diameter and the number of stems, the species showed similar trends possibly because the measurements occurred at a very early stage when trees were not completely established.

Table 27: Mean height, diameter and number of stems of four species irrigated with treated wastewater

Species	Height (m)	Diameter(cm)	Stem number
Acacia angustissima	1.6±0.1a	1.4±0.1a	2.2±0.2a
Afzelia africana	0.3±0.0b	0.5±0.1a	1±0.0a
Gliricidia sepium	1.7±0.2a	1.9±0.2a	2.9±0.3a
Leucaena. Hybrid LxL	2.4±0.3a	1.9±0.2a	2.0±0.3a

Tree management

A second plantation was established in mid-March 2006 to support tree management studies (coppicing trials). The species used were the same as in the main experiment. Complete randomized blocks each composed of three plots were used. Each plot had 16 plants. Tree growth measurements were done in mid-January 2007 and tree species displayed similar growth trends as in the main experiment since they were established roughly at the same time. However, because the overall survival rate was less than 55 % in this case, the coppicing trial was delayed, and the plantation will be converted to economic studies at tree maturity.

Work package 3: Tree Water–use and soil water status

Sap flow rate was estimated from sap flow measured using Stem Dynagauge type SGB35 (Flow32 Dynamax Inc, USA). Data was collected on one species per block for both the main experiment and the stock quality experiment. Due to lack of resources, sap flow was not measured on the management experiment. On each tree, the gauge was installed on a stem having an axial length of at least the gauge height which was smoothed and cleared of branches. Each gauge on tree stem was insulated against solar radiation, wind and rain by covering it with aluminum foil. Measurements were made every 1-minute and 15-minute mean values stored on a DNX10 Datalogger (Flow32, Dynamax, USA). The measurement on each replicate lasted for four days before the equipment was moved to another replicate. The diameters of the stems were also measured and further entered along with the parameters of the sensors into a spreadsheet of Excel 2003 to calculate the sap flow.

Changes in soil water content were monitored using HH2 Moisture meter profile probe of 1 m length, Type PR1 (Delta-T Devices Ltd, England) in the same blocks used for tree transpiration measurements as described above. Monitoring was done with two access tubes per block for both experiments every day in the morning, midday and afternoon.

A weather station was installed in the site of the experiments during the transpiration and soil water content measurements period. The weather station was equipped with sensors measuring the following variables: air and soil temperatures, solar radiation, relative humidity, wind speed and rain. Measurements were made every 1-minute and 15-minute mean values stored on a 21X Micrologger (Campbell Scientifuc Ltd, England).

Good data were obtained for only two replicates of the stock quality experiment because many of the gauges did not work properly. As a consequence no statistical analysis was performed. For the same reason, no good data were obtained for *A. mangium* (Figure 12). Based on the two replicates, *L. hybrid* showed the highest transpiration rate (1.09 L cm^{-2} day^{-1}), followed by *L. leucocephala* (0.93 L cm^{-2} day^{-1}), *G. sepium* (0.93 L cm^{-2} day^{-1}) and *A. angustissima* (0.61 L cm^{-2} day^{-1}).

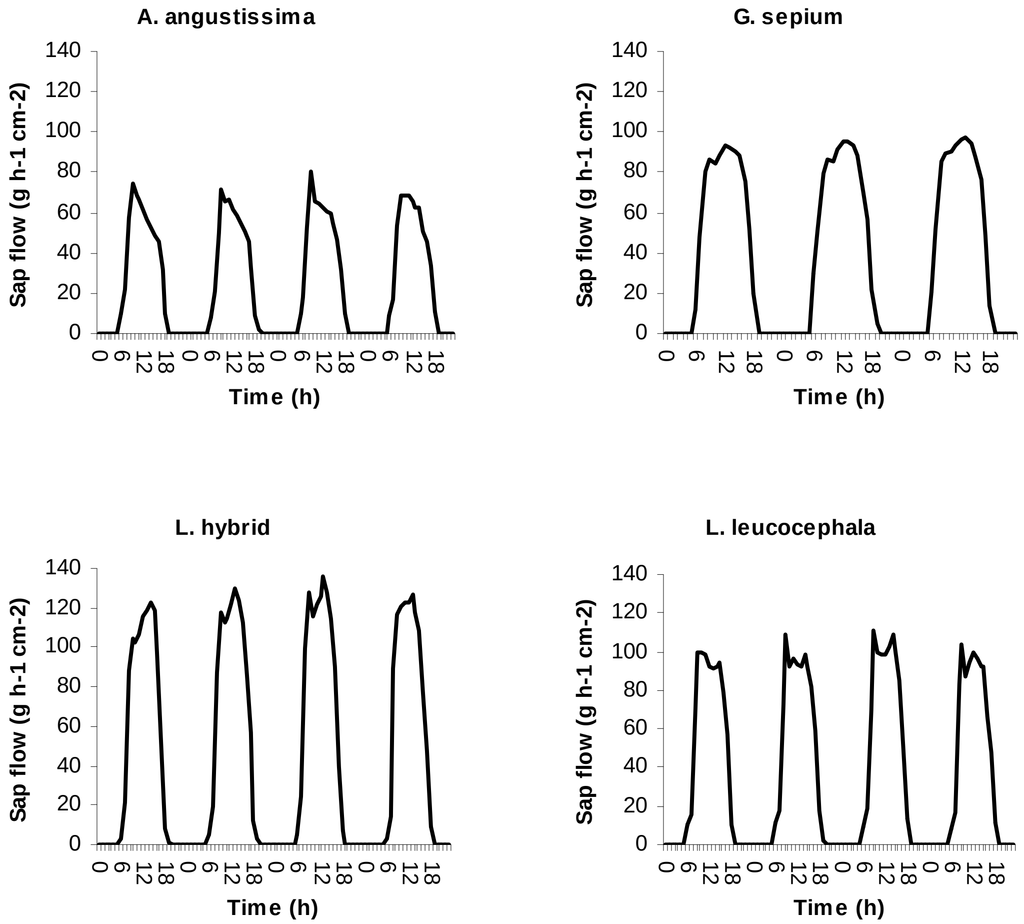


Figure 12: Sap flow rates of four irrigated fast growing species in Burkina Faso

As expected, sap flow followed the same pattern as solar radiation and wind speed, whereas the other weather variables present opposite trends (Figure 13).

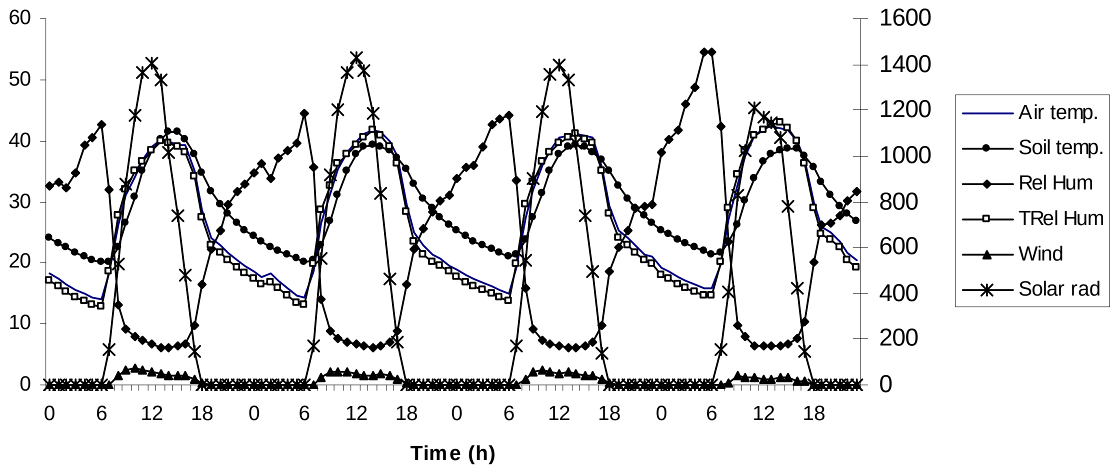


Figure 13. Daily pattern of temperature in ^{o}C (air and soil), relative humidity in % (Rel Hum and TRel Hum), solar radiation and wind speed in m s^{-1} of the study site during the measurement of sap flow in Burkina Faso

Other activities

Julia Wilson and Bob Munro (coordinating staff from CEH) spent 2 weeks training INERA team in Ouagadougou during the beginning of February 2007. This training focused on use of equipments for determination of sapflow, soil moisture and meteorology, and the related data processing, and use of scanners and software for leaf area determination.
Mahamadi Dianda spent a 2- week visit at CEH in late September, receiving training on AM fungi with Dr Kevin Ingleby.

Overview

Some activities have been delayed because there were some difficulties in the functioning of the wastewater and irrigation equipment, which had further influenced the initial planning of the project actions. INERA is making considerable effort to definitely overcome these difficulties in view of the next phase of use and dissemination activities. Also the delayed activities will be resumed along with this second phase after the project closure.

Partner 5: University Abdou Moumouni, Niger

Sanoussi Atta, Zoubeirou M. Alzouma, Germaine Ibro, Marafa Dahiratou Ibrahim, Mahamane Sani Laouali, Mahamane Saadou.

INTRODUCTION

During this final year of the project, the main activities were the evaluation of coppicing of planted trees in order to measure their capacity of regeneration after three months for fodder or fuelwood production. Additionally, a documentary was produced in collaboration with a private radio-television company in order to disseminate the results of the project.

I. TREE GROWTH, MANAGEMENT, AND MICROSYMBIONTS

1.1. Experiment: After being inoculated with appropriate inocula in the nursery (see report 2006), trees were transferred to the field on August 13, 2006. From August to February, trees were watered with purified waste water through a drip system at two-day intervals with 6 litres of purified water tree^{-1}. But since March, with the high temperatures, the frequency of irrigation was increased, and each tree received 6 litres day^{-1}. The density of planting was 1m x 1m, corresponding to 10,000 trees ha^{-1}. On July 11, 2007, trees were coppiced at 0,5 m height in order to estimate their fodder and fuelwood production. Thereafter trees were coppiced at three month intervals in order to determine their capacity of regeneration.

1.2. Results: The results indicated that after two years of growth, the production of fodder (leaves) varied greatly between the species, from 4,130 kg of dry matter (d.m.) for *Gliricidia sepium*) to 18,400 kg of d.m. for *Acacia crassicarpa* (Table 28). But this production was influenced by the microsymbiont inoculation, being mainly a mycorrhizal effect. The highest production of fodder was recorded with mycorrhizal inoculation for all species. For *Acacia angustissima*, the production of fodder was about 11,600 kg of d.m.; 7,010 kg of d.m.; 5,080 kg of d.m. and 5,260 kg of d.m. for plants inoculated respectively with mycorrhiza (M), rhizobia (R), R+M, and control. The corresponding production was 41,300 kg; 18,670 kg; 12,670 kg; 18, 400 kg of d.m. for *Acacia crassicarpa*, and 7,800 kg; 5,110 kg; 3,700 kg ; 4,130 kg for *Gliricida sepium*. There was also a variation among species in the fuelwood production (Table 28). For the control, this production varied from 420 m^3 (steres) for *Acacia angustissima* to 560 m^3 for *Gliricidia sepium*. For all species, the production of fuel wood was also higher for plants inoculated with mycorrhiza, about 600 m^3 for *A. angustissima*, 620 m^3 for *G. sepium* and 640 m^3 for *A. crassicarpa*.

Three months of growth after coppicing, the capacity of regeneration of the different tree species in term of fodder and fuelwood production was very important, but varied according to species (Table 29). For the control, the fodder production was about 2,031 kg for *A. angustissima*, 6,621 kg for *A. crassicarpa* and 4,914 kg for *G. sepium*. The corresponding production for plants inoculated with mycorrhiza was 4,826 kg; 9,048 and 8,784 kg. This production was lower for the plants inoculated with rhizobia or the combination of rhizobia and mycorrhiza for *A. angustissima* and *A. crassicarpa* (Table 29).

Table 28: Production of fodder and fuel wood of tree species after 2 years of growth

Treatments	*Acacia angustissima*	*Acacia crassicarpa*	*Gliricidia sepium*
a) Production of fodder (kg of dry matter)			
Control	5,260b*	18,400b	4,130
+ Mycorrhiza (M)	11,600a	41,300a	7,800
+ Rhizobia (R)	7,010b	18,670b	5,110
+ R + M	5,080b	12,670b	3,700
b) Production of fuel wood (m^3)			
Control	420	460	560
+ Mycorrhiza (M)	600	640	620
+ Rhizobia (R)	480	460	360
+ R + M	480	500	600

*: data of the same column with the same letter are not statistically different at 0.05 probability level.

Table 29 Production of fodder and fuelwood of tree species, 3 months after coppicing.

Treatments	*Acacia angustissima*	*Acacia crassicarpa*	*Gliricidia sepium*
a) Production of fodder (kg of dry matter)			
Control	2,031b*	6,621	4,914
+ Mycorrhiza (M)	4,826a	9,048	8,784
+ Rhizobia (R)	3,123ab	5,635	9,392
+ R + M	3,863ab	5,665	7,715
b) Production of fuel wood (m^3)			
Control	56.25a	18.75	65.00
+ Mycorrhiza (M)	46.25ab	35.94	67.50
+ Rhizobia (R)	25.56bc	27.50	97.00
+ R + M	36.25c	21.25	67.50

*: data of the same column with the same letter are not statistically different at 0.05 probability level.

But for *G. sepium*, the highest production of fodder was recorded with the inoculation with rhizobia, about 9,392 kg of d.m. The production of fuel wood varied from 19 m^3 for *A. crassicarpa* to 65 m^3 for *G. sepium* (Table 29). For *A. angustissima*, there was a significant difference in the fuelwood production among the treatments. The highest production was recorded for the control and the plants inoculated with mycorrhiza, around 50 m^3. For the other species, no significant differences were recorded among the treatments, although the production was higher for plants inoculated with mycorrhiza for *A. crassicarpa* and with rhizobia for *G. sepium*, 36 m^3 and 97 m^3 respectively.

II. DISSEMNINATION OF KNOWLEDGE

In order to disseminate the main results of the project, a documentary was done in collaboration with Radio Télévision Dounia, a private organ of communication. This documentary reported the system of waste water treatment and the main results of tree growth in nursery and in field condition according to the microsymbiont inoculation. Results of coppicing were also presented.
A copy of this documentary is provided with this report.

III. PROBLEMS AND DIFFICULTIES

The main problem met during this last year of the project was the waste water pump which broke down. Therefore a new one was bought in order to continue the experiment of coppicing trees.

Partner 6: IRD

Tatiana Krasova-Wade and Marc Neyra*

Work package 4 (Microsymbionts and Nitrogen-Fixation)

Nodule analysis

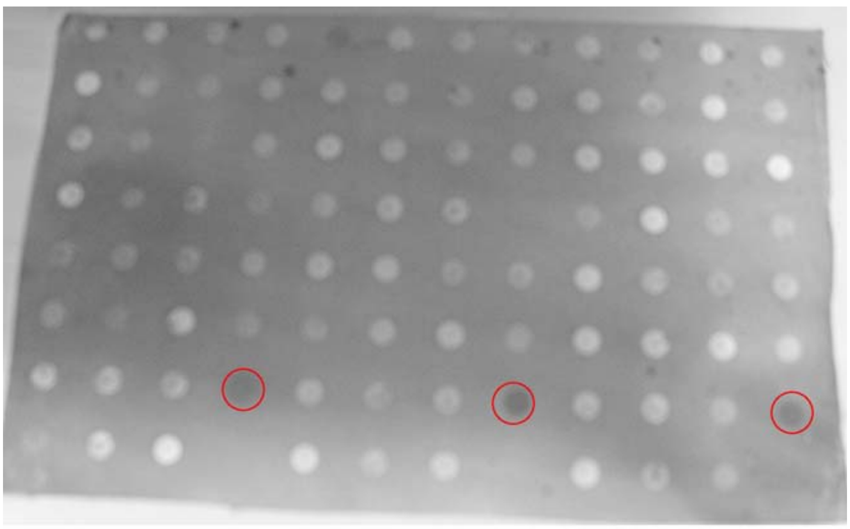
During this year, analysis of nodules collected in field studies by partners 3, 4 and 5 has been partially done and is detailed in the respective reports of these partners.

Development of a probing technique for nodules analysis

To obtain a nodule collection for the hybridization tests, plants of *Acacia mangium* were inoculated in sterile soil conditions in the greenhouse of the experimental station in Senegal with Aust 13C alone or in association with *Glomus intraradices*, *G. mosseae* and *G. monocarpus*. Total DNA of 30 nodules per treatment was extracted as described previously with GES reagent. The DNA was denatured by incubation at 100°C for 10 min and cooled in ice for 10 min before dotting to nylon (+) membrane 0,45 µm (Biodyne PLUS, PALL) at rate of 50 to 100 µl of extract with the Dot Blotting Fisher Bioblock Scientific system. The total DNA of Aust 13C as control was dotted with three dilutions 1 µg, 62,5 ng and 15,63 ng. The membrane was hybridized with the corresponding specific probe at Tm 52°C. The conditions of probe hybridization were described in the last annual report. The revelation was done by addition of the chemiluminescence substrate CSPD producing a distinct luminescent signal that is recorded on X-ray film.

Results

An example is shown in Figure 14: DNA extracts of 90 nodules corresponding to the three treatments were dotted to the membrane. 30 nodules for the treatment A, 27 for the treatment B and 28, for the treatment C showed positive reactions with the probe (Figure 14 and **Table 30**).



Treatment A Treatment B Treatment C

Figure 14 Photo of membrane hybridized with the Aust 13C probe corresponding to Aust 13C strain

Table 30 Example of results of hybridization reaction of nodule DNA with Aust 13C probe

+	+	+	+	+	+	-	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	-
+	+	+	+	-	+	+	+	+	+	+	+
+	+	+	Aust 13C	-	+	+	Aust 13C	-	+	+	Aust 13C
+	+	+		+	+	+		+	+	+	
Treatment A				Treatment B				Treatment C			

Legend: treatment A, nodules obtained on plants inoculated with Aust 13C strain; treatment B, nodules obtained on plants inoculated with Aust 13C and the three endomycorhiza strains ; treatment C, nodules obtained on plants inoculated with Aust 13C and *G. mosseae* and *G. monocarpus*; (+), positive signal; (-), negative signal; red circles correspond to DNA extracted from pure culture of Aust 13C, from left 1 µg, 62,5 ng and 15,63 ng.

Training of scientists from developing countries

Falaye Kanté (partner 3, Univ. Mali) spent four months in LCM in Dakar for nodule analysis, and Kady Sanon, (partner 4, INERA) spent four months in LSTM in Montpellier for training in molecular analysis of mycorrhizal fungi.

Establishment of partnership with farmers' organisations.

Although beneficial effects of inoculation of trees and crops with selected micro-organisms has been known for many years and is currently used in different parts of the world, inoculation technology is not developed in Africa. At the same time as the socio-economic global approach on the adoption of peri-urban wastewater recycling and fuelwood and fodder systems of production by local and rural communities, it seems important to set up the conditions for dissemination and technology transfer. For this, we developed a partnership with representative farmers' organizations regrouped under the umbrella of ROPPA (Réseau des Organisations Paysannes et des Producteurs Agricoles d'Afrique de l'Ouest, who federates millions of farmers through twelve west African countries): CPF (Confédération Paysanne du Faso) in Burkina Faso, CNOP (Coordination Nationale des Organisations Paysannes) in Mali and PFP (Plate Forme Paysanne) in Niger, and, through a complementary funding from the French foreign Ministry, with FUPRO (Fédération des Unions de Producteurs) in Benin, and CNCR (Conseil National de Concertation et de Coopération des Ruraux).in Sénégal. Workshops have been held, associating farmers, scientists and extension services. Some very positive participative field inoculation trials have been set up and the feasibility of production of mycorrhizal fungi in small units supervised by farmer’s organizations is being examined.

Publications

The results obtained on the optimization of DNA extraction procedure have been published in: Krasova-Wade T, Neyra M (2007) Optimization of DNA isolation from legume nodules. Lett. Appl. Microbiol. 45: 95-99

Contract number: ICA4-CT-2002-10017	Year 5 2006-2007

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)	1	
Number of articles/books (published)		
Number of other publications	3	
2. *Training*		
Number of PhDs	2	
Number of MScs	4	
Number of visiting scientists	8	
Number of exchanges of scientists (stays longer than 3 months)	8	
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