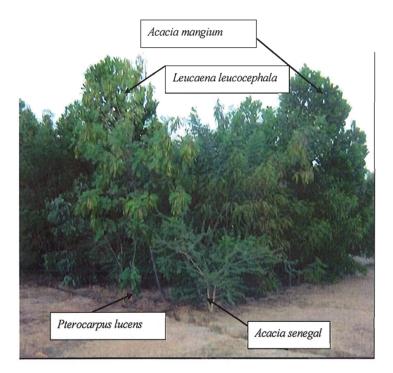
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INCO-DC: International Cooperation With Developing Countries (1998 – 2002)

Contract number: ICA4-CT-2002-10017

Fourth Annual Report: 1/12/2005 - 30/11/2006

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, fuel wood, microsymbionts

Centre for Ecology & Hydrology Library Service Lancaster

Contract number: ICA4-CT-2002-10017.

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

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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.

Fourth Annual Report: 1/12/2005 – 30/11/2006

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BREATHING AND STATISTICS

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 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 common species are being used in all three countries. Nursery screening trials were conducted and plants were then out planted to the irrigation sites. Due to the delays in Wp1, trials are running behind schedule and a one year extension to the project was granted by the EU. Trees are growing quickly under the irrigated conditions and many species are performing well. No significant problems have been detected. Species which were selected for their performance in the nursery are not necessarily the best performers in the field plots.

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.

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.

However nursery and field studies in Mali, Niger and Burkina Faso have given much less clear results. These differences may be linked to the use of unsterile soil for tests in these countries and the consequent colonisation of roots by wild-type mycorrhizal fungi and rhizobia. Further investigation, including critical evaluation of root infection rates and soil inoculum potential is required, and studies are ongoing. However, wild-type mycorrhizal fungi and rhizobia appears to be having a significant positive impact on tree growth.

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.

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 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 will continue in all countries to meet the deliverables.

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. The growth of trees will be followed in the field to determine whether the predicted effects of different shoot: root ratios result in different growth rates under irrigated conditions, or not.

Work package 8 Pest monitoring and management

No significant problems have been detected.

Scientific Annual Report

Work package 1 Water treatment and irrigation

All the treatment and irrigation facilities are up and running. Partners benefited from **SCP**'s site visit in late 2005, especially in terms of maintenance of the facilities, and in identifying problems with the systems and possible solutions. Now that partners have functioning irrigation systems and tree trials planted, they have been able to concentrate on increasing understanding of tree water demands, optimising the day to day running of the irrigation systems, and measuring the quality of the water after treatment.

Deliverables 1.1, 1.2, 1.3 and 1.4 achieved.

Ongoing: Monitoring of human health aspects and quality of treated water will be an ongoing process in each country. Maintenance and improvement of the irrigation systems will also be an ongoing process.

Work package 2 Tree growth and management

Burkina Faso has overcome the delays in setting up their irrigation system and studies of tree growth with irrigation are now in progress in Mali, Niger and Burkina Faso.

Assessments in **Mali** confirm those reported last year. In Experiment 1, 18 months after planting, *Leucaena leucocephala* was the tallest species at 6.04 m tall, with a root collar diameter (rcd) of 8.02 cm, while *Khaya senegalensis* was the shortest at 3.9 m, but with a similar rcd of 8.18 cm. In Experiment 2, which used a wider range of species but did not test effects of inoculation, *Acacia mangium* was the best performer, reaching 6.16 m, with a rcd of 9.18 cm. It was noted that although *Acacia angustissima* grew well, it was easily water-stressed, and therefore may not be a suitable species for these locations.

In **Burkina Faso**, a supplementary study of irrigation was set up in pots, pending the completion of the irrigation system. Species varied in their responses to water logging or intermittent watering, providing useful information concerning the adaptability of species to irrigation systems and their tolerance of drought.

In Niger, assessments after 12 months revealed *Leucaena leucocephala* as the best performing species, with a height of 2.7m. *Gliricidia sepium* performed less well than in Mali.

L. leucocephala has been a good all round performer, it has performed well both in glasshouse trials in the UK and in field trials in each country.

Deliverables 2.1 achieved, Ongoing 2.2 – 2.4 Planned 2.5

Work package 3 Tree water use and soil water status

Studies this year have focused on the field site in **Mali**. Evapotranspiration was calculated. The highest values were in July, averaging 5.5 mm day⁻¹. Measurements of soil water content showed that surface soil layers were drier than measurements at depth in January. Whereas, in August (rainy season), the surface was wetter. Sap flow varied between species and treatments. *Acacia angustissima* was easily water-stressed.

Deliverable 3.1, 3.2, 3.3 ongoing

Work package 4 Microsymbionts and N fixation

Glasshouse studies in the **UK** continued to show that inoculation was favourable to growth when plants were irrigated with either tap water or simulated sewage. Uninoculated plants grew poorly, and trees could only benefit from the added nutrients if they were mycorrhizal. Measurements of soil microbial activity (through substrate-induced respiration (SIR)) showed that inoculated plants generally had higher measurements of SIR from a wider range of substrates than uninoculated plants, especially when supplied with nutrient rich irrigation water.

In **Mali**, the field trials demonstrated a significant effect of double (+M+R) inoculation in the tree nursery on tree height and rcd, while there was an interaction between tree species and inoculation on number of branches. Root samples have been collected for assessment of mycorrhizal infection in the field. In **Niger**, effects of inoculation on tree growth are inconsistent and variable between species. Field trials in **Burkina Faso** are still at an early stage.

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.

Deliverables: 4.1 completed Planned 4.2, 4.3, 4.4, 4.5, 4.7

Work package 5 Economics and quality of produce

In **Mali**, the socioeconomic survey was conducted in 504 villages. Wood and charcoal supplied practically all the energy requirements – there were no alternatives available. The two most important constraints in the supply chain are shortage of wood supply and distance (and time) required for collection. Wood supply is becoming more organised, with increasing population.

In order to inform the management of the irrigation sites, **Niger** developed and circulated a questionnaire to determine the optimum size of timber for sale. Deliverable 5.1 completed

5.2, 5.3, 5.4, 5.5 ongoing 5.6 – 5.8 planned

Work package 6 Soil and plant nutrition

Work is ongoing in all countries. In **Mali**, studies showed that pesticide levels were not significant, but that there was a build up of ammonium and turbidity after the plantation. In Burkina Faso (reported under WP1) microbiological analyses showed that the water treatment was successful in reducing levels of bacteria. Analyses will continue in all countries to meet the deliverables.

Deliverables 6.1 - 6.5 ongoing

Work package 7 Planting stock quality

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. The growth of trees will be followed in the field to determine whether the predicted effects of different shoot: root ratios result in different growth rates under irrigated conditions, or not. Deliverable7.1 completed

1

7.2 ongoing

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 -8.3 ongoing

Future plans

Plans for the final year of the project focus on completing field and laboratory assessments, evaluation of the advantages and disadvantages of irrigated woodlots, and the dissemination of results by publications, meetings, posters etc.

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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.

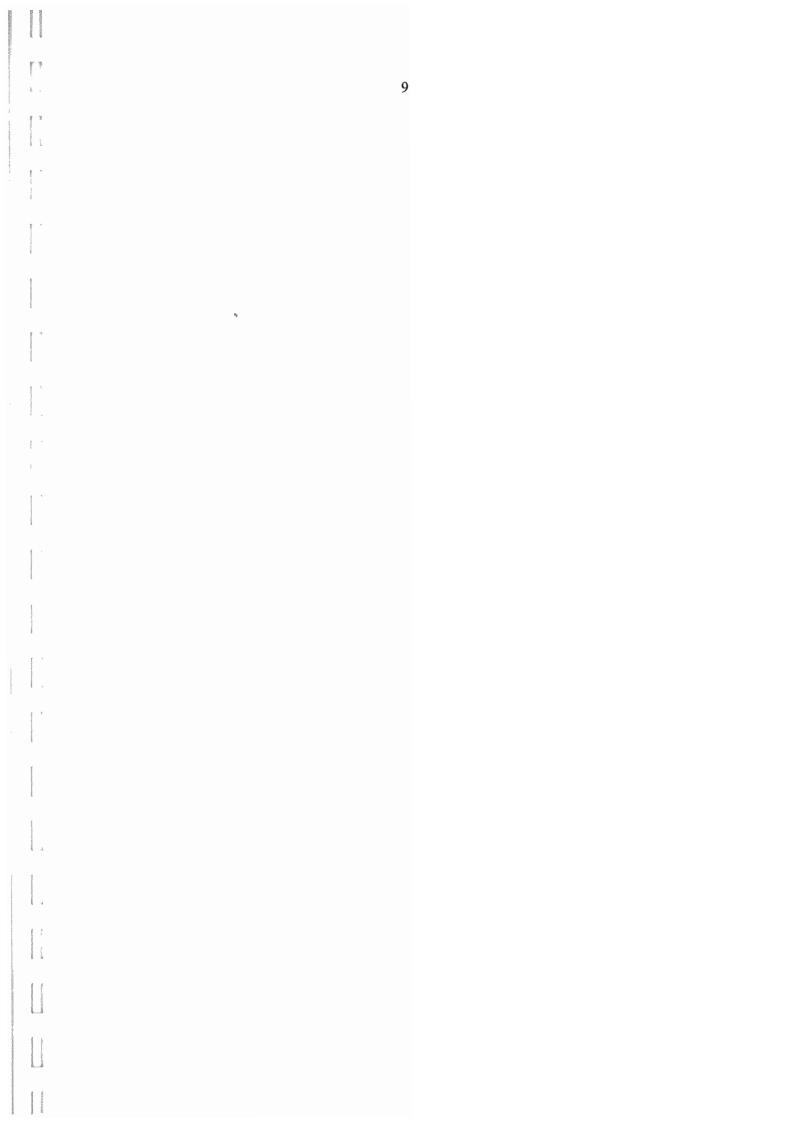
This year, no coordination meeting was held as finances did not permit (the project has been extended by one year). However, local partners have held joint discussions, and there has been regular contact by email.

The report from the visit of SCP to partners, held in November/December 2006 is in Annex 1.

A final project meeting in September 2007 is planned to be held in the UK.

Management problems

As indicated previously, cash-flow is a continuing problem for many partners.



Partner 1 Centre for Ecology and Hydrology, UK

Julia Wilson, Kevin Ingleby, Robert C Munro

Summary of Progress

- WP3: continued support and advice was provided to IER in operation of sap flow and soil water equipment and in analysis of the data
- WP4: The glasshouse experiment continued during 2006, with 1 of the 3 tree species removed from the experiment to allow better spacing for the remaining trees. This years results show that both *Khaya senegalensis* and *Senna siamea* only showed a growth response to the irrigation treatment when they were mycorrhizal. The results also suggest that *Senna siamea* may have greater mycorrhizal dependency than *Khaya senegalensis*.
- WP4: Soil microbial activity in soils in which *Senna siamea* was grown, was assessed by measuring CO₂ respiration of whole soil samples in 96-well microtitre plates after addition of a range of C substrates. The results indicate that microbial biomass and activity was greatest when trees were inoculated with *Glomus mosseae* and given the irrigation treatment.

Activities

WP3: Tree water use and soil water status

It had been planned to transfer the sap flow and soil water equipment from IER Mali to INERA in Burkina Faso in the later months of 2006. However, external commitments of Partner 1 meant that Partner 1 staff would be unavailable to provide training to INERA until 2007. Thus it was agreed that the equipment would remain with IER throughout this reporting period. Continued support and advice in running the equipment was provided over this period.

WP4: Glasshouse experiment

The glasshouse irrigation experiment has continued at CEH during 2006. In January 2006, the fast-growing *Leucaena leucocephala* trees were removed from the experiment as they had already been pruned twice to restrict their vertical growth, and had far outgrown the 32 litre tubs. This allowed for better spacing of the remaining trees in the glasshouse. The experiment was thus reduced to 48 tubs, which were arranged in 4 replicate blocks: the 2 remaining tree species (*Khaya senegalensis* and *Senna siamea*) arranged in a split plot design, with each tree species plot containing the 3 inoculation and 2 'irrigation' treatments (+ or - added nutrients; see previous report for detail). All treatments received additional tap water, on demand. During the winter months, the trees made little growth and irrigation was reduced to 250 ml per tub per week: from March 2006, irrigation resumed at 500 ml per week and was increased to 1 litre per week in May 2006.

Tree growth

Although growth of the larger, inoculated trees has begun to slow as they have become increasingly pot-bound, measurements of stem diameter made in September 2006 continued to show significant treatment effects (Figure 1).

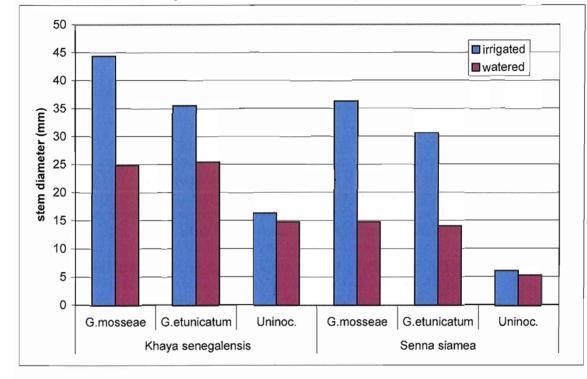


Figure 1 Effect of irrigation treatment on growth (stem diameter) of inoculated and uninoculated *Khaya senegalensis* and *Senna siamea* trees, 29 months after inoculation.

Similar effects to those reported previously are still present: inoculation has increased the growth of both tree species, with growth of those trees inoculated with *Glomus mosseae* better than those inoculated with *Glomus etunicatum* (P<0.001). Although irrigation has also increased the overall growth of both tree species (P<0.001), a significant inoculation*irrigation interaction (P<0.001) showed that this effect was absent in uninoculated plants. This indicates that both tree species could only show a growth response to the irrigation treatment when they were mycorrhizal. Although there was no significant tree*inoculation*irrigation effect, Figure 1 suggests that this response was greater in *Senna siamea* than *Khaya senegalensis* which may indicate that this species has greater mycorrhizal dependency. This Figure also suggests that the difference between uninoculated control plants and inoculated plants was much greater for *S. siamea* than for *K. senegalensis*, which may also indicate a greater mycorrhizal dependency.

Microbial activity of soils

Soil microbial activity was assessed by measuring CO_2 respiration of whole soil samples after addition of a range of C substrates and calculating substrate induced respiration (SIR) rates. Measurements were made using the MicroResp system (Campbell et al., 2003), which comprises two 96-well microtitre plates; the first a

deep well plate containing the soil samples and C substrate; the second a detection plate with an indicator dye (cresol red) set in agar for colorimetric measurement. Immediately after mixing of soil and C substrate, the plates were assembled together with a rubber gasket providing interconnecting holes between the corresponding wells, and then incubated for 6 hours. Carbon sources were selected according to availability and their occurrence in the soil as root exudates. Root exudates were targeted because they are considered to be a highly diverse group of compounds with slow utilisation rates that offer a high degree of discrimination (Campbell et al., 1997). The C substrates used were: malic acid, citric acid, D-glucose, D-galactose, Laspartic acid, L-asparagine and arabinose. Soil moisture content for each soil was determined and dose rates for each C substrate calculated. A deionised water control was included so that colorimetric readings from the detection plates could be normalised. SIR rates for the substrates were then calculated. Initially, 4 of the experimental treatments were chosen for examination: Senna siamea trees inoculated with Glomus mosseae or uninoculated and irrigated or not irrigated. For each treatment, soil was sampled from 2 of the 4 blocks.

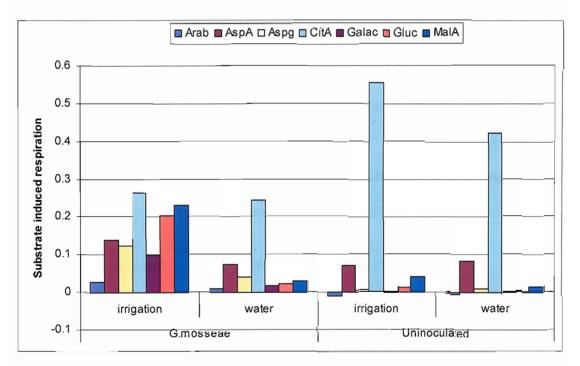


Figure 2 Substrate induced respiration rates ($\mu g \operatorname{CO}_2 - \operatorname{C} g^{-1} h^{-1}$), using 7 different C substrates, of soils from under *Senna siamea* trees inoculated with *Glomus mosseae* or left uninoculated, and given the irrigation treatment (additional nutrients) or watered normally.

The results are shown in Figure 2. The SIR rates for the citric acid substrate were significantly (P<0.001) greater than the other C substrates and were greater (P=0.003) in soils from uninoculated trees. This contrasted with the SIR rates for the remaining six C substrates, where SIR rates were greater in inoculated trees (P<0.001) and irrigated soils (P<0.001). Examination of inoculation x irrigation interactions showed that SIR rates for five of the C substrates (arabinose, asparagine, galactose, glucose and malic acid) were significantly (P=0.003) greater in soils from inoculated trees

which were given the irrigation treatment. A similar non-significant trend was seen for the sixth substrate (aspartic acid).

It is thought that differences in SIR response profiles between soils are related to the amount of organic C mineralised by the microbial communities in the soil (Degens & Harris, 1997). However, little is known of the mechanism of the SIR response to individual C substrates or which microbes are involved. Degens & Harris also found that an increase in the soil SIR rate following addition of glucose reflected the total microbial biomass of the soil, rather than the presence of any one group of microbes. This presumably was also shown by the other 5 C substrates that reacted in a similar manner to glucose.

The results suggest that inoculation and greatly improved tree growth had a greater effect on SIR rates than the irrigation treatment. Improved tree growth resulted in greater root density and therefore presumably the organic content in the soil. Presence of mycorrhizal fungi on the inoculated trees may have increased the amount of mineralised organic C available to other microbes. It is probable that greater SIR rates in the irrigation treatment were caused by increased levels of soluble nutrients like nitrogen in the soil, which stimulated bacterial growth and activity. This may have counteracted negative effects of the high levels of zinc applied in the irrigation water, as Campbell et al. (2003) found that zinc-rich sludge reduced SIR response in soils.

The results also suggest that additional C substrates should be included in the test in order to obtain a broader SIR response profile which may provide greater discrimination.

Progress against activities defined in technical annex

- WP3: transfer of equipment to Burkina Faso was delayed for logistical reasons
- WP4: the glasshouse experiment was continued during the year and yielded further useful data on the effects of irrigation and AM inoculation on growth of the trees and soil respiration.
- WP4: pot cultures of the AM fungal isolates have been maintained in the CEH glasshouse, and supplied to partners on demand.

Forward look

- WP3 to provide training in use of sap flow equipment to Burkina Faso, and develop irrigation model
- WP4 practical work has been completed. Results will be written up for publication

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

Mr Daouda Sidibé, Dr Kalifa Traoré, Mr Broulaye Koné

Work package 1: Water treatment and Irrigation

The objective of this work package is to treat and valorise rice irrigation's waste water from Siribala irrigated perimeter for fodder and fuel wood production.

An experimental site (4 ha) was set up near a village called Siribala situated 30 km from Niono. The site (1 ha) is located at 14°4' N, 6°03' W and at an altitude of 274.3 m, and is irrigated with water from the drain of Minimana and is protected with a wire netting fence. The irrigation system was installed earlier in the project, and a site visit was made by the irrigation sub-contractor during the previous reporting year. Resulting from this site visit, many follow up actions were made during 2005-6, especially concerning the depth of the irrigation canal and the management of artificial basin. Many exchanges have been made between researchers such as taking appropriate care of irrigation infrastructures (irrigation canals, dykes, artificial basin...) and equipment (water pump, PVC tubes, and flood gates).

Irrigation is done twice a week, commencing at 08.00h. Until the plants were 3-4 months old, the irrigation period was of 2 hours duration, which included 15 minutes of preparing the irrigation system (opening the Minimana drain, verifying the drain's state). Since then, the irrigation period has been extended to 2 hours 10 minutes.

The pumping rate is $84 \text{ m}^3 \text{ hour}^{-1}$, thus about 168 m^3 water is distributed over the plot during the pumping period.

Work package 2: Tree growth and Management

2. Field experiments: 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. This is an ongoing experiment, described in detail in previous reports. It was planted into the field at the end of May 2005.

Materials and Methods

As previously described, the experiment has a factorial design, with four tree species *Gliricidia sepium*, *Leucaena leucocephala*, *Acacia angustissima* and *Khaya senegalensis* x two nursery inoculation treatments ; inoculation with mycorrhizas (and rhizobium – for legumes), or a control with no inoculation. There are 5 randomised blocks, each containing one plot of each species, with inoculated and uninoculated subplots. Each sub-plot contains 16 trees arranged 4 x 4. Only the central 4 trees are measured. There is 1 m between trees within a row, and 2 metres between the rows. The first measurements of biophysical parameters started at the end of June 2005, one month after planting. Since then, tree height, root collar diameter, diameter at 1.30m

(dbh – diameter at breast height) and the number of branches have been measured monthly.

Data Analyses: Data have been analysed using MINITAB statistical software (Release 13 for Windows).

Results and discussion

Growth parameters of the species used in the first experiment will be presented in this report. These are the results of the analysis of variance of tree growth parameters measured on 28 November 2006 which corresponds to **one year and 6 months** since planting.

Table 1 shows output from the analysis of variance for mean root collar diameter per species and per treatment. There is a high significant effect of tree species and inoculation treatment with respectively p values = 0.000 and 0.002. The root collar diameters of *Gliricidia sepium* (7.95 cm), *Leucaena leucocephala* (8.02 cm), and *Khaya senegalensis* (8.18 cm) were significantly higher than that of *Acacia angustissima* (4.61 cm).

Figure 3 shows that tree species *Gliricidia sepium*, *Leucaena leucocephala* and *Khaya senegalensis* grow better than *Acacia angustissima* and the double inoculation with rhizobium and mycorrhizas had a positive effect on tree growth compared to the control treatment (Figure 4). The basal diameter, height and the number of branches of inoculated trees are significantly different from those of the control treatment respectively at p values of 0.002; 0.001 and 0.000.

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Source	DF	SS	MS	F	Р
Species	3	356.5	118.83	55.51	0.000
Treatment	1	20.94	20.94	9.78	0.002
Interaction	3	4.07	1.36	0.63	0.594
Error	152	325.39	2.14		
Total	159	706.9			

Table 1 Analysis of Variance (two-way ANOVA) for the mean diameter (cm) at the base per species and treatments

Table 2 shows the result of the analysis of variance for the diameter at breast height of tree species and per treatment. There is a significant difference between tree species and treatments with p values of 0.000 and 0.001 respectively. *Leucaena leucocephala* has the highest diameter followed by *Khaya senegalensis* and *Gliricidia sepium*. *Acacia angustissima* has the smallest diameter.

Table 2 Analysis of Variance (two-way	ANOVA)	for the mear	diameter	at 1.30m
(dbh) per species and treatments				

(
Source	DF	SS	MS	\mathbf{F}	Р
Species	3	132.69	44.23	40.03	0.000
Treatment	1	12.97	12.97	11.74	0.001
Interaction	3	5.61	1.87	1.69	0.171
Error	152	167.93	1.1		
Total	159	319.19			

Table 3 shows the result of the analysis of variance for the mean height of tree species and treatments with p values of 0.000 and 0.000 respectively. Three groups can be observed: *Leucaena leucocephala* is the tallest (6.04m) which is significantly different from the height of the other trees. *Gliricidia sepium* constitutes the second group with a height of 5.13m, while *Khaya senegalensis* and *Acacia angustissima* are the smallest at 3.91 and 4.11 m respectively.

Source	DF	SS	MS	F	P
Species	3	1125487	375162	70.03	0.000
Treatment	1	191823	191823	35.80	0.000
Interaction	3	6030	2010	0.38	0.771
Error	152	814335	5357		
Total	159	2137674			

Table 3 Analysis of Variance (two-way ANOVA) for the height (m) per species and treatments

Looking at Table 4, we can see the highly significant difference found between the number of branches of different tree species (p = 0.000) and also between treatments (p = 0.000). Figure 3 shows that *Leucaena leucocephala* has the highest number of branches (60 branches) compared to the other species. *Acacia angustissima* and *Gliricidia sepium* follow with 25 and 21 branches respectively. *Khaya senegalensis* with only 4 branches has the lowest mean number of branches.

It appears clearly from Figure 3 that the double inoculation has a positive effect on the number of branches of tree species. The mean number of branches of inoculated trees is significantly higher than non-inoculated trees with 32 and 24 branches respectively. However, there is a species x inoculation treatment interaction to be taken into account when interpreting the data.

Table 4 Analysis of Variance (two-way ANOVA) for the number of branches per species and
treatments

Source	DF	SS	MS	F	Р
Species	3	68203.5	22734.5	274.56	0.000
Treatment	1	2205.2	2205.2	26.63	0.000
Interaction	3	1429.3	476.4	5.75	0.001
Error	152	12586.0	82.8		
Total	159	84424.0			_

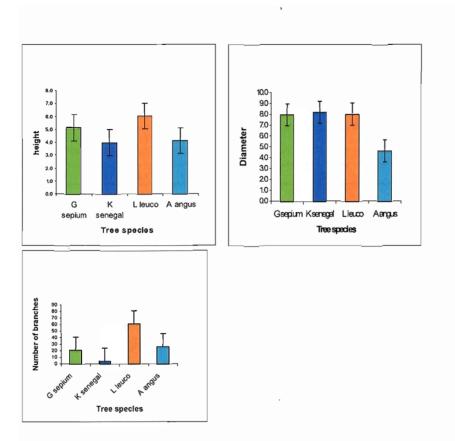


Figure 3 Tree height (m), diameter (cm) and number of branches, 18 months after planting, by tree species

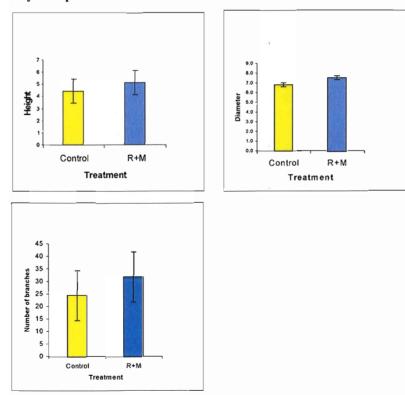


Figure 4 Tree height (m), diameter (cm) and number of branches, 18 months after planting, by inoculation treatment

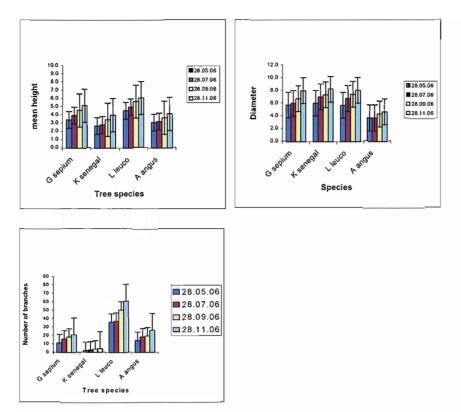


Figure 5 Growth in tree height (m), diameter (cm) and number of branches, between May and November 2006, by tree species

Figure 5 shows the growth of the tree species used in the first trial at different dates of measurement. It appears clearly that *Leucaena leucocephala* is the best performing species taking into account all the parameters measured, including the number of branches. This species is followed by *Gliricidia sepium. Acacia angustissima* is better than *Khaya senegalensis* concerning the height and the number of branches while Khaya is better concerning the increase in diameter. The growth rate of this local species is surprising because this species is considered to be very slow growing. The fast growth in diameter and height (4 metres in one year and half) found here is certainly due to irrigation. Some phenological characteristics such as flowers and fruits of trees are being observed. For instance, Leucaena has many flowers and fruits only five months after planting. The double inoculation has a positive impact on all tree species used since all the measured parameters show significant differences between inoculation and control treatment.

The trial is pictured in Plate 1. Compare this photograph with Plate 2 in last year's report, taken of the same trial.



Plate 1 First tree species x inoculation trial at Minimana

2.2. Experiment 2

Objective: to screen a wider range of tree species than tested in Experiment 1. This is an ongoing experiment, described in detail in previous reports.

Materials and methods

Seeds of the ten following tree species were 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. Field planting started at the end of May 2005 (like the first experiment) and was completed in early June.

This experiment is a very simple design, testing different tree species, all receiving inoculation (+M, with +R if appropriate (for legumes)). One tree per species was planting in each block, all randomised. 30 blocks were planted. Once a month, height, root collar diameter and number of branches are measured, on 10 blocks only.

Results and Discussion

Table 5 shows the result of the ANOVA of root collar diameter for tree species. The block effect was also tested and found to be not significant. Highly significant differences (p = 0.000) were found between species, so that four groups can be seen from Table 5:

- The first group (group A) which is the best performing group is formed by the species like *Gliricidia sepium*, *Acacia mangium* and *Leuceana leucocephala*.
- Acacia auriculiformis and Acacia crassicarpa are the second group (B).
- Acacia senegal, Acacia angustissima, Calliandra calothyrsus and Khaya senegalensis constitute the third group (C).

• The last group with the smallest diameter is formed by the local species *Pterocarpus lucens*.

Species	rcd (cm)	ht (m)	No. branches \pm
	± SE	± SE	SE
Acacia angustissima	4.03 ± 0.3 C	4.96 ± 0.20 B	29 ± 3 B
Acacia auriculiformis	$7.38\pm0.4\ B$	5.20 ± 0.16 B	51 ± 3 A
Acacia crassicarpa	$6.25\pm0.6~BC$	4.43 ± 0.38 B	$37 \pm 4 B$
Acacia mangium	$9.18\pm0.5\;A$	$6.16\pm0.04~A$	63 ± 4 A
Acacia senegal	5.13 ± 0.3 C	$3.4 \pm 0.22 \text{ C}$	55 ± 3 A
Calliandra calothyrsus	3.81 ± 0.3 C	3.51 ± 0.20 C	$16 \pm 2 BC$
Gliricidia sepium	9.56 ± 0.9 A	$5.22\pm0.29~\mathrm{B}$	$37 \pm 4 B$
Khaya senegalensis	3.63 ± 0.4 C	$2.35 \pm 0.26 \text{ D}$	6 ± 4 C
Leucaena leucocephala	$9.03\pm0.4\;A$	5.63 ± 0.33 A	57 ± 6 A
Pterocarpus lucens	$1.17\pm0.1~D$	$0.97 \pm 0.06 \; \text{E}$	$5 \pm 1 \text{ C}$

Table 5 Root collar diameter (rcd), height and number of branches of different tree species, analysed by ANOVA

Table 5 shows the result of the ANOVA for height (m) per species. High significant differences (p = 0.000) were found between species. Five distinct groups can be seen:

- The first group (A) is formed by *Acacia mangium* and *Leuceana leucocephala* showing that these two species are the best performing when you look at their growth in height.
- Gliricidia sepium, Acacia auriculiformis, Acacia angustissima and Acacia crassicarpa are members of the second group (B).
- The third group is composed with Acacia senegal and Calliandra calothyrsus.
- The forth group is *Khaya senegalensis* and
- The last group (E) is *Pterocarpus lucens* presenting the lowest growth in height.

Concerning the number of branches (Table 5), four groups of tree species with statistically significant differences can be distinguished with p = 0.000. The numbers of branches of *Acacia mangium*, *Leuceana leucocephala*, *Acacia senegal* and *Acacia auriculiformis* (Group A) are significantly higher than the other species, followed by the group B with species like *Gliricidia sepium*, *Acacia crassicarpa* and *Acacia angustissima*. The third group is formed by *Calliandra calothyrsus* which is an intermediate species between group B and C. The last group with lowest number of branches is formed by two local species *Khaya senegalensis* and *Pterocarpus lucens*. The number of branches of *Khaya senegalensis* is among the lowest which is in conformity with the data obtained in the first experiment.

Figure 6 shows the growth rate of the 10 tree species used in the screening trial, 12 - 18 months after planting (bars indicate \pm SE). The height growth rates of *Acacia* mangium, Leuceana leucocephala and Gliricidia sepium are significantly higher than those of the other species. *Khaya senegalensis* and *Pterocarpus lucens* have the lowest growth rate.

Gliricidia sepium, Acacia mangium, Leuceana leucocephala have the best performance in terms of diameter. These species are followed by *Acacia crassicarpa* and *Acacia auriculiformis.* It is well known that local species such as *Pterocarpus lucens, Khaya senegalensis* and *Acacia senegal* are slow growing species but from these irrigated experiments this thought could be undermined since the two last species have done surprisingly well in 18 months. The irrigation appears to boost the growth performance of local species. This trial is pictured in Plate 2.

It is worthwhile to say that *Acacia mangium* excels in comparison with other species, 18 months after planting. In the nursery phase the performance of this species was not impressive and this species was not selected for the main experiment, but this screening experiment shows that this is one of the best performing species. *Acacia angustissima* which was chosen shows less good performance in the field – it appears to have a very high demand for water and is easily water-stressed. *Acacia mangium* seems to be better adapted than other Australian Acacias in our irrigated condition in Siribala.

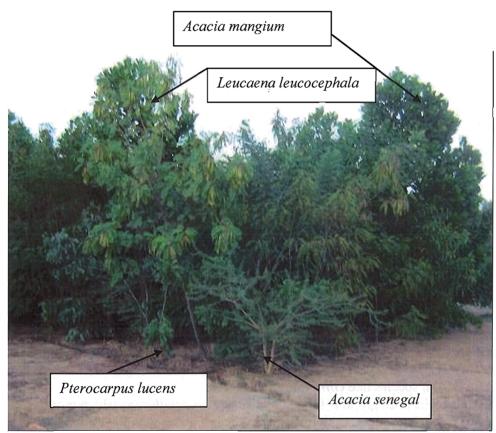
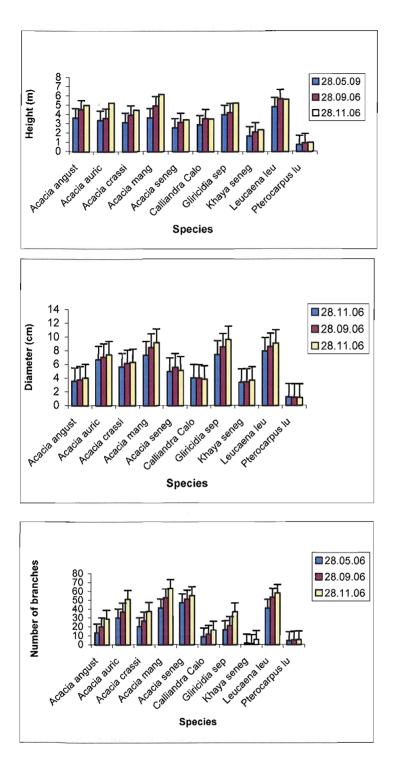
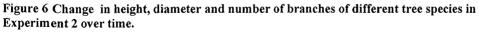


Plate 2 Growth of plants in Experiment 2 at Minimana





Remainder of irrigated space: Two types of trials were set up in August 2005 in the remainder of the irrigated space: A trial in which the growth of three species

(Gliricidia sepium, Acacia angustissima and Leuceana leucocephala are compared and another one where the growth of Khaya senegalensis is measured.

- Trial with *Gliricidia sepium*, *Acacia angustissima* and *Leuceana leucocephala*: The experimental design is randomised complete block with 4 repetitions. The number of factors is two (tree species and treatments). Two levels of treatment have been done: the inoculation with mycorrhizas and the inoculation with rhizobia. The inoculation was done at the nursery phase. There are 4 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 between trees within a row, and 2 metres between the rows.
- 2. Trial with *Khaya senegalensis*: The experimental design is randomised complete block with 4 repetitions. There is only one factor which is treatment. Two levels of treatment have been done: the inoculation with mycorrhizas and the control. There are 4 blocks, each containing one plot of 16 trees arranged 4 x 4.

Data Collection:

From January 2006 up to now parameters such as height of trees, root collar diameter, diameter at 1.30m and the number of branches are measured monthly. These data are available but are not yet analysed and then will not be presented in this report.

Coppicing: The assessments of fuel wood and fodder will start in March 2007. In order to perform these activities, many exchanges have been done between the staff involved in the project in Mali. The coppicing protocol has been elaborated.

Work package 3: Tree water-use and soil water status.

3.1. Meteorological data

Reference 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. These conditions are generally determined by the temperature (in sunny warm weather the loss of water by evapotranspiration is greater than in cloudy and cool weather), relative humidity (in humid areas the high humidity of the air reduces the evapotranspiration demand), solar radiation (the evapotranspiration process is determined by the amount of energy available to vaporize water), wind speed (by replacing humid air by drier air, a small variation of wind speed may result in larger variations in evapotranspiration rate) and location (altitude above sea level, latitude because atmospheric pressure is a function of the site elevation). The ET_0 provides a standard to which evapotranspiration during the whole year or in other region can be compared. It also allows the comparison of evapotranspiration of different plants.

Figure 7 shows mean values of ET_0 during the January-November study period (March-June period is missing because of a technical problem). The measurements have high correspondence to the mean solar radiation energy available during the clear days. Cooler temperature in January is characterized by lower solar radiation compare to the July-October period where they are both higher.

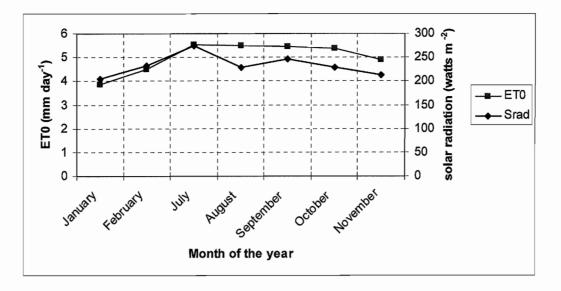


Figure 7 Monthly mean reference crop evapotranspiration measured during the study period at Siribala in 2006

ET and mean monthly atmospheric conditions are summarized in Table 6. Hot air coupled with a high wind speed may lead to an increase of plant transpiration and a great demand for water. During October – November, wind speeds were reduced and ET was lower, despite high air temperatures.

	mm day ⁻¹	Wm ⁻² Solar	m.s ⁻¹	% Air R	°C	mm
Month	ET	radiation	wind speed	Humidity	Air temp.	Rain
January	3.84	204.81	0.93	28.14	22.47	0.00
February	4.49	232.73	0.88	28.63	25.59	0.00
July	5.51	274.74	0.71	69.31	29.84	63.60
August	5.49	227.63	0.76	77.24	28.33	257.80
September	5.44	246.98	0.59	76.29	28.37	133.30
October	5.37	227.55	0.41	64.48	29.18	12.30
November	4.87	211.71	0.45	55.74	25.54	0.00

Table 6 Summary of calculated ET and measured atmospheric conditions and rainfall at Siribala during 2006

Relative humidity is high during July –September. In January and February the temperature are cooler and relative humidity is reduced.

3.2. Soil Water Content

The soil moisture measurements were done at the same time of day and the same distance from trees. During the sap flow measurements, the soil moisture measurements were determined daily and at other times, the measurements were done twice a week. For each measurement period we did 18 measurements per treatment and a total of 144 measurements for the experiment (18 measurements X 4 species X 2 types of inoculation)

The mean soil water content during the study period is reported in Table 7.

				Deptl	h (mm)		
Month	Species & treatment	100	200	300	400	600	1000
January	GsTO	0.131	0.141	0.162	0.492	0.242	0.459
January	AaTO	0.071	0.005	0.020	0.141	0.099	0.326
January	LleuTO	0.144	0.098	0.201	0.342	0.156	0.306
January	KsTO	0.146	0.041	0.120	0.256	0.228	0.456
January	LleuR+M	0.054	0.131	0.179	0.289	0.362	0.052
January	Gs R+M	0.050	0.011	0.063	0.089	0.382	0.426
January	Ks R+M	0.101	0.103	0.112	0.273	0.216	0.460
January	AangR+M	0.092	0.093	0.147	0.346	0.098	0.245
February	GsTO	0.089	0.096	0.159	0.434	0.087	0.216
February	AaTO	0.136	0.149	0.160	0.650	0.256	0.546
February	LleuTO	0.064	0.021	0.049	0.180	0.077	0.294
February	KsTO	0.162	0.126	0.270	0.564	0.176	0.310
February	LleuR+M	0.075	0.071	0.113	0.165	0.153	0.365
February	Gs R+M	0.057	0.108	0.151	0.189	0.261	0.133
February	Ks R+M	0.050	0.050	0.101	0.091	0.344	0.257
February	AangR+M	0.095	0.069	0.101	0.130	0.272	0.452
March	GsTO	0.095	0.095	0.151	0.372	0.091	0.206
March	AaTO	0.145	0.111	0.132	0.297	0.239	0.407
March	LleuTO	0.090	0.009	0.005	0.159	0.108	0.307
March	KsTO	0.143	0.110	0.228	0.399	0.160	0.310
March	LleuR+M	0.099	0.084	0.126	0.245	0.129	0.332
March	Gs R+M	0.063	0.110	0.163	0.288	0.353	0.083
March	Ks R+M	0.074		0.069	0.115	0.377	0.428
March	AangR+M	0.154	0.127	0.146	0.310	0.247	0.449
June	GsTO	0.300	0.222	0.274	0.585	0.354	0.479
June	AaTO	0.111	0.108	0.137	0.104	0.075	0.290
June	LleuTO	0.237	0.130	0.262	0.313	0.176	0.304
June	KsTO	0.250	0.047	0.106	0.173	0.478	0.527
June	LleuR+M	0.157	0.106	0.157	0.227	0.276	0.116
June	Gs R+M	0.171	0.052	0.105	0.107	0.408	0.267
June	Ks R+M	0.200	0.127	0.151	0.142	0.270	0.467
June	AangR+M	0.220	0.193	0.188	0.233	0.241	0.433
July	GsTO	0.181	0.136	0.149	0.354	0.302	0.461
July	AaTO	0.133	0.061	0.056	0.183	0.078	0.294
July	LleuTO	0.156	0.137	0.272	0.580	0.179	0.339
July	KsTO	0.202	0.051	0.116	0.331	0.489	0.521
July	LleuR+M	0.084	0.131	0.186	0.413	0.391	0.041
July	Gs R+M	0.158	0.038	0.081	0.172	0.395	0.427
July	Ks R+M	0.179	0.125	0.146	0.367	0.231	0.500
July	AangR+M	0.154	0.128	0.163	0.494	0.092	0.182
August	GsTO	0.299	0.174	0.203	0.375	0.107	0.622
August	AaTO	0.373	0.334	0.396	0.553	0.434	0.718
August	LleuTO	0.176	0.021	0.101	0.090	0.094	0.364
August	KsTO	0.233	0.167	0.305	0.387	0.205	0.354
August	LleuR+M	0.192	0.112 `	0.134	0.176	0.178	0.369
August	Gs R+M	0.127	0.164	0.227	0.282	0.419	0.059
August	Ks R+M	0.227	0.068	0.141	0.095	0.663	0.503
August	AangR+M	0.217	0.138	0.158	0.189	0.286	0.804
November	GsTO	0.172	0.122	0.095	0.277	0.166	0.105
November	AaTO	0.236	0.172	0.143	0.261	0.085	0.061
November	LleuTO	0.134	0.170	0.386	0.186	0.125	0.264
November	KsTO	0.749	0.696	0.142	0.129	0.056	0.206
November	LleuR+M	0.169	0.120	0.080	0.198	0.006	0.129
November	Gs R+M	0.140	0.131	0.114	0.248	0.050	0.119
November	Ks R+M	0.172	0.144	0.098	0.304	0.057	0.271
November	AangR+M	0.134	0.157	0.134	0.285	0.073	0.125

Table 7: Monthly soil water content (m³m⁻³) during the study period at Siribala in 2006 associated with plots of *Gliricidia sepium* (GS), *Acacia angustissima* (Aa), *Leucaena leucocephala* (Lleu), and *Khaya senegalensis* (KS) with (R+M) or without (TO) inoculation

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Soil water content is analyzed during two contrasted period: January with cooler temperature and August with higher temperatures when the trees are expected to transpire much water. Table 8 shows that in January the soil water content varied only according to soil depth; rhizobium + mycorrhizas and plant species did not have significant effects on soil water uptake.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Spec	3	0,045539	0,045539	0,015180	1,07	0,484 x
RM	1	0,003485	0,003485	0,003485	0,27	0,653 x
Dep	5	0,454941	0,454941	0,090988	10,73	0,033 x
Spec * RM	3	0,040585	0,040585	0,013528	1,63	0,225
Spec*Dep	15	0,135145	0,135145	0,009010	1,08	0,438
RM * Dep	5	0,038887	0,038887	0,007777	0,94	0,485
Spec*RM*de	ep15	0,124581	0,124581	0,008305	**	
Error	0	0,000000	0,000000	0,000000		
Total	47	0,843164				

Table 8 ANOVA of soil water content during the month of January at Siribala in 2006

In August, where the climate is hot one observes all most the same as previously mentioned. However, in this period the profile is uniformly humid (Table 9).

Table 9 ANOVA of soil water content during the month of August at Siribala in 2006

Source	DF	Seq SS	Adj SS	Adj MS	F	Р	
Spec	3	0,284498	0,284498	0,094833	4,54	0,261 x	
RM	1	0,027889	0,027889	0,027889	0,78	0,447 x	
Dep	5	0,503334	0,503334	0,100667	5,35	0,173 x	
Spec * RM	3	0,087578	0,087578	0,029193	1,44	0,272	
Spec*Dep	15	0,180419	0,180419	0,012028	0,59	0,840	
RM * Dep	5	0,135646	0,135646	0,027129	1,33	0,303	
Spec*RM*de	p15	0,305126	0,305126	0,020342	**		
Error	0	0,000000	0,000000	0,000000			
Total	47	1,524490					

In January, for all the species, with or without inoculation, the soil was drier from the soil surface to 30 cm depth (Figure 8). The driest soil was observed in the *Acacia angustissima* control plot and the R+M *Gliricidia sepium* plot. The other species showed almost the same dehydration pattern. The profile became more humid in the deeper soil layers.

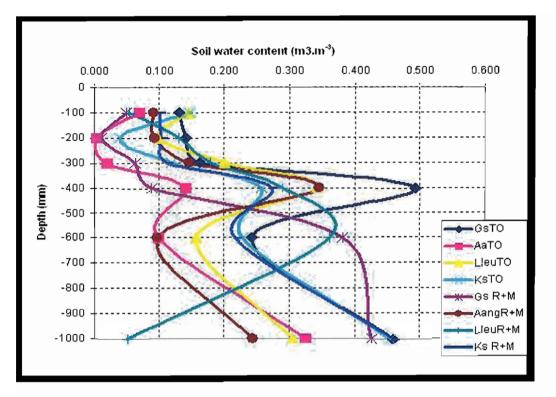
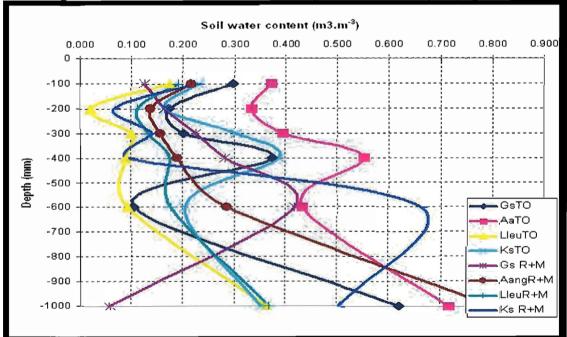


Figure 8 Vertical profiles of soil water content (m³.m⁻³) in January in 2006, at Siribala in the experimental plot. Data are averages of 18 measurements for each depth.



As in January, the upper soil layers in August were drier than the deeper layers. One observes greater soil water content in the *Acacia angustissima* control plot (Figure 9).

Figure 9 Vertical profiles of soil water content (m³.m⁻³) in August in 2006, at Siribala in the experimental plot. Data are average of 18 measurements for each depth.

According to the numerous sampling and analysis which have been done by our laboratory (soil-water-plant laboratory) in Sotuba, the soil field capacity varies in this area from 0.1 m³.m⁻³ for very sandy soil in upper land to 0.45 m³.m⁻³ in clayey irrigated soil like those from Siribala at our experiment site.

In rainy season, particularly in August when the rain events are regular occurrences, irrigation is stopped because of soil saturation. We did not observe any negative reactions of trees to soil saturation.

3.3. Sap flow measurement

In 2006, sap flow of four species (*Leucaena leucocephala, Gliricidia sepium, Acacia angustissima, and Khaya senegalensis*) were monitored. Data from July in the beginning of the rainy season and August when the profile is wet have been used to compare these species. Trees had been planted since May 2005.

Four gauges (SGB 13, SGB 16 and SGB 19, SGB 25, Dynamax Inc, Houston, TX, USA) were used for the sap flow measurement. The gauges were placed in the plots as follows: diameter of 25 mm (*Leucaena leucocephala*), 19 mm (*Khaya senegalensis* R+M, control of *Khaya senegalensis*, control of *Gliricidia sepium*); 16 mm (*Gliricidia sepium* R+M, control of *Acacia angustissima*) and 13 mm (*Acacia angustissima* R+M, *Leucaena leucocephala* R+M).

Comparison between exotic and indigenous species, with and without inoculation is shown in Figure 10& Figure 11 in July at the beginning of the rainy season. These measurements were conducted simultaneously. The maximum of sap flow is always observed at midday (with inoculation or without inoculation). *Khaya senegalensis* and *Gliricidia sepium* which show a small sap flow quantity without inoculation show the opposite trend with inoculation. For *Acacia angustissima* the sap flow amount is greater when not inoculated. At this period *Leucaena leucocephala* seems to be less sensitive to inoculation.

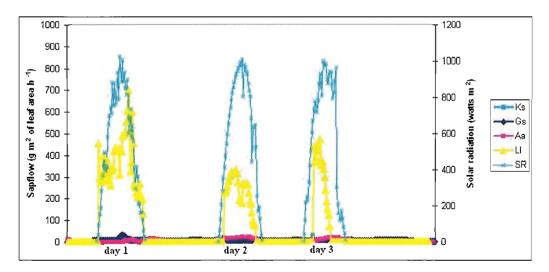


Figure 10 Comparative mean water use by four tree species without inoculation and the flux of solar radiation (SR) in three successive days in July, the beginning of the rainy season in Siribala (Mali).

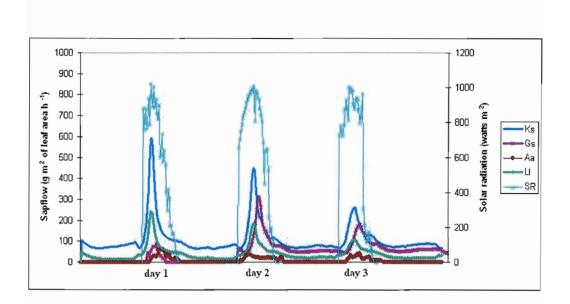


Figure 11: Comparative mean water use by four tree species inoculated with rhizobium plus mycorrhiza and the flux of solar radiation (SR) in three successive days in July, the beginning of the rainy season in Siribala (Mali).

It appears that the indigenous species (*Khaya senegalensis*) used more water per unit leaf area than the exotic ones (*Gliricidia sepium*, *Acacia angustissima*, *Leucaena leucocephala*) when inoculated.

In August when the climate is favourable, the trend is very clear and there is no difference between treatments. Figure 12 & Figure 13 show the amount of sap flow.

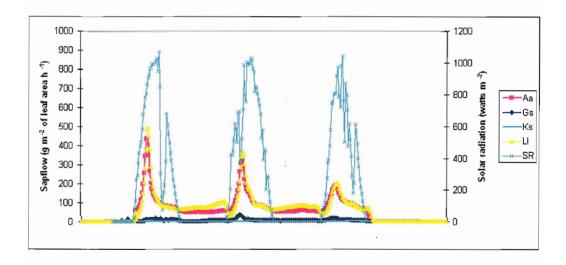


Figure 12 Comparative mean water use by four tree species without inoculation and the flux of solar radiation (SR) in three successive days in August, the rainier month of the wet season in Siribala (Mali).

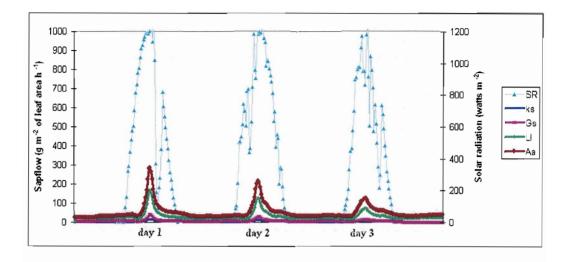


Figure 13 Comparative mean water use by four tree species inoculated with rhizobium plus mycorrhiza and the flux of solar radiation (SR) in three successive days in August, the rainier month of the wet season in Siribala (Mali)

With or without inoculation, the sap flow amount is greater for *Leucaena leucocephala* and *Acacia angustissima* than for *Gliricidia sepium* and *Khaya senegalensis* although it's smaller with inoculation.

3.4. Discussion

The measurement performed in this study may normally be smaller than those from whole plants because of a problem of shading and other environmental factors as pests, diseases, yellowing leaves (Deans, 1994). Ansley et al. (1994) reported that natural shading causes sap flow reductions of less than 20%.

Sap flow is high at midday as is the solar radiation. This fact can be explained by the great radiation flux which emphasizes transpiration and photosynthesis.

In July, both ETo and solar radiation are higher which can result in high water demand. This situation probably explains why the sap flow amount reaches for instance 700 g m⁻² of leaf area h-1 for *Leucaena leucocephala*. Wind speed and solar radiation are also higher in this period. Many researchers reported the relation between sap flow amount and meteorological parameters (Herzog et al., 1997, Diawara et al., 1991, Allen and Grime, 1995). The sap flow amount ranging from 180 to 300 g m⁻² of leaf area h⁻¹ obtained in July is comparable to those obtained by Deans (1994) who reported a value of 204 g m⁻² of leaf area h⁻¹.

The high relative humidity from July to September may explain the low sap flow amount in August, mainly in the inoculated plots. In August the soil water content is high, solar radiation is low, and the transpiration is low. These situations combine with rhizobium and mycorrhizas may explain the low sap flow amount which can be explained by stomatal opening leading to improvement of water use efficiency. The works of Pamela et al., (2003) mentioned the relation between plant transpiration rate and stomatal conductance and soil moisture content. The sap flow amounts in our study are in line with those obtained by Deans (2004) who reported values ranging from about 50 to 700 g m⁻² of leaf area h⁻¹ when studying water use for four species in similar semi arid areas. Brenmer et al. (1991) also obtained results ranging from 30 to 90 g m⁻² of leaf area h⁻¹. These values are within the range of our values which varies from 20 to 700 g of leaf area m⁻² h⁻¹.

Our observations indicate that with inoculation, *Acacia angustissima* grows well, using more water than the other species, leading to water shortage in the upper layers. However, as well as having a high water use, this species is also easily water stressed, and a slight water deficit is enough to cause wilting of this species.

Conclusion:

In August when the weather is favourable, the inoculation has great effect on the sap flow amount of all the species studied. The sap flow amount which is less than 300g m^{-2} of leaf area h^{-1} with the double inoculation (R+M) is more than 500g m^{-2} of leaf area h^{-1} without inoculation. However, the actual mycorrhizal and rhizobial status of the roots of the trees used for sap flow measurements need to be checked, as some time had elapsed since the nursery treatments were imposed and naturally occurring inoculum may have infected roots since that time. It appears that the least welladapted species in semi-arid zone of Siribala is *Acacia angustissima* – which has high water use and is easily stressed. *Leucaena leucocephala* needs also water but less than *Acacia angustissima*. In relatively humid areas this species could be advised because it produces a great amount of biomass.

Work package 4: Microsymbionts and N-fixation

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 will be 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 in this reporting period because the data that will be gathered are absolutely necessary for the interpretation of our results.

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

Work package 6: Soil and Plants nutrition,

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 plantation. Soil samples will be taken just before the coppicing activities. Plant component of all the species used in the experiments have been collected and oven dried. Plant component will be sampled just before coppicing trees. All these samples will be analysed for their chemical content in this reporting period.

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 have been presented in last year annual report.

Dissemination activities:

- Field Visits: Siribala city council, women association and breeders association have visited the experimental site of Minimana twice this year.

- The head of Forestry Research Programme of IER has visited this year the experimental site.

- The project (Objectives, results) has been presented at the committee program of IER, held in June in Bamako. The committee program is a big workshop where all the stakeholders of agricultural research in Mali are invited.

- The results of the project are disseminated to local population through broadcasting of the local radios of Siribala and Niono.

Dissemination plan for this reporting period:

Prior to coppicing:

- Stake holder's 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. A team of Malian National TV will visit the site, and the images, the objectives, the results of the project will broadcasted.

After coppicing:

- 1. Stockholder's meeting will be organised at the end of project in Ségou or Niono;
- 2. Scientific publications and posters will be produced.

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Partner 3 University of Mali

Dr. Inamoud Ibny YATTARA, FAST University of Bamako Mali MSc. Youssouf CISSE, IER Bamako, Mali Msc Fallaye KANTE, University of Bamako, Mali Mr Bakary Samaké Technicien FAST, Université de Bamako Mali Mr. Hamed BATHILY FAST University of Bamako, Mali

Summary

Water, soil and plant nutrients. The main analyses were performed in Mali by two laboratories. Water contents control by « Laboratoire de la Qualité des Eaux » de la Direction Nationale de l'Hydraulique, and pesticide residues by « Laboratoire de toxicologie et de contrôle qualité Environnementale » du Laboratoire Central Vétérinaire à Bamako.

Soil and plant nutrient samples were sent for analysis to the lab of IPR/IFRA at Katibougou (Mali) and the Laboratoire de biopédologie de IRD, Dakar Senegal).

Microsymbionts and N-fixation The main results concerned inoculum potential assessment and molecular characterization of established experiments at Minimana under irrigated conditions. Detail of results concerned data obtained at 9, 12 and 15 months after planting three field trials.

Pest monitoring and management. A survey of plant health was performed. One notes that plants were invaded by crickets or locusts identified as *Micratauria microtaurides*. But no significant damage occurred because the problem was overcome by rapid intervention of local population and authorities, project technician and technical service agencies.

Economics. According to questionnaire performed by different partners, economic studies on utilisation of wastewater for fuel wood and fodder production are undertaken according the recommendations made at the last meeting in Burkina Faso. Main constraints of wood supply, and consumption of energy are detailed below.

Formal Training:

At this date Mr Fallaye KANTE registered for Ph.D degree at ISFRA in Mali, is carrying out molecular studies at the laboratory of partner IRD- Laboratoire Commun de Microbiologie (LCM) in Dakar (Sénégal).

In the framework of established experiments under irrigation, the main objectives according to specific work package were

- to utilise microsymbionts to minimise the need for fertiliser application and to verify their persistence and effectiveness using molecular tools at 9, 12 and 15 months after planting

- to continue the socioeconomic studies
- to analyse soil, water and plants for their nutrient contents and bacterial occurrence
- to investigate pests under irrigation conditions
- to receive training in modern molecular microbiological methods
- to disseminate results of these studies to the user communities (GOs, NGOs,

farmers, peri-urban landowners etc.) via participatory workshops and visits to sites.

Work Package 4: Microsymbiont and N-Fixation.

To determine the microsymbionts inoculum potential of irrigated soils and to characterize microbial diversity, two experiments (1 and 2) previously described above were used. The data presented here are supplementary to those presented by partner 2, from the same experiments, but the observations are taken at different times.

Effects of inoculation on growth According to previous protocols, inoculation of selected tree species was performed using both rhizobial and mycorrhizal strains (R+M). In this study, assessment of rhizobial infection before planting was done using experiment 1 and 2. Measurements of plant growth and nodulation were performed three times in: February, May and September 2006.

Experiment 1.- Plant growth. In February (9 months after planting), no significant effect of inoculation on A. angustissima and Khaya senegalensis was noted. But significant effects were obtained on height, and root collar diameter of Gliricidia sepium, and height of Leucaena leucocephala.

In May and September 2006, 12 and 15 months after planting, no significant effect of inoculation was found on growth of *Acacia angustissima*, *Gliricidia sepium Leucaena leucocephala* and *Khaya senegalensis*.

Nodulation. No significant effect of inoculation on plant nodulation was found.

Experiment 2. Plant growth parameters In February, nine months after planting, we noted increase of root collar diameter of *Gliricidia sepium* and *Leucaena leucocephala*, and increase of height of all tree legumes (Table 10).

In May, 12 months after planting, a significant effect of inoculation was related to the increase of collar diameter of *Gliricidia sepium* and *Leucaena leucocephala*, increase of height of all tree legumes was noted.

In September, 15 months after planting, no significant effect of inoculation on plant growth was noted

Nodulation. Nine months after planting (February 2006), inoculation induced high nodulation of *Acacia angustissima*. Fifteen months after transplantation (September 2006) no significant effect of inoculation on plant nodulation was found. But nodulation of *Gliricidia sepium* is higher than the others.

Tree species	Parameter					
_	Diameter Height		NNod	NW		
	(cm)	(cm)	(plt^{-1})	mg ⁻¹		
A. angustissima	2,200a	253,000b	1 39,000b	401,700b		
G. sepium	5,400b	257,400b	45,667a	136,267a		
L. leucocephala	4,600b	292,800b	12,667a	93,967a		
K. senegalensis	2,000a	95,600a				

Table 10 showing variation of plant growth parameters and nodulation (9 MAP)

Table 11 showing variation of plant growth parameters (12 MAP)

Tree species	Parameter				
	Diameter	High			
	(cm)	(cm)			
A. angustissima	3,500a	337,000b			
G. sepium	8,200b	378,700bc			
L. leucocephala	7,200b	410,300c			
K. senegalensis	3,000a	131,100a			

> Experiment 3

At concern all periods (MAP), inoculation of selected tree species with specific strains had no significant effect on plant growth and nodulation

Molecular Characterization

Rhizobia diversity studies were performed using nodules. DNA was extracted from nodules collected from plants in experiment 1 and 2. DNA quality was checked using gel electrophoresis. PCR of rDNA IGS 16S-23S was performed in our lab at FAST. Main molecular studies were carried out at the laboratory of LCM (Partner 5) at Dakar using protocol described by Krasova Wade *et al.*, 2005. For each experiment, relevant results were illustrated using types of genetic profiles.

Results showed that no strain used as inoculum was found in the nodules. Indigenous strains (A, B and C) nodulated all legume trees species. Indigenous strains corresponding to IGS type A nodulated *Acacia angustissima*, *Gliricidia sepium* and *Leucanna lauceanhola*. Their frequency varies according to tree species, but is high

Leucaena leucocephala Their frequency varies according to tree species, but is high, ranging from 50% up 81,25%. Strain C frequency is 33% up 50%. Those results were obtained using limited enzymes only *Hae*III and *Msp*I. Therefore

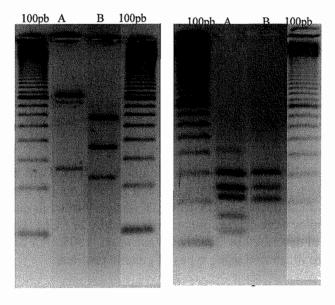
there interpretation could be taken with care. Table 12 and Figure 14 below illustrate the main results obtained with *G. sepium*.

	Types of genetic profiles				
Treatments	Introduced strains	Indigen	ous strains		
	GsK4	А	В		
Μ	0	7	0		
R	0	6	3		
Total	0	13	3		

Table 12 Number of IGS profile types of 16S-23SDNA obtained by PCR-RFLP of G. sepium nodules

number of nodules used = 15

Figure 14 Profiles of IGS types A et B Hae III and Msp I



Hae III

Msd I

WP5 economics

The survey of economics studies was done according to methodology and questionnaire discussed in Burkina Faso during the last meeting. Relevant data on fuel and fodder were collected in 504 villages using investigation method (sample survey). Six (6) levels were concerned: Householders, wood and fodder providers and sellers, technical services (ON, Research Centre for IER.). Supporting Consul and NGOs, Private Actors, Field experiments in Minimana,

In those villages several meetings and discussions were held with rural community and different groups concerned by fuel and fodder production. Main constraints of wood supply, and consumption of energy are given below.

At all the levels, i.e. in the urban district of Niono, in the villages around the establishment of the tests of the project "Ubenefit", one notes that the problems of wood and fodder arise with acuity. The availability of wood (for all the uses) and of fodder becomes increasingly rare compared to the needs, thus creating a strong pressure of human and animals on the environment. Indeed, wood and fodder are increasingly rare in quantity and quality, especially at certain periods of the year (i.e. rainy season). The price of wood is high, the demand for wood being higher than the amount on offer. Consequently consumers find it difficult to obtain wood. Although fodder is not the subject of monetary exchanges, its availability is posed with acuity for feeding the livestock and oxen for ploughing. One can imagine the impact which this situation can have on the physical environment, and the stability of the socioeconomic environment (see cultural) medium. The supply chain of wood in the commune of Niono in general is confronted with the various types of constraints: scarcity (37,6%), distance to be traversed (31,5%), quantity and quality (15%), etc as indicated in Table 13 below.

Constraints	Frequency	Percent (%)
Scarcity	80	37,6
Outdistance 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	7	3,3
biodiversity		
Increase in population	3	1,4
Purchase	6	2,8
Implication of men	1	,5
Repression by the forest	3	1,4
agents		
Total	213	100,0

Table 13: Constraints of wood supply

The pressures of the human and animal populations become increasingly strong on the soils, however individual plantations for the production and exploitation of wood are rare. In 1987, wood consumption of the zone of the office of Niger was regarded as 'rural' type. The cities were too small to generate the organization of real systems of provisioning; in addition, the standard of living of the populations did not make it possible to carry out purchases of wood or to pay loggers. Charcoal had few uses and each family cut their own wood at the edge of the village. Since then, this situation has developed considerably. Indeed, the population increased and this increase is related to the development of the size of the exploitations, the rehabilitations in progress and the extensions envisaged attract many candidates to the "colonat", originating in the close areas, even more distant (together of Mali, Burkina Faso, etc). The wood consumption related to the energy production domesticates would be thus

of constant increase. It should be said that this fuel represents, for the moment, almost the only source of energy usable and covers practically 100% of the needs (charcoal included). Table 14 below shows the yearly consumption of wood energy of per capita (tons) rural and urban area in the zone of intervention of the Ubenefit project.

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

Table 14. Yearly consumption of woody energy per capita (tons)

In the zone of the project, time necessary to provision households with wood and fodder is high. Indeed, in Niono, for example, the average distance traversed by the carters is 30 km with the extreme ones going from 60 to 70 km. On average, the number of days necessary is 3 days. The general average is of 34,4 km distance to travel and 2.7 days to obtain a wood cart, with the fodder attached to the two with dimensions similar to the cart. One always needs two asses and the kitchen utensils to make a success of such adventures undertaken by people in the urban centres. In Niono, the small households consume on average 4 "charretés" of wood, the households of average size 6, spring cleanings 8. With regard to the prices, they vary according to the periods, qualities and required types of wood. With regard to the wood for heating, a stere costs 7500 FCFA in dry season and 10000FCFA in the rainy season. The charcoal comes from the cities of Fana and Konobougou, on the road of Ségou, while going in Niono. The bag of 100 kg costs 2500 FCFA during the dry season and 3500 during the rainy season. The wood of service for the construction of the roofs and hangars costs 25 FCFA the "perchette", known under the technical name of "gaulette"; the prices of the forks or wood being used as cross-piece during construction vary between 1500 FCFA and 2000FCFA. These construction woods are rare in the zone of the project Ubenefit. It comes from the centres in Mali like Konobougou, Fana, Doila, Segou. As for fodder, one can note that the continuous increase in number of animals poses a problem of space management between agriculture and the breeding, of the crucial problems of food for the animals. Approximately 120.000 heads of bovines are in the three zones of Niono, Molodo, and Débougou, creating problems of food, environmental pollution and also conflicts related to the cohabitation for which solutions must be found.

A film and photographs were elaborated to document step by step this study. According to questionnaire performed by partners in charge of this work package, At this period we expected coppicing trees could done in February 2006.

Work Package 6: *Water, soil and plant nutrition Water analysis*

Water was sampled and analysed for chemical, heavy metal and pesticide residues during May and November 2006 (12 and 18 months after planting). Water contents were investigated at the lab of LQE in Bamako, Mali, using three types of water: irrigated water at Minimana (used channel or water from rice field), water collected after plant irrigation (called collecteur, output) and in Typha channel (considered as control because purified with Typha).

 Table 15 Results of irrigation water analysis for chemical contents in three sites: Rizière: Tank receiving water from rice field; Minimana: New channel (input, water used for plant irrigation); Typha: Old channel (control: water was supposed purified by biological system as Typha).

 Collecteur (output : tank receiving water after irrigated plant).

	Types of water samples							
		(I	Date)					
Water contents	Typha	Rizière	Minin		Collecteur			
	Control	Rice field	(Used c	hannel)	(Outflow)			
	05/06	(input)	05/06	11 /07	05/07			
N02	05/06	05/06	05/06	11/06	05/06			
N02 N03 ⁻	0,01	0,00	0,01	0,01	0,01			
NH4 ⁺	0,30	0,20	0,70	5,00	0,30			
	0,33	0,58	0,45	0,10	2,42			
OrthoPO ₄ ³⁻	0,13	0,08	0,20	?	0,24			
SO4	0	0	0,3	0	2			
pH	6,82	6,79	6,85	6,99	7,13			
Fe ²⁺	3,18	1,13	0,96	?	2,98			
Ca ²⁺	5,6	4,8	4	11,2	4			
Mg ²⁺	0,5	1	1,5	2,9	0,5			
Na ⁺	5,3	5,5	5,9	6,9	5,9			
K ⁺	2,4	2,8	2,6	3	3,5			
Mn	0	0	0	?	0,07			
Cl	2,5	2	1,5	3,5	4			
Cu ²⁺	0,07	0,09	0,07	?	0,10			
DBO5	1	2	3	8	2			
DCO	3	4	6	70	5			
Conductivité (µS/cm)	61	56	5,7	100	53			
Turbidité	16	61	39	24	325			
Couleur	17	182	158	78	500			
Dureté	16	8	16	40	12			
Bicarbonate	36	30	32	59	27			
Chrome	0	0	0	?	0			
Matière solide totale	46,26	42,47	43,23	94,76	40,19			
Alcalinité	30	24	26	49	22			
Indice de Rymar	12,61	12,24	13,03	10,26	12,87			

Pesticide residues detection. Presence of pesticide residues in irrigation water (Minimana channel) was checked for risk assessment. All analysis were performed in the Laboratoire de toxicologie et de contrôle la Qualité Environnementale du Laboratoire Central Vétérinaire (LCV) de Bamako, Mali. Table 16 below, summarises the methodology and results obtained.

The report provided by the laboratory shows that water sampled do not contain detectable pesticides residues at the specified "LOD".

Standard pesticides	Methods	Detect	ors	LOD
used	DFG	NDP	ECD	(ppb)
Deltamethrine	X	1	X	1
Dimethoate	X	i L	X	0,3
Lindane	X		X	0,0008
Chlorpyrifos	X		X	2,3
Cyanophos	X		X	0,9
Lamda cyhalothrine	X		X	4
Malathion	X		X	1
Fenitrothion	X		X	0,1

Table 16 showing standard used pesticides, method and detectors allowing to obtain the results

Plant nutrient contents

Sampled plant sheet were dried and sent to the Laboratoire de biopédologie at Dakar for analysis.

Package 8: Pest monitoring and Management

According to methodology defined during the last meeting in Burkina Faso, pest and diseases were investigated in the two experimental design at six, nine and twelve months after planting. The objective is to define the risks of attack by pests or diseases caused on tree species under irrigated conditions.

Soil and plant roots were sampled and analysed for nematode investigations in the lab of IPR/IFRA. In each plot, composite sample or main sample was performed using five sub-samples collected and mixed as defined by protocol adopted by different involved partners.

In each plot, 4 plants were concerned for nematode roots investigations.

Plant health was observed regularly. We observed that plants 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. In fact, chemical product Drusbans (1%, 11itre of Drusbans at 15cc in 100 litres of water) has been utilized by technical staff.

According to last observations on aerial parts of plants performed in November 2006 by field technicians no damage was detected. Results of recent soil, root analysis for

pest have not been done because of the transfer of our Colleague Diafar CISSE from IPR-IFRA.

Formal Training:

Mr Fallaye KANTE registered for Ph. D degree at ISFRA in Mali, is now the laboratory of partner IRD- Laboratoire Commun de Microbiologie (LCM) in Dakar (Sénégal). In the frame work of his thesis, he also has been supported by AUF. This project allows Youssouf Cisse from IER charged with economics to go on his thesis at ISFRA.

Liaison with partners: According to discussion between local partners IER and FAST trees coppicing could be done after cold season after February 2006. From partner of Niger Univ. Abdou Moumouni exchanged were established about questionnaire on economics.

Our collaborator Diafar Cisse from IPR/IJRA who is charged of Pest and plant sanitary has moved from laboratory of IPR to an agriculture service. He initiated soil and plant analysis for pest, nematode and plant survey. Since 6 months ago he has not provided any result.

According to the project plan we expected that Youssouf Cisse who is charged with economic work plan and Inamoud I. Yattara responsible of the project could participate to next meeting at UK

Problem encountered

Our colleague Diafar Cissé who is charged with work package pest and plant is moved from IPR/IFRA for others tasks. Therefore, some analysis results are not available. Plant sheet and soil samples were sent to Laboratoire de biopédologie (Dakar) for analysis.

Molecular analysis are now performed by Fallaye KANTE at the LCM Dakar. Plant coppicing is late according to our previous plan.

We expected receiving money for going on and achieving the remaining tasks.

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Partner 4 report INERA

Mahamadi Dianda, Jules Bayala and Kadidia Sanon

Work package 1: Water treatment and irrigation

Waste water purification system

The waste water treatment equipment was totally installed and tested at the beginning of December 2005. This test coincided with the visit of SCP staff (represented by Mme Bouroulet) to INERA with the aim of assessing the functioning of the water treatment system and training local partners. According to her evaluation the purified waste water presented good characteristics. However, the system stopped functioning in late December because the sole pump that deserves the equipment with waste water collapsed due to plastic materials carried with the waste water. SCP and the local waste water treatment specialists assisted INERA for seeking solutions. It took us a long time to replace this component by a new pump that needed to be commissioned from Europe. Finally the pump has been reinstalled allowing the monitoring of the treated waste water quality to resume.

Irrigation

This part of the system was completely installed in January 2006. The work was voluntarily delayed waiting for running treated waste water system in view to optimize the adjustment of the irrigation system. However, because the treated waste water was not available due to the difficulties outlined above, the irrigation system was installed without any water flow.

There were many criticisms by the SCP about the functionality of the irrigation component which appeared to not be adapted to the field topography. Basically, a much steeper slope should have been realized to allow the purified waste water to run downhill (gravity). Thus, there are many errors inherent to initial misconception of the system that further may account for the major difficulties in irrigation activities. Currently, the irrigation activities are conducted manually using an independent motor pump (with fuel) to supply the treated waste water to the plantation.

Assessment of treated waste water quality

For the first time since the installation of the waste water purifying system at the University of Ouagadougou, the quality of treated waste water was assessed in order to verify if it functions adequately and contributes to reducing pollution. Thus, a monitoring of waste water quality was carried out over a 4-week period lasting from 20th June to 10th July 2006 by a student from France.

Materials and methods

Waste water was randomly sampled at weekly intervals from each of the four basins of the system, i.e. (i) crude waste water arriving in the system-homogenizing basin, (ii) anaerobic basin, (iii) facultative basin and (iv) treated effluent flowing out of the disinfecting basin. Sampling was done for four weeks and samples were analyzed for physical parameters (temperature of waste water when sampled at the site, conductance, oxygen and pH), chemical and biochemical characteristics (DBO5, DCO, NH4⁺, NO3⁻), and microbial parameters (*E. coli*, CTT and SF). The data were analyzed statistically by GLM procedures (SAS software package) considering

sampling dates of waste water and basins as factors. Where appropriate, simple comparisons procedures were used to compare treatments means.

Results and discussions

Physical parameters: The temperature of waste water increased significantly with time and varied between ponds at the experimental site (all P<.001) (Figure 15). The concentration in O_2 also increased with time during the study period (p<.01). However, no significant difference was found between ponds in O_2 concentration (p>.05). The increase in O_2 concentrations when the temperature increases is not an expected phenomenon since the solubility of oxygen in water is known to decrease with ambient weather heating. This may be due to the fact that the temperature measurements and those in O_2 concentration were done in quite different conditions (i.e. immediately in the site during sampling, and at the laboratories later after sampling, respectively).

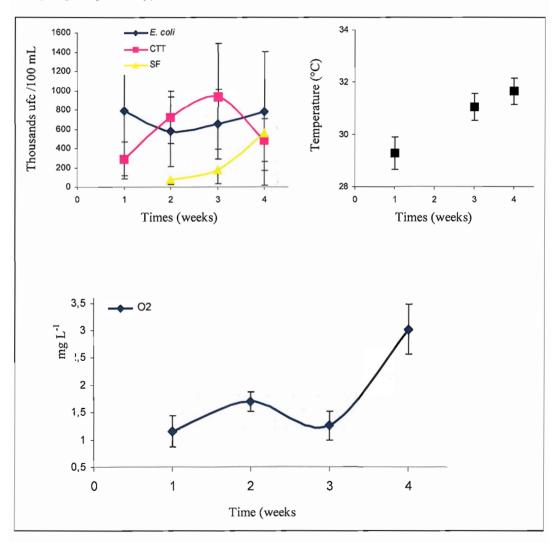


Figure 15. Average values of microbial pollutants, oxygen concentration and temperature of waste water pounds at the University of Ouagadougou

Chemical and biochemical parameters: Amongst the parameters assessed, DBO5 which represents the biochemical potential requirements in O₂ for processing C compounds, and nitrate ions (N03⁻) showed significant variation with time (All P<.05). Variation patterns of these parameters may be related to those of the physical characteristics of waste water. There were significant variations between the basins in DCO (chemical requirement in O_2) (p<.05). Large variation also occurred between the lagoons in P (p<.05), and NH4+ nutrient concentrations (p<.01). The concentration in NH4+ was reduced by 50% for treated waste water compared with crude waste water (p<.05). However P and NH4+ concentrations showed the highest values in the intermediate lagoons. Although interpreting these trends is not evident, we assume that the accumulation of these nutrients may have been favoured by irregular waste water supply due to the pump breakdowns. The seasonal Harmattan wind also contributed considerably with tree litter falling in the pounds transported by this wind. Though parts of this biomass have been manually removed, a certain unknown quantity has remained in the largest pounds and may have interfered with the waste water purification processes.

Microbial parameters: The waste water treatment equipment significantly affected *E. coli* population of waste water (p<.001) (Table 17). *E. coli* germs decreased consistently through the system, being 99% less in treated waste water than in crude waste water. Populations of other germs (CTT and SF) were not significantly affected by the purifying system (p>.05).

In conclusion, these measurements provide indications that the waste water equipment efficiently improves the quality of water used for irrigation by reducing the pollution relative to *E. coli* (99%) and ammonium (50%), while increasing certain nutrient concentrations (Table 1). The data confirm the good characteristics of the purified waste water as reported by the representative of SCP during her on site evaluation trip at Ouagadougou. However, further investigations are needed to better document and understand the underlying processes of waste water treatment for optimization of the purifying system.

Basin	Physical parameters			Mic	robiological anal	ysis	
	Temperature (°C)	Conductivity µS m ⁻¹	O ₂ (mg L ⁻¹)	pН	<i>E. coli</i> (ufc) (thousands)	CTT (ufc) (thousands)	SF (ufc) (thousands)
Crude wwater	 31,9 ± 0,6	240 ± 17	1,2 ± 0,4	7.1	2130 ± 366	750 ± 65	1000 ± 611
Exit of anaerobic basin	31,2 ± 0,8	253 ± 17	1,8 ± 0,7	7.6	635 ± 167	1220 ± 464	67 ± 33
Exit of aerobic basin	29,5 ± 0,8	246 ± 9	2,2 ± 0,4	9.1	28 ± 24	385 ± 274	7±7
Treated wwater	30,1 ± 0,7	259 ± 11	1,9 ± 0,4	7.7	13 ± 9	85 ± 34	7±7

Table 17 Parameters of waste water in ponds at the	he waste water purifying system inst	alled at the University of Ouagadougou

Basin	DBO5	DCO	MES	Total P	PO4 ³⁻	NH_4^+	NO ₃ ⁻
		~		mg L ⁻¹			
Crude waste water Exit of anaerobic basin Exit of aerobic basin Treated waste water	15 ± 7 26 ± 15 14 ± 9 10 ± 7	61 ± 6 77 ± 1 94 ± 14 46 ± 13	61 ± 49 44 ± 7 82 ± 38 28 ± 2	$\begin{array}{rrrr} 1.7 \pm & 0.2 \\ 2.5 \pm & 0.1 \\ 2.4 \pm & 0.5 \\ 1.4 \pm & 0.1 \end{array}$	0,9 ± 0,2 0,3 ± 0,3 0,7 ± 0,2 0,7 ± 0,3	7,7 ± 0,7 8,7 ± 1,9 3,4 ± 0,7 3,8 ± 0,5	1,1 ± 0,2 1,1 ± 0,3 1,6 ± 0,4 1,5 ± 0,5

с.

Work package 2: Tree growth and management

Preliminary experiments

At the preceding coordination meetings of the project, it was agreed that preliminary irrigation experiments were needed to address some essential scientific questions not explicitly detailed in the work packages. INERA previously carried out irrigation experiments along with inoculation with microsymbionts on 20 local and introduced species, using well water for irrigation. Although these experiments allowed the assessment of growth responses of species both to irrigation and inoculation, the result remained incomplete since data on mycorrhizal status of plants were lacking. Samples of fine roots that were taken to estimate the extent of root colonization by mycorrhizal fungi were accidentally lost. Therefore, a small trial was carried out as previously detailed (except that tap water was used instead of well water) to generate more complete data on the mycorrhizal status of inoculated species in our environment.

Materials and methods

The experiment dealt with the responses of 20 species to irrigation and inoculation with mycorrhizal inoculants (inoculated vs. uninoculated plants). There were two levels of irrigation, i.e. "permanently" wet and "temporarily" wet. In the former irrigation option, plants were grown in pots containing permanent levels of water delineated by holes made 4-5 cm below the soil surface in the pots, whilst in the later option treatment consisted of common pots with holes made 2-3 cm above the base. The pots were daily watered twice with tap water in excess. Observations have shown that between the two water supplies, the soil surface tends to dry out only in pots with basal holes. The design was a split plot (with irrigation in main plots) with 3 blocks. A single mycorrhizal strain (*Glomus* sp.) was used for inoculation. Seeds were pre-treated following the recommendations of the suppliers. After germination the seedlings were transplanted in pots containing 2 kg of sand. The experiment was ended 3 months after inoculation for the assessment of growth and symbiotic characteristics.

Results and discussions

Plant height and collar diameter: The inoculation treatment showed no significant effect on the plant growth characteristics (p>.05; data not shown). "Permanently" wet irrigation regime generally depressed height growth in species compared with the "temporarily" wet treatment (p<.05; Figure 16a). However this effect varied depending on plant species. Eight species (*Afzelia africana, Acacia angustissima, Acacia crassicarpa, Pterocarpus erinaceus, Khaya senegalensis, Acacia mangium, Senna siamea and Ziziphus mauritiana*) displayed similar height growth in both irrigation treatments (Figure 16a). These species may naturally have the ability to withstand harsh conditions relative to both drought and water logging. On the other hand, growth reduction by excess watering was the greatest in two species (Leucaena hybrid and Leucaena leucocephala) which could require particular management once established in plantations and irrigated with treated waste water. There were significant differences between species in collar diameter with varying trends according to the water regime (Figure 16). The collar diameter was greater under excess watering regime in some species (Ziziphus mauritiana, Pterocarpus erinaceus) (Figure 16b). The plant tap root length followed a similar pattern as for height growth (Figure 16c).

Plant biomass: Shoot and root DWs and total plant biomass varied significantly between species, and also within species depending on the irrigation regime (p<.01; Figure 17). According to the total biomass accumulation and trends of its partitioning between shoots and roots, we may assign the tested plant species into four distinct groups. The first group was composed of species clearly not adapted to permanent water supply, and consequently accumulated the greatest biomass (either shoot, root or total DWs) in temporarily wet conditions. This group includes the majority of the tested species such as *Gliricidia sepium*, *Leucaena spp., Antada africana, Pterocarpus spp., Calliandra and Albizia lebbeck, Afzelia africana, Acacia angustissima, Acacia crassicarpa, Pterocarpus erinaceus, Khaya senegalensis, Acacia mangium, Senna siamea* and Ziziphus mauritiana (Figure 17).

The second group contrasts with the 1st one, and contain species such as *Eucalyptus* and *A. crassicarpa* that showed a consistent best growth performance, with regard to shoot, root and total DWs, under flooded conditions. *A. mangium* and *Z. mauritiana* are considered as representative of the 3rd group that consists of species sharing a similar ability of growth in both irrigation regimes. A last group is proposed for *Afzelia africana* that displayed a particular biomass allocation trend: the seedlings of this species had similar total DWs with different shoot and root allocations depending on the irrigation treatment (Figure 17).

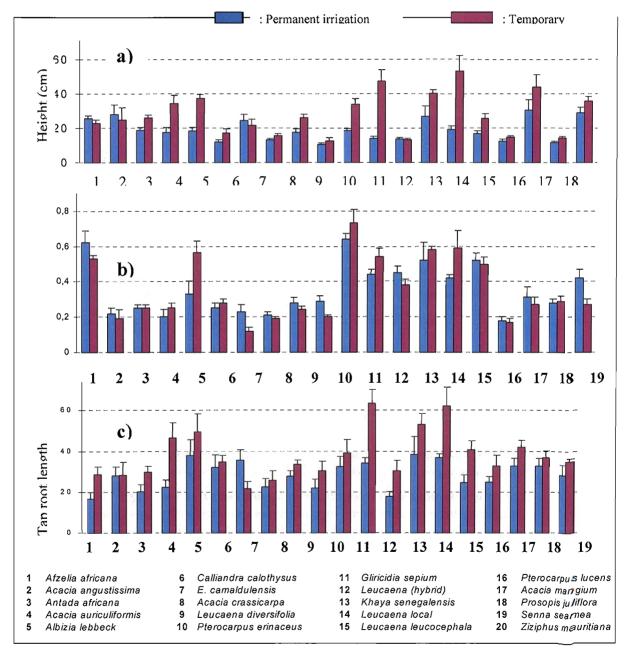


Figure 16. Height, collar diameter and tap root length of twenty forage and wood producing species grown under normal and excessive watering regimes

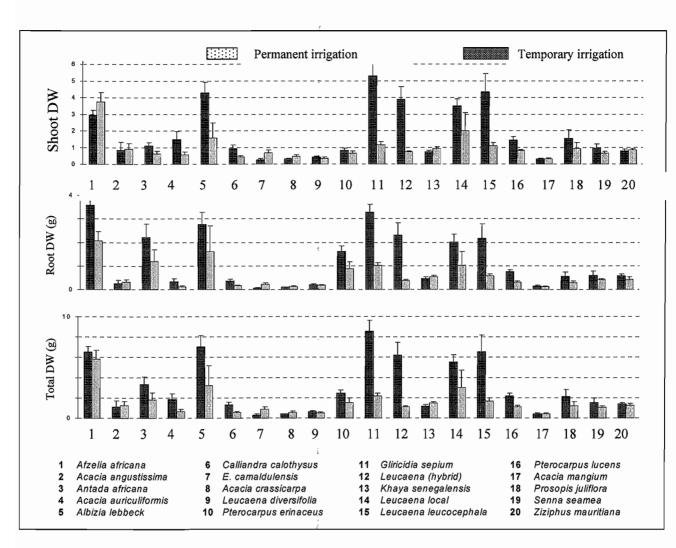


Figure 17. Accumulation of shoot, root and total plant biomass in twenty forage and wood producing species grown under normal and excessive watering regimes

The remaining species not previously listed in groups are considered as intermediate species.

If relevant, this grouping of species at the nursery stage is expected to influence plant establishment and their growth traits in further field experiments.

Leaves and symbiotic characteristics in leguminous tree seedlings: There were no significant differences between inoculated and uninoculated plants in nodule characteristics and leaf number (p > .05, data not shown). Moreover the mycorrhizal status of plants also showed no significant variation amongst the tested factor (p > .05) (Table 18), probably due to high variability in the data. However, nodulation and number of leaves on stems were greater for seedlings temporarily irrigated than those grown in permanently wet conditions (Table 19). Leaf shedding occurred in most species roughly one month after the experiment was set up and onwards which may have contributed to these differences. There was also large variation within tree species depending on the irrigation regime (p < .01) (Table 19). With the exception of *Gliricidia sepium* for which significant nodules DW was recorded, nodulation in the

other species appeared negligible since nodules were very small and weighed little (Table 19). With regards to N_2 fixation, such result suggests that most species fixed very small amounts of N_2 in the soil used for this experiment.

 Table 18 Average percent mycorrhizal colonization in twenty forage and wood producing species

Species		Mycorrhizas
·	n	(% root colonization)
Afzelia africana	70	28,4 ± 3,3
Acacia angustissima	89	$27,6 \pm 2,5$
Antatda africana	70	34,3 ± 2,9
Acacia auriculiformis	70	37,1 ± 3,0
Albizia lebbeck	69	$32,2 \pm 3,1$
Calliandra calothysus	68	36,2 ± 3,3
Eucalyptus sp.	60	26,4 ± 3,4
Acacia crassicarpa	69	29,7 ± 2,6
Leucaena diversifolia	69	22,0 ± 2,0
Pterocarpus erinaceus	60	29,0 ± 2,8
Gliricidia sepium	80	28,9 ± 2,4
Leucaena (hybrid)	69	33,5 ± 3,2
khaya senegalensis	89	26,5 ± 2,8
Leucaena (local)	60	38,6 ± 3,3
L. leucocephala	100	27,8 ± 2,1
Pterocarpus lucens	59	28,8 ± 3,2
Acacia mangium	79	30,3 ± 2,4
Prosopis juliflora	70	40,9 ± 3,4
Senna seamea	78	27,4 ± 2,8
Ziziphus mauritiana	70	34,1 ± 3,1

Irrigation	Species	Leaves	Nodules			
treatment		number	number	DW (mg)		
Temporarily w	vet					
	Acacia angustissima Antatda africana Acacia auriculiformis Albizia lebbeck Calliandra calothysus Acacia crassicarpa Leucaena diversifolia Pterocarpus erinaceus Gliricidia sepium Leucaena (hybrid) Leucaena (local) L. leucocephala Pterocarpus lucens	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$9 \pm 3 \\ 3 \pm 1 \\ 8 \pm 5 \\ 11 \pm 3 \\ 12 \pm 5 \\ 1 \pm 0 \\ 3 \pm 1 \\ 20 \pm 4 \\ 27 \pm 6 \\ 22 \pm 9 \\ 15 \pm 3 \\ 19 \pm 5 \\ 17 \pm 4$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$		
	Acacia mangium Prosopis juliflora	12 ± 0 24 ± 4	1 ± 1 4 ± 2	$\begin{array}{rrrr} 0 & \pm & 0 \\ 40 & \pm & 10 \end{array}$		
Permanently	wet					
	Acacia angustissima Antatda africana Acacia auriculiformis Albizia lebbeck	19 ± 4 8 ± 1 9 ± 2 10 ± 1	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$		
	Calliandra calothysus Acacia crassicarpa Leucaena diversifolia	7 ± 1 8 ± 1	$ \begin{array}{rcrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{ccc} 0 & \pm & 0 \\ 0 & \pm & 0 \end{array}$		
	<i>Eterocarpus erinaceus</i> Gliricidia sepium	8 ± 1 10 ± 1 7 ± 1	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$ \begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$		
	Leucaena (hybrid) Leucaena (local) L. leucocephala	12 ± 2 13 ± 3 11 ± 2	1 ± 0,83 5 ± 4,58 11 ± 4,74	$ \begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$		
	Pterocarpus lucens Acacia mangium Prosopis juliflora	13 ± 1 10 ± 1 16 ± 5	9 \pm 4,15 0 \pm 0 2 \pm 1,64	$\begin{array}{cccccccccccccccccccccccccccccccccccc$		

Table 19. Number of leaves of number, nodules and dry weights in seedlings of fifteen forage and wood producing species grown in excess vs. standard irrigation regimes

Main experiment

The main field experiment has been set up with the four species retained by INERA according to the plan agreed on by all partners (2 m and 1 m spacing between and within lines, respectively). The trial was installed as a Latin Square design with 4×4 plots each comprising 4×4 plants. The data analyzed were collected only on the inner

2 x 2 plants of each plot. The results showed that *Afzelia africana* had the lowest growth on the site (Figure 18). *G. sepium* and *Leucaenas* were the best performers on the site, displaying similar collar stem diameters. However, *G. sepium* had greater stems per trunk than *Leucaena* which grew the best in height. Due to this trend at the earlier stage of the growth, the species are expected to exhibit large differences in wood and fodder production at maturity.

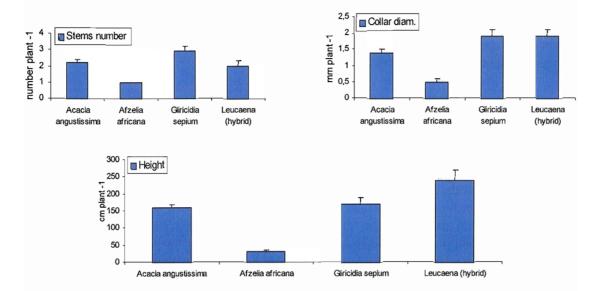


Figure 18 Number of stems, height growth and collar diameter of four forage and wood producing species irrigated with treated waste water

Tree management

A second plantation similar to the main experiment (except that there are 3 blocks) was established to support tree management studies (coppicing trials).

Work package 7: Planting stock quality

7.1. Material and methods

7.1.1. Study site

The study was conducted in a nursery in an open area of the courtyard of Département Productions Forestières (DPF) of Institut de l'Environnement et de Recherches Agricoles (INERA) in Ouagadougou, Burkina Faso, West Africa (12°22' N and 1°30' W and at an altitude of 306 m.a.s.l). The plantation was done in a plot 500 m far from the courtyard of the department.

7.1.2. Experimental design

7.1.2.1. Nursery phase

The methods of this phase have already been reported in 2005's activity report.

7.1.2.2. Plantation phase

For the individuals to be transplanted, the nursery was established mid-June 2005 and the planting took place at the end of December 2005. The design was a split split plot design with single plant as the experimental unit. Species was assigned the main plot treatment, inoculation to the subplot treatment, the substrate to sub-sub-plot treatment and container size to the experimental unit. Species used were the same as in the nursery (*Acacia angustissima, Acacia mangium, Afzelia africana, Gliricidia sepium, Leucaena hybrid,* and *Leucaena leucocephala*) and inoculation had two levels: a double inoculation with both Rhizobium and Mycorrhizas and non-inoculated plants serving as controls. The types of substrates and containers were as described in the nursery phase. Each species was repeated 3 times.

7.1.3. Data collection and handling

In September 2006, i.e. 14 months after planting, one plant out of two was uprooted and separated in three components: leaves, wood and roots. The roots were washed before drying all the three plant components until constant weight was obtained at 60°C. Before uprooting the trees, their height and diameter at 1.30 m were measured. For the plantation phase, only the ratio shoot: root was calculated in addition to the weight of all components, the height and diameter at 1.30 m of the plants. The data were subjected to ANOVA using Genstat Release 8.11 (Rothamsted Experimental Station) Software package. Due to high rate of mortality in *Afzelia africana*, the data of this species was not included in the analysis for the plantation phase.

7.2. Results

7.2.1. Nursery phase

The results of this phase have already been reported in 2005's activity report.

7.2.2. Plantation phase

7.2.2.1. Plant dimensions

No interaction between factors was observed. Out of the four factors, only species exerted a significant effect on both the height and the diameter (all P<0.001). Two groups were distinguished for height with *A. mangium*, *A. angustissima* and *G. sepium* forming the first group and the second group was composed of the two leucaenas. Within each of these two groups the species did not differ from each other (Table 20). For the diameter, *G. sepium* did not differ from *A. angustissima* but both displayed a significantly lower diameter compared to the rest. *A. mangium* did not differ from *L. leucocephala* but was different from *L. hybrid* whereas the two leucaenas did not differ significantly (Table 20).

Species	Height (m)	Diameter (cm)			
A. angustissima	3.65±0.12a	4.07±0.22a			
A. mangium	3.57±0.16a	6.16±0.48b			
G. sepium	3.69±0.15a	4.00±0.31a			
L. leucocephala	5.94±0.38b	7.75±0.60bc			
L. hybrid	5.99±0.23b	8.42±0.44c			
s.e.d	0.31	0.61			

 Table 20 Mean height (m) and diameter at 1.30 m (cm) of five introduced species in Burkina Faso,

 West Africa

s.e.d= average standard error of differences

7.2.2.2. Plant components weights

Again no interaction was observed between the studied factors while only species showed a significant effect on plant components weights, except for leaf weight (Table 21). Thus, *L. leucocephala* showed a significant higher root weight compared to *A. angustissima* and *A. mangium* (P<0.01) whereas this species did not differ from *G. sepium* and *L. hybrid*. The rest of the species did not differ significantly from each other (Table 21). For wood, *L. leucocephala* differed significantly from *A. mangium* (P<0.05) but not from the rest of the species which in turn did not differ from one anther. Finally, two groups were revealed with the acacias displaying the higher shoot: root ratio compared to the rest (P<0.001).

Table 21 Mean weight (kg) of plant components and shoot: root ratio of five introduced species in
Burkina Faso, West Africa

Species	Roots	Wood	Leaves	Shoot: root ratio
A. angustissima	0.37±0.10a	2.89±0.54ab	1.12±0.21a	15.52±1.90a
A. mangium	0.24±0.7a	2.55±0.52a	1.27±0.25a	20.95±2.60a
G. sepium	0.74±0.16ab	3.28±0.46ab	0.73±0.09a	6.33±0.78b
L. leucocephala	1.25±0.28b	5.79±1.28ab	1.63±0.35a	6.22±0.56b
L. hybrid	0.87±0.17ab	4.84±0.88ab	1.20±0.28a	7.48±0.57b
s.e.d	0.24	1.11	0.35	2.17

s.e.d= average standard error of differences

7.3. Discussion-Conclusion

Based on the results of nursery phase, the species were roughly grouped as followed: in one group the two acacias, in a second the two leucaenas and in a third *A. africana* and *G. sepium*. The two acacias showed the lowest values in diameter, height and plant weight. At this stage, these two species appeared to the least suitable for irrigation system because the main goal is to obtain fast growth of the aboveground part. In line with the results of growth parameters and their variability, no clear trend was noted for pot size factor when examining quality indexes while double inoculation seemed to improve the seedling stock quality of all species. In general, *G. sepium* gave the best seedlings for non-irrigated system followed by *A. africana*, the two leucaenas and the two acacias when using sand as substrate. For irrigated system, one might think that the way to interpret these indexes is the opposite of the rain fed system. If so, then the two acacias are the best for irrigated system followed by the two leucaenas, *A. africa* and *G. sepium*. Such interpretation seems to be reasonable because at plantation stage again the two acacias displayed the highest shoot: root ratio (Table 21) indicating a higher allocation of growth resources to the aboveground part of the plants and that is what was expected in the present study. Higher shoot: root ratio in the acacias at nursery stage was maintained suggesting that this may be suitable as desirable characteristic for stock quality assessment for irrigated system.

In turn, the fact that inoculated plants performed better than non-inoculated as well as plants grown in big pots and in ordinary nursery substrate gave highest values of plant growth parameters, these effects have disappeared in plantation. Such effects seem to be easily modifiable by natural conditions and do not constitute reliable indicators for seedling stock quality assessment if we do not include the nutrition aspects.

WP 8. Pest monitoring and management

The monitoring of pests and diseases were done at seedling stage in the nursery, in bare field soils, and in the field again after the establishment of the plantations. At no stage, were significant signs of diseases noticed, except scores of dieback in blocks and attacks by termites.

Other activities

INERA provided the mycorrhizal inoculants to all the partners (Mali and Niger).

INERA organised the 3rd annual meeting held in Ouagadougou (3-5 May 2005). INERA have received a staff member of SCP (Bouroulet Françoise) in December 2005 who evaluated the waste water purifying system installed, and trained technicians and students.

Students have been supervised for their fieldwork of short training (one for 45 days from University of Bobo-Dioulasso) and degrees (one for MSc from University of Toulouse, France, 2 for BSc from University of Ouagadougou, Burkina Faso).

Future plans

- Management activities will start at the beginning of next year.
- Socio-economic studies also will be resumed according to the new proposal made for this WP.

Constraints

Apart from the technical problems outlined above, our activities are threatened by financial limitation. Most variables that need to be monitored in the field including the quality of wastewater treatment, pest monitoring soil and plant analyses, etc., require

the contribution of specialized laboratories and may bring about huge expenses. Therefore, we will certainly experience difficulties to cover all topics listed. Furthermore, we have commissioned for a film on all activities of the project as a proper tool for dissemination. Even though up to two third of the scenario have been realized, we have been forced to stop the process due to lack of financial resources. Overall we are thinking of revising and managing the number of activities we have planned to do in a way to get the maximum out of them.

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Partner 5 Université Abdou Moumouni, UNIV A. MOUM, Niamey, Niger

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

SUMMARY OF PROGRESS

- The physical and chemical composition of waste water was analysed
- The result of inoculation in plant growth in field indicated beneficial effects only for *Leucaena leucocephala*.
- A socio economic questionnaire was elaborated in order to determine the preferred size of timber for fuel wood and hence the best time of pruning.

WP 1. WATER IRRIGATION AND TREATMENT

Plant irrigation

After growing in the nursery, trees of the first experiment, which aims to compare the growth of species under irrigated conditions were transferred to the field on August 13, 2005. From August to February, trees were watered with waste water through a drip system at two day intervals with 6 litres of purified water/tree. But since March, with the high temperatures, the frequency of irrigation has increased.

Analysis of waste water quality

Samples of purified waste water used for tree irrigation were collected twice a week in order to determine its quality. The following nutrients were analyzed : total N, NH_4^+ , NO_2^- , NO_3 , total and available P, Al, Cd, Cr, Co, Cu, Mn, Mo, Ni, Na, Zn, SO_4^2 , pH and conductivity.

Results:

The results indicated that the pH of purified water varied slightly over time, from 8.2 to 8.5 (Figure 19). These slight variations could be attributed to the green algae which are present in the effluent.

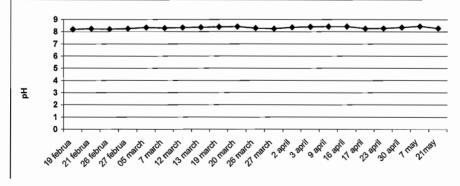


Figure 19 Variation of waste water pH with time

The electric conductivity of waste water was low, around 450 micro siemens (Figure 20) indicating also a low mineralization of effluent.

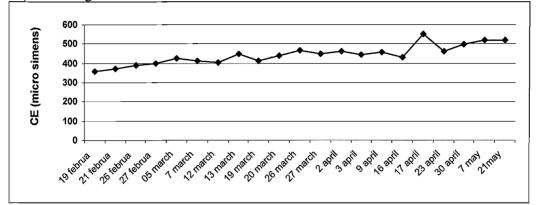


Figure 20 Variation in wastewater conductivity over time

The variation in total and ammoniacal nitrogen in the treated water used for tree irrigation is shown in Figure 21. The total N is Kjeldhal nitrogen and represent the amount of organic and ammoniacal nitrogen. The result of analysis indicates a very low proportion of organic matter in the effluents. This is the consequence of effluent filtration by different layers of gravel in the basin. However the amount of nitrogen in the water is enough for feeding trees.

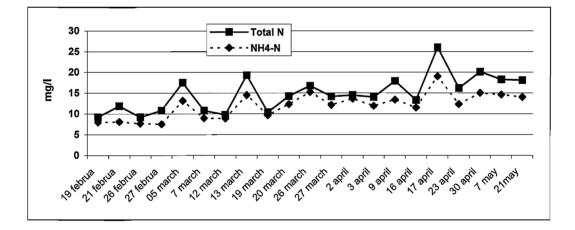


Figure 21 Variation in total and ammonium N with time

The proportion of available phosphorus is relatively low compared to that of total P (Figure 22). This indicated that the time of mineralization of organic P is not sufficient.

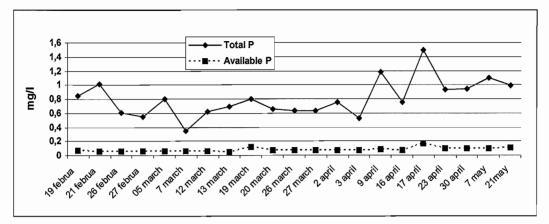


Figure 22 Change in total and available phosphorus in waste water with time

The level of potassium and calcium in the waste water remained steady during the study (Figure 23) indicating the buffering effect of the storage basin.

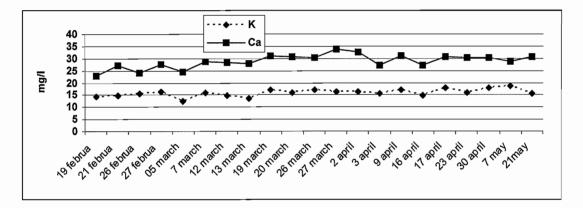


Figure 23 Variation in potassium and calcium with time

The proportion of heavy metals is very low in the effluent (Figure 24), indicating that there is no risk of contamination of trees by these elements. Therefore there is no risk of contamination by heavy metals of tree leaves which can be used as forage.

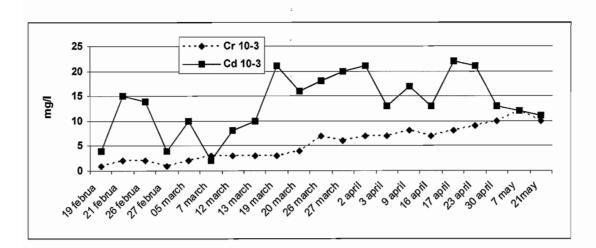


Figure 24: Change in heavy metqls in waste water over time

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WP 2 AND 4. TREE GROWTH, MANAGEMENT, AND MICROSYMBIONTS

Field experiment 1.

Objective:

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

Material and method:

Seed of five species (*Acacia angustissima, A. crassicarpa, A. seyal, Gliricidia sepium and Leucaena leucocephala*) were inoculated in nursery with microsymbionts. The trees were inoculated with mycorrhiza on April 11, 2005. The inoculation with rhizobial strains (4 ml of inoculum/tree) was done on May 5, 2005 with the following inoculum strains :

- Acacia angustissima : 11c + ORS 324;
- A. crassicarpa : 11c;
- *A. seyal* : ORS 3324;
- *Gliricidia sepium* : GSK4.
- Leucaena leucocephala : LDK4;

Thereafter, trees were transferred to the field on August 13, 2005 and since then irrigated with waste water through a drip system. From August to February, trees were watered at two days interval with 6 litres of purified water/tree. But since march, with the high temperatures, the frequency of irrigation have increased.

Measurement:

The following measurements for plant growth were carried out on trees at weekly intervals: the collar diameter and the total height of the tree. The measurements where done on 15 trees per species and per treatment (control, mycorrhiza, rhizobium, rhizobium+ mycorrhiza).

Results:

Table 22 shows the tree collar diameter and total height, one year after planting in the field.

Table 22 Tree collar diameter and total tree height of five species inoculated with microsymbionts, 12 months after planting

	Tree collar diameter (mm)				Total plant height (cm)					
	A. angustis.	A. crassi	G. sepium	Leucaena	A. seyal	A. angustis.	A. crassi	G. sepium	Leucaena	A. seyal
С	30,12 [§]	38,58	30,75b	31,21a	30,56	201,7b	190,8	182,4bc	204,4a	171a
м	32,38	37,57	30,98b	29,48a	28,29	195,9ab	157,9	140,7b	244,2ab	162,5a
R	29,42	37,74	18,80a	38,17b	34,03	173,2a	169,6	84,4a	280,4b	201,5Þ
R+M	32,77	42,07	31,96b	39,75b	35,31	219,5b	195,8	194,7c	351,0c	196,2Þ
Mean	31,52	38,99	28,12	34,65	32,05	197,6	178,5	150,6	270,0	182,8

(C = control; M= + mycorrhiza; R= + rhizobium; R+M = + rhizobium+ mycorrhiza).

[§] : data in the same column with the same letter are not significantly different at 0.05 probability level.

After one year of tree growth, there was a difference of tree height within the species (Table 22). The mean tree height varied from 150 cm (Gliricidia sepium) to 270 cm (Leucaena leucocephala). The remaining species had an intermediate height. No significant effect of inoculation was detected on tree diameter for A. crassicarpa. But the tree inoculation, mainly by rhizobium and combination of rhizobium and mycorrhiza have increased the total tree height of both A. seyal and Leucaena leucocephala. The mean collar diameter varied also greatly among species (Table 22), from 28.12 mm for Gliricidia sepium to 40 mm (A. crassicarpa). But the effect of inoculation on the diameter of the tree varied according to the species. No significant effect of inoculation was recorded after one year of growth in field for all the three Acacias (angustissima, crassicarpa and seyal). But the inoculation, mainly by rhizobium and combination of rhizobium and mycorrhiza has increased significantly the diameter of the tree for Leucaena leucocephala. For this species, the beneficial effect of inoculation on the tree diameter has become important since the 8th month after planting (Figure 25). For the remaining species, the effect of inoculation on the collar diameter during the year of growth in field was not evident.

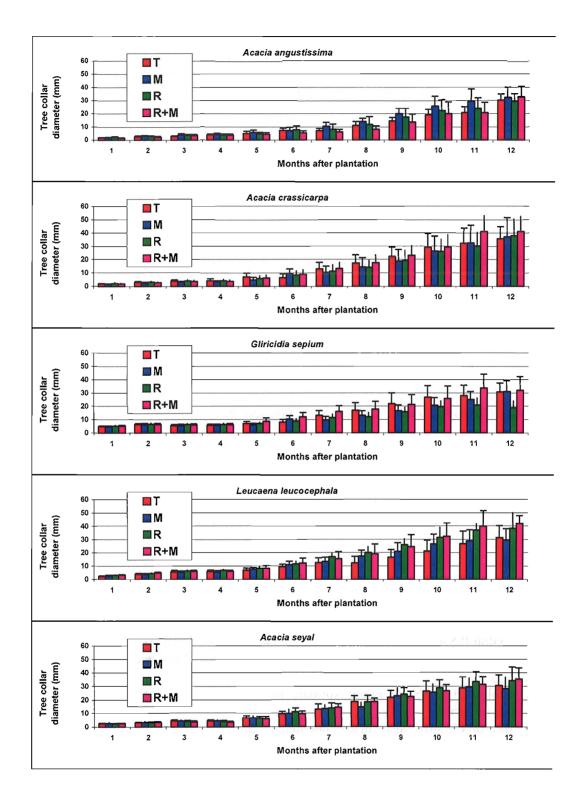


Figure 25 Effect of inoculation in nursery on tree root collar diameter after planting. (T= control; M= + mycorrhiza; R= + rhizobium; R+M = + rhizobium+ mycorrhiza).

Field experiment 2.

The nursery phase for the second experiment has also begun. The objective of this experiment is to do a quick screening of the trees that have already produced, just to make sure that the species which were selected for experiment 1 are the best species

Eleven tree species are used for this experiment:

- five (5) exotic species : Acacia angustissima, A. auriculiformis, A. crassicarpa, A. mangium, Gliricidia sepium.

- six (6) local species: A. nilotica, A. raddiana, A. seyal, Bauhinia rufescens, Leucaena leucocephala and Piliostigma reticulatum

WP 3. TREE WATER USE AND SOIL WATER STATUS

Samples of soils were also collected from the site in order to detect the changes in soil nutrient and organic matter status pre and post irrigation/plantation. The physical and chemical characteristics of soil will therefore be analyzed.

WP 4 : ECONOMICS AND QUALITY OF PRODUCE

In order to determine the stage of pruning for fuel wood specie a questionnaire was elaborated by Mrs Germaine Ibro. The objective of this questionnaire was to collect information with the wood sellers and the households about the diameter of tree according to the specie and the utilization. In addition the price of wood varied also according to its diameter. The questionnaire is in appendix I.

Training on waste water treatment

On December 2nd and 3rd, 2005, Françoise Bouroulet from SCP trained three persons from University Abdou Moumouni on waste water treatment and valorisation:

- M. Seydou ISSAKA, Faculté des Sciences, Département de Biologie

- Mrs Dahiratou MARAFA, Ecole Normale Supérieure, Département de Sciences naturelles

- M. Halilou OUMAROU, Ecole Normale Supérieure, Département de Sciences de la Vie et de la Terre.

Mrs Bouroulet gave also many documents and a CD ROM on waste water management.

Mr Mahamane Sani Laouali made a scientific visit at Ecole Technique de Nancy (France)

FUTURE PLAN OF ACTIVITIES:

- Analysis of soil samples of the site for different nutrients such as C, N, P, K, Na, Mg and Ca;

- Pruning of the different tree species according to their utilisation, fuel wood or forage;

- Analyse of the forage quality for species chosen for fuel wood;

- Analyse of pre and post purified waste water;

- The results of M. Ibro's questionnaire will be used to determine the diameter of tree prunings preferred for different uses

- Dissemination workshop on the utilisation of waste water to produce fuel and wood from fast growing species – stakeholders will be invited to this workshop during which they will be shown the techniques of water purification, plant inoculation etc.

- A short film will be made of the project's activities

Appendix I. Questionnaire : Enquête pour l'estimation du diamètre de coupe

L'enquête pour l'identification du diamètre de coupe se fera auprès des vendeurs de bois et des consommateurs.

Le but étant de collecter des informations empiriques pouvant appuyer les données connues à travers la littérature.

Le diamètre du bois varient en fonction du type d'utilisation, et de l'espèce c'est pourquoi, les enquêteurs sillonneront les villes de Niamey, Ouagadougou, et Bamako pour mesurer les diamètres par espèces et préciseront les utilisations qui en sont faites.

Le diamètre sera mesuré à l'aide d'un mètre ruban à 3 niveaux (le bas, le milieu, et le haut) pour ensuite calculer la moyenne lors du traitement des données.

Les diamètres seront mesurés aussi en fonction des utilisateurs (la grande famille préfèrera les grands diamètres alors que la petite famille se contente du petit diamètre).

Questionnaire aux vendeurs de bois

Quel type de bois vendez-vous ?

1 = bois d'œuvre

2 = bois de chauffe

3 = bois de service

Pour le bois d'œuvre ou de service donnez les informations suivantes

Espèces	Type d'utilisation	Diamètre de coupe

Mesure des diamètres par type d'utilisation

Espèces	Type d'utilisation	Diametre1	Diametre2	Diametre3

Pour le bois de chauffe donnez les especes utilisées et leurs diametres

Espèces	Type d'utilisation	Diametre1	Diametre2	Diametre3
				,

Questionnaire aux consommateurs

Quels sont les diamètres préférés par type d'utilisateur

Type d'utilisateur	Diamètre		Dépenses journalière affectée au bois	
	Diametre1	Diametre2	Diametre3	

Code type d' utilisateur

- 1= petite famille < 4 personnes
- 2= famille moyenne 5 à 10 personnes
- 3= grande famille > 11 personnes
- 4= Restaurateurs
- 5= Bouches

Partner 6 IRD

Tatiana Krasova-Wade and Marc Neyra (IRD)

Francoise Bouroulet (SCP)

Work package 1 (Water treatment and irrigation)

The report of SCP's visit in November, December 2005 is provided in Annex 1.

Work package 4 (Microsymbionts and Nitrogen-Fixation)

Nodule analysis

The principal task planned for IRD for this period was the analysis of the nodules collected by partners 3, 4 and 5 in field studies in Burkina-Faso, Mali and Niger. Unfortunately, due to the delays for establishing experimental trials, the nodules have been only partially brought to Dakar, and we just began this analysis which will thus be completed during the next period.

Development of a probing technique for nodule analysis

Rhizobial strains Aust 13C and Aust 11C have been used as inoculants in Mali, Burkina Faso and Niger, for inoculation of *A. mangium*, *A. crassicarpa* and *A. auriculiformis*. In order to allow the development of a rapid and low-cost technique for identifying these strains in the hundreds of nodules collected in the field and thus propose to the other partners an alternative method of the PCR-RFLP, strain-specific DNA probes have been designed on the basis of 16S-23S rDNA IGS region sequence analysis. The theoretical specificity of the probes was checked by alignment of their sequences with those available in the laboratory and in international GenBank database by using the algorithm BLAST (Altschul *et al.*, 1997).

The probes have been synthesized and labelled by Eurogentec (Belgium) with 5', 3' and internal Dig-labelling. Their lyophilised pellets have been resuspended in sterile water to obtain 200 μ M of storage solution. Purity and quantity of the final solutions were checked by spectrophotometry at 200 to 340 nm and the final quantities were calculated as:

DNA (
$$\mu$$
M) = (DO 260 nm x 10⁻⁷) / 1,

where l is the length of the oligonucleotide. The melting temperatures (T_m) , theoretical hybridization temperature, were calculated according to the formula of Boehringer's manual:

 $T_m = 81.5 + 16.6 \text{ x} (\log_{10} [\text{Na}^+]) + 0.41 \text{ x} (\%\text{G}+\text{C}) - (600/\text{I})$

where 1 is the length of the oligonucleotide and $[Na^+]$ is the concentration of sodium ions in the last washing solution.

The characteristics of the probes are presented in Table 23.

Table 23 Designed probes and their properties

Probe	Target strain	Sequence 5' - 3'	% G+C ^a	lp	Т т
Aust 13C	Aust 13C	CGCTTGTTCATCGCGGCTCATCG	61	23	52°C
Aust 11C	Aust 11C	GGTGAGCGGGTTGTAAATGATCCC	54	24	50°C
² C/C and		liganizate at ide blig the length of the aligani	valaatida		

^aG/C content of the oligonucleotide; ^bl is the length of the oligonucleotide

The specific hybridization conditions have being determined empirically by probe hybridization on strain DNA on the basis of calculated hybridization temperature as a starting point. We use the following procedure:

Membrane preparation by "Dot blotting"

The DNA extracted from the strains is denatured by incubation at 100°C for 10 min and cooled in ice for 10 min before dotting to nylon (+) membrane 0,45 μ m (Biodyne PLUS, PALL) at a rate of 1 μ g per dot with Dot blotting Fisher Bioblock Scientific system. The DNA is fixed on the membrane while heating to 80 °C during 30 minutes.

Probe hybridization

Pre-hybridization

This step aims to equilibrate the membrane with hybridization solution and to block any a-specific sites. The membranes are incubated with 10 ml of the hybridization solution (5 xSSC, 750 mM NaCl, 75 mM sodium citrate, 1 % w/v blocking reagent, 0,1 % w/v N-laurylsarkosine sodium salt, 0,02 % w/v SDS) at 68°C for 2 h.

Hybridization

The hybridization solution is replaced by 10 ml of the hybridization solution containing 2 to 10 pmol of labelled probe. The hybridization is down by night at the hybridization temperature T_m .

Washings

The objective of this step is to eliminate any a-specific hybridization and to protect the probe/target specifically formed hybrids.

The membranes are washed twice for 5 min in a first washing solution (2 x SSC, 300 mM NaCl, 30 mM Citrate de sodium, 0,1 % w/v SDS) at hybridization temperature and twice for 5 min in a second washing solution ($0,1 \times$ SSC, 15 mM NaCl, 1,5 mM sodium citrate, 0,1 % w/v SDS) at hybridization temperature.

Hybridization revelation

This is an immuno-enzymatic revelation: antibody anti-digoxigenin coupled with alkaline phosphatase. It is realized by a colorimetric reaction (formation of a blue precipitation on the membrane at the dot containing targeted DNA, due to oxido-reduction reaction with the BCIP/NBT).

The membranes are rinsed with 50 ml per membrane of buffer B1 (0,1 M Tris-HCl 0,15 M NaCl) for one minute at room temperature. The solution is then replaced by

50 ml per membrane of buffer B2 (0,1 M Tris-HCl, 0,15 M NaCl 0,5 %, w/v blocking reagent) and the membranes are incubated for 30 minutes at room temperature. After a rapid rinsing in B1, the membranes are incubated in 10 ml per membrane anticorps solution1:5000 v/v (Anti-Digoxigenin-AP, Boehringer Mannheim), then rinsed twice for 15 minutes in 50 ml of B1. Then they are incubated for 3 minutes in 50 ml of B3 (0,1 M Tris-HCl, 0,1 M NaCl , 0,05 M MgCl₂). The revelation is down in the dark in plastic bags containing 100 μ l of BCIP/NBT 1:100 v/v solution (Boehringer Mannheim) in 5 ml B3. Positive reaction appears as blue precipitation at the DNA dots.

Results

Specific signals have been obtained with the corresponding target strains (1 μ g of total DNA per spot) for the both probes at the calculated Tm. The signal with the probe Aust 13C is more intense than with the probe Aust 11C and begins to appear 30 min after the revelation and is obvious after 4 hours. The signal with the probe Aust 11C appears 1 h 15 after the revelation. Its intensity is not improved after 20 h of exposition. This may be resolved by Tm reduction by 2°C. These tests will be done soon.

Training of scientists from developing countries

Falaye Kanté (partner 3, Univ. Mali) finished in December a six months stay (from July to December 2005) in LCM in Dakar, during which he was trained on characterization of DNA extracted from nodules, and went again in Dakar in November 2006 for a new four months stay, during which he will do molecular analysis of samples collected in Mali.

Planning meeting

Inamoud Yattara ((Université of Bamako, Mali, Partner 3) and Alzouma Mayaki Zoubeirou (Université Abdou Moumouni, Niger, Partner 5) met together with Tatiana Krasova-Wade and Marc Neyra in Dakar on April 26 to plan the future actions.

Work to be done in the next months

During the extension year of the project, we shall realize the work which was devoted to IRD. In particular, in collaboration with partners 3, 4 and 5, we shall study the effects of wastewater on rhizobial survival and effectiveness in field studies. Nodules will be analyzed, by using PCR-RFLP analysis, and for the trials using Aust 11C and 13C, by applying the probes described above on DNAs extracted from nodules.

ANNEX 1 REPORT ON SCP'S VISIT

PROGRAMME DE FORMATION - NOV-DEC 2005

POURQUOI RÉUTILISER DES EAUX USÉES TRAITÉES ?

L'IMPORTANCE DE LA GESTION DES EAUX USÉES AU NIVEAU D'UN PAYS, AU NIVEAU D'UNE RÉGION, ANALYSES D'EXEMPLES

LE MAROC

LA JORDANIE

LA FRANCE (ILE DE PORQUEROLLES)

COMMENT GÉRER LES RISQUES SANITAIRES LIÉS À LA RÉUTILISATION DES EAUX USÉES TRAITÉES?

LES ADAPTATIONS POSSIBLES

EPURER DE FAÇON PLUS OU MOINS POUSSÉE :

SYSTÈMES DE TRAITEMENT INTENSIF (LITS BACTÉRIENS ET DISQUES BIOLOGIQUES, BOUES ACTIVÉES)

SYSTÈMES EXTENSIFS : INFILTRATION PERCOLATION, FILTRES PLANTÉS À ÉCOULEMENT VERTICAL OU HORIZONTAL, LAGUNAGE ANAÉROBIE, LAGUNAGE À MICROPHYTES, LAGUNAGE À MACROPHYTES, LAGUNAGE INTENSIF

ADAPTER LE MODE D'IRRIGATION (IRRIGATION GRAVITAIRE, IRRIGATION LOCALISÉE, IRRIGATION PAR ASPERSION)

ADAPTER LE TYPE DE CULTURES

COMMENT METTRE EN ADÉQUATION LA PRODUCTION DES EAUX USÉES TRAITÉES ET LES BESOINS LIÉS À LA VALORISATION ?

Les différents modes de stockage

Stockages aériens (réserves)

Stockages souterrains, recharge de nappe

Quels sont les problèmes agronomiques que peut poser la valorisation agricole des eaux usées traitées

la recherche d'un équilibre agronomique : qualité des eaux-type de sol-besoins de la plante

La salinité

Les déséquilibres de fertilisation

Les éléments traces (métaux lourds) - toxicité en chlore

- Quelques conditions de réussite d'un projet de valorisation agricole des eaux usées traitées
- La recherche d'une dynamique durable conciliant les aspects réglementaires, les aspects sociaux, les aspects économiques, le partage des responsabilités

Liste des critères à prendre en considération

Analyse d'un exemple à Gaza

La démarche « stratégie de traitement- valorisation des eaux usées traités » au niveau de l'étude de faisabilité

Analyse d'un exemple : ville de Elmarsa province d'Elyaounne Maroc

i

Contrat n°ICA4-CT-2002-10017 Projet « UBENEFIT » : projet de traitement des eaux usées domestiques et de leur utilisation pour l'irrigation de productions forestières et fourragères

Rapport de mission du 23 novembre au 9 décembre 2005

au Mali, Niger et Burkina Faso

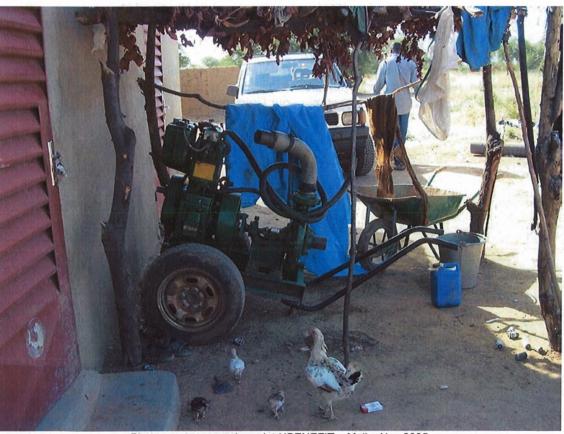


Photo : motopompe du projet UBENEFIT - Mali - Nov 2005



La mission a été réalisée par Françoise BOUROULET du 23 novembre au 9 décembre 2005.

Remerciements :

Je tiens à remercier très chaleureusement tous les partenaires du projet UBENEFIT, ainsi que leurs proches collaborateurs et partenaires d'autres organismes (Faculté des Sciences de Niamey, Université de Ouagadougou), pour leur accueil, leur disponibilité et leur efficacité dans l'organisation et le bon déroulement de cette mission.

1. Objectif et déroulement de la mission

1.1. Objectif : expertise et formation

L'objectif de la présente mission consistait à :

- visiter les trois sites expérimentaux mis en place dans le cadre du projet UBENEFIT (un site expérimental de 1 ha par pays),

- évaluer le fonctionnement des stations de traitement des eaux usées, en soulignant les succès et difficultés rencontrés,

- évaluer le fonctionnement des systèmes d'irrigation des plantations d'arbres fourragers et à production de bois de chauffe,

- former les partenaires du projet, leurs équipes et collaborateurs, aux principes et techniques de réutilisation des eaux usées traitées, notamment extensives, ainsi qu'aux conditions de leur utilisation en agronomie pour l'irrigation de cultures.

1.2 Programme de la mission

Me 23 Nov	Trajet aller Marseille-Paris-Bamako
Je 24 Nov MALI	Visite de terrain à Minimana près de Séribala sur le site expérimental.
Ve 25	Retour de Minimana, préparation du programme de formation
Sa 26	Journée de formation à l'EIR de Sotuba- Bamako
Di 27	Collecte et mise en forme des documents de formation à remettre aux participants
Lu 28	Travail en groupe sur l'expérimentation de Minimana : retour d'expérience, analyses, recommandations, protocoles de suivi. Trajet Bamako-Niamey
Ma 29 Nov NIGER	Contacts préliminaires et prise de RDV téléphoniques Préparation et organisation de la formation
Me 30	Visite de terrain sur le site expérimental de la Faculté des Sciences de Niamey Réunion de travail avec les partenaires

Je 1 Dec	Formation «Réutilisation des eaux usées »- Partie 1
Ve 2	Réunion de travail à la Faculté des Sciences – 2 ^{ème} Visite sur le site expérimental Formation «Réutilisation des eaux usées »- Partie 2
Sa 3	Fin de la formation et synthèse finale Trajet Niamey - Ouagadougou
Di 4 Dec BURKINA FASO	
Lu 5	Réunion de travail avec les partenaires. Visite sur le site expérimental de la Faculté Préparation de la formation
Ma 6	Formation «Réutilisation des eaux usées »- Partie 1
Me 7	Formation «Réutilisation des eaux usées »- Partie 2
Je 8	a series in the second s
	Retour Paris et Marseille

1.3. Mission de Formation

Les séances de formation ont fait l'objet de conférences (théoriques et illustrées à partir d'exemples de projets de stations d'épuration des eaux usées et de leur valorisation en agronomie, mis en œuvre par la SCP). Les conférences ont été suivies de débatsdiscussions nourris.

Un CD-rom contenant toutes les présentations Powerpoint ainsi que les textes (articles et documents de référence) ont été diffusés aux partenaires du projet UBENEFIT et à des organismes associés (ENS de Niamey, Faculté des Sciences de Ouagadougou...).

Le programme de la formation dispensée figure en annexe.

Les journées de formation ont concerné :

- 8 personnes au MALI IER/PRF Daouda SIDIBE Koné BROULAYE Youssouf CISSE IER / Laboratoire SEP Kalifa TRAORE

> FAST – Laboratoire de microbiologie - Université du Mali Hamed BATHILY Bakary SAMAKE Fassé SAMAKE Inamoud YATTARA

- 3 personnes au NIGER

Seydou ISSAKA Faculté des Sciences, département de Biologie Dahiratou MARAFA : ENS, département de Sciences naturelles Halilou OUMAROU : ENS, département de SVT

- un très grand nombre de professeurs, chercheurs et étudiants de la Faculté de Ouagadougou, ingénieurs et techniciens de l'INERA et de l'EIER.

La sensibilisation à l'hygiène et la sécurité des ouvriers, techniciens et chercheurs au contact des eaux usées traitées sont déjà très bonnes.

Les principales questions abordées lors des discussions ont été :

- nécessité de vaccinations pour protéger les travailleurs (anti-tétanos...),

- traitement spécifique des éléments traces (métaux lourds toxiques) afin de ne pas induire de pollution lors de la valorisation agronomique (pollution des sols et des produits récoltés) – existence de végétaux limitant ces pollutions ?

Exemple : traitement des eaux issues de tannerie, d'abattoir, de brasserie, d'industrie textile.



Photo : participants aux séances de formation à Ouagadougou - visite sur le terrain - déc 2005

2. Synthèse, analyses et propositions suite à la visite des sites expérimentaux

2.1. Site expérimental de Minimana au MALI

Partenaires accompagnateurs pour la visite de terrain : Inamoud YATTARA, Daouda SIDIBE, Kalifa TRAORE.

2.1.1. Etat de fonctionnement de la station d'épuration et de l'irrigation

Le site expérimental de 1 hectare clôturé se situe à Minimana, au nord de Markala. Une habitation a été construite pour le gardien du site (poste permanent à temps plein) avec une pièce de rangement et de sécurisation du matériel.

L'irrigation s'effectue à partir d'une pompe mobile, avec prise dans le canal de drainage (drain de Minimana). Des algues rouges (Azola) sont visibles).

L'épuration se fait par épandage direct des eaux usées sur la plantation, avec récupération, dans un canal collecteur (canal de drainage), des eaux ayant percolé dans le sol. Le sol joue le rôle de filtre vivant.

L'eau **chemine par gravité** à partir d'un tuyau PVC de 200 m de long, posé à 50 cm de hauteur au dessus du sol et recouvert de végétaux pour limiter l'exposition aux fortes chaleurs. Une ligne d'arbres a également été plantée pour ombrager cette canalisation (primaire).

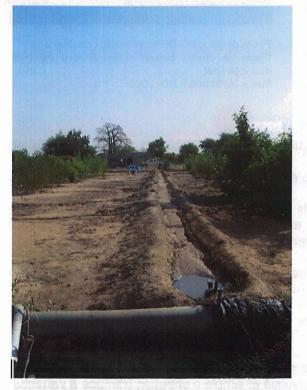


Photo de gauche : canalisation primaire d'amenée d'eau à partir du pompage dans le drain Minimana.



Photo de droite : filiole en terre – irrigation en gravitaire au pied de l'arbre – Mali – Nov 2005.

L'eau passe ensuite, par un jeu de vannes, dans des canaux en terre surélevés (secondaires) et parvient jusqu'au pied de chaque arbre grâce à un **réseau dense** (astucieux et sophistiqué) de sillons en terre (tertiaires), qui nécessitent un entretien très régulier (curage des canaux en terre et désherbage chaque mois).

Chaque secteur de plantation est entouré d'un réseau de sillons et canaux en terre de collecte des eaux de drainage. Celles-ci parviennent après un long cheminement dans un canal vers un **bassin collecteur final**.

Le fond de celui-ci est réputé étanche car il a été damé lors des travaux de terrassement à l'optimum proctor.

En raison des épisodes de pluie de la saison d'hivernage, le bassin était rempli d'herbacées lors de la visite. Il était prévu de les faucarder très rapidement.





Photo de gauche : filioles et canaux de drainage Photo de droite : bassin collecteur des eaux de drainage final Mali – Minimana – Nov 2005

Les arbres ont été plantés en août, après un élevage de 5 mois en pépinière (semis en mars), correspondant à différents essais.

L'arrosage fonctionne bien, il est abondant. L'ensemble de la plantation est irriguée en moins de 2 heures (forte pression).

La croissance des plants est très bonne. Ils présentent un bon état végétatif (plants bien verts). L'état sanitaire de l'expérimentation est très satisfaisant : les arbres ne présentent aucun signe d'attaque parasitaire ou cryptogamique, ni aucun signe de défoliation.

Les densités de plantation sont élevées et ne correspondent pas aux densités usuelles de plantations forestières production de bois (de chauffe ou de fourrage). Les résultats de production devront être analysés avec la plus grande prudence, en raison des situations de compétition existant entre les arbres. A ces densités, les plantations (expérimentales) ne sont pas pérennes.

Remarque : le dispositif adopté, qui est très satisfaisant, s'éloigne du dispositif initialement proposé par la SCP en 2003, qui était plus rustique et simple d'entretien (rampes à vanettes, irrigation à la raie avec modelage du sol en ados et plantation de deux rangées d'arbres de

part et d'autre du canal d'amenée d'eau) et exposait moins l'agent d'irrigation à l'effluent.

Néanmoins, les mesures de sécurité sont bien suivies : port de bottes et de gants. Les suivis sont bien réalisés (tenue de cahiers de bord).

2.1.2. Améliorations à apporter au dispositif d'irrigation/épuration

* Le ruissellement des eaux excédentaires est récupéré 15 à 30 minutes après l'apport d'eau dans les sillons de drainage en terre qui entourent les parcelles plantées. La percolation latérale dans les sillons de drainage dure environ 2 heures après l'apport d'eau.

Il est important de limiter le ruissellement immédiat de l'excédent d'eau et, au contraire, de « forcer » l'eau d'irrigation à percoler lentement dans le sol. Pour cela, les sillons d'amenée d'eau (tertiaires) seront fermés des deux côtés (actuellement fermés que d'un côté), afin de permettre à l'eau de s'infiltrer dans le sol.

Il est recommandé que les sillons et canaux de drainage soient creusés à au moins à 50 cm de profondeur.

* Le **bassin de stockage** des eaux drainées n'a pas d'utilisation actuelle. Il n'y a pas de demande d'utilisation de ces eaux pour du maraîchage. Par contre, afin d'éviter de perdre l'eau juste par évaporation, il peut être envisagé de réaliser un (ou des) bassin(s) d'aquaculture (tilapia...) en séparant ce grand bassin en 4 compartiments (par murets d'argile compactée), et reliés entre eux par des surverses.

Les surverses peuvent être aménagées de la façon suivante : superposition d'une double rangée de planches en bois, l'espace entre planches étant rempli d'argile. En ajoutant ou supprimant des planches, on peut augmenter ou diminuer le niveau de l'eau.

Un suivi régulier des volumes d'eau et de la qualité de l'eau devra être réalisé à ce niveau (niveau de salinité, taux de matières en suspensions...).

* Il est proposé de faire **l'inventaire des pesticides** utilisés pour la riziculture, afin d'avoir une idée précise des matières actives présentes dans l'eau pompée dans le drain de Minimana et de déterminer les métaux lourds en présence sur le site expérimental. Cette détermination orientera les analyses des eaux à faire (avant et après irrigation) et servira éventuellement à expliciter le suivi physiologique de la plantation.



Photo : l'équipe du Mali - Nov 2005

Rapport de mission 23 Nov - 9 Dec 2005 - Projet UBENEFIT - SCP

2.2. Site expérimental de la Faculté des Sciences de Niamey

Partenaires accompagnateurs pour la visite de terrain : Prof SAADOU, Sanoussi ATTA et Sani LAOUALI.

2.2.1. Etat de fonctionnement de la station d'épuration et de l'irrigation

Les constructions des bassins de la station d'épuration correspondent au projet initial (conception SCP 2003).

Il a été procédé à quelques aménagements par rapport au projet initial, notamment dans le choix du substrat déposé dans le fond du bassin d'infiltration.

Le système d'épuration est constitué de 4 éléments :

- un bassin de lagunage anaérobie 70 m3 (3 m de profondeur)
- un bassin de stockage
- un bassin d'infiltration-percolation de 240 m²
- une bâche de reprise avant irrigation.

L'eau est amenée en amont par tuyau PEHD, placé en hauteur (à 50 cm du sol) et recouvert de terre pour éviter les risques de brûlure par le soleil.

Tous les bassins sont bétonnés, à l'exception du bassin d'infiltration-percolation qui est recouvert d'une membrane résistante aux UV, retenue sur les bords par des murets en béton. L'ensemble du dispositif est **étanche**.

Le bassin d'infiltration est constitué de graviers et de tout venant (constitué de gravier fin et de sable). L'épaisseur du substrat au fond du bassin est d'environ 2m. Il ne fonctionne actuellement pas comme un massif d'infiltration-percolation car il est recouvert en permanence d'une lame d'eau d'environ 50 cm (cf discussion ci-dessous).

Néanmoins, l'eau qui sort actuellement du bassin d'infiltration est claire, sans matières en suspension.

Le choix du système d'irrigation s'est porté sur l'irrigation localisée au goutte à goutte. La pompe est commandée par une électro-vanne. L'eau traitée bénéficie d'une filtration par disque.

Un tuyau de type goutte-à-goutte utilisé par le maraîchage a été mis en place avec des orifices régulièrement espacés de 20 cm.

L'extrémité de chaque tuyau en fin de ligne a été bouchée mais pourra facilement être ouverte pour purger le système lors de l'entretien du système d'irrigation.

Le système d'irrigation fonctionne bien.

Un cabanon va être prochainement construit afin de protéger la pompe et le système électrique des fortes insolations et poussières.

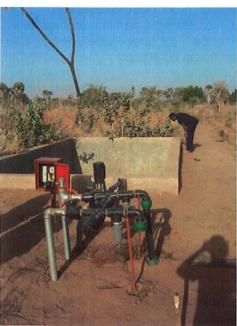


Photo de gauche : pompe et filtration à disque



Photo de droite : plantation et irrigation localisée

Les végétaux ont été mis en place et se développent bien (plantation d'arbres réalisées l'été 2005, après un élevage deux mois en pépinière). La surface plantée et irriguée correspond à environ 500 m².

Le protocole de plantation correspond à une **forte densité** : 2m sur le rang et 1 m entre les rangs. Les conditions « classiques » d'exploitation forestière ne sont pas réunies (espacement trop faible entre les arbres). Les résultats devront être généralisés avec prudence dans le cas de plantations forestières.

Parmi les espèces plantées, *Acacia seyal* présente de longues et nombreuses épines. Son port est très étalé (développement multi-tronc).

Gliricidia sepia et *Leucaena leucocephal* présentent une croissante en hauteur importante (développement avec un tronc). Une taille de formation sera nécessaire dans les mois qui viennent. Les coupes de feuilles et branches pour la valorisation en fourrage ou en bois de chauffe seront réalisées au bout de 15-20 mois (protocole à préciser).

En raison d'un apport d'eau calculé au plus près des besoins des plantes, apporté en petites quantités et fréquemment, et en raison d'une forte capacité de drainage du sol sableux, le risque de salinité est très limité.

2.2.2. Difficultés rencontrées et propositions d'amélioration des systèmes d'épuration et d'irrigation

a) fonctionnement du bassin d'infiltration

* Le bassin présente un fonctionnement « biaisé » en termes d'infiltration-percolation, du fait de la **présence permanente d'une lame d'eau de plus de 50 cm**.

Il convient de procéder à des aménagements pour retrouver un fonctionnement « normal » de bassin d'infiltration-percolation « satisfaisant » et durable.

Rapport de mission 23 Nov - 9 Dec 2005 - Projet UBENEFIT - SCP

* Les **séparations** prévues dans le grand bassin d'infiltration n'ont pas été réalisées. Le rôle des séparations est d'alimenter en eau successivement trois portions de bassin, afin d'alterner phases en eau et phases au sec (ressuyage-repos – substrat visible et à l'air libre). La lame d'eau ne devrait en théorie pas dépasser 25 cm (idéal 20-30 cm).

--→II est proposé de **poser sur le substrat de graviers des séparations** telles que des planches en bois (de type batardeau, retenues par des pieux enfoncés dans le substrat) ou élément béton préfabriqué ou tôle ondulée, tout en veillant à ne pas endommager la membrane en place.

* La **granulométrie grossière** du substrat actuellement implanté risque d'entraîner une percolation trop rapide pour pouvoir bénéficier d'une épuration tertiaire poussée. Une solution pourrait être d'ajouter du substrat fin sableux au substrat existant, sur une hauteur de 20 cm supplémentaires en récupérant du sable local (présent sur le site de la Faculté des Sciences) et en le lavant pour le débarrasser de fines.

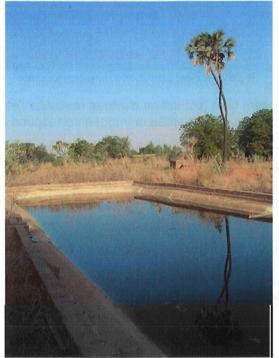


Photo: bassin d'infiltration/percolation recouvert d'une lame d'eau de 50 cm - Niamey - déc 2005

b) communication entre bassins

Actuellement, l'eau circule directement du **bassin de lagunage anaérobie** au bassin de stockage avec un traitement primaire sommaire et écourté. Une circulation préférentielle de l'eau et des zones mortes dans les angles des bassins sont observées. Ces deux bassins sont odorants, présentent des mousses en surface (détergents) et des larves de moustique s'y sont développées.

Les partenaires proposent d'installer un programmateur afin d'alimenter le bassin anaérobie en plusieurs périodes dans la journée, espacées de 2h (temps estimé de décantation des MES), afin qu'il joue pleinement son rôle de décanteur-digesteur pendant le temps de rétention souhaité de 1 jour. Il faut vérifier le volume pompé /jour.



Photo : bassin anaérobie - Faculté des Sciences - Niamey - déc 2005

Le **bassin de stockage** délivre actuellement de l'eau en continu, grâce à un tuyau PE affleurant à la surface, réduisant le volume et débit d'eau qui transite.

--→II est proposé d'enlever le tuyau et de fermer la sortie du bassin de stockage, afin de permettre d'évacuer l'eau par bâchées. Ces arrivées d'eau par bâchées sur le bassin de filtration permettront d'obtenir une diffusion la plus homogène possible sur la surface du filtre et une submersion temporaire.



Photo : bassin de stockage - Niamey - déc 2005

Rapport de mission 23 Nov - 9 Dec 2005 - Projet UBENEFIT - SCP

Le **bassin récupérateur** (bâche de reprise finale) est envahi d'algues vertes visibles à l'œil nu qui pourraient entraîner un colmatage des orifices des tuyaux d'arrosage. Il est proposé de couvrir le bassin récupérateur à l'aide d'une plaque métallique afin de limiter leur prolifération.

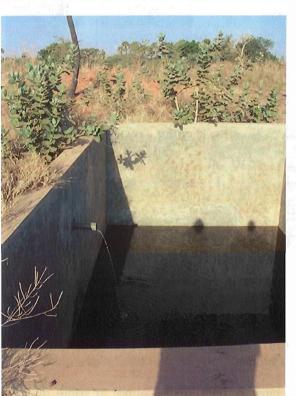


Photo : bassin récupérateur - Niamey - Déc 2005

2.2.3. Préconisations d'entretien pour une épuration par infiltration-percolation

a) Entretien régulier (tous les mois ou 2 mois)

Noter le temps de disparition de la lame d'eau, le degré de colmatage de la surface du filtre, hauteur d'eau sur la plage d'infiltration.

Elimination des flottants et vérification du niveau des boues primaires Vérification des affouillements et maintien du nivellement de la plage d'infiltration Vérification de la qualité des rejets et du fonctionnement du système de goutte-à-goutte Faucardage de la végétation des talus de remblai et de la proximité des bassins Curage et enlèvement des feuilles et branches des canaux d'amenée d'eau

b) Autres opérations d'entretien :

Les accumulations organiques du massif filtrant doivent être ratissées et évacuées selon une périodicité à ajuster empiriquement.

Le changement des 5 à 10 premiers cm de substrat est à prévoir tous les 3-4 ans. La vidange des boues du lagunage anaérobie est à prévoir 1 fois par an. Les boues primaires peuvent être utilisées pour constituer du compost en mélange avec d'autres déchets verts.

Rapport de mission 23 Nov - 9 Dec 2005 - Projet UBENEFIT - SCP

Des analyses des teneurs en nitrates¹ (et éventuellement en germes pathogènes) des eaux traitées sont à effectuer régulièrement.

Il est rappelé la nécessité de travailler en sécurité avec des bottes et en se lavant les mains après mesures effectuées sur les arbres. Une vaccination anti-tétanos est fortement recommandée.



Photo : plantation expérimentale de Niamey - Dec 2005

2.3. Site expérimental de Ouagadougou

Partenaires accompagnateurs pour la visite de terrain : Dianda MAHAMADI Joseph WETHE : EIER Jean KOULIDIATI : Université de Ouagadougou

Le site expérimental est installé sur un terrain mis à disposition du projet UBENEFIT par l'Université de Ouagadougou (1,5 ha au total), jouxtant les terrains de l'EIER et de l'INERA.

Le projet de traitement des eaux usées tel que proposé par la SCP au printemps 2004 a été modifié et adapté par les partenaires locaux.

¹ Un massif filtrant fonctionnant de manière optimale produit des nitrates et toute baisse de concentration en sortie (à l'échelle du mois) reflète un manque d'oxygène donc une dégradation du traitement. Cela peut être réalisé facilement à l'aide de papiers indicateurs.

Eléments du projet de la SCP	Eléments de la construction actuelle de la station de traitement des eaux usées (conforme au DCE de juin 2004)	Photos (état actuel – déc 2005)
	Construction d'un petit réseau d'égout à écoulement gravitaire (collecte de toutes les eaux usées de la cité universitaire)	
Une bâche de relevage	Une bâche de relevage en béton armé	
	Un bassin d'homogénéisation en béton armé 0,5 m3	

Rapport de mission 23 Nov - 9 Dec 2005 – Projet UBENEFIT – SCP

p 14/20

Un bassin de lagunage anaérobie de 150 m3 (5 x 10, prof 3 m)	Un bassin de stabilisation anaérobie en perré maçonné (prof 4 m)	
Un bassin de lagunage aérobie facultatif de 800 m3 (20x20m, prof 2m)	Un bassin de stabilisation aérobie facultatif en perré maçonné et reprise ciment (prof 1,5 m)	Au premier plan : lagune aérobie Au second plan : bassin de désinfection

Un bassin aérobie de maturation de 500 m3 (20x25, prof 1m)	Un bassin dit « de désinfection » 30 m3 : 2 chicanes parallèles de 3 tronçons chacune (3x1x1m) x 5 m de long Tronçons remplis en amont de gros blocs de latérite, puis en aval d'un lit de graviers grossiers de plus en plus fins (La mise en place de sable non lavé, de granulométrie plus fine, a entraîné un trop fort ralentissement du débit des eaux).	
Une bâche de stockage de 100 m3 (10x10, prof 1m) pour des lâchers par bâchées (stockage = 1 jour)	4m3 chacun en béton armé	
Irrigation gravitaire à la raie – modelage en ados et utilisation de vannes (martelières)	Irrigation gravitaire – installation d'un mini réseau d'irrigation avec conduite de distribution PVC diam 100 mm Faisceau de canaux en terre compactée (raie)	
Tout le système (traitement + irrigation) fonctionne totalement en gravitaire à partir de la bâche de relevage, Station de traitement des eaux usées construite en remblais	Système de traitement construit en déblais (dans le sol naturel) et fonctionnant en gravitaire jusqu'au basin de désinfection	

Rapport de mission 23 Nov - 9 Dec 2005 – Projet UBENEFIT – SCP

p 16/20

2.3.1. Etat de fonctionnement de la station d'épuration et de l'irrigation

Lors de la visite, la station d'épuration venait juste d'être mise en eau (fin novembre) et bénéficiait des premiers tests de fonctionnement. Les problèmes d'étanchéité étaient réglés. Son fonctionnement était bon avec une épuration très satisfaisante observée dans le bassin de lagunage aérobie (présence d'algues vertes microscopiques conférant une couleur verte à l'effluent).

Le système d'irrigation n'était pas encore fonctionnel. La pose des conduites d'amenée d'eau était en cours. Le terrain a été nivelé par endroits (mais pas modelé en ados), quelques raies creusées.

Le premier essai planté était arrosé à l'aide d'une motopompe (mode d'irrigation non observé lors de la mission).

Les arbres concernant les deux essais principaux du projet UBENEFIT n'étaient pas encore plantés mais allaient l'être sous peu. Les plants étaient « en attente » à la pépinière de l'INERA, dans un substrat constitué à 100% de sable, avec une croissance maîtrisée, volontairement « réduite » (limitée).

2.3.2. Difficultés rencontrées lors de la mise en service de la station d'épuration des eaux usées et du système d'irrigation

- La pompe de la bâche de relevage est actionnée manuellement en raison du volume très important des eaux usées provenant du réseau d'égout (volume supérieur à celui estimé pour la conception du projet UBENEFIT qui se basait sur un volume utile de 100 m3/jour pour irriguer 1 hectare de plantation d'arbres). Une surverse a été installée avec évacuation des eaux excédentaires dans les canaux du système de récupération des eaux pluviales.
- Problème de lenteur de la vitesse de filtration des eaux dans le bassin de désinfection, probablement dû au colmatage des pores du sable non lavé. Des essais sont en cours pour trouver la meilleure granulométrie du substrat.
- Le bassin de désinfection comprend un bassin final de recueil des eaux de <u>4 m3</u>, à <u>écoulement continu</u> et à 20 cm de la surface du sol, ce qui a pour résultat une charge et donc une pression très faibles pour une utilisation en irrigation. Le volume du bassin final autorise un stockage d'eau très faible par rapport aux besoins quotidiens estimés pour 1 ha de plantation de ligneux. Par ailleurs, la faible hauteur du niveau d'eau par rapport au sol entraîne une charge très faible.
- Conduite de distribution de relativement grand diamètre (100 mm) et enterrée à environ 15 cm dans le sol : embouts PVC encore non installés lors de la visite, qui devraient atteindre la surface du sol pour alimenter les raies. Les facteurs diamètre élevé et profondeur de la canalisation par rapport au niveau du sol renforcent la nécessité d'une pression amont élevée, afin que les raies soient alimentées en eau de façon homogène et jusqu'à leur extrémité, ce qui n'est pas le cas dans la situation présente.

Cette difficulté est renforcée par le fait que la pente du terrain est faible, voire à contre-pente : les lignes de plantation ont été aménagées pour optimiser l'irrigation à la raie.

2.3.3. Propositions pour améliorer le système d'irrigation

Rapport de mission 23 Nov - 9 Dec 2005 - Projet UBENEFIT - SCP

Il n'apparaît pas possible, dans les conditions :

- de très faible pression de l'eau d'irrigation disponible en sortie de bassin de désinfection et de l'écoulement permanent (absence de stockage d'eau et de mise en charge),
- de hauteur de la conduite d'amenée d'eau par rapport au niveau du terrain naturel (surface du sol),

de conduire une irrigation à la raie totalement en gravitaire.² L'utilisation d'une pompe s'avère inévitable.

En effet, de gros travaux de terrassements et de modelage du sol ne sont pas envisageables compte-tenu de la faible profondeur de sol utile (20 cm) avant de parvenir à des horizons limono-argileux pauvres et compacts.

De même, après étude, un système d'irrigation souterraine n'est finalement pas envisageable, en raison de la nécessité d'une filtration très fine (micro-tamisage qui nécessite une forte pression de l'eau : condition non remplie), de son coût élevé de fourniture et de mise en place, de la quasi-impossibilité d'entretien lors du colmatage des tuyaux.

a) 1^{ère} solution

 Transformer le massif filtrant du bassin dit de désinfection (en enlevant tous les blocs et graviers) en bassin de stockage d'une capacité de 30 m3 et d'environ 1 m de profondeur. L'écoulement actuellement en continu en sortie du bassin sera bloqué. Une surverse vers le milieu naturel devra être installée.

Attention : cette solution technique (qui ne correspond pas à une épuration tertiaire poussée) ne garantit pas une eau totalement exempte de germes bactériens.

- L'irrigation d'un ha de plantation nécessitera le passage du personnel 3 fois par jour, qui veillera au pompage de l'eau et à la distribution de l'eau à la raie en gravitaire, par tiers de surface. Des précautions particulières doivent être prises par l'utilisateur en raison de la présence possible de germes bactériens.
- L'équipement actuel pourra être utilisé ou légèrement adapté. Le surcoût sera faible, si ce n'est l'achat d'une pompe et son alimentation en énergie (électrique, solaire, motopompe ?). Le personnel devra veiller à sa sécurité lors des manipulations de bouches/vannes.

b) 2^{ème} solution

Objectifs :

- obtenir de l'eau dépourvue de matières en suspension
- irriguer en micro-irrigation les plantations

Modifications à apporter au bassin de désinfection qui constitue un massif filtrant à écoulement vertical :

 Transformer le bassin de désinfection en enlevant les blocs et graviers et en le remplissant de 0,50 m de sable lavé à granulométrie fine (d₁₀ de 0,3 à 0,4 mm) : attention cependant au risque de colmatage par les algues provenant du bassin amont,

 $^{^2}$ Il est généralement recommandé un débit de 30 l/s pour une irrigation gravitaire à la raie « main d'eau ».

- Equiper le bassin d'une pompe et pratiquer l'irrigation au goutte à goutte après pose de tuyaux au sol,
- Equiper les extrémités d'antennes d'une vanne de purge.

En l'absence de l'installation d'une réserve complémentaire (type « château d'eau »), le stockage de l'eau en sortie de bassin de désinfection sera de 4 m3 et nécessitera en théorie 25 passages du personnel par jour pour irriguer un hectare de plantation (présence permanente, risques de surverse...).

Le surcoût de cette solution est important : achat de la pompe, achat et pose des tuyaux de goutte à goutte sur l'ensemble du site expérimental.

La percolation latérale à travers ce filtre horizontal peut aboutir à une désinfection de l'effluent (traitement tertiaire), mais l'efficacité de la désinfection doit être surveillée.

b) 3^{ème} solution variante

Le bassin dit de désinfection sera utilisé comme un bassin de stockage de 30 m3 (cf 1^{ère} solution).

Attention : cette solution technique (qui ne correspond pas à une épuration tertiaire poussée) ne garantit pas une eau totalement exempte de germes bactériens.

Une pompe sera installée, l'eau filtrée à partir d'un filtre à sable ou micro-tamis puis utilisée pour une irrigation localisée en goutte à goutte. C'est la solution la plus onéreuse.

Autres améliorations ponctuelles proposées :

- Il est préconisé de racheter une seconde pompe de secours pour la bâche de relevage.

- Afin d'éviter le développement d'algues vertes dans le bassin de désinfection, il y a possibilité de le recouvrir avec une plaque métallique.

- Il pourra être envisagé dans un second temps de mettre en place des surverses pour le bassin aérobie facultatif, soit à destination du milieu naturel (pente basse vers le fond du site expérimental côté INERA), soit à destination du canal de collecte des eaux pluviales.

3. Conclusion

Chaque site expérimental a soulevé des questionnements et problématiques particulières, nécessitant des aménagements plus ou moins importants pour un fonctionnement durable du système de traitement des eaux usées et d'irrigation des plantations, qui ont été discutés au cas par cas.

Annexe :

estimation des coûts additionnels d'irrigation pour le site de 0 (sur la base des prix France, en € HT fin 2005)	Duagadougou
a) 1 ^{ère} solution : irrigation en gravitaire	
achat d'une motopompe diesel de 30 m3/h à 6 bars : environ embouts de départ, colliers de prise en charge, divers :	8 000 € 300 €
OU	
pompe électrique de 30 m3/h à 6 bars à 380 V triphasé : embouts de départ, colliers de prise en charge, divers :	4 000 € 300 €
b) 2 ^{ème} solution : irrigation localisée au goutte à goutte	
une pompe électrique 15 m3/h à 4 bars 50 rangs de 100m : 5000 ml de gaine jetable à 0,10 €/ml Un goutteur de 1l/h tous les 30 cms, soit 3 l/h par ml 15 m3/ha/h Fonctionnement de la pompe pendant 6h (si réserve)	3 000 € 5 000 €
OU	
une pompe électrique 20 m3/h Tuyau PEBD diam 16 mm, avec goutteur intégré,	3 500 €
monté avec vannes : 0,50 €/ml Un goutteur de 2 l/h tous les 50 cms, soit 4 l/h/ml Fonctionnement de la pompe pendant 5h (si réserve)	2 500 €
nour un filtro à achle ou temis, il est nécessaire d'avair au mains 2 hars	

pour un filtre à sable ou tamis, il est nécessaire d'avoir au moins 2 bars. Filtre à tamis 1 500 €

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Contract number: ICA4-CT-2002-10017

Year 4 2005-2006

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

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

2. Training

Number of PhDs Number of MScs Number of visiting scientists Number of exchanges of scientists (stays longer than 3 months)

3. Achieved results

Number of patent applications Number of patents granted Number of companies created Number of new prototypes/products developed Number 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 Financial contribution by industry Industrial partners:- Large SME¹

5. *Comments* Other achievements (use separate page if necessary)

Totals (cumula	tive)	
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