

Report

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

Contract number: ICA4-CT-2002-10017

Final report 1/12/2002 – 30/11/2007

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

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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.

Final Report: 1/12/2002 – 30/11/2007

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Compiled by J. Wilson

Abstract

The overall objective was to develop low-tech water treatment and irrigation systems and test their use for the production of fuel wood and fodder in semi-arid sub-Saharan Africa.

Sites for waste water treatment plants and irrigation systems were identified and the systems were constructed in Burkina Faso, Mali and Niger. Project partners were trained in how to use and maintain the systems. The irrigation sites were characterised in advance of tree planting, and soil and water analyses are being conducted regularly.

Tree species with potential for use in irrigated conditions in each country were identified and experimental designs for the trials produced. Some species were used in common in all three countries. Nursery screening trials were conducted and plants were then planted at the irrigation sites. Trees grew quickly under the irrigated conditions and many species performed well. Species which were selected for their good performance in the nursery were not necessarily the best performers in the field. Biomass production was determined and the effects of different coppicing treatments on regrowth were assessed. Parameters of planting stock quality were assessed and incidence of pest attacks determined.

Partners were also trained in the use of sap flow and associated equipment. Considerable variation between tree species in water use was noted, together with differential effects according to the inoculation history of the plants.

Working in controlled glasshouse conditions, using sterilised soil media, considerable variation in effectiveness of different mycorrhizal strains on different tree species was detected. When plant response to simulated irrigation water was measured, uninoculated plants grew very poorly irrespective of whether they are receiving the simulated irrigation water or not. Thus mycorrhizal infection enabled plants to make use of the nutrients in the irrigation water. Initially, nursery and field studies gave much less clear results. However, assessments during the final year of the project demonstrated positive effects of inoculation on tree growth in Mali and Niger. These observations were reinforced on the rhizobial side by molecular studies, using strain-specific probes for nodule analysis.

In Ouagadougou it was 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 and Mali.

Dissemination to stakeholders has been conducted and is continuing. The irrigation sites are continuing to be run. An important development is the formation of a partnership with representative farmers' organizations regrouped under the umbrella of ROPPA (Réseau des Organisations Paysannes et des Producteurs Agricoles d'Afrique de l'Ouest, who federates millions of farmers through twelve west African countries, with the objective of bringing symbionts to farmers.

Summary Final 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 which were unavailable locally and delays in purchase of expensive items due to cash flow problems. Staff have been trained in how to use and maintain the systems. Some modifications and refinements and repairs have been necessary, but systems are functioning in each country. The irrigation sites were characterised in advance of tree planting, and soil and water analyses are being conducted regularly.

Work package 2 Tree growth and management

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

Work package 3 Tree water use and soil water status

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

Work package 4 Microsymbionts and N fixation

Working in controlled glasshouse conditions, using sterilised soil media, the UK partner has identified considerable variation in effectiveness of different mycorrhizal strains on different tree species. After the initial screening phase, selected tree species were taken on to the second phase of the study in which plant response to simulated irrigation water is being measured. Uninoculated plants grew very poorly irrespective

of whether they are receiving the simulated irrigation water or not. The response of inoculated plants to irrigation varied with inoculant and tree species.

Initially, nursery and field studies in Mali, Niger and Burkina Faso gave much less clear results. However, assessments during this final year of the project have demonstrated positive effects of double inoculation (with rhizobium and mycorrhizas) on tree growth in Mali and Niger. Averaged across species, fuelwood production by inoculated plants was 67% greater than uninoculated, and fodder production was 35% greater. These effects indicate that inoculation in the nursery gets plants off to a good start and that indigenous inocula are either ineffective or deficient.

These observations are reinforced by molecular studies, using strain-specific probes for nodule analysis, which have been successfully tested against the inoculants, studies on samples collected from the field experiments in Mali suggest that the inoculant strains are absent – other types are present. This suggests that either the original inoculation was unsuccessful, or that the inoculants have been out-competed by indigenous strains.

Work package 5 Economics and quality of produce

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

Work package 6 Soil and plant nutrition

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

Work package 7 Planting stock quality

Studies have been conducted in Burkina Faso and Mali.

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

was considerable variation between species in growth. At the time of planting, there were considerable differences in shoot: root ratios between species.

Work package 8 Pest monitoring and management

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

Establishment of partnerships with farmers' organisations

An important development for the extension of the application of symbionts to farmers, is the formation of a partnership with representative farmers' organizations regrouped under the umbrella of ROPPA (Réseau des Organisations Paysannes et des Producteurs Agricoles d'Afrique de l'Ouest, who federates millions of farmers through twelve west African countries): CPF (Confédération Paysanne du Faso) in Burkina Faso, CNOP (Coordination Nationale des Organisations Paysannes) in Mali and PFP (Plate Forme Paysanne) in Niger, and, through a complementary funding from the French Foreign Ministry, with FUPRO (Fédération des Unions de Producteurs) in Benin, and CNCR (Conseil National de Concertation et de Coopération des Ruraux).in Sénégal. Workshops have been held, linking farmers, scientists and extension services. Some very positive participative field inoculation trials have been set up and the feasibility of production of mycorrhizal fungi in small units supervised by farmer's organizations is being studied.

INERA Mali produced a proposal for a follow on project 'Responsabilisation du producteur pour une gestion durable des déchets liquides domestiques en milieu urbain Sahélien'

Main publications

Publications and conference papers

Krasova-Wade T, Neyra M (2007) Optimization of DNA isolation from legume nodules. Lett. Appl. Microbiol. 45: 95-99

Submitted

2008 : Valorisation des eaux usées pour l'irrigation des espèces forestières destinées à la production de bois de chauffe et du fourrage au Niger. Paper submitted to *Sécheresse*

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A manuscript on the results on hybridization probes is in preparation.

A number of other publications are planned or in the early stages of preparation.

PhD

2008 Fallaye KANTE (Mali)

2008 Youssouf CISSE (Mali)

MSc

2007 Abdoulaye Harouna (Niger) MSc Botany and Phytocology University of Ouagadougou

Consolidated Scientific Report

Objectives and context of project

The project aimed to produce low-input, sustainable, high yielding, irrigated permanent fuel wood and fodder production systems that are adaptable for wide-scale use in the peri-urban zones of dry land cities. Such plant production systems require the use of irrigation to drive tree growth during the long dry season. Industrial and wastewater would be recycled for this process, being purified to WHO agricultural standards to diminish health risks. The purification systems developed would be low-technology low-cost systems appropriate to the site conditions and maintainable in developing countries. The water would supply irrigation to experimental multi-species trials of fast growing fuel wood and fodder trees.

European waste water treatment and irrigation specialists would work with local partners to determine the most appropriate and efficient systems of treatment and irrigation, following site surveys. The established purification, irrigation and production systems would serve as sites for information dissemination to stakeholders such as NAR's, NGO's, farmers' and women's groups and to local authority officers. Water quality would be monitored to ensure that the purification systems were effective.

These irrigation trials, established in the peri-urban zones of Bamako, Ouagadougou and Niamey would be the core of the project. To reduce the requirements for fertilizers, studies would focus on exotic N-fixing species, but locally important indigenous species would also be included in the study. Nitrogen fixation would be maximised by inoculation with strains which had been previously proved to be effective, and rhizobial skills would be transferred to local scientists through PhD and technician training. Nutrient acquisition would be further enhanced by testing and application of mycorrhizal fungi. Particular attention would be paid to the specific requirements of plants and their symbionts growing in irrigated conditions, where environmental conditions affect root:shoot ratios. Seedling quality attributes would be assessed before planting. Growing trees in dense species-poor plantations may give rise to pest problems and these would be monitored.

These studies would be coupled with socioeconomic surveys to determine the effects of these plantations.

Activities

Activities were structured in 8 work packages, viz;

1. Water treatment and irrigation
 - a. construction of experimental wastewater treatment facilities in peri-urban Mali, Burkina Faso and Niger
 - b. fine tuning and optimisation of systems
 - c. use of systems to irrigate plantations
 - d. training of local researchers
 - e. use of sites for educational, demonstration and dissemination purposes

This work package underpinned all the field activities, and delays had knock-on effects throughout the rest of the project. Subcontractor SCP visited each site and

developed the specifications for each irrigation system (these were not uniform across all countries, but designed specifically for each location). Local partners (partners 2, 3, 4 & 5) obtained permissions for utilization and development of the sites, and organised the purchase and import of the necessary materials, and the construction of the facilities, their daily running and repairs and improvements. Once the sites were up and running, SCP revisited the sites and provided training and also assisted with design modifications where appropriate. Once the systems were functioning, trees produced under other WP's were planted out to these sites and their assessments were conducted under other WPs. In each country, waste water analyses were conducted at regular intervals to determine whether the treated water reached the appropriate WHO standards.

Sites were at Siribala in Mali (using waste water from rice irrigation), in Ouagadougou in Burkina Faso (using waste water from the University Zogona campus), and on the University campus in Niger, again using waste water from the University.

Overall, this was a considerable, expensive and time consuming task.

2. Tree growth and management
 - a. Establishment of replicated trials on the irrigation sites of indigenous and exotic tree species
 - b. Testing of coppicing
 - c. Evaluation of tree performance

A number of replicated trials were established at each location by partners 2 - 5 in their respective countries. Design of the trials was to a common format adopted by all countries. Many of the tree species used were in common (exotic fast growing N-fixing species), with variations of local tree species in addition and as appropriate. Common tree species planted across all sites were *Acacia mangium*, *A. auriculiformis*, *A. crassicarpa*, *A. senegal* and *Leucaena leucocephala*. Tree performance in conjunction with different inoculation and coppicing treatments was determined.

Many activities under this work package are very closely linked with activities under WP4 (see later).

3. Tree water use and soil water status
 - a. Quantification of tree water use
 - b. Estimation of irrigation requirements
 - c. Training of local researchers

Training of partners 2, 4 & 5 in assessment of tree water use and soil water status was provided by partner 1. Equipment (sap flow sensors, data loggers, meteorological station, soil water sensors, scanner, computer software and laptop computer) was provided by partner 1 and was installed first in Niger and then moved in sequence to Mali and Burkina Faso. Training followed the movement of equipment. Partners 2, 4 and 5 operated the equipment over a period of several weeks, comparing the water use of different tree species and assessing soil water status.

4. Microsymbionts and N fixation
 - a. Testing of rhizobial and mycorrhizal strains' effects on tree growth

- b. Determination of inoculum potential of irrigated sites
- c. Determine persistence of rhizobial strains
- d. Training of local researchers

Partner 6 identified a wide range of appropriate effective rhizobial strains and provided them to project partners (2, 3, 4 & 5) for use in inoculation studies, providing tree species – specific strains for the tree species which were planted in common. For studies of rhizobial persistence, partner 6 optimised DNA isolation and extraction from nodules and designed specific DNA probes for the selected isolates. Analyses of nodules was done in collaboration with partners 3, 4 & 5, who sent staff to Senegal for training by partner 6. Partner 6 also established partnerships with farmers’ organisations and set up participative field inoculation trials and evaluated the feasibility of mycorrhizal fungal production by farmers’ organisations.

In the UK, Partner 1 conducted controlled glasshouse experiments evaluating the responses of different tree species to inoculation under different irrigation (nutrient input) conditions. In Mali, Burkina Faso and Niger, partners 2, 3, 4 & 5 respectively tested the effects of inoculation on growth of a range of tree species.

5. Economics

- a. Conduct surveys of householders, end users, suppliers and vendors – to evaluate quantities of fuelwood used, preferences, values, limitations etc

Partner 5 led the design of questionnaires, and partners 3, 4 and 5 conducted in depth surveys in Mali, Burkina Faso and Niger respectively.

6. Soil and plant nutrition

- a. Conduct soil and plant analyses for each site, repeated at intervals

These studies were conducted by partners 2, 4 and 5, covering the irrigation trials in each country.

7. Planting stock quality

- a. Determine attributes of planting stock most appropriate to irrigated site conditions.

These studies were led by partner 4.

8. Pest monitoring and management

- a. Observe, monitor and control pests and diseases, and evaluate risks associated with fast growing plantations

Detailed activities focussed in Mali, using the expertise of partner 3.

Results achieved

Work package 1 Water treatment and irrigation

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than planned for a variety of reasons, including delays to obtaining permits for construction, delays associated with importation and shipping of components which were unavailable locally and delays in purchase of expensive items due to cash flow problems. Staff were trained in how to use and maintain the systems. Some modifications and refinements and repairs have been necessary, but systems are functioning in each country. The irrigation sites were characterised in advance of tree planting, and soil and water analyses are being conducted regularly.

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Initially, nursery and field studies in Mali, Niger and Burkina Faso gave much less clear results. However, assessments during the final year of the project have demonstrated positive effects of inoculation on tree growth in Mali and Niger.

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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, where the most detailed studies were conducted, previous effects of inoculation, substrate and pot size detected in a pot experiment, were no longer evident after planting out, however there was considerable variation between species in growth. At the time of planting, there were considerable differences in shoot: root ratios between species.

Work package 8 Pest monitoring and management

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

Problems encountered

The main problems encountered were in connection with the selection of the sewage treatment and irrigation sites, the purchasing of the capital items for these sites and in the maintenance of the sites. Several problems were encountered during the project;

- sites had to be negotiated for
- high up front capital costs
- importation of materials expensive and slow
- repeated problems with pumps (lack of durability, susceptibility to clogging).

These problems had knock on effects through the rest of the project, and delayed many aspects of the work. The high up front costs consumed much of the initial project funding, starving other work packages of funds, and partners who did spend on other work packages then found that they had insufficient funds to purchase essential items for the irrigation systems. Although the mechanisms of EU funding had been explained to partners, they still found it difficult to plan their activities in relation to the EU funding profile. To overcome some of the problems, partner 1 advanced funds to some partners, covering the associated risks. Permission to extend the project by one year enabled results to be obtained from the irrigation sites, but publications in the scientific literature are not yet complete. Partners are continuing to work on these and on dissemination to the local community using their own resources.

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

Technology implementation plan

The etip has been completed. All results are in the public domain.

Publications and conference papers

Krasova-Wade T, Neyra M (2007) Optimization of DNA isolation from legume nodules. *Lett. Appl. Microbiol.* 45: 95-99

Submitted

2008: Valorisation des eaux usées pour l'irrigation des espèces forestières destinées à la production de bois de chauffe et du fourrage au Niger. Paper submitted to *Sécheresse*

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A manuscript on the results on hybridization probes is in preparation.

A number of other publications are planned or in the early stages of preparation.

Conclusions

The project has brought together teams in sub-Saharan Africa with partners in Europe, and built on previous projects funded by the EU. Results of the irrigation and inoculation trials are promising. However, there is a big step to be made between these small-scale trials and plantings which will make a real difference to fuelwood supply and it will be a considerable challenge to overcome.

Management Report

Organisation of the collaboration

The project was originally coordinated by JD Deans. He retired in late 2004 and was replaced by Dr Julia Wilson.

Management of the project has been through coordination meetings and regular email communication with all participants and scientific visits and exchanges between partners. Extension of the project by one year was helpful in terms of providing sufficient time to assess tree growth on the irrigation sites, but created problems in terms of organising meetings because of limited funds. To conserve funds, the meeting at the end of year 4 was postponed until the end of year 5. Unfortunately the visa problems mentioned elsewhere prevented this 5th year meeting being held. The lack of meetings has reduced the incentive to complete publications. The project coordinator combined visits to partners in Mali, Niger and Burkina Faso with sapflow training visits.

Meetings were held as follows:

20 – 24 January 2003, Niamey, Niger. Start up coordination meeting

4 - 7 May 2004 Bamako, Mali. Annual coordination meeting

1 – 3 May 2005 Ouagadougou, Burkina Faso Annual coordination meeting

September 2006, no coordination meeting (postponed until 2007) was held as finances did not permit (the project has been extended by one year). However, local partners held joint discussions, coordinated by Dr Marc Neyra.

SCP visited partners in Burkina Faso, Niger and Mali, in 2002 and again in November/December 2006

September 2007 Edinburgh: cancelled due to visa problems

Exchanges

In 2003, Falaye Kante (University of Bamako) spent the year at the laboratory of partner 6 (IRD) in Dakar, and Alzouma Zoubeirou (Universite Abdoumouni, Partner 5) visited for 2 months. Kady Sanon (INERA, partner 4) also visited these laboratories.

In 2004, Ousmane Sacko (partner 3, Univ. Mali) and Alzouma Zoubeirou (Universite Abdoumouni, Partner 5) spent 8 and 6 months respectively in the laboratory of partner 6 in Dakar.

In 2005, Falaye Kante, (University of Bamako) spent 6 months at the laboratory of partner 6 (IRD) in Dakar.

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

Partner 1 Centre for Ecology and Hydrology, UK

Julia Wilson, Kevin Ingleby, Robert C Munro

1. Summary

- A glasshouse experiment was conducted in 2 phases during 2004-5. The first phase tested 7 arbuscular mycorrhizal (AM) inoculants for effectiveness with 4 agroforestry tree species (2 N-fixing species and 2 non N-fixing species).
- AM inoculation improved the growth of all 4 tree species, but *Senna siamea*, *Leucaena leucocephala* and *Khaya senegalensis* showed a greater response to inoculation than *Gliricidia sepium*.
- Growth and AM colonisation of all 4 tree species was greatest when inoculated with isolates of *Glomus mosseae* and *Glomus fasciculatum*.
- The second phase examined the response of 3 tree species (*S. siamea*, *L. leucocephala* and *K. senegalensis*) and 3 AM inoculants (*G. mosseae* 1, *Glomus etunicatum* 1 and uninoculated plants) from the 2004 experiment, to an irrigation treatment designed to simulate wastewater recycling in West Africa.
- Inoculated trees of *S. siamea* and *K. senegalensis* showed a positive growth response to irrigation which was related to increased leaf P concentration and AM colonization on the roots.
- *G. mosseae* mycorrhizas promoted the growth of *S. siamea* and *K. senegalensis* trees in the irrigated soils, whereas *G. etunicatum* mycorrhizas only promoted the growth of *S. siamea*.
- Inoculated *L. leucocephala* showed little growth response to the irrigation treatment but assimilated more N than *S. siamea* and *K. senegalensis*.
- The results indicated that *S. siamea* and *K. senegalensis* showed greater mycorrhizal dependency than *L. leucocephala* and demonstrated that tree growth responses mediated by AM fungi can alter dramatically with changes in soil conditions.
- The key factors in these responses appeared to be the trees' N-fixing capabilities and degree of mycorrhizal dependency.
- It is concluded that selection of appropriate tree species/AM fungal combinations is a critical factor in the development of agroforestry practices which use recycled wastewaters.
- AM cultures were maintained in the CEH glasshouse during the course of the project
- A 2 week period of training in arbuscular mycorrhizal techniques was provided to Mahamadi Dianda from Burkina Faso.
- Equipment was provided and partners in Niger, Mali and Burkina Faso were trained at their irrigation sites in methods of measuring soil moisture and sap flow.

2. Activities

2.1. Glasshouse experiments at CEH

The objectives of the experiment were to:

- (a) test a range of AM inoculants for effectiveness with 4 multipurpose tree species (2 N-fixing species and 2 non N-fixing species), and
- (b) examine the effect of an irrigation treatment simulating wastewater being recycled in West Africa on established tree species x AM fungal associations

The experiment was conducted in 2 phases: the first phase, set up in 2004, screened AM inoculants with different tree species, the second phase set up in 2005 examined the response of selected tree species x AM inoculant combinations to the irrigation treatment.

Screening experiment in 2004

Tree species

Gliricidia sepium Dakar 6/03, ILG50 (ex. Mali)

Leucaena leucocephala Dakar 6/03 (T2C Odonto)

Senna siamea CNSF 1154 (Bobo Prov.)

Khaya senegalensis CNSF 1156 (Mondon Prov.)

Mycorrhizal inoculation

Pot cultures of 7 AM inoculants were grown for 4 months using *Pennisetum* sp. and *Sorghum* sp. as host plants

Glomus aggregatum isolate 1

Glomus aggregatum isolate 2

Glomus fasciculatum isolate 1

Glomus mosseae isolate 1

Glomus etunicatum isolate 1 (International Bank for the Glomeromycota (BEG) isolate 176)

Gigaspora albida isolate 1 (BEG isolate 172)

Gigaspora albida isolate 2 (BEG isolate 173)

Germinated seedlings were inoculated in pots containing a sterilized soil mixture and the appropriate AM inoculum. Control pots received autoclaved inoculum. All *L. leucocephala* and *G. sepium* seedlings were inoculated with *Rhizobium* cultures known to be effective for the tree species. Four weeks after inoculation, 10 seedlings of each tree species (4) x mycorrhizal inoculation treatment (8) were transferred to 1.5 litre pots containing a sterilized soil mixture, see Figure 1.



Figure 1 Part of the glasshouse experimental set up for tree species x mycorrhizal studies

During 2004, assessments of stem diameter were made every 2 weeks and in October 2004, 24 weeks after inoculation, soil cores were removed from all the pots for assessment of root concentration and mycorrhizal colonisation. Shoots of those plants not selected for inclusion in the irrigation experiment were removed and leaf and stem dry weights were also determined.

Mycorrhizal inoculation significantly benefited shoot and root growth of all 4 tree species. Growth benefits were most pronounced for *L. leucocephala* (Figure 2, Table 1), *K. senegalensis* (Figure 3, Table 1) and *Senna siamea* (Figure 4, Table 1) and least pronounced for *G. sepium* (Figure 5, Table 1). Overall levels of mycorrhizal colonisation were high in *S. siamea* (66 %), *K. senegalensis* (61 %) and *G. sepium* (61 %), but significantly lower in *L. leucocephala* (51 %). All parameters of tree growth were positively correlated with mycorrhizal colonisation (Table 2), although correlation coefficients for *G. sepium* were noticeably lower. These results suggested that *G. sepium* was least responsive to mycorrhizal inoculation with the seven AM isolates. Uninoculated, non-mycorrhizal seedlings of *G. sepium* were also able to sustain a better growth rate than non-mycorrhizal seedlings of the other three tree species, which indicates that *G. sepium* was also the least dependent on mycorrhizal colonisation to sustain growth.

Table 1 Shoot growth, root growth and mycorrhizal colonisation of inoculated and uninoculated *Leucaena leucocephala*, *Khaya senegalensis*, *Senna siamea* and *Gliricidia sepium* seedlings, 24 weeks after inoculation

Tree species		Inoculation treatment								P value
		G.agg. 1	G.agg.2	G.fas.1	G.mos.1	G.etu.1	Gi.alb.1	Gi.alb 2	Uninoc.	
<i>Leucaena leucocephala</i>	Stem diam. ¹ (mm)	9.11a ²	6.78b	9.03a	9.50a	8.55a	3.82d	4.87c	3.66d	<0.001
	Stem dry wt. ¹ (mg)	1052a	501b	959a	-	-	26c	79c	-	<0.001
	Leaf dry wt. ¹ (mg)	560a	308b	403ab	-	-	38d	139c	-	<0.001
	Myc. col. ¹ (%)	59c	46d	67b	75a	64bc	0.6e	46d	0e	<0.001
	Root conc. ¹ (cm/40cc)	242a	126c	191b	252a	121c	18e	47d	42d	<0.001
<i>Khaya senegalensis</i>	Stem diam. (mm)	6.31bc	5.86c	6.87ab	7.29a	6.36abc	3.95d	4.21d	3.73d	<0.001
	Stem dry wt. (mg)	725a	593a	853a	-	-	192b	198b	-	<0.001
	Leaf dry wt. (mg)	1089a	995a	1278a	-	-	573b	602b	-	<0.001
	Myc. colon. (%)	87a	79b	80b	79b	57c	1.7e	45d	0f	<0.001
	Root conc. (cm/40cc)	662a	518ab	578ab	510ab	564ab	226c	484b	114c	<0.001
<i>Senna siamea</i>	Stem diam. (mm)	4.51ab	4.23b	5.11a	4.33b	4.11bc	2.33d	3.67c	2.07d	<0.001
	Stem dry wt. (mg)	558a	332b	507ab	-	-	49c	276b	-	<0.001
	Leaf dry wt. (mg)	1651b	1670b	2261a	-	-	320c	1419b	-	<0.001
	Myc. col. ³ (%)	71b	72b	82a	79ab	60c	12d	84a	0e	<0.001
	Root conc. ⁴ (cm/40cc)	-	-	-	-	-	-	-	-	-
<i>Gliricidia sepium</i>	Stem diam. (mm)	10.0abc	9.4bc	11.3a	11.2ab	9.8abc	8.3c	9.3c	8.9c	<0.001
	Stem DW (mg)	257b	179b	445a	486a	234b	132b	237b	142b	<0.001
	Leaf DW (mg)	393b	202bcd	628a	605a	266bcd	133cd	306bc	111cd	<0.001
	Myc. col. (%)	69c	61d	79b	85a	74bc	3.1e	58d	0f	<0.001
	Root conc. (cm/40cc)	151bc	66e	191a	182ab	104d	20f	125cd	31f	<0.001

¹ For statistical analysis, square root transformations were performed on stem dry wt., leaf dry wt. and root conc.; angular transformations were performed on mycorrhizal colonisation; untransformed means are shown in this table

² Letters indicate significant differences within each row as determined by ANOVA and Fishers's LSD test when $P < 0.05$

³ Mycorrhizal colonisation of *S. siamea* was assessed using a different method to that used for the other tree species

⁴ Root concentration of *S. siamea* was not determined

Figure 2 Growth of *Leucaena leucocephala* seedlings after inoculation with 7 different fungi (Bars indicate \pm SE)

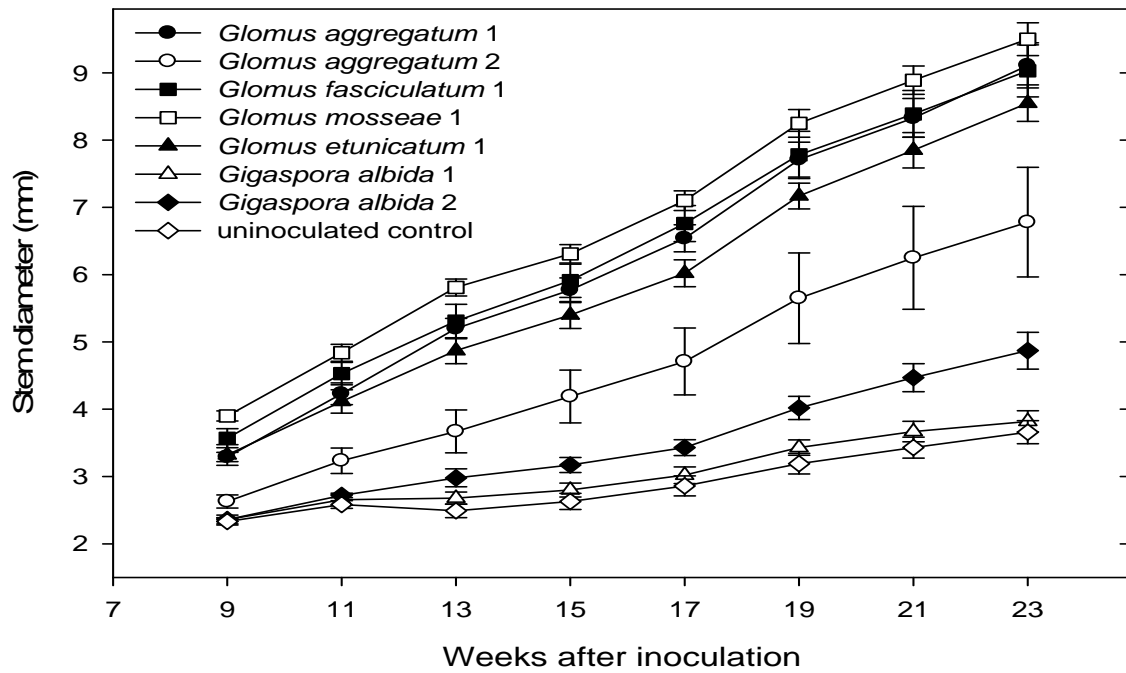


Figure 3 Growth of *Khaya senegalensis* seedlings after inoculation with 7 different fungi (Bars indicate \pm SE)

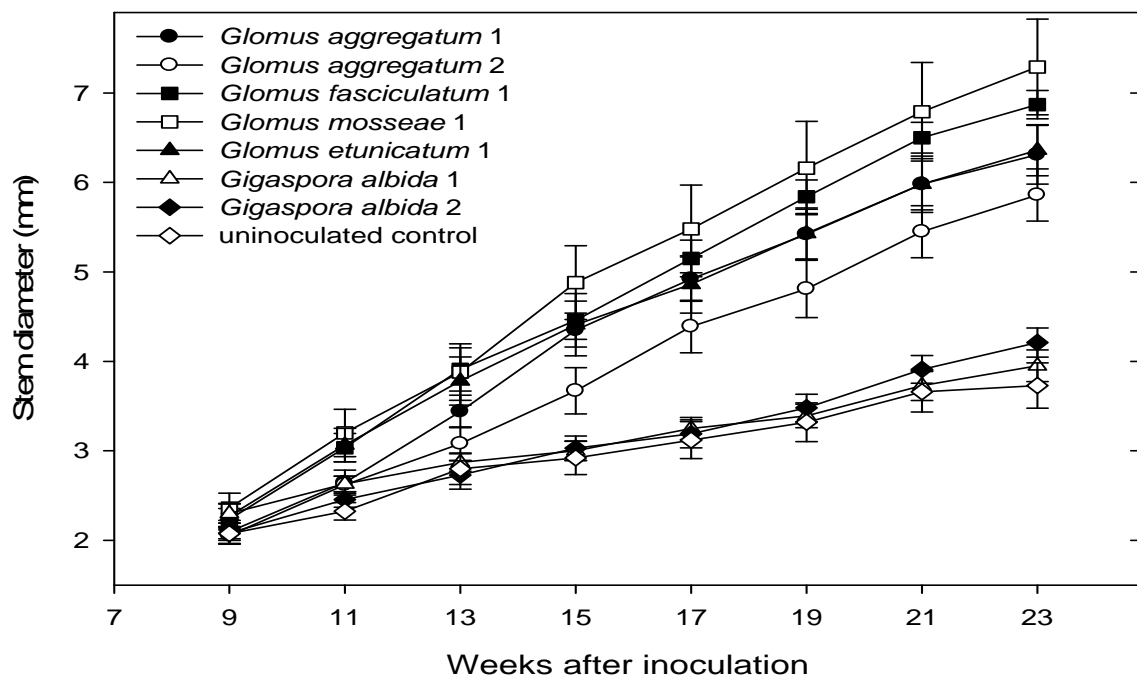


Figure 4 Growth of *Senna siamea* seedlings after inoculation with 7 different fungi (Bars indicate \pm SE)

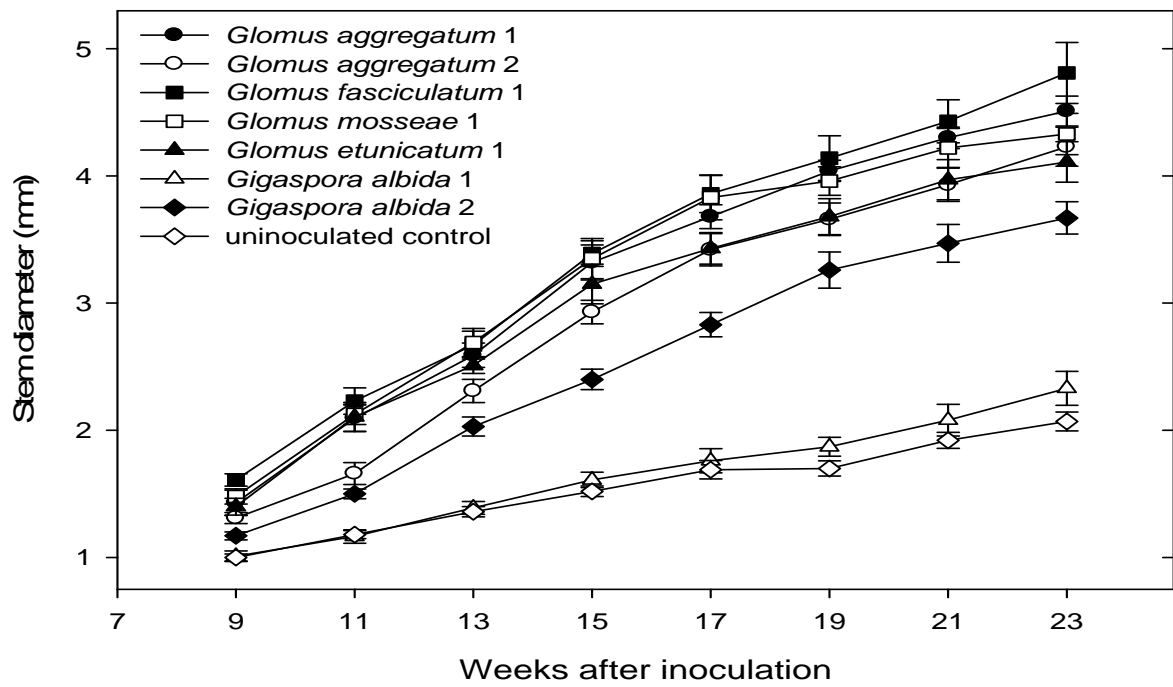


Figure 5 Growth of *Gliricidia sepium* seedlings after inoculation with 7 different fungi (Bars indicate \pm SE)

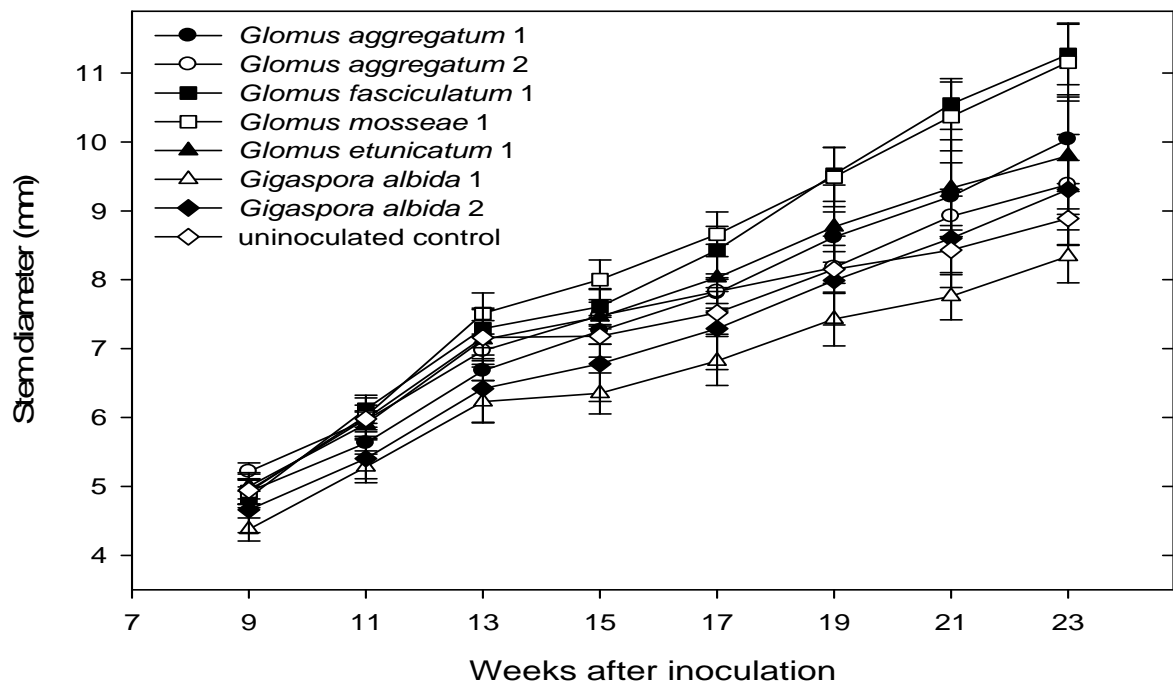


Table 2 Correlation coefficients (r) between mycorrhizal colonisation and growth of the 4 tree species, 24 weeks after inoculation (n=80 for stem diameter and n=50 for dry wt. measurements)

	<i>S. siamea</i>	<i>L. leucocephala</i>	<i>K. senegalensis</i>	<i>G. sepium</i>
Stem diam.	0.724***	0.734***	0.715***	0.389***
Stem dry wt.	0.661***	0.729***	0.652***	0.474***
Leaf dry wt.	0.809***	0.740***	0.594***	0.523***

*** significant at $P < 0.001$

Generally, all 4 tree species grew best when inoculated with the *G. mosseae* 1 and *G. fasciculatum* 1 isolates, and these 2 isolates also formed the highest levels of colonisation (80 and 77 % respectively). However, several significant tree species x AM inoculant interactions were seen: growth of *L. leucocephala* and *K. senegalensis* was best when inoculated with the *G. mosseae* 1, whereas growth of *S. siamea* and *G. sepium* was best when inoculated with the *G. fasciculatum* 1. *Gi. albida* 2 and *G. aggregatum* 1 were both most effective and formed highest levels of mycorrhizal colonisation with *S. siamea*, and least effective with *L. leucocephala*. Growth of all 4 tree species was poorest for the uninoculated trees and those inoculated with the *Gi. albida* 1. After 24 weeks, uninoculated trees remained non-mycorrhizal while those inoculated with isolate *Gi. albida* 1 had formed very few mycorrhizas (4.4%).

Irrigation experiment in 2005

Three tree species were selected from the screening experiment: *L. leucocephala* (a N-fixing legume), *S. siamea* (a non-nodulating legume) and *K. senegalensis* (a non-legume). Three inoculation treatments were also selected: 2 of the effective inoculants - *G. mosseae* 1 and *G. etunicatum* 1 (an isolate for which molecular markers were available) and the uninoculated control.

In October 2004, 25 weeks after inoculation, 8 plants of each of the 9 treatments (3 tree species x 3 inoculation treatments) were potted into 32 litre tubs containing the same sterilized soil mixture as used in the pots (Figure 6). Irrigation treatments commenced in January 2005, with 4 plants allocated to each irrigation treatment.



Figure 6 Plants set up for the irrigation treatments

(1) **Irrigated:** watered normally and supplied with a nutrient solution (Ingestad 1971) adding additional NH_4NO_3 and ZnSO_4 to increase levels of N and Zn. The modified solution contained 132, 9.4 and 32.5 mg/litre of NPK and 1.46 mg/litre of Zn respectively.

(2) **Not irrigated:** watered normally and supplied with additional water as appropriate to compensate for nutrient additions.

Assessments of stem diameter were continued until the end of the experiment in November 2005, when nutrient concentration (N, P, K, and Zn) in leaf and root material and soil nutrients (available $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, $\text{PO}_4\text{-P}$, K and Zn) were determined.

Inoculated trees of all 3 species continued to grow better than the uninoculated trees (Figure 7, 9). None of the uninoculated trees showed any response to irrigation, whereas responses of the inoculated trees differed according to the tree species and AM inoculant: *S. siamea* trees inoculated with both fungi showed improved growth with irrigation (Figure 7), whereas only *K. senegalensis* trees inoculated with *G. mosseae* 1 responded to irrigation (Figure 8). Growth of *L. leucocephala* showed little response to irrigation with either AM inoculant (Figure 9).

Figure 7 Growth of inoculated and uninoculated *Senna siamea* trees in 2005 during application of irrigation treatment (Bars indicate \pm SE)

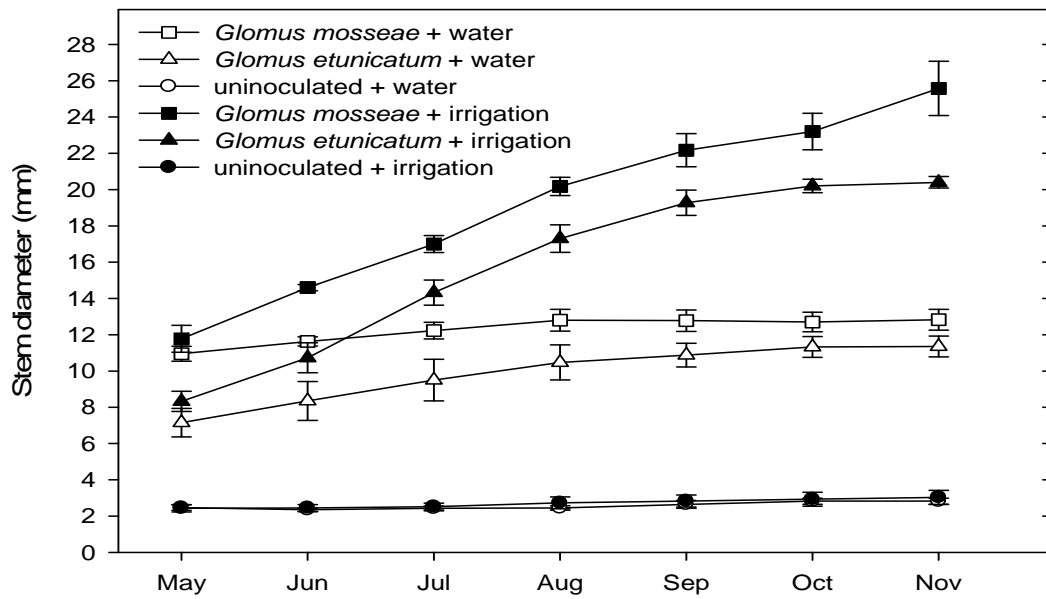


Figure 8 Growth of inoculated and uninoculated *Khaya senegalensis* trees in 2005 during application of irrigation treatment (Bars indicate \pm SE)

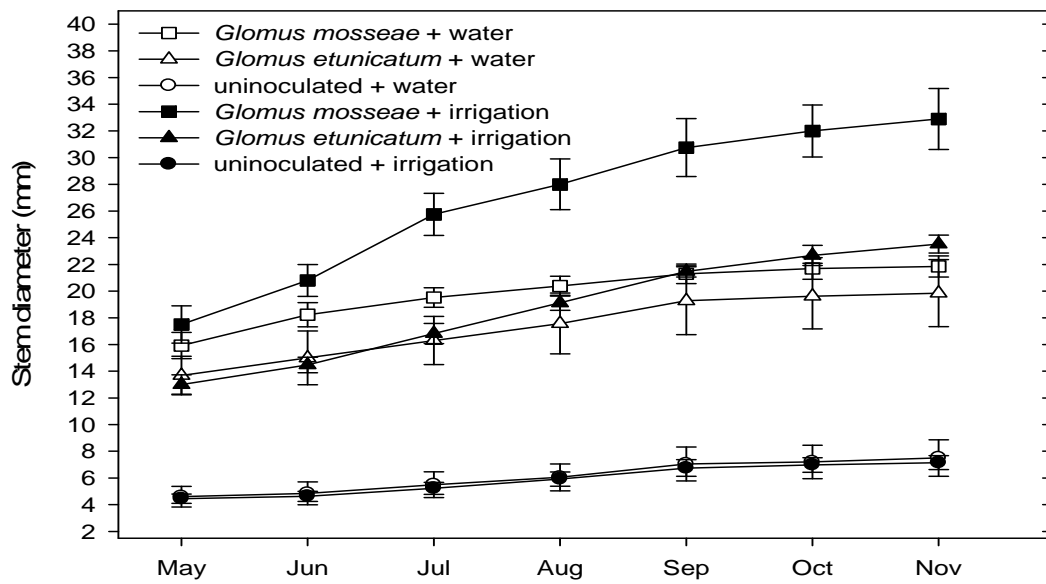
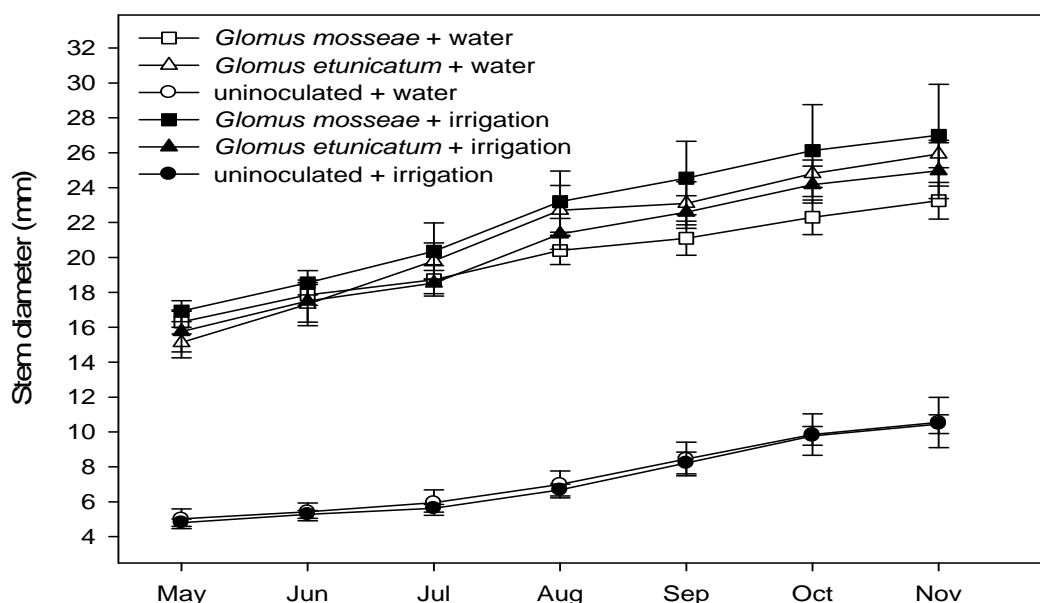


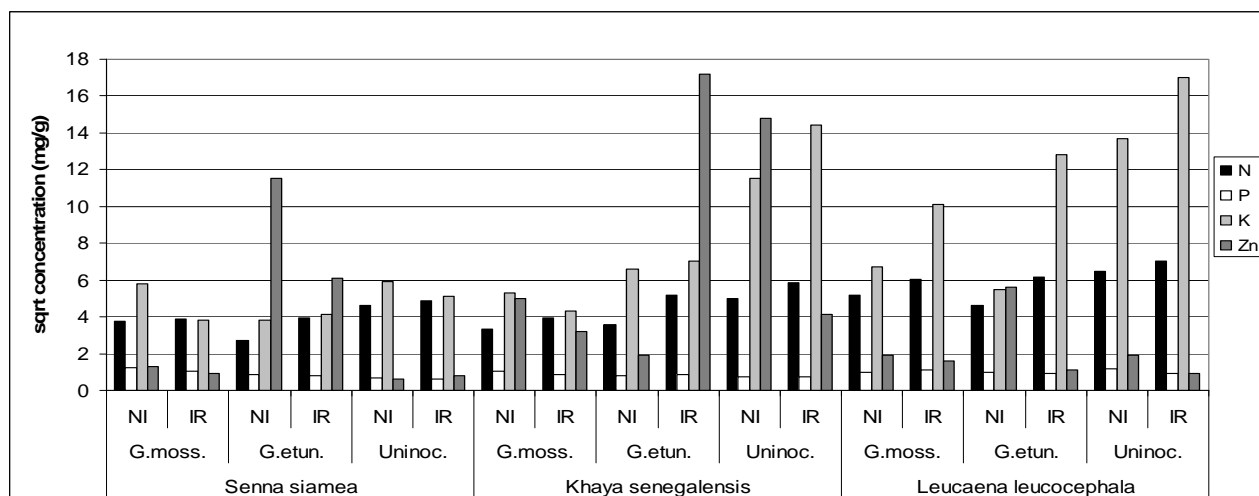
Figure 9 Growth of inoculated and uninoculated *Leuceana leucocephala* trees in 2005 during application of irrigation treatment (Bars indicate \pm SE)



Leaf nutrients (Figure 10)

Overall levels of N were greater in leaves of *L. leucocephala* than in those of *K. senegalensis* and *S. siamea* ($P < 0.001$). Levels of N were also greater in the uninoculated trees than in the inoculated trees ($P < 0.001$), and in the irrigated trees compared with those that received only water ($P = 0.002$). Overall levels of P were also greater in leaves of *L. leucocephala* than the other 2 tree species ($P = 0.004$). Although overall levels of P were greater in trees inoculated with *G. mosseae* 1 compared with those inoculated with *G. etunicatum* 1 and the uninoculated trees ($P < 0.001$), a significant tree x AM inoculant interaction ($P = 0.016$) showed that this effect was only evident for trees of *K. senegalensis* and *S. siamea* and not for *L. leucocephala*. Overall levels of K were greater in leaves of *L. leucocephala* than in leaves of *K. senegalensis*, which in turn were greater than those found in leaves of *S. siamea* ($P < 0.001$). A significant tree x AM inoculant interaction ($P = 0.012$) showed that levels of K were greater in leaves of uninoculated *L. leucocephala* and *K. senegalensis*, but that no difference was seen for *S. siamea*. A significant tree x irrigation interaction ($P = 0.006$) showed that levels of K were greater in leaves of *L. leucocephala* receiving irrigated water, but that no effect was seen in trees of *K. senegalensis* and *S. siamea*. No significant effects of tree species, AM inoculant or irrigation treatments were found on Zn concentration.

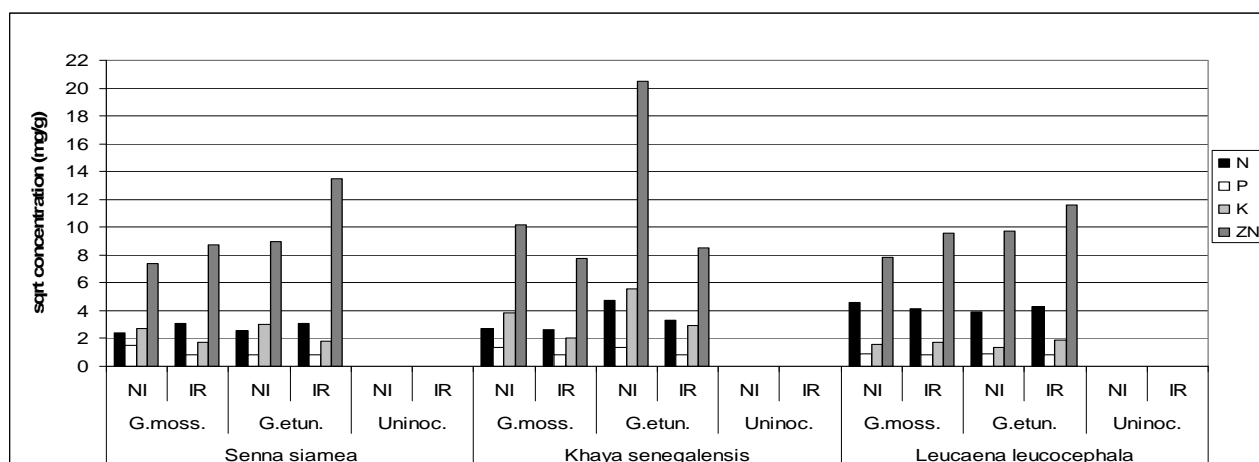
Figure 10 Leaf nutrient content of inoculated and uninoculated *Senna siamea*, *Khaya senegalensis* and *Leucaena leucocephala* trees after application of irrigation treatments in 2005



Root nutrients (Figure 11)

Root nutrient concentrations were only examined in inoculated trees, as insufficient root material was recovered from the uninoculated control trees for chemical analysis. Overall concentrations of N were greatest in roots of *L. leucocephala*, and higher in *K. senegalensis* than *S. siamea* ($P < 0.001$). Concentrations of N were also greater in trees inoculated with *G. etunicatum* 1 compared to those inoculated with *G. mosseae* 1 ($P = 0.024$), although a significant tree x AM inoculant interaction ($P = 0.002$) indicated that this effect was only present in *K. senegalensis* trees. A significant tree x irrigation interaction ($P = 0.011$) also showed that levels of N were greater in roots of *K. senegalensis* trees that received only water. Concentrations of P were also greatest in roots of trees that received only water ($P = 0.002$). Overall concentrations of K were greatest in roots of *K. senegalensis*, and higher in *S. siamea* than *L. leucocephala* ($P < 0.001$). More K was also present in roots of trees that received only water ($P = 0.002$), although a significant tree x irrigation interaction ($P = 0.003$) indicated that this effect was most evident in roots of *K. senegalensis*. No significant effects of the treatments were found on Zn concentration, although overall levels were much higher than those found in the leaf material.

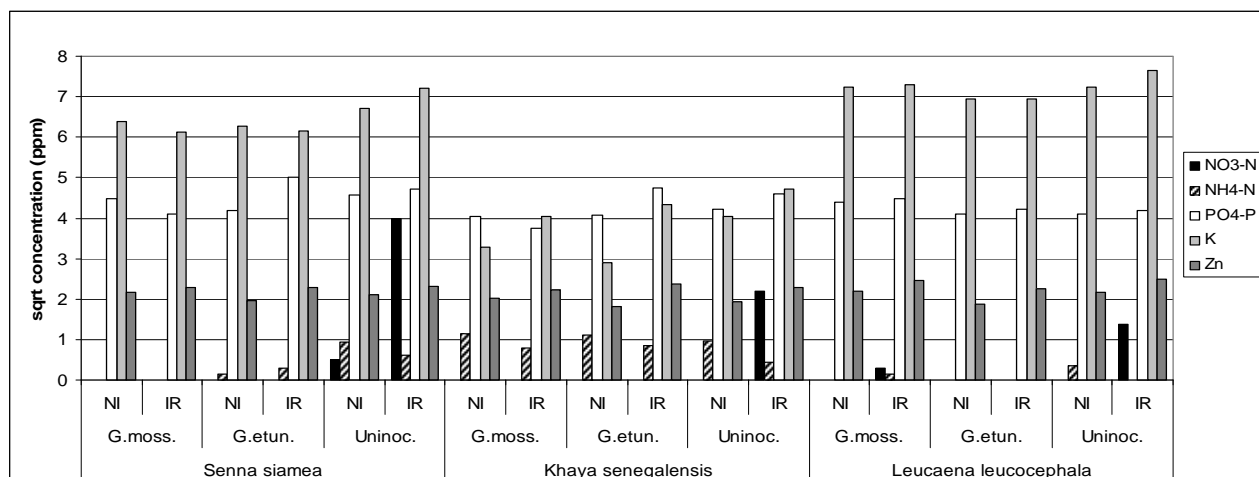
Figure 11 Root nutrient content of inoculated and uninoculated *Senna siamea*, *Khaya senegalensis* and *Leucaena leucocephala* trees after application of irrigation treatments in 2005



Soil nutrients (Figure 12)

Chemical analysis of the soils showed that greatest concentrations of $\text{NO}_3\text{-N}$ occurred when uninoculated trees of all 3 species were growing in irrigated soil ($P < 0.001$). Irrigated soils from under uninoculated *S. siamea* also had greater levels of $\text{NO}_3\text{-N}$ than those from *K. senegalensis* and *L. leucocephala* ($P = 0.036$), and overall levels of $\text{NO}_3\text{-N}$ were lowest in soils of *L. leucocephala* ($P < 0.001$). In contrast to soil $\text{NO}_3\text{-N}$, higher concentrations of $\text{NH}_4\text{-N}$ were found in soil from trees which received only water ($P = 0.031$), although a significant tree x irrigation interaction ($P = 0.023$) indicated that this effect was only present in soil from under *K. senegalensis* trees, where levels of $\text{NH}_4\text{-N}$ were greater than those in soils from under *S. siamea*, which in turn were greater than those in soils from under *L. leucocephala* ($P < 0.001$). More $\text{NH}_4\text{-N}$ was also found in soils of uninoculated *S. siamea* than in soils from inoculated trees ($P = 0.002$). Concentration of K was greater in soil from under *L. leucocephala* than in soil from *S. siamea* which, in turn, was greater than *K. senegalensis*. Concentrations of K were also greater in soil from uninoculated trees compared with inoculated trees ($P = 0.006$) and in soil from irrigated trees compared with trees receiving only water ($P = 0.023$). Concentrations of Zn were greater in irrigated soil ($P < 0.001$). There were no significant effects of any of the treatments on $\text{PO}_4\text{-P}$ concentration in the soils.

Figure 12 Soil nutrients in tubs of inoculated and uninoculated *Senna siamea*, *Khaya senegalensis* and *Leucaena leucocephala* trees after application of irrigation treatments in 2005



Mycorrhizal colonisation present on the tree roots at the start of the irrigation experiment was positively correlated with levels of P in the leaves, and negatively correlated with levels of PO₄-P in the soil (Table 3). Mycorrhizal colonisation was also negatively correlated with levels of Zn in the leaves.

Table 3 Correlation coefficients (r) between mycorrhizal colonisation of trees at start of irrigation experiment in November 2004 and nutrient content of leaf, root and soil samples in October 2005 (n=36)

	AM colonisation
Leaf N	-0.152
P	0.566***
K	-0.111
Zn	-0.366*
Root N	-0.277
P	0.219
K	-0.258
Zn	-0.278
Soil NO ₃ -N	-0.285
NH ₄ -N	-0.037
PO ₄ -P	-0.455**
K	0.071
Zn	0.101

*, **, *** significant at $P < 0.5$, 0.01 and 0.001 respectively

The results showed that *S. siamea* and *K. senegalensis* showed a positive growth response to irrigation, whereas *L. leucocephala* did not. In addition, uninoculated trees of *L. leucocephala* grew better than those of *S. siamea* and *K. senegalensis*. These growth responses were related to increased P concentration in the leaves, which was lower in uninoculated *S. siamea* and *K. senegalensis* trees, but not in *L. leucocephala*. Furthermore, mycorrhizal colonisation at the start of the irrigation experiment was positively correlated with P concentration in the leaves and negatively correlated with phosphate concentration in the soil, which indicated that P

uptake was generally better in mycorrhizal plants. As poor growth of non-mycorrhizal plants and increased shoot growth and P concentration of mycorrhizal plants are characteristics of plants with high mycorrhizal dependency, the results suggest that *S. siamea* and *K. senegalensis* showed a greater degree of mycorrhizal dependency than *L. leucocephala*.

The most surprising result was the difference in response of *G. etunicatum*-inoculated *S. siamea* and *K. senegalensis* trees to irrigation. Associated with *S. siamea*, *G. etunicatum* mycorrhizas were effective in the uptake of nutrients from the irrigation water and promoted plant growth: associated with *K. senegalensis*, they were not. This behaviour contrasted with that of *G. mosseae* mycorrhizas which were able to promote the growth of both tree species in the irrigated soils. Several recent studies have demonstrated that specificity exists in host plant-AM fungal responses and that these can be observed in field inoculation experiments and at the level of plant community (e.g. Adjoud et al. 1996; van der Heijden et al. 1998). These results support this and indicate that host plant-AM fungal responses can be extremely sensitive and exhibit a high level of specificity.

Although levels of N in leaves, roots and soil indicate that N uptake was much better in *L. leucocephala* than in *S. siamea* and *K. senegalensis*, trees of *L. leucocephala* showed little or no growth response to the irrigation treatment. High concentrations of leaf P in *L. leucocephala* leaves suggests that growth was not P-limited (Stribley 1980). Wallender (1995) proposed that under high levels of available N, ectomycorrhizal pine seedlings were forced to assimilate N and consumed more C. It is therefore possible that the *L. leucocephala* trees reacted in a similar manner and were unable to regulate N uptake, hence consuming C which otherwise would have been allocated for shoot growth. High levels of NO_3 and NH_4 in irrigated soils from uninoculated trees can be attributed to the smaller size of these trees rather than to enhanced N uptake by inoculated plants. N concentration was also greater in leaves of irrigated, uninoculated plants, so it appears that the AM fungi did not play a key role in N uptake by the trees and showed no preference for NH_4 over NO_3 .

The irrigation treatment resulted in higher levels of Zn in the soil, but there was very little indication that this had any effect on tree growth or Zn uptake as evidenced by Zn concentration in plant tissue. It should be noted however, that Zn concentration in the leaves was negatively correlated with mycorrhizal colonisation and, as Zn concentration in the roots of uninoculated trees was not determined, comparisons with levels found in the roots of *G. mosseae* and *G. etunicatum*-inoculated trees could not be made.

Overall, the experiments demonstrated that tree growth responses mediated by AM fungi can alter dramatically with changes in soil conditions. Under normal soil conditions, when both P and N were limiting growth, most of the AM isolates benefited growth of the four tree species, whereas in soil containing high levels of available N, the AM isolates showed a differential response according to the host tree species. The key factors in the response of the different tree species to irrigation appeared to be their N-fixing capabilities and their degree of mycorrhizal dependency. Although N-fixing trees were able to accumulate and recycle more N, thereby increasing the quality of fodder produced and reducing the amount of NO_3 flushed into groundwaters, this would appear to be at the cost of sustainable, long-term

growth and the efficient recycling of nutrients in the irrigation waters. It is concluded that selection of appropriate tree species/AM fungal combinations is a critical factor in the development of agroforestry practices which use recycled wastewaters.

2.2. Maintenance of pot cultures

During the course of the project, pot cultures of AM inoculants were maintained in the CEH glasshouse. Cultures of AM inoculants were also exchanged with project partners engaged in mycorrhizal work.

2.3. Training

Training in sap flow was provided as follows:

by JD Deans and Julia Wilson, Niger, April 2004

by Julia Wilson and Bob Munro, April 2005 Mali, and March 2007 Burkina Faso

During October 2007, CEH hosted the visit of Dr Mahamadi Dianda for training in arbuscular mycorrhizal techniques focusing mostly on the taxonomy of AM spores (2 weeks).

3. Dissemination

A paper has been prepared describing results of the glasshouse experiment and will be submitted for publication in *Biology and Fertility of Soils*.

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

Mr Daouda Sidibé, Dr Kalifa Traoré, Mr Broulaye Koné

1. Objectives

The objectives of the project in Mali were to treat and evaluate the use of waste water from rice irrigation, taken from the Siribala irrigated perimeter, for irrigating trees for fodder and fuel wood production.

2. Activities

Work package 1: An experimental site (4 ha) was chosen near Siribala village, situated at 30 km from Niono. The site is situated at 14° 4' N, 6°03' W and at an altitude of 274.3 m. This site was chosen because of the presence of a new drainage canal which was not overgrown by invasive aquatic plants such as Typha and Azolla, following advice of the subcontractors SCP of France.

The experimental site was delimited. Then the topographic, morpho-pedological and climatic data were recorded by Partner 2 and sent to the SCP team in France who developed an irrigation system plan (see SCP s' report produced in November 2003) which was implemented. Following the design produced by SCP, Partner 2 installed the irrigation system in the field which included site facilities (earthworks, house, guard room, dam building) and equipment (water pump engine and irrigation materials) as shown in **Figure 13**. PVC tubes were placed on the primary embankments, and adjustable floodgate were placed on the secondary embankments. Trenches were dug out in order to facilitate drainage of water to plants.



Figure 13: PVC tube (200 m length) placed on the primary embankment and adjustable floodgate on the secondary embankment (left); water pump engine and the filter in the drain of Minimana.

The role of Partner 2 at this site, was dealing with water treatment and irrigation, tree growth and management, soil and plant nutrition and tree water-use and soil water status. Soil and water samples were collected at different periods in the irrigation canal and given to Partner 3 (University of Bamako) for chemical analyses.

Experimental plots (1 ha) were delimited and surrounded with wire netting to ensure the security of tree species and irrigation materials.

Work package 2 and 4: Tree growth and Management/ Microsymbionts

Experiment 1 compared the growth of tree species (*Gliricidia sepium*, *Leucaena leucocephala*, *Acacia angustissima* and *Khaya senegalensis* inoculated with Mycorrhizas + Rhizobium or not) under irrigated field conditions in Burkina Faso, Niger and Mali.

Experiment 2 concerned a quick screening of 10 tree species (*Acacia crassicarpa*, *Acacia mangium*, *Acacia auriculiformis*, *Leucaena leucocephala*, *Gliricidia sepium*, *Calliandra calothyrsus*, *Acacia angustissima*, *Acacia senegal*, *Pterocarpus lucens* and *Khaya senegalensis*) to assess their performance relative to species used in Experiment 1. The seeds of the Australian tree species and inoculum were purchased at the AgroForester Tropical seeds in USA by Partner 2 and inoculum was sent to Partner 4 (Burkina Faso) for mass production to be used by all the countries. The biotic ingredients of this inoculum were: Endomycorrhizal (VAM) spores; minimum 40 spores/cc of blended *Glomus brasilianum*, *Glomus clarum*, *Glomus deserticola*, *Glomus intraradices*, *Glomus monosporus*, *Glomus mosseae* and *Gigaspora margarita*.

To avoid cross contamination during the inoculation, sufficient space was maintained between treatments and pots were raised off the ground during nursery studies (Figure 14).



Figure 14 Layout of the pots placed on sheds in the nursery at Minimana (right in experiment 1 and left in experiment 2).

Biophysical parameters such as height of trees, root collar diameter, diameter at 1.30m and the number of branches were measured monthly and data analysed using MINITAB statistical software and recently using STATISTIX 7.0, 2000 analytical software, USA.

Work package 3: To measure tree water-use and soil water status, Dr Julia Wilson and Bob Munro from CEH Edinburgh visited Mali for training Malian staff (Daouda Sidibé, Kalifa Traore, Bakary Diassana and Broulaye Koné) from 16 to 30 April

2005. The training program was based on sapflow, meteorological and soil moisture measurements techniques (Figure 15).



Figure 15: Training in the use of sap flow equipment (left) and meteorological station at the Minimana experimental site.

Work package 6: Before planting in 2003 soil samples were taken and sent to partner 4 for analysis. Further soil samples were taken in 2007 after coppicing and sent to our laboratory for the same analyses. The two results will be compared in order to assess changes in soil chemical characteristics.

3. Results achieved

After one year and 6 months of growth in **experiment 1**, the result of the analysis of variance for the mean basal diameter per species and per treatment showed a high significant difference between tree species and inoculation treatments ($p = 0.000$ and 0.002 respectively) on tree growth. The basal diameters of *Gliricidia sepium* (7.95 cm), *Leucaena leucocephala* (8.02 cm), and *Khaya senegalensis* (8.18 cm) were significantly greater than that of *Acacia angustissima* (4.61 cm).

The double inoculation with rhizobium and mycorrhizas had a positive effect on tree growth compared to the control treatment (Figure 16). The diameter, height and the number of branches of inoculated trees were significantly greater than those of the control treatment at p values of 0.002 ; 0.001 and 0.000 respectively.

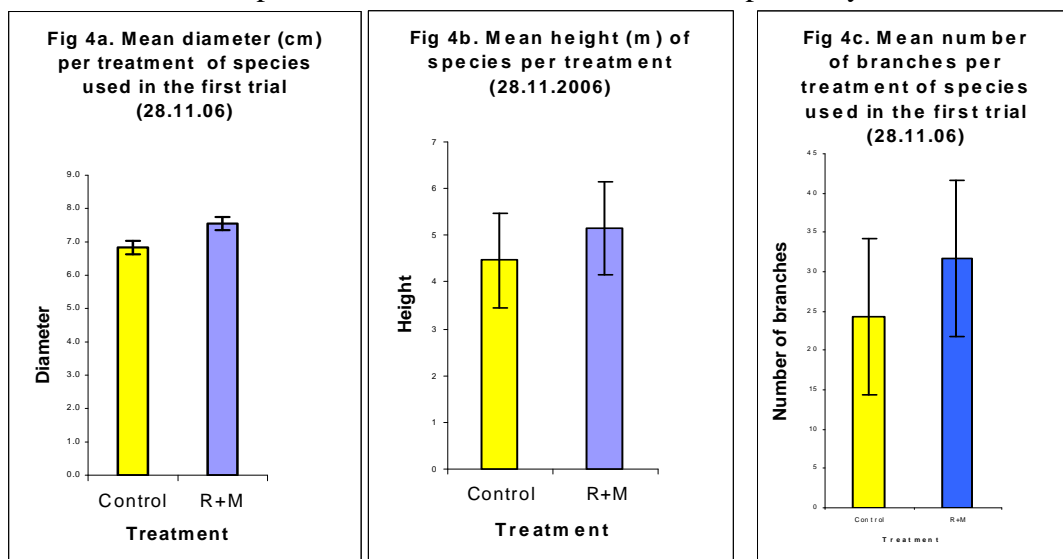


Figure 16: Effects of inoculation treatment on tree growth in Experiment 1 after 18 months. IR+M = inoculated with mycorrhizas and rhizobium, Control = not inoculated

different dates of measurements. It appears clearly that *Leucaena leucocephala* is the most performing species taking into account parameters measured mainly the number of branches (Figure 5).

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 growth in diameter. The growth rate of this local species is surprising because this species is known as a very slow growing species. The fast growth in diameter and height (4 meters in one year and half) found here is certainly due to irrigated condition

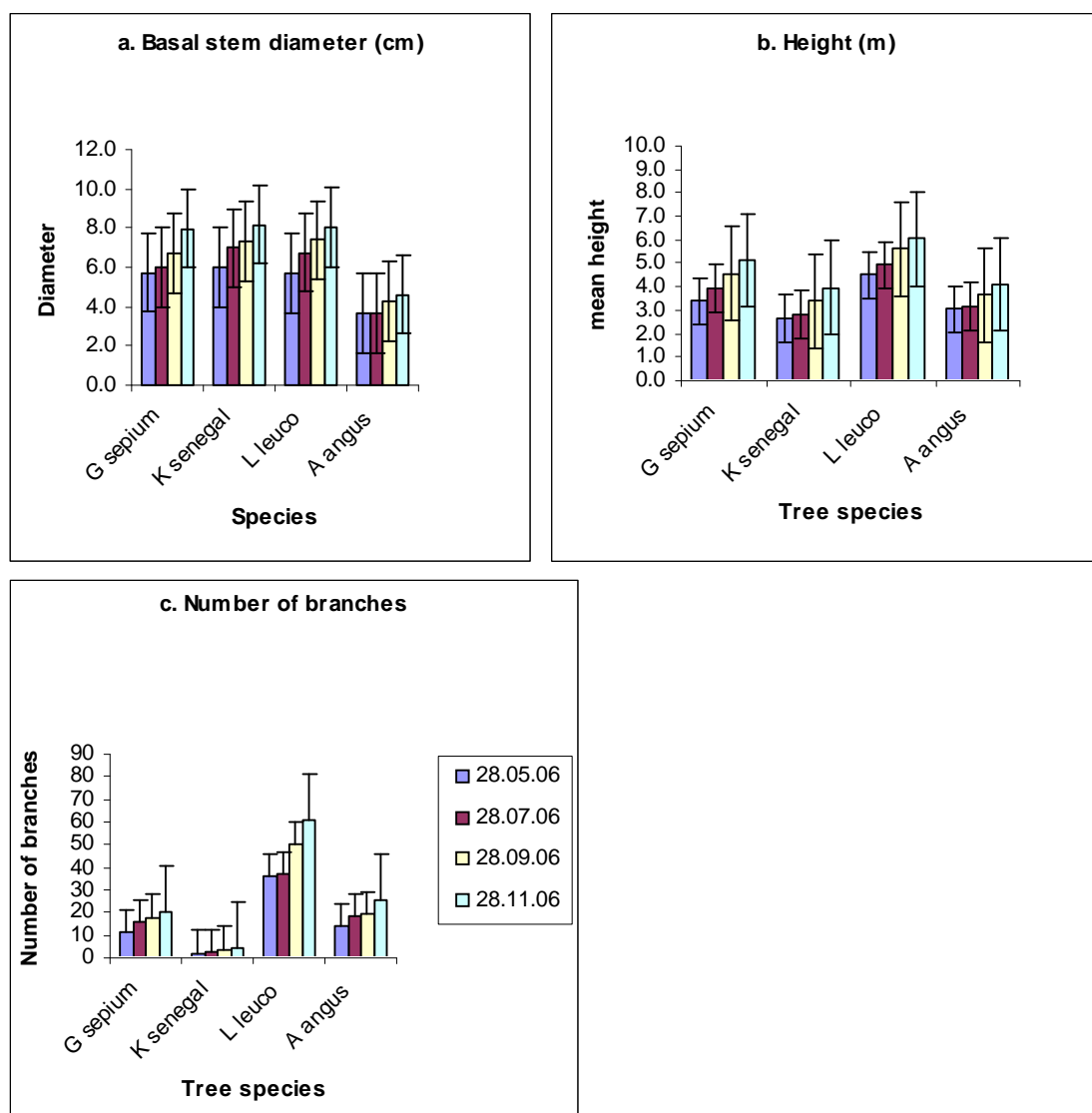


Figure 17: Change in basal stem diameter, height and number of branches of the different tree species in Experiment 1

In experiment 2, the results of the analysis of variance showed very high significant differences ($p = 0.000$) between species for basal stem diameter and plant height. The best performing group was formed by *Acacia mangium*, *Leucaena leucocephala* and

Gliricidia sepium with a mean diameter at the base of 9.25 cm and a mean height of 6 m.

In terms of the number of branches, four groups with statistically significant differences between the numbers of branches of tree species can be distinguished with $p = 0.000$. The number of branches of Group A (*Acacia mangium* ($n = 63$), *Leuceana leucocephala* ($n = 57$), *Acacia senegal* ($n = 55$) and *Acacia auriculiformis* ($n = 51$)) were significantly higher than Group B with species like *Gliricidia sepium* ($n = 37$), *Acacia crassicarpa* ($n = 37$) and *Acacia angustissima* ($n = 29$). The third group is formed by *Calliandra calothyrsus* ($n = 16$) which is an intermediate species between group B and C. The last group with fewest branches is formed by two local species *Khaya senegalensis* ($n = 6$) and *Pterocarpus lucens* ($n = 0.5$). The number of branches of *Khaya senegalensis* is among the lowest which is in conformity with the data obtained in the first experiment

Work package 3. To assess and understand the adaptation factors of these numerous species, climatic data, soil water status and water used (sapflow measurement) data were collected.

The crop evapotranspiration (ET_0) was calculated using the FAO software CROPWAT 5.7 because the major parts of the effects of various weather conditions are incorporated into its estimation. So, parameters such as temperature, relative humidity, solar radiation, wind speed, and location (altitude and latitude) have been measured (Table 4).

Table 4: Climatic data in a semi arid region in Siribala (Mali).

Month	mm day ⁻¹ ET	Wm ⁻² Solar radiation	m.s ⁻¹ windspeed	% Air R Humidity	°C Air temperature	mm 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

The ET_0 provides a standard to which evapotranspiration during the whole year or in other regions can be compared. It also allows comparison of evapotranspiration of different species. For instance, Figure 18 shows the mean measurement of the ET_0 during the January-November study period. The measurements have high correspondence to the mean solar radiation energy available during the clear days. Cooler temperatures in January were characterized by lower solar radiation compared to the July-October period where they were both higher. This situation combined with high temperatures and high wind speed may lead to an increase in plant transpiration and a great demand for water. However the presence of a particular calm condition characterized by a wind speed less than 0.5 m s^{-1} during the October-November period should be mentioned because it had an effect on irrigation frequency which decreased.

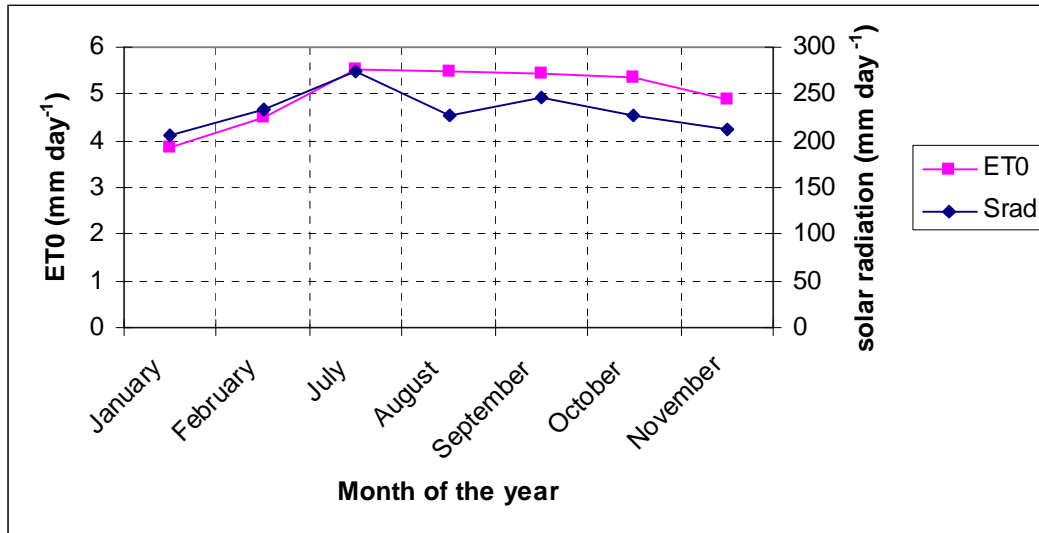


Figure 18: Monthly mean reference crop evapotranspiration measured during the study period at Siribala in 2006.

The measurements of soil water content concerned four species corresponding to four treatments with or without inoculation (rhizobium +/- mycorrhizas) at various depths (100 to 1000 mm). Soil water content was determined during two contrasting periods: January with cooler temperatures and August with high temperatures when the trees might be expected to transpire more water. The results showed that there were no significant differences in soil water content according to species ($p= 0.484$ in January and 0.261 in August) but it varied significantly according to depth ($p= 0.033$). In January or in August, for all the species, with or without inoculation, the soil water content is smaller from the soil surface to 30 cm depth (Figure 19) than in deeper soil layers.

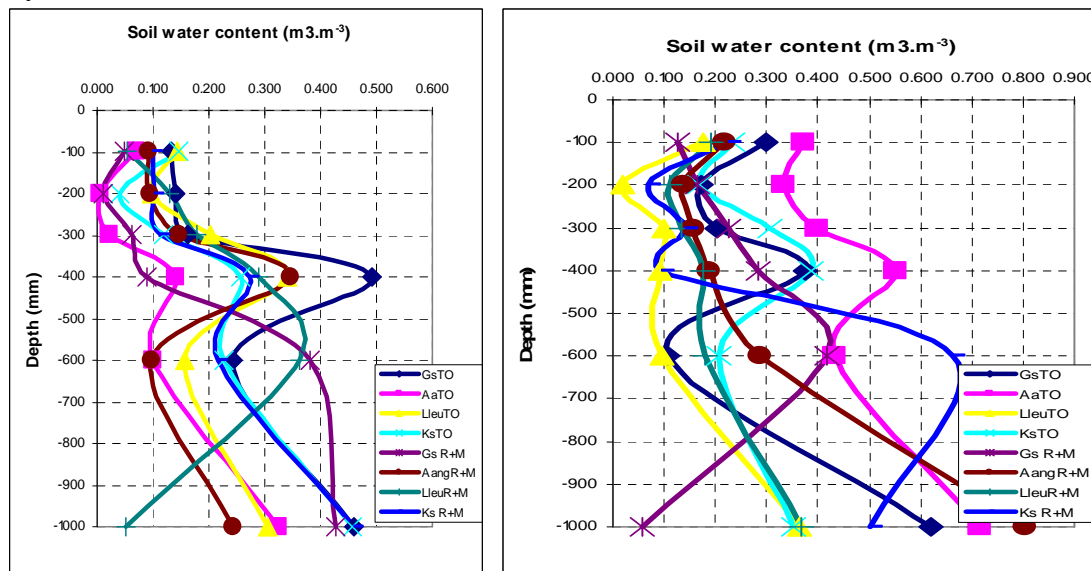


Figure 19 Vertical profiles of soil water content ($m^3 m^{-3}$) in January (left) and August (right) in 2006, at Siribala. Data are means of 18 measurements for each depth. TO = uninoculated plants, R+M = plants inoculated with rhizobium and mycorrhizas. GS = *Gliricidia sepium*, Aa = *Acacia angustissima*; Lleu = *Leucaena leucocephala*, Ks = *Khaya senegalensis*.

Sapflow of four species (*Leucaena leucocephala*, *Gliricidia sepium*, *Acacia angustissima*, and *Khaya senegalensis*) with or without inoculation were monitored in experiment 1. Data from July in the beginning of the rainy season and August when the profile is wet have been used to compare these species Figure 20, Figure 21 .

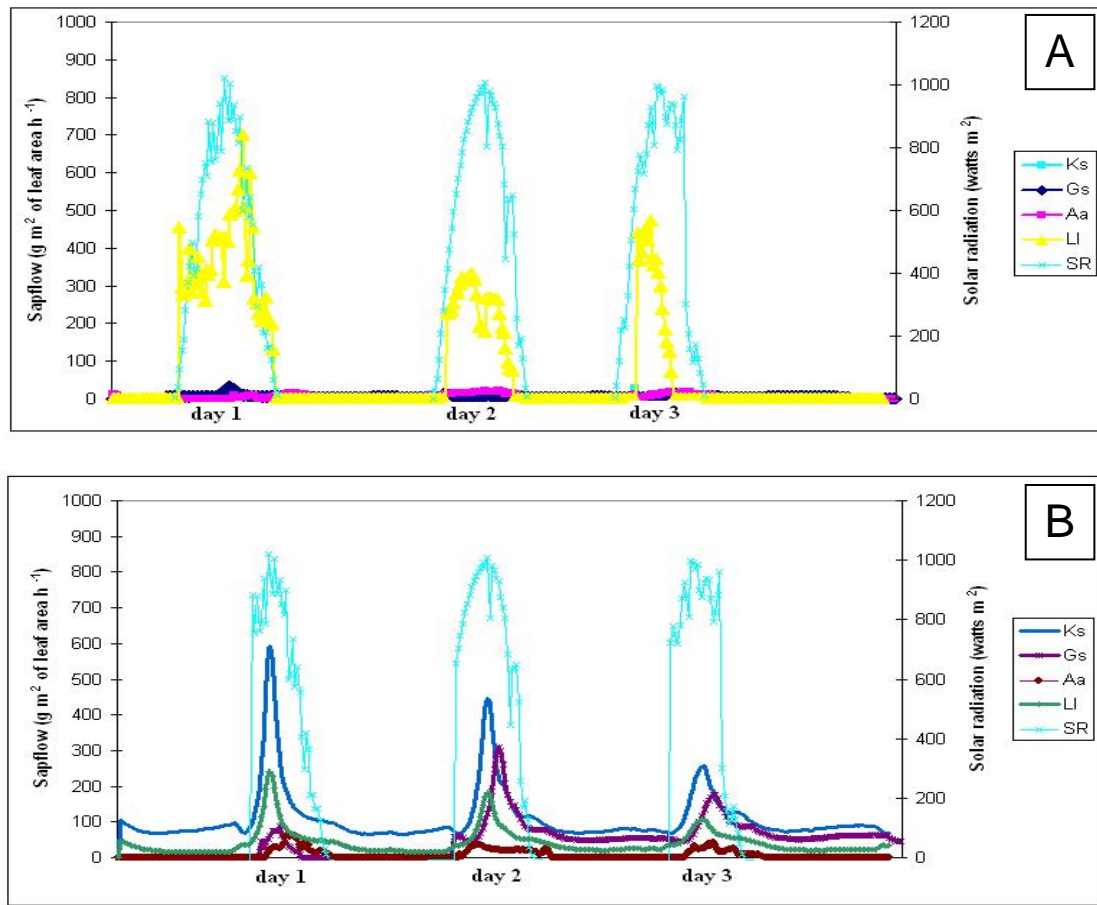


Figure 20: Comparative water use of four tree species without (A) and with (B) rhizobial and mycorrhizal inoculation and the flux of solar radiation (SR) on three successive days in July at the beginning of the rainy season at Siribala, Mali.

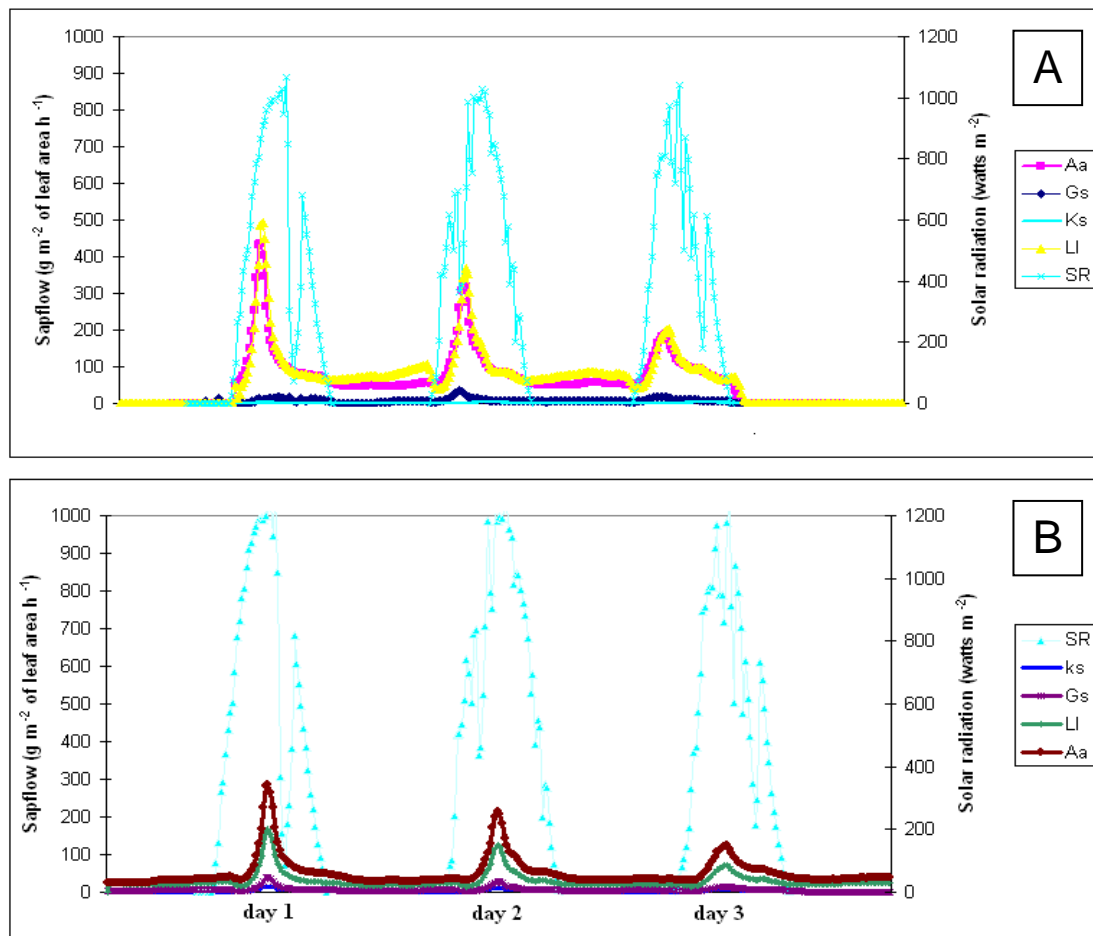


Figure 21 Comparative water use of four tree species without (A) and with (B) rhizobial and mycorrhizal inoculation and the flux of solar radiation (SR) on three successive days during the rainy season in August at Siribala, Mali.

Seasonal variation was found in terms of relative transpiration of different species and the effects of inoculation. It appears that the indigenous species (*Khaya senegalensis*) used more water than the exotic ones (*Gliricidia sepium*, *Acacia angustissima*, *Leucaena leucocephala*) when inoculated at the beginning of the dry season. In August, during the rainy season, the sapflow was greater with *Acacia angustissima* and *Leucaena leucocephala*. This remark is also valuable for this two species without inoculation in July.

The maximum of sap flow was always observed at midday (with or without inoculation) as does the solar radiation. This fact can be explained by the great radiation flux which emphasizes transpiration and photosynthesis. Solar radiation was higher in July than in August (Figure 18) which can result in high water demand. This situation probably explains why the sapflow amount reaches for instance 700 g m^{-2} of leaf area h^{-1} for *Leucaena leucocephala*. Wind speed and solar radiation were also higher in this period.

The high relative humidity from July to September (Table 4) may explain the low sapflow amount in August, mainly in the inoculated plots. In August, the soil water content is high (Figure 19), and the solar radiation is low.

It appears that the **least adapted** species in semi-arid zone of Siribala is *Acacia angustissima*. *Leucaena leucocephala* also has a high water use, but less than *Acacia angustissima*. In relatively humid areas this species could be advised because it produces a **great** amount of biomass.

Work package 6. The examination of the profiles shows that the textures of these soils are loamy-sand on the surface horizons and gradually more clayey in-depth with a presence of more or less tender ferruginous concretions. For these reasons these soils are classified as “ferrugineux tropicaux lessivés” (CPCS, 1967) or ultisols (Soil taxonomy, 1998).

The amounts of carbon, nitrogen, phosphorus, Ca, and Mg are low as is usual in the highly weathered soils from tropical regions of Africa (Table 5).

Table 5 Soil C,N,P content and pH in various profiles in Minimana

Profiles	Depth	N (g kg ⁻¹)	C (g kg ⁻¹)	C/N	P(total) (mg kg ⁻¹)	pH (H2O)	pH (KCl)
Profile n°1 (P1)	0 - 20	0,61	4,52	7,38	259,17	5,2	5,0
	20 - 60	0,34	2,31	6,87	248,76	4,3	3,7
	60 - 110	0,24	1,64	6,73	231,23	4,9	4,2
	110 - 195	0,15	0,44	2,90	226,48	5,5	5,1
Profile n°2 (P2)	0 - 20	0,43	3,30	7,64	226,48	4,6	4,3
	20 - 40	0,34	1,77	5,25	213,65	4,6	3,7
	40 - 120	0,24	1,03	4,23	204,97	4,9	3,8
	120 - 200	0,15	0,52	3,42	205,89	5,0	4,4
Profile n°3 (P3)	0 - 25	0,71	5,80	8,16	212,20	4,1	3,8
	25 - 55	0,34	2,87	8,43	206,00	4,1	3,5
	55 - 120	0,25	1,78	7,21	202,95	4,5	3,6
	120 - 185	0,20	1,24	6,22	204,97	5,0	4,4

Fuel wood and fresh fodder production

After two years, coppicing was done on all the species at two heights: 25 cm and 50 cm above the soil surface in order to assess the regeneration capability for each species. The amounts of fuelwood and fodder were determined.

Experiment 1

Fodder production and fuelwood production are significantly different between species (Table 6).

Table 6 Dry fuelwood and fodder production (t ha⁻¹) by various species inoculated with or without rhizobium and mycorrhiza in semi arid region in Siribala (Mali).

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

Lleu (*Leucaena leucocephala*), Gsep (*Gliricidia sepium*), Aang (*Acacia angustissima*), Ksen (*Khaya senegalensis*),

Figure 22 shows this trend. Inoculation had a positive effect on plant production ($p = 0.0087$ for fuelwood production and $p = 0.0294$ for fodder production). Fuelwood production is 67% greater than the control (29.150 t ha⁻¹ against 17.450 t ha⁻¹) and fodder production is 35% greater (23.750 t ha⁻¹ against 17.600 t ha⁻¹).

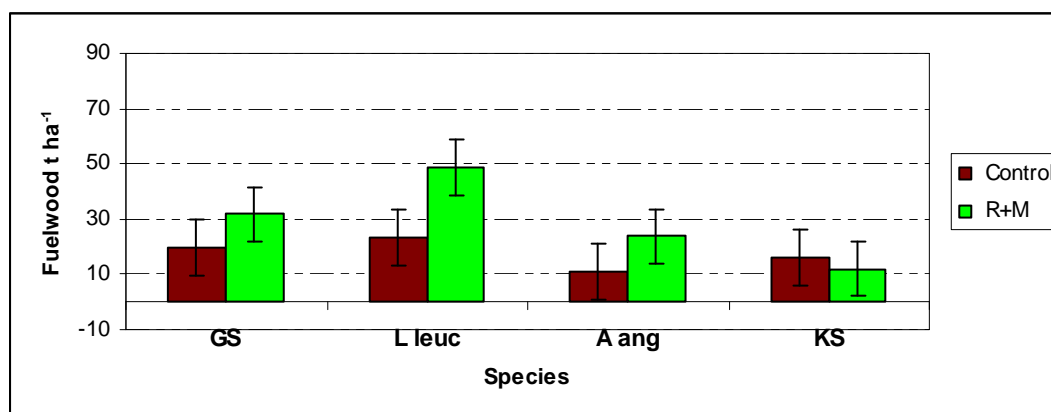


Figure 22: Dry fuelwood production by four species inoculated or not with rhizobium and mycorrhiza in semi arid region in Siribala (Mali).

The production rates obtained are comparable to those reported by the organization TREES FOR THE FUTURE (2006) which mentioned 60 t ha⁻¹ for *Leucaena* in 3 years plantation.

Fodder production follows the same tendency (Figure 23).

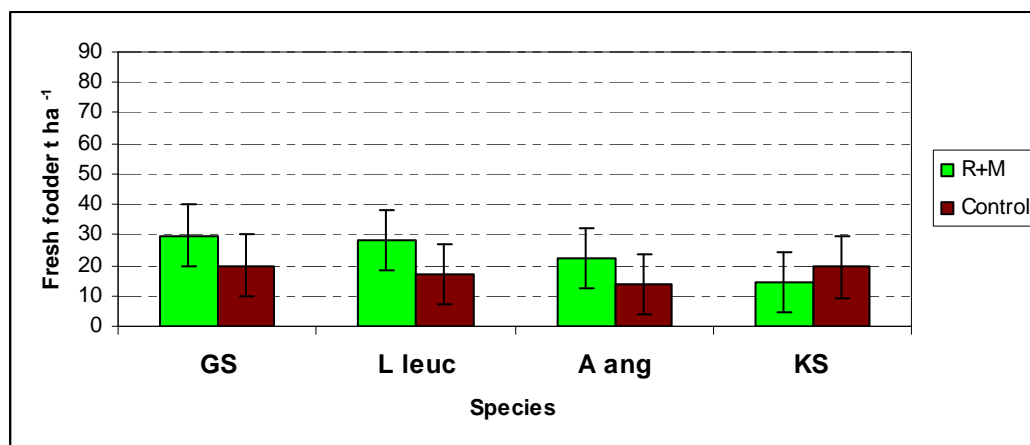


Figure 23: Fresh fodder production of four species inoculated or not with rhizobium and mycorrhiza in semi arid region in Siribala (Mali).

The results show that these species are very sensitive to competition. In the experiment 2 because of the number of species (10) and the small spacing between trees the production for the same species diminished considerably. For instance *Khaya* in experiment 2 produced only 0.5 t ha⁻¹ of fuelwood when mixed compared with 14 t ha⁻¹ in pure plantation in Experiment 1. However benefit can be gained by planting many species because of the diversity gained in fodder and the management of risk (such as plant health and climatic constraints).

Experiment 2

The means of dry fuelwood and fodder were unaffected by coppicing height (respectively $P = 0.5799$ and 0.9240 with a probability level of 5%) concerning a cutting height of 25 cm measured from soil surface (respectively 2.9638 t and 4.1153 t), compare to those of 50 cm (respectively 3.2491t and 4.2010 t). However, the dry fuelwood production differed significantly according to species (Table 7) and it ranged from 1 kg ha⁻¹ to 7.6916 t ha⁻¹. *Pterocarpus lucens* had the smallest production (only 1 kg ha⁻¹ of dry fuelwood and 56 kg ha⁻¹ of fodder) because it genetically grows very slowly but also because it doesn't tolerate competition. *Leucaena leucocephala* and *Acacia mangium* had significantly greater dry fuelwood production followed by *Gliricidia sepium*, *Acacia auriculiformis* and *Acacia angustissima*. There are 4 species (*Acacia crassicarpa*, *Acacia senegal*, *Calliandra calothyrsus* and *Khaya senegalensis*) in which the dry fuelwood means are not significantly different from one another. One surprising and interesting thing is the production of *Khaya senegalensis* which is well known as a very slow growing species (Yengue and Callot, 2002). In our screening experiment where 10 species are mixed, the production of this species can be evaluated up to 5 t ha⁻¹ and 7.7 t ha⁻¹ respectively for fuelwood and fodder, after only 2 years.

With 14.333 t ha⁻¹, *Acacia mangium* had the greatest fodder production which is double that of its dry fuelwood. *Acacia auriculiformis* which produced less wood (3.7912 t ha⁻¹) produce much fodder (6.5667 t ha⁻¹).

To summarize, the fuelwood and fodder production are greater for *Acacia mangium*, *Gliricidia sepium*, *Leucaena leucocephala*, *Acacia auriculiformis*. But the best is *Acacia mangium* which produces much wood and much fodder.

Table 7. Fuelwood and fodder production (t ha⁻¹) of various species in semi arid region in Siribala (Mali).

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

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

Conclusion:

Acacia mangium has the greatest fodder production which is double that of its dry fuelwood. *Acacia auriculiformis* which produce less wood (3.7912 t ha⁻¹) produced much fodder (6.5667 t ha⁻¹). In the screening experiment *Acacia mangium* seems well adapted to the site conditions and is one of the best performing species than the other Australian Acacias in our irrigated condition in Siribala. *Acacia angustissima* which was chosen shows a poorer performance in the field, with high water demands and small production (2 t ha⁻¹ of fuelwood and 1.7 t ha⁻¹ of fodder).

The inoculation with rhizobium and mycorrhiza had a positive effect on plants production. It is 67% greater than the control (29.150 t ha⁻¹ against 17.450 t ha⁻¹) for fuelwood production and 35% for fodder production (23.750 t ha⁻¹ against 17.600 t ha⁻¹).

References:

YENGUE J.-L., CALLOT Y. (2002) L'arbre et la ville dans la région de Maroua (Extrême-Nord-Cameroun). Science et changements planétaires / Sécheresse. Volume 13, Numéro 3, 155-63, Septembre 2002, Notes originales.

TREES FOR THE FUTURE (2006).

www.treesftf.org/resources/pops/Leucaena%20FR.pdf

Partner 3 FAST, University of Bamako, Mali

Objectives

The overall objective in Mali is to use irrigation to continuously produce fodder and fuelwood in the peri-urban zones of Niono city in semi-arid sub-Saharan Africa by installing low cost, low technology systems of industrial and domestic wastewater purification and their effluent used to irrigate fast growing plantations

Workpackage 4: Microsymbionts and Nitrogen fixation

Main objective is to utilise microsymbionts to minimise the need for fertiliser application and to verify their persistence and effectiveness using molecular tools.

Two sites : Sotuba (Bamako) and Minimana (Siribala, Niono) were chosen to produce tree species.

At Sotuba, eleven (11) tree species (4 indigenous and 7 exotic) were tested in nursery (Table 8) in order to select the best for forage and food production at Minimana under irrigated conditions. Seeds of local tree species *Acacia senegal*, *Acacia seyal*, *Pterocarpus lucens* and *Khaya senegalensis* were harvested at two sites in Mali (NIONO and CINZANA). Seeds of exotic tree species were obtained by the team of IER. The experiment was set up at Sotuba using a randomised complete block design with 5 replications and four treatments were used: (R: Inoculation with Rhizobia; M: Inoculation with mycorrhiza; R+M: Rhizobia + Mycorrhiza; T: Control (without inoculation)).

Table 8: Tree species and rhizobia strains used in nursery and field experimentation at Sotuba and Minimana (Mali).

Tree species	Rhizobial strains	Mali
<i>Acacia crassicarpa</i> , <i>Acacia mangium</i> , <i>A.cacia auriculiformis</i>	13C + 11C	•
<i>Leucaena leucocephala</i>	LDK4	•
<i>Gliricidia sepium</i>	GSK4	•
<i>Calliandra calothyrsus</i>	KWN35	•
<i>Acacia senegal</i>	CIRADF 300 ; CIRADF30 ; CIRADF 302	•
<i>Acacia seyal</i>	ORS 3324 ; ORS 3327	•
<i>Pterocarpus lucens</i>	13c + 11c	•
<i>Khaya senegalensis</i>	M	Burkina Faso

At Sotuba four tree species: *A. angustissima*, *Gliricidia sepium*, *L. leucocephala* and *L. Khaya senegalensis* were selected according their performance in term of growth and inoculation effect (R+M). *A. angustissima* followed by *Leucaena leucocephala* were the best performing leguminous trees.

At Minimana site (Siribala) selected tree species were produced in the nursery using two treatments. All plants were irrigated using waster water originated from rice

fields and collected in a new channel. Experimental designs were similar in each country.

Assessment of the value of inoculation was done using experiment 1 and 2 in nursery and field. According to previous protocols, measurements of growth parameters: height, root collar diameter, biomass production, nodulation were performed for the following 4 tree species: *A. angustissima*, *Gliricidia sepium*, *L. leucocephala* and *Khaya senegalensis*,

In the nursery, data were collected at 3, 5 months after sowing in plastic bags containing soil from the Minimana site

After planting in field trials, measurements were performed at 4, 9, 12, 15, 24 and 27 months after planting.

The data collected were analysed by one-way ANOVA using Minitab. Separate analyses were run for each tree species. Results from the nursery stage of these studies are summarized in Table 9 and Table 10.

Table 9: Growth of trees with and without inoculation in the nursery, 5 months after sowing (Experiment 1), just prior to planting in the field

Tree species	Treatment	Root Collar Diameter (cm)	Height (cm)	Dry biomass (g)		Nodules per plant	
				shoot	roots	number	dry weight
<i>A. angustissima</i>	R + M	0,515 a*	50,850 a	4,35 a	2,37a	24,000 a	195,333 a
	Témoin	0,555 a	56,950 b	7,13 a	3,98 a	45,667 a	182,333 a
<i>Gliricidia sepium</i>	R + M	1,010 a	52,700 a	5,80 a	2,13 a	30,333 a	114,667 a
	Témoin	1,020 a	47,828 a	8,58 a	3,88 a	52,333 a	349,667 b
<i>L. leucocephala</i>	R + M	0,740 a	59,650 b	6,13 a	4,18 a	22,333 a	235,333 b
	Témoin	0,710 a	44,450 a	5,13 a	5,10 a	10,667 a	95,000 a
<i>Khaya senegalensis</i>	M	0,610 b	27,500 a	1,65 a	0,62 a		
	Témoin	0,540 a	26,300 a	1,27 a	0,49 a		

* within a species, values superseded by different letters are not significantly different at p = 0.05.

In experiment 1 (Table 9), double inoculation (R+M) improved the height of *L. leucocephala* and mycorrhizal inoculation improved the root collar diameter of *K. senegalensis*. Inoculation had no significant effect on biomass production, root collar diameter of leguminous trees and nodulation of *A. angustissima* and *Leucaena leucocephala*. Height of *Acacia angustissima* and nodule dried biomass of *G. sepium* were better without inoculation (T).

Table 10: Growth of trees with and without inoculation in the nursery, 5 months after sowing (Experiment 2), just prior to planting in the field

Tree species	Root collar diameter (cm)	Height (cm)	Dry biomass (g)		Nodules	
			Aerial	Roots	Number	Dry weight
<i>A. angustissima</i>	0,530 a	52,350 c	9,000 ab	6,217 b	33,000 a	233,667 a
<i>Gliricidia sepium</i>	1,100 c	46,300 b	14,733 b	7,133 b	34,333 a	291,000 a
<i>L. leucocephala</i>	0,690 b	58,350 d	7,900 ab	6,800 b	13,667 a	288,000 a
<i>Khaya senegalensis</i>	0,605 ab	23,950 a	3,600 a	1,217 a		

In experiment 2 (Table 10) relevant biomass, height and nodulation were recorded. *Gliricidia sepium* and *Leucaena leucocephala* grew better than the other species.

In the field, the effect of inoculation on plant growth parameters and nodulation at different periods is given in Table 11 and Table 12. Per treatment, 10 trees were used for height and collar diameter measurements and three trees were used for nodulation studies. Data collected were analysed using ANOVA.

- **Experiment 1.- Plant growth parameters.** In February (9 months after planting), no significant effect of inoculation on the *A. angustissima* and *Khaya senegalensis* was noted. But significant effects were obtained for height, and collar diameter of *Gliricidia sepium*, and height of *Leucaena leucocephala*. (Table 11)

In May and September 2006, 12 and 15th months after planting respectively, 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, 9 months after planting, we noted increased root collar diameter of *Gliricidia sepium* and *Leucaena leucocephala* relative to the other species, and increased height of all tree legumes.

In May, 12 months after planting significant effects of inoculation is related to the increased collar diameter of *Gliricidia sepium* and *Leucaena leucocephala*, (Table 12), increased height of all tree legumes were noted. But 15 months after planting, no significant effect of inoculation on plant growth was noted

Nodulation. Nine (9) months after plantation (February 2006), inoculation was associated with improved height growth of *Acacia angustissima*. At 12 and 15 months after transplanting (September 2006) no significant effect of inoculation on plant nodulation was found.

- **Experiment 3.** At all time periods, inoculation of selected tree species with specific strains had no significant effect on plant growth and nodulation.

At 21 and 24 months after planting, the effect of inoculation on plant growth and nodulation was not assessed, because of flooding at the site.

Table 11 Plant growth 9 months after planting in the field, in Experiment 1.

Tree species	Parameter			
	Diameter (cm)	Height (cm)	NNod (plt ⁻¹)	Nod weigh mg ⁻¹
<i>A. angustissima</i>	2,200a	253,000b	139,000b	401,700b
<i>G. sepium</i>	5,400b	257,400b	45,667a	136,267a
<i>L. leucocephala</i>	4,600b	292,800b	12,667a	93,967a
<i>K. senegalensis</i>	2,000a	95,600a		

Table 12 Plant growth, 12 months after planting in the field, in Experiment 2.

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

Rhizobia molecular characterization.

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

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

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

Workpackage5. Economics & quality of produce

Questionnaires were used to collect data and information and main results on utilisation of wastewater for fuelwood and fodder production at different levels: villages, associations, households were developed in French. Data are presented in the annual report. Some of the exotic species received good evaluations from local populations. The majority of the population thought that waste water quality and quantity was good. Waste water was used for a variety of activities, but assessing the quantity of water for each use was not done. Most wood, for all purposes, is collected locally. Small amounts are purchased, or come from other localities. The main constraints to wood supply are scarcity, distance, quantity and quality. Annual consumption for domestic use varies considerably, from .26 - .87 T ha⁻¹ y⁻¹.

Information on costs, margins and prices of existing production chains and systems have been obtained, and several meetings were held with end users, NGOs, womens' groups, which were documented by film and photographs.

Workpackage 6: Water, Soil and plant nutrition

The objective is to analyse irrigation water (wastewater), soil and plants for nutrient contents and bacterial occurrence

Analyses of nutrient content, heavy metals and presence of pesticide residues in wastewater used for food and fuelwood production at Minimana site were performed at least twice each year, samples were taken before and after irrigation, usually in February, May and September/October.

For analysis water were sampled at three places: Minimana where experiments were carried out: water used for plant irrigation at **Minimana** (in a new channel), water coming from ricefield considered as input to the new channel (**ricefield**, collected before plant irrigation), water collected after plant irrigation output of new channel (named **Collector**).

In another site, water of an old channel with *Typha* plant is analysed as control because water was supposed purified by biological systems such as typha.

Results (see annual reports) highlighted the bad quality of water, based on color, turbidity, DCO and orthophosphate concentration and some times iron. However, in general, water physical and chemical contents conform to WHO standards. For pesticide residues, there were no water detectable pesticide residues at the specified "LOD". No risk in relation to plant irrigation were found. However, these results should be interpreted with caution because of the limited number of analyses.

Analysis of collected soil and plants samples for nutrient contents were undertaken by the Laboratory of IPR/IFRA. Because of unavailability (accident, moving) of responsible of this workpackage main results are not provided.

Workpackage 8: Pest Monitoring and Management

The objective is to define the risks of attack by pests or diseases caused on exotic trees species under irrigated conditions

Before setting up all experiments at *Minimiana*, soil used in nursery was investigated for nematodes, termite occurrence and attacks by insects and other predators. In nursery and field trials investigations were performed using experiments 1 and 2 by searching nematodes, termites occurrence, attacks by insects and others predators. Pests and plants climate constraints and tolerance were taken into account.

In nursery. at 3 and 5 months of plant growth pests, nematodes were investigated in soil of plastic bags and in plant roots. Observations on aerial parts of plants were performed.

In field trials 6 and 9 months after plantation, soil and plants roots were sampled and analysed for nematodes investigation 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 assessed for nematodes roots investigation.

In all cases, plant health were observed regularly and particular surveys of exotic plant growth and pests was done.

Main results for lab investigations pointed out occurrence of nematodes in the soil and plants roots as summarised below.

At 3 months primary main results pointed out:

- morphological good aspect of plants, no attacks by pest, green plants are observed
- no phytophageous nematodes in the roots.
- occurrence of nematodes species in the soils.
- occurrence of nematodes in uninoculated *Gliricidia sepium* and *Khaya senegalensis* tree species

At 5 months after growth just before transplantation in field trial no phytosanitary problems were detected in the 2 experiments. We found no nematodes in the roots of *Gliricidia sepium* contrary to observations at 3 months. We explained that fact by a possible effect of plastic bags used in nursery. Plastic bags could affect the survival of nematodes. Also, at 5 months after growth *Gliricidia* has good lignification and could develop resistance against nematode penetration in the tissues.

In nursery soil, in plastic bags contains the lowest number of nematodes when inoculation of plants with (Rx M) was applied. Field soil had 4800 nematodes per litre of soil just before the planting.

Nine (9) and Twelve (12) months (February and May) after planting before coppicing, under irrigation conditions all observations made during dry season, showed adverse effect of attacks by termites on two tree species. Those attacks lead to the reduction of numbers of *Leucaena leucocephala* trees and poor survival of *Calliandra calothyrsus* in both experiments 1 and 2.

Twenty four 24 months after planting corresponding to dry season (May 2007), we also observed that all experiments were invaded by crickets or locusts identified as *Micraturia microtaurides*. But no significant damage was noted because the problem was overcoming by rapid intervention of local population and authorities, project technician and technical service agencies.

After trees coppicing 27 month after plantation (MAP), September 2007, in the rainy season the experimental site was flooded by water. All plots planted with *Acacia angustissima* were inundated by water causing the death of this tree species: film and CD are available to document this aspect.

Conclusion: In field, according to observations on plant health in experiment 1 *Gliricidia sepium*, *Khaya senegalensis* followed by *Leucaena leucocephala* are more resistant than the others to termites and pest in irrigation conditions. In Experiment 2, local trees were well adapted. In all experiments *Acacia angustissima* and *Calliandra calothyrsus* seemed the most affected by termites and climate as environmental factors.

Meetings and visits

Coordination meetings 2 (Mali and Burkina Faso)

5 Meetings with Local local groups and rural population in Minimana site where plantations were set up

Several meetings and discussions were held with rural community and different groups concerned by fuel and fodder production in 3 villages.

Visites to experimental site at MINIMANA (Siribala).

Delegation led by Dean of the department of Biology at FAST, University of Bamako ONG AAVNU,

Association KAFO des femmes de Siribala
Visites des autorités municipales de Siribala
Association DEMBAGNOUMAN des femmes de Siribala et de Laminibougou
Association KAVURAL des Eleveurs peulhs
SCP and all partners involved in the project

Collaboration was developed at different levels and covered topics such as training, implementation of field activities, coordination, dissemination of results, local considerations, sample analysis , publications.

National Institutes and NGOs

IER

IPR/IJRA

ONG AAVNU

ISFRA

LQE, LCV

Local communities

Association KAFO des femmes de Sérribala

Visites des autorités municipales de Sérribala

Association DEMBAGNOUMAN des femmes de Sérribala et de Laminibougou

Association KAVURAL des Eleveurs peulhs

Training

Fallaye KANTE at (FAST) and Youssouf CISSE (IER) registered for Ph. D degrees at ISFRA in Mali are completing their theses,

Dr Inamoud YATTARA, Dr Fassé SAMAKE, Bakary SAMAKE and Hamed BATHILY were trained in wastewater treatments by Partner SCP.

In the framework of training this project allows to partner 3 to train 6 persons and to be supported by AUF. Fallaye Kante has been supported by AUF for 4 months at LCM.

Publications (draft)

- 3 articles for publication
- 1 film
- 1 CD
- A Film and photographs were elaborated to document step by step the economics study. According to questionnaire performed by partners in charge of this workpackage

Problems encountered:

Some data as plant sheet and soil samples content analysis are not available because of Diafar CISSE from IPR/IFRA is moved. Due to the great number of samples, analyses of nodules is not yet completed.

Partner 4 report INERA

Mahamadi Dianda, Jules Bayala, Kadidia Sanon and Sibiri Jean Ouedraogo

Objectives of the project

This project aimed to produce a low input, sustainable, irrigated high yielding, fuelwood and fodder production system in the peri-urban zones of dryland cities. A plant production system that guarantees to be permanently productive in the Sahel, requires the use of irrigation to drive growth during the long dry season. Thus, domestic wastewater was recycled and purified to WHO agricultural standards using low cost, low technology systems which are maintainable in DCs. The water was then supplied to experimental one hectare multi-species trials of fast growing fuelwood and fodder trees.

Research methodology

1. Study sites

Two plantations of tree species with high potential for fodder and fuelwood production were established near Ouagadougou. The main one was established and irrigated close to the wastewater treatment installations in the area of the university at Zogona campus. The site was previously cropped to sorghum and contained scattered mature trees of *Senna siamea*, *Khaya senegalensis*, and *Mangifera indica* Figure 24a. The second plantation dealt with a field screening trial at the research station of CREAM (INERA/ Kamboinsé), about 15 km north of Ouagadougou. Studies in nurseries were conducted in an open area at the Département Productions Forestières (DPF) of INERA in Ouagadougou, about 500 m far from the main experimental site. The average monthly rainfall and evapotranspiration trends during the decade 1990-2000 in Ouagadougou are given in Figure 25.

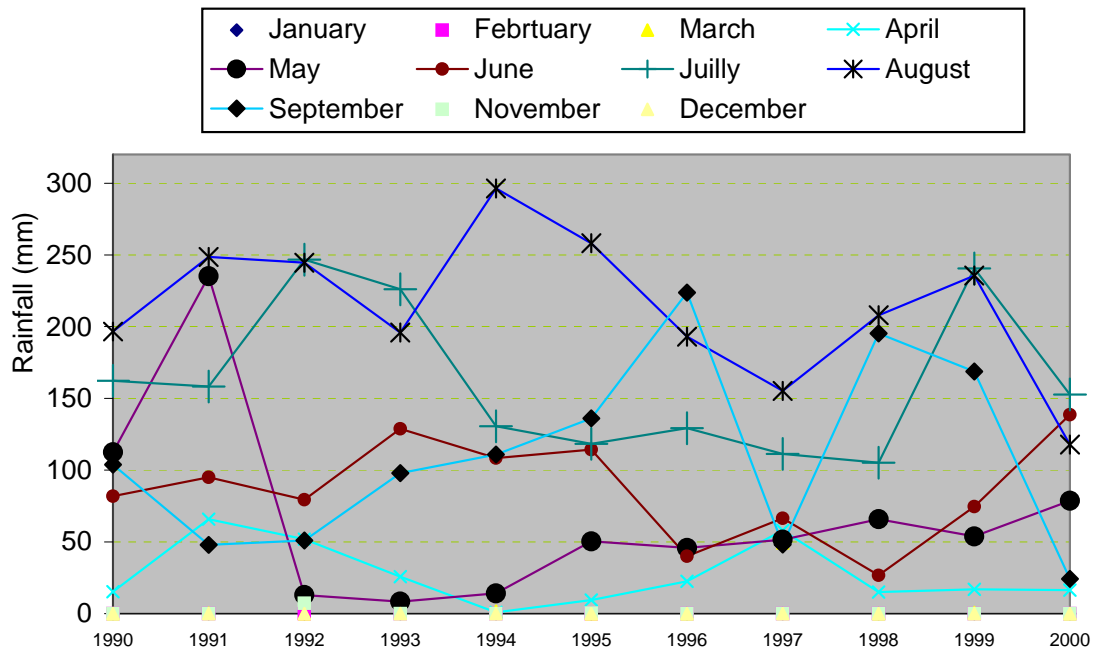
2. Workpackage 1. Water treatment and irrigation

Two important sources of wastewater have been investigated with the assistance of the SCP subcontractor as potential sites for constructing wastewater purification and irrigation systems. One consisted of both Industrial tannery wastewater (TAN-ALIZ) and domestic wastewater from a prison (MACO). The other source, which was exclusively made of domestic wastewater, was from the University of Ouagadougou. The wastewater was used (especially by women) to produce vegetables without any treatment Figure 24b. At this time no wastewater treatment facilities were available on the campus. The latter site was chosen on the basis of soils and wastewater analyses. SCP subcontractor further assisted the local partners to design and install the wastewater treatment equipment. Sampling was done for four weeks and samples were analysed for physical and microbial parameters to monitor the quality of the treated wastewater.



Figure 24 View of (a) the experimental site and (b) the production of vegetables with non-treated wastewaters at the university of Ouagadougou before the start of the project activities; (c) and (d) Fodder and fuelwood tree species plantation irrigated with treated wastewater; (e) uprooting trees to assess stock quality; (f) Nodules (arrows) on the roots of a freshly uprooted *Acacia mangium* tree

a)



b)

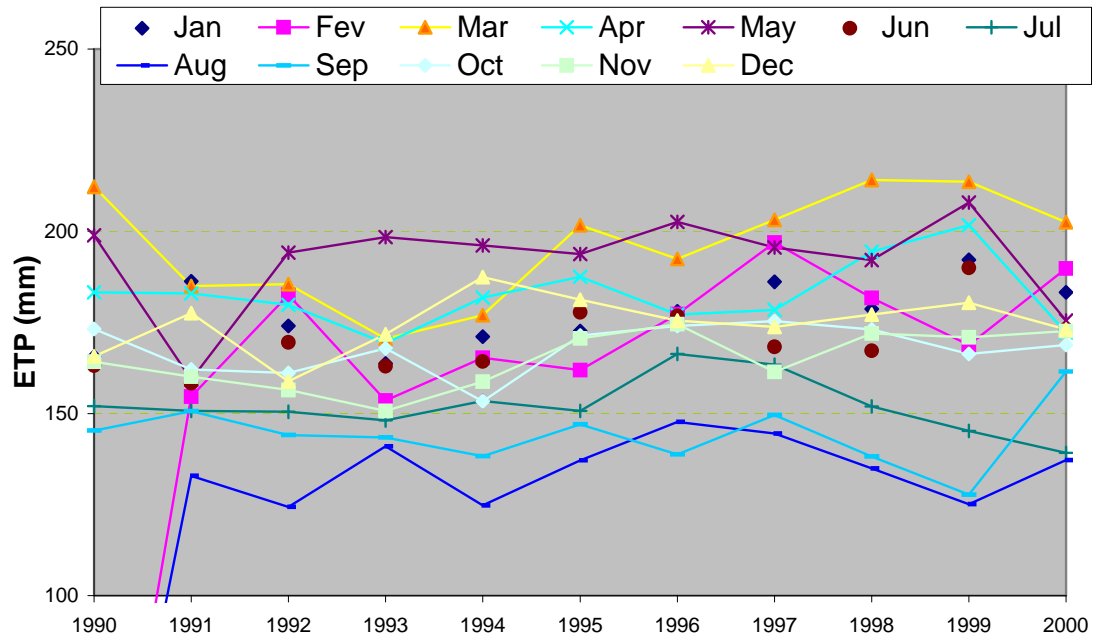


Figure 25 Mean monthly rainfall (a) and ETP (b) during the decade 1990-2000 at Ouagadougou

3. Workpackage 2. Tree growth and management

3.1. Preliminary Experiments

At the launching of UBENEFIT project (meeting of Niamey, Niger), there was an agreement that developing country partners should conduct preliminary irrigation trials in order to screen the potential species as well as microsymbionts, and select candidate species for the main experiments using the treated wastewater. Thus INERA carried out irrigation and inoculation experiments with 20 local and introduced species.

Screening experiments have been carried out both in the nursery and the field. In either case, the seeds were pre-treated following the recommendations of the suppliers. Seed germination rate was almost 100 % for all species except for *A. auriculiformis* and *A. crassicarpa* (< 50%). After germination the seedlings were transplanted in pots containing 2 kg of sand and abundantly watered using well water. Growth and nodulation data were analysed using GLM ($P < .05$) procedures followed by the comparison of the means using Duncan or Scheffe tests with SAS Software package (SAS Institute, inc.).

3.1.1 Experiment 1: Potential growth of irrigated species for fodder and fuel wood production

The trial dealt with the response to irrigation of 20 species having great potential for fodder and/or fuel wood production. Each species was considered as a treatment and was composed of 20 plants. The design was a complete randomised blocks and watering was done twice daily using well water. Plant height and number of leaves were monitored monthly.

3.1.2 Experiment 2 Response of local fodder producing species to irrigation and inoculation with AM fungi

3.1.2.1. Nursery phase: This experiment was conducted with the material locally available (endomycorrhizas, fuel and fodder species). In this trial, the combined effect of both irrigation and inoculation with arbuscular mycorrhizal fungi (AMF) (*Glomus aggregatum*) was evaluated using six local fodder and fuel wood tree species (*Afzelia africana*, *Khaya senegalensis*, *Prosopis africana*, *Senna siamea*, *Ziziphus mauritiana*, *Pterocarpus lucens*). The treatments were the combinations of the six species and the inoculation factor with two levels (inoculated and non inoculated with AMF). Ten plants were allocated to each treatment in a complete randomised blocks design. Four months after planting height, diameter, biomass and nodulation variables were measured.

3.1.2.2. Field phase: Screening of field-grown fodder and wood producing leguminous tree species under irrigated conditions

This trial was carried out aiming at further screening in the field 10 of the N₂ fixing species (*Calliandra calothyrsus*, *Leucaena leucocephala* (local), *Leucaena*

leucocephala, *Leucaena hybrid*, *Leucaena diversifolia*, *Acacia mangium*, *Gliricidia sepium*, *Albizia lebbeck*, *A. crassicarpa* and *A. auriculiformis*) already tested in the nursery. The seedlings of each species were either inoculated (with both AMF and *Rhizobium*) or left non inoculated (control plants) when produced in the nursery. The experiment had 20 treatments replicated three times using a split plot design. Tree species were allocated to 7 m x 4 m plots. The inoculation treatment within tree species were randomly affected to sub-plots each of 9 plants (3 rows of 3 trees) planted at 1 x 1 metre spacing. The experiment was established in mi-May 2005 at the experimental station of the CREAM of Kamboinsé. From planting until the onset of the wet season, the plots were irrigated twice weekly by pumping water from a river located nearby the site. The measurements of tree growth (height, diameter and number of branches) were done late October 2005 at the beginning of the dry season.

INERA was in charge of producing the endomycorrhizal inoculant to be used for the experiments of all the partners. It was suggested during the meeting of Bamako (2nd year project meeting) to use mixed inoculants, which compared to a single strain inoculant, has better potential to be adapted to flooded sites. Therefore IER provided the inoculant that was multiplied by INERA for all the partners.

3.1. 3. *Experiment 3: Effects of Substrate type, pot size and inoculation with microsymbionts on the growth of six forage and wood producing N₂ fixing tree species*

Treatments were made of the combination of four factors as following:

1. Two substrates: pure sand and normal nursery substrate in Burkina Faso that consists of a mixture of arable soil, sand and manure in the proportions of 2:1:1
2. Two inoculation levels: non-inoculated plants and double inoculated plants with *Rhizobium* provided by IRD and AMF (mixed inoculant)
3. Six species: *Acacia angustissima*, *Acacia mangium*, *Azelia Africana*, *Gliricidia sepium*, *Leucaena hybrid*, *Leucaena leucocephala*
4. Two container sizes: small plastic pots and big plastic pots.

3.1.3.1. *Nursery trial*: The design was a split split plot with three blocks. Seeds were pre-germinated and then transplanted to containers at the end of June 2005. All treatments were watered twice a day using tap water. The measurements of plant growth were done three months after planting. At the beginning of September 2005, the plants from each treatment were removed and plant height, root collar diameter were measured. Shoot systems were severed at the root collar, and then all plant parts were dried separately to constant weight and weighed.

3.1.3.2. *Plantation trial*: For the individuals to be transplanted in the field the nursery experiment described above was replicated in mi-June 2005 and the plantation took place at the end of December 2005 nearby the wastewater treatment station and irrigated with treated wastewater. The plants were established at 2 m and 1 m spacing between and within lines, respectively. The design was the same as described above in nursery studies. The treatments were arranged in a four-order split design and replicated 3 times. Species was randomly attributed to main plot, inoculation to the sub-plot, the substrate to sub-sub-plot and container size to fourth order plot. Thus, 8

plants within blocks represented each tree species. The whole trial was established in duplicate to allow destructive observations. Plant growth was monitored in early August 2006 and also in mi-September 2006.

3.2. Main experiment

The main field experiment was set up in early March 2006 with the four species retained by INERA, i.e. *Acacia angustissima*, *Gliricidia sepium*, *Leucaena* hybrid Lx L and *Azalia africana*. Species were inoculated with *Rhizobium* and AM fungi and grown for three months in the nursery. The plantation was established according to the plan agreed by all partners (2 m and 1 m spacing between and within lines, respectively). The layout was a Latin square with experimental plots comprising 16 plants (4 rows each of 4 plants). Plant growth traits (height, diameter, number of branches) were measured in August 2007 only on the inner 2x2 trees of each plot.

3.3. Tree management

A second plantation similar to the main experiment (except that 3 blocks were used) was established in mi-March 2006 to support tree management studies (coppicing trials). Tree growth measurements were done in mi January 2007. Soil profiles were realized at randomized positions to describe the soil structure. From these profiles, soil samples were collected for chemical analyses before any irrigation activities.

4. Workpackage 3. Tree water use and soil water status

Sap flow rate was estimated from sap flow measured using Stem Dynagauge type SGB35 (Flow32 Dynamax Inc, USA). Data was collected on one tree per species per block for both the main experiment and the preliminary experiment (experiment 512 a & b below). The measurement on each replicate lasted for four days before the equipment was moved to another replicate. The diameters of the stems were also measured and were entered together with the parameters of the sensors into a spreadsheet of Excel 2003 to calculate the sap flow.

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

A weather station was installed in the site of the experiments during the transpiration and soil water content measurements period. The weather station was equipped with sensors measuring the following variables: air and soil temperatures, solar radiation, relative humidity, wind speed and rain.

5. Workpackage 4. Microsymbionts and N-fixation

5.1. Preliminary experiments in the nursery

5.1.1. Experiment 1: Response of leguminous fuel and fodder species to the double inoculation in irrigated conditions

An experiment was conducted to address the growth response of 10 local and exotic N₂-fixing species (*Acacia angustissima*, *Acacia mangium*, *Azelia africana*, *Gliricidia sepium*, *Leuceana diversifolia*, *Leuceana hybrid*, and *Leuceana leucocephala*) to inoculation with AMF and *Rhizobium*. To each species was applied (1) inoculation with AMF (mixte inoculant) alone, (2) inoculation with both AMF and *Rhizobium* and (3) inoculation with *Rhizobium* strain alone, (4) a control with no application of neither AMF nor *Rhizobium*. The seedlings were raised on a sand soil in pots and treatments were randomly arranged in 10 blocks. Four months after planting the following variables were assessed: height, collar diameter, shoot dry weight, root dry weigh, the number of nodules and dry weight of the nodules.

5.1.2. *Experiment 2: Responses of tree species - microsymbionts associations to excessive watering*

An experiment was carried out to address the responses of 20 species to two different watering regimes and inoculation with mycorrhizal inoculants (inoculated vs. uninoculated plants). There were two levels of irrigation using tap water, i.e. “permanently” wet and “temporarily” wet. The pots are watered twice daily with tap water in excess. The design was a split plot (with irrigation in main plots) with 3 blocks. After germination the seedlings were transplanted in pots containing 2 kg sand.

6. **Workpackage 5. Economics and quality of produce**

6.1. *Supply of fuelwood and charcoal and fodder*

The survey for fuelwood, charcoal and fodder supply was conducted by placing interviewers on 12 main roads that were identified by INSD (1987) and are used to introduce these products in the town of Ouagadougou. On these roads, 36 interviewers were recruited and placed 3 by road. The survey lasted one week and was done permanently all day long with one interviewer posted from 6 h to 14 h, the second from 14 h to 20 h and the third from 20 h to 6 h. The data collected were the number of pedestrians, cyclists, carters, and cars of different sizes that transport wood and charcoal.

6.2. *Retailers of fuelwood and charcoal*

Ten quarters have been selected and within each quarter 5 retailers were retained for the survey. With each of the five retailers, 5 bundles of firewood per price were weighed and three piles of charcoal were weighed similarly.

6.3. *Household survey on fuelwood, charcoal and fodder uses*

Three main areas can be distinguished in the town of Ouagadougou, i.e. the old quarters, the residential zone and the new peripheral zones. In each of these three areas, 5 quarters were selected and within each quarter 20 households were surveyed, giving a total of 300 households. The questionnaire on fodder used was administered where there was stockbreeding.

6.4. *Retailers of fodder*

The questionnaire of this survey was administered in three markets of livestock of the town of Ouagadougou. In each of the three markets, 20 retailers were interviewed.

7. Workpackage 6. Soil and plant nutrition

8. Workpackage 7. Planting stock quality

Plant growth traits in the preliminary trials (experiments 3.1.3.1. and 3.1.3.2. described above) were used to derive Sturdiness quotient, Quality index and Shoot:root ratio. These indices were further used to appreciate the quality of the seedlings.

From the morphological characteristics of seedlings in the nursery phase trials (experiment 3.1.3.1.) the seedling quality and performance attributes were assessed through three main indexes: Shoot:root dry weight ratio, sturdiness quotient (height (cm)/root collar diameter (mm)), Dickson's Quality Index (Dickson et al., 1960). The values of the generated indexes were subjected to ANOVA General Linear Model (GLM) using Minitab Software package.

In September 2006, i.e. 14 months after plantation, one plant out two was uprooted and separated in three components: leaves, wood and roots. All the three plant components were dried until constant weight (Annex 11, Fig.1e). The height and diameter at 1.30 m were measured. For the plantation trial, only the ratio Shoot:root was calculated. The data were subjected to ANOVA using GenStat Release 8.11 (Rothamsted Experimental Station) Software package. Due to high rate of mortality in *Azelia africana*, the data of this species was not included in the analysis.

Results

Workpackage 1. Water treatment and irrigation

Experimental site acquisition

At the beginning of January 2004, a consultation held between INERA and the university of Ouagadougou, was concluded by a formal authorisation of the University allowing the implementation of the activities of the project on a site located at the campus of Zogona. On the official authorisation of the university, it is stated that the two collaborators (INERA and the university) can continue their collaboration on the experimental site of UBENEFIT project beyond the lifetime of the project.

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

Installation of the wastewater purification system

The construction of the station for the wastewater treatment was delayed due to technical and administrative difficulties. Thus, the installation of the wastewater treatment equipment was totally completed and was tested only in early December 2005. During the same moment SCP visited INERA to evaluate system and train local partners.

The monitoring of waters quality showed that the equipment successfully improved the quality of the wastewater. Especially the density of microbial populations was decreased in treated wastewater compared with crude wastewater (Annex 2; Table 3). An additional test carried out has shown that the treated effluent was free of various harmful agents (ascaridae, cysts of *Entamoeba coli*, *Giardia Lamblia intestinalis*, etc.) which were found in the crude wastewater. According to the recommendations of WHO (OMS, 1998) the results of the microbiological analyses indicated that the treated wastewater had relevant properties for use in irrigation activities (B category) such as for tree plantations.

Workpackage 2. Tree growth and management

Preliminary Experiments

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

After three months of irrigation, four species (*Gliricidia sepium*, the two *Leucaena* sp. and *Afzelia African*) displayed greater height than the rest of the. *G. sepium* and *A. africana* displayed the highest number of leaves. The results showed acacias and *Pterocarpus* sp. to display the slowest growth and therefore these species should benefit from more management care (such as inoculation treatments) to accelerate their juvenile growth.

Experiment 2: Response of nursery-grown local fodder species to irrigation and inoculation with AM fungi

Nursery trial: Four months after planting, differences were revealed among species for both the height and the diameter with *Prosopis*, *Khaya* and *Afzelia* being the best performing species. *Pterocarpus* displayed the lowest values for the two growth variables. In general, endomycorrhization exerted a beneficial effect on the diameter and the biomass of some species (*Khaya*), unfavorable effects in other cases (*Prosopis*, *Senna*, *Pterocarpus*), and neutral impacts for *Afzelia*, which is a typical ectomycorrhizal species. However, the growth performance was not entirely representative of the effects of endomycorrhization due to the fact that roots of some individuals got through the pots and penetrated in the soil underneath. Such behaviour was typical of local species that used a main tap root that usually pierced the pots.

Field trial: At the end of the rainy season, large differences in growth characteristics occurred between tree species whilst the inoculation factor did not show any effect on tree growth. The observed variation in growth however was attributed to the inherent ability of species to tolerate water stress because the whole site was flooded during the experimental period. The local leucaena and *A. crassicarpa* showed the highest height followed by *Gliricidia sepium*. The local leucaena displayed the highest diameter followed by *A. auriculiformis* and *Gliricidia*. The leucaena from Hawaii and *A. crassicarpa* tended to develop higher number of branches than the other tree species.

Experiment 3: Effects of Substrate type, pot size and inoculation with microsymbionts on the growth of six forage and wood producing N₂ fixing tree species

a) **Nursery phase:** Two months after transplanting there were large differences between treatments in nodule number and dry weight. The details of the results are presented. Significant inoculation x substrate x tree species interactions, and inoculation x tree species x pot size interactions occurred for both nodulation variables assessed. In general *Leucaena* sp. didn't form nodules in the pure sand unless they were inoculated with the Rhizobium mixture, probably suggesting that bacteria able to nodulate these species are lacking in this substrate. Nodule number and dry weight was higher in *Gliricidia sepium* compared with the other nodulating species. However, *Gliricidia sepium* performed the best in nodules formation when plants were inoculated with *Rhizobium* on the pure sand in the small pots. The inoculum was apparently more infective than the indigenous bacteria in this soil where the plants were probably nutrient-limited. *Acacia angustissima* showed a nodulation pattern similar to *Leucaena* sp. except that it didn't form nodules in the substrate mixture.

In general, all tree species were mycorrhizal at the harvest time except *Azadirachta indica* which is known to be a non-endomycorrhizal species. The mycorrhizal infection of species was not altered neither by the pot size nor the inoculation treatment. There were significant tree species x substrate interactions. In general, the mycorrhizal infection was several times higher in the sandy substrate compared with the conventional nursery soil. However, these differences were the greatest in *A. angustissima* and *L. hybrid*.

b) **Plantation phase:** Data collected in August 2006 showed that the two leucaenas species performed better in height growth (4.0 m) and diameter (> 3 cm). Out of the various species studied, *Azadirachta indica* showed the poorest growth with heights about 0.3 m. In general, most species performed the best in height and diameter when raised on the conventional nursery soil compared with the sandy soil. The inoculation treatments didn't appear to affect the growth trends of the various tree species. However they did affect the form of the trees in terms of numbers of stems formed by each plant. Three groups were distinguished by the number of stems: in the first group (*L. hybrid* LxL and *Azadirachta indica*) the species only had one stem tree⁻¹. The second group comprised *A. mangium* and *L. leucocephala* which formed multi-stemmed trees only on the sandy soil, and similar number of stems tree⁻¹ in all inoculation treatments. The last group was constituted by *A. angustissima* and *G. sepium* which responded differently to inoculation treatments depending on the soil substrate. The inoculated trees of *A. angustissima* showed almost two times as much number of stems on the ordinary nursery substrate, compared with the control, whereas on the sandy soil only the control trees had multiple leader stems. A similar pattern of stem development relative to inoculation treatments occurred in *Gliricidia*.

From analyses of tree growth measures carried out in September, only species exerted a significant effect on the height. *A. mangium*, *A. angustissima* and *G. sepium* formed a group with a lower height (3.7 m) than that of a second group composed of the two leucaenas (6 m). For the diameter, the two leucaenas also performed the best (7 to 8.6 cm) whatever was the substrate used. *A. mangium* showed a similar good performance when raised on the ordinary nursery soil. The use of AM Fungi and rhizobia significantly improved the diameter of the trees raised on the conventional soil for all

species. The use of these selected microsymbionts was also proved efficient in improving the nodulation status of trees in *A. mangium*.

Main experiment

Out of the four species included in this trial, *Afzelia* was the slowest growing species (less than 0.4 m height and 0.6 cm in diameter). The three other species (*A. angustissima*, *G. sepium* and *Leucaena* hybrid) displayed similar growth performance when irrigated with treated wastewater. For the diameter and the stems numbers, the species showed similar trends possibly because the measurements were carried out very early when trees were still getting established.

Tree management

All species displayed growth patterns very similar to those reported above for the main experiment. However because the survival of most species was very low, the management trial (coppicing studies) was delayed waiting for the tree maturity to convert it to economic studies.

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

Good data were obtained for only two replicates of the preliminary experiment because many of the gauges did not work properly. For the same reason, no good data were obtained for *A. mangium*. *L. hybrid* showed the highest transpiration rate ($1.09 \text{ L cm}^{-2} \text{ day}^{-1}$), followed by *L. leucocephala* ($0.93 \text{ L cm}^{-2} \text{ day}^{-1}$), *G. sepium* ($0.93 \text{ L cm}^{-2} \text{ day}^{-1}$) and *A. angustissima* ($0.61 \text{ L cm}^{-2} \text{ day}^{-1}$). Solar radiation and wind speed follow the same pattern as the sap flow whereas the other weather variables present opposite trends. This is in conformity with what was expected.

Wp 4. Microsymbionts and N-fixation

The mixed AMF inoculants provided by IER were multiplied by INERA for all partners

Preliminary experiments in the nursery

Experiment 5.1.1: Response of leguminous fuel and fodder species to the double inoculation in irrigated conditions

After four months of growth, two species (*Calliandra calothyrsus* and *Acacia angustissima*) were left out of the analysis due to their low survival rates. On average, acacias performed poorly with similar growth whereas *Gliricidia* showed the best performance. Applying either *Rhizobium* or endomycorrhizas improved plant growth while double inoculated plants performed poorly. Some species like *Gliricidia*, *Leucaena diversifolia* and *L. hybrid*, responded better to endomycorrhization alone or to inoculation with *Rhizobium* alone. Growth of *L. leucocephala* and acacias was better without inoculation. However the response to the double inoculation was more important to that of the single inoculation with either *Rhizobium* or endomycorrhizas. Differences in growth appeared to be difficult to explain due to the fact that the inoculants were not tested before to ascertain their efficiency.

Experiment 5.1.2

The inoculation treatment showed no significant effect on plant growth characteristics. “Permanently” wet irrigation regime generally depressed height growth in species compared with “temporarily” wet treatment. Eight species (*Azelaia africana*, *Acacia angustissima*, *Acacia crassicarpa*, *Pterocarpus erinaceus*, *Khaya senegalensis*, *Acacia mangium*, *Senna seamea* and *Ziziphus mauritiana*) displayed similar height growth in both irrigation treatments. These species may naturally have the ability to withstand harsh conditions (drought and flooding). Growth reduction by excess watering was the greatest in two species (*Leucaena (hybrid)* and *Leucaena leucocephala* which should require particular management once established in plantations and irrigated with treated wastewater.

There were significant differences between species in diameter with varying trends according to the water regime. The values were the greatest under excess watering regime in some species (*Ziziphus mauritiana*, *Pterocarpus erinaceus*). For the other species, values were similar whatever the irrigation treatment, or the greatest for temporarily wet treatment. The plant tap root length followed similar pattern as for height growth. Shoot and root DWs and total plant biomass varied between species, and also within species depending on the irrigation regime. According to the total biomass accumulation and trends of its partition between shoots and roots, the tested species were arranged into four distinct groups: The first group is composed of species clearly not adapted to permanent water supply, and consequently accumulate the greatest biomass in temporarily wet conditions. This group includes the majority of the tested species (*Gliricidia sepium*, *Leucaena*, *Antada africana*, *Pterocarpus*, *calliandra*, *Azelaia africana*, *Acacia angustissima*, *Acacia crassicarpa*, *Pterocarpus erinaceus*, *Khaya senegalensis*, *Acacia mangium*, *Senna siamea* and *Ziziphus mauritiana*). The second group contrasts with the 1st one, and contained 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 *Ziziphus* 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 *Azelaia 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. The remaining species not previously listed in groups are considered as intermediates species. If relevant, this grouping of species at the nursery stage is expected to influence plants establishment and their growth traits in field experiments.

Leaves and nodulation in leguminous tree seedlings: There were no significant differences between inoculated and uninoculated tree species in nodule characteristics and leaf number. Nodulation and number of leaves on stems were greater for seedlings temporarily irrigated than those grown in permanently wet conditions. Leaf shedding occurred in most species broadly one month after the experiment set up and on ward, which may have contributed to these differences. There was also large variation within tree species depending on the irrigation regime. With the exception of *Gliricidia sepium* for which significant nodules DW was recorded, nodulation in the others species appeared only qualitative since nodules were very small and weighted

light. With regards to N₂ fixation, this suggests that most species do not fix significant amount of N₂ in the soil used for this experiment.

Work package 5: Economics: Characterization of the traditional production systems of fuelwood and fodder for the town of Ouagadougou

Supply of wood: The results revealed that the most important transportation means for wood in decreasing order were bicycles, carters, pedestrians, big trucks, small trucks and small cars. The road Ouaga-Ouahigouya displayed the highest number of cyclists. The highest number of carters was registered on the road Ouaga-Kamboinsé whereas no big truck was encountered during the period of study on this way. This may be partly due to the poor conditions of the road for such big trucks and the limited availability of the resource on this direction. The road of Ouaga-Saponé, which is tarred and leads to areas where wood resource is still available (classified forests), was very frequented by a number of articulated trucks. On the same way, private vehicles and people back from duty travels play an important role in supplying themselves and therefore the town.

The quantity of fuelwood introduced in Ouagadougou was worked out for the whole year. We can notice an increase by 83,200 tons in comparison with the 41,804 tons reported by Zida (1991).

The same categories of actors have been registered for charcoal supplying to the town of Ouagadougou on the same 12 roads. Bicyclists were again the most frequent on all the 12 roads with 810 passages in one week.

Suppliers of fodder: Different types of fodders were encountered: annual grasses, annual leguminous, leaves of ligneous plants, crop residues and industrial byproducts. No specific difference was found in the transportation means compared with those used for fuelwood and charcoal transportation. Again cyclists were the most frequent on the roads transporting fodder to Ouagadougou. Trucks do not transport fodder alone but animals and fodder at the same time.

Fuelwood and charcoal uses in households: Bendogo was the quarter where we registered the highest number households using wood as main energy source (57 households) followed by Paspanga with 40 households and the residential quarter (1200 logements) with 20 households. Therefore we can notice in the new quarter (Bendogo), people are still having a behaviour closer to that of people from the village whereas inhabitants of the old quarter (Paspanga) are using gas in higher proportion. In residential area (1200 logements) people tend to replace the wood by the gas. The study showed that there are other factors that have an influence on the use of wood, for instance: education level of the decision maker of the household, the size of the household, frequency of “tô” (main dish in Burkina made from flour of cereals), kitchen position in the courtyard, the type of house and the religion.

Fodder uses in households: The main types of fodder in decreasing order are grasses, crop residues, cereal bran, tree leaves, cotton seeds. Tree fodder was not mentioned frequently because the survey period was not the most appropriate for this type of fodder. Another reason may be due to the fact that the pruning of trees even for fodder is prohibited by forest services.

Out of 75 households interviewed per zone, 62 households practice stock breeding in Paspanga, 49 households in Bendogo and only 4 households in the residential quarter (1200 logements). Thus, as the quarters become more modern people tend to abandon animal breeding in town. The most frequent types of animals are sheep (46 cases), goats (29 cases), and cattle (16 cases).

Commercialization of fuelwood and charcoal: Two types of actors are involved in commercializing fuelwood and charcoal: the suppliers and the retailers. If the suppliers are dominated by men, the retailing is an activity conducted both by men (~50%) and women (~50%). This activity does not require any specific training so that we found people of a large range of instruction levels. However, retailing of fuelwood and charcoal appeared to be done mainly by people of 40 to 50 year old. A small percentage (12%) of the actors involved in fuelwood commercialization is composed of the members of professional organizations. Such organizations aim at improving the working conditions of these actors.

In general, retailers are supplied twice a month, rarely 3 or 4 times. They pay forest taxes of 1000 to 4000 F CFA per year for fuelwood, and 800 to 3000 F CFA for charcoal. Additionally, they also pay state taxes varying between 1250 to 2000 F CFA. The hiring of their working place costs 1000 to 2000 F CFA and the authorization to do the activity costs 2000 F CFA per year.

The fuelwood is sold at 50 to 500 F CFA the bundle but the price per kg does not vary much. The charcoal is sold in piles of 50 F CFA and 100 F CFA. Based on their weights, the equivalents are 60 F CFA kg⁻¹ and 110 F CFA kg⁻¹, respectively. The daily income evolves between 1,500 and 200,000 F CFA for fuelwood, and 4,500 to 200,000 F CFA for charcoal. These figures constitute an indication that the activity of commercializing fuelwood or charcoal yield substantial incomes for those involved in making them capable to face the health, schooling and food charges of their families.

Commercialization of fodder: As for the animals, the fodder is sold on the same markets and therefore the survey was done in the markets of Tanghin, Tampouy and Gounghin. Two types of retailers were identified. The specialized retailers practice this activity as their main income generating activity and they represent 88% of the sample. The seasonal retailers are mainly pupils on holidays for who this activity constitutes a way to get financial resources to pay their school fees and their books.

Buyers encountered were from all social levels of the city. Almost all of them buy the ration of the day for their animals because of storage and conservation problems mainly for fresh fodder.

The prices evolved according to the season. At harvest the residues of peanut cost 50 or 75 F CFA kg⁻¹. The price becomes 100 F CFA during the dry cool period (Mi-December to the end of February) and reaches 175 F CFA kg⁻¹ during the dry hot period (March- June). The cost of one pile of the grass fodder evolves from 25 F CFA during the rainy season to 50 F CFA during the dry season.

On average, the retailers of fodder earn 142,800 F CFA per year. Most of them (97%) invest this income in buying food for their family as well as school fees, health care, tools for their work, etc.

Workpackage 7: Planting Stock Quality

Nursery phase: *G. sepium* displayed the lower values of the sturdiness quotient followed by *A. africana*, the two leuceana and the two acacias. The values were higher for the ordinary nursery substrate compared to the sand in *A. africana*, *A. angustissima*, *G. sepium*, *L. hybrid*, and *L. leucocephala*. No inoculation effect was noted for *A. Africana*, *A. angustissima*, and *L. leucocephala* whereas *A. mangium* showed a higher value when inoculated. *G. sepium*, and *L. hybrid* showed higher values when inoculated but only with the ordinary nursery substrate whereas *L. hybrid* showed the opposite on the sand substrate. No pot effect was noted for *A. Africana*, *A. angustissima*, *G. sepium*, *L. hybrid* and *L. leucocephala*. In turn, *A. mangium* displayed lower value when raised in small pots.

The two acacias displayed the highest shoot:root ratios followed by *G. sepium*, *L. leucocephala*, *L. hybrid* and *A. africana*. *A. africana* did not show significant difference according to the level of inoculation for shoot:root ratio on sand while the value of this parameter was higher on the ordinary nursery substrate. In turn, *A. mangium* showed significant difference according to the level of inoculation for shoot:root ratio on sand while the difference was not significant on the ordinary nursery substrate. *G. sepium*, *L. hybrid* displayed higher shoot:ratio on ordinary nursery substrate compared to the sand and *G. sepium* had higher value in this parameter when inoculated compared to non-inoculated plants. Shoot:root value was significant lower in big pots for *A. africana*. Inoculation induced a significant higher value of shoot:root ratio on ordinary nursery substrate when inoculated and the opposite on the sand substrate for *A. angustissima*. *A. africana* gave significant lower value of shoot:root ratio in big containers compared to small ones. *L. leucocephala* showed the opposite but only on the ordinary nursery substrate. No significant effect of container size was observed for this parameter with *A. angustissima*, *A. mangium*, *L. hybrid*, *L. leucocephala*.

The two acacias displayed the lowest Dickson's Quality Index values followed by the two leuceanas, *G. sepium* and *A. africana*. The value of quality index was higher for inoculated plants compared with the non-inoculation in *A. africana*, in ordinary nursery substrate when compared with sand substrate, in big containers when compared with small ones in *A. angustissima*. No significant effect of substrate and container size was noted on the quality index for *A. africana*. There were significant interactions between substrate and container size for *A. mangium*, *G. sepium*, *L. hybrid*. Thus Dickson's Quality Index values were higher in big pots with the ordinary nursery substrate whereas no significant difference was found between big and small pots with sand. Similarly, there was significant interaction between substrate and inoculation for *G. sepium*. Therefore Dickson's Quality Index value was higher in inoculated plants with the ordinary nursery substrate whereas no significant difference was found between big and small pots with sand. Quality index value was also higher in big pots when the plants of *G. sepium* were inoculated with no difference between

container sizes when not inoculated. A significant interaction was noticed between substrate, inoculation and container size in *L. leucocephala*. Thus significant higher values of Dickson's Quality Index were observed in ordinary nursery substrate for inoculated plants as well as in big pots for inoculated plants.

Plantation phase

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

At both nursery and plantation stages the two acacias displayed the highest shoot:root ratio 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.

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1. OBJECTIVES

• **Context**

In most of the Sahelian countries such as Niger, wood is the main source of energy for more than 90% of households. The need for fuel wood is increasing in cities like Niamey due to the increase of the urbanization. For example, in this town, the quantity of fuel wood has increased from 110,000 tons in 1983 to 133,000 tons in 1990. This has had a substantial impact on forest resources and is not sustainable. Furthermore, the need for fodder is also very important because of intensive livestock farming around big cities.

• **General objectives of the research.**

The overall objective is to use irrigation to continuously produce fodder and fuel wood in the peri-urban zones of 3 cities in semi-arid sub-Saharan Africa. Low cost, low technology systems of industrial and domestic wastewater purification will be installed and their effluent used to irrigate fast growing plantations. The system will generate novel sources of fuel wood and fodder, create local wealth and employment, improve the local environment, limit urban spread and sustainably increase efficiency of water use while reducing pollution and human health risks.

• **Specific scientific and technological objectives.**

1. To design and install low cost, low technology industrial and domestic wastewater purification systems that are appropriate to local site conditions in peri-urban environments in Mali, Burkina Faso and Niger.
2. To purify the wastewaters to WHO standards such that they can be recycled for agricultural or forestry purposes thus sustainably increasing efficiency of water use.
3. To utilise the wastewater to irrigate plantations of fast growing N-fixing trees thus enabling sustainable continuous production of fuel and fodder in peri-urban areas of 3 semi-arid zone cities.
4. To calculate irrigation needs from measurements of soil water and plant water use.
5. To maximise fuelwood and fodder production by optimising tree management for wood and leaf production respectively.
6. To identify the most productive tree species/provenances for fuel and fodder production in the semi-arid zones of the 3 Sahelian countries.
7. To assess the quality and potential for marketing of the produced fuelwood and fodder.
8. To provide a complete economic analysis of the irrigation/fodder/fuelwood production systems and assess the socio-economic and marketing constraints which could hinder adoption.

9. To utilize microsymbionts to minimise the need for fertiliser application and to verify their persistence and effectiveness using molecular tools.
10. To train DC scientists in wastewater treatment, molecular biology, soil/plant water relations and the use of modern data loggers and instrumentation.
11. To disseminate results of these studies to the user communities (GOs, NGOs, farmers, peri-urban landowners etc.) via participatory workshops and visits to sites.

2. ACTIVITIES

2.1. Construction of the waste water treatment

The activities of the project began with the build of the station for waste water treatment near the student accommodation at the University. Thus, the waste water treated is domestic water coming from the city. The system of waste water purification is a lagoon composed of 4 basins: i) an anaerobic basin of 70 m³ volume; ii) a basin for waste water storage; iii) a basin of infiltration-percolation of 240 m² and iv) a basin of irrigation. The basin of infiltration-percolation contained about 1 m of gravel and 30 cm of sand and its bottom recovered by a prefabricated tarpaulin in order to keep treated waste water. Thereafter the treated waste water is stored in a last basin for plant irrigation through a drip system.

- *The plan for the build of the station was provided by Société Canal de Provence (France)*
- *The station was built by the society GTR (Niger)*

2.2. Analysis of the soil of the experimental site

In order to determine the nutrient status of irrigation sites, soil samples were collected on May 21, 2005. Three profiles, P1, P2 and P3 were done at approximately 2 m depth according to the heterogeneity of the site. The samples collected from different horizons of each profile were analyzed at the soil lab of ICRISAT (Niger) for physical composition (sand, loam and clay) , and nutrient content - C, N, P, K, Na, Mg, Ca.

2.3. Analysis of chemical composition of water

Three types of water samples, waste water before and after purification and tap water, were collected in 2004 in order to determine their chemical composition. Samples were collected on 3 successive dates (April 30, May 1th and May 2th) for the following determinations:

- Be, Al, V, Cr, Mn, Co, Ni(60), Cu, Zn, As, Se, Cd, Sb, Ba and Pb : this analysis was performed by the Centre of Ecology and Hydrology of Edinburgh;
- Ca, Mg, Na, K, Mn, Zn, NH₄-N, Fe, Cu, Al, NO₃-N, pH and electric conductivity analyzed at the Sahelien centre of ICRISAT at Sadoré (Niger).

In addition, samples of treated waste water used for tree irrigation were collected twice a week in order to study the variation of their chemical composition with time. The following nutrients were analyzed at ICRISAT Center: total N, NH₄⁺, NO₂⁻, NO₃⁻ , total and available P, Al, Cd, Cr, Co, Cu, Mn, Mo, Ni, Na, Zn, SO₄²⁻, pH and conductivity.

2.4. Growth of plants in the nursery

2.4.1. Selection of species

In the first experiment conducted in 2003, the growth capacity of twelve (12) tree species selected for fuel and wood production was determined :

- exotic species : *Acacia angustissima*, *A. auriculiformis*, *A. crassicarpa*, *A. mangium*, *Calliandra calothyrsus*, *Gliricidia sepium*. Seeds of these species were obtained from AgroForester Tropical Seeds in USA;

-local species: *A. nilotica*, *A. raddiana*, *A. seyal*, *Bauhinia rufescens*, *Leucaena leucocephala* and *Piliostigma reticulatum* which are of particular interest for Niger were also chosen for the study.

The soil used in nursery was a mixture of ½ manure and ½ riddled sand distributed in pot of 1 liter. The pots were isolated from soil by wooden stands.

After pre-treatment, seeds were germinated and young plants transferred to the nursery in pots.

2.5. Inoculation of plants with microsymbionts in nursery

In order to study the effect of microsymbiont inoculation on plant growth, plants were inoculated with rhizobium and mycorrhizas on November 12, 2003. 4 types of treatments were applied:

(i) control;

(ii) inoculation with mycorrhizas : the common specific inoculum *Glomus aggregatum* was used at a rate of 10 g of inoculum per pot to inoculate all species;

(iii) inoculation with rhizobia: about 5 ml of a cell suspension of rhizobia (10^8 cells/ml) of the following specific strains were injected with a syringe into the pot at the base of the plant:

- *A. crassicarpa*, *A. auriculiformis* and *A. mangium* inoculated with strain 13c;
- *Leucaena leucocephala* inoculated with strain LDK4.
- *Gliricidia sepium* inoculated with strain GSK4.
- *Calliandra calothyrsus* inoculated with strain KW35.
- *A. nilotica* inoculated with strain CIRADF 304;
- *A. raddiana* inoculated with ORS 1096;
- *A. seyal* inoculated with ORS 3324.

(IV) inoculation with rhizobia and mycorrhizas.

Bauhinia rufescens and *Piliostigma reticulatum*, which are considered as non N-fixing species were inoculated only with mycorrhizas.

Forty eight (48) pots/species were used for each treatment.

The installation of the nursery was finished on October 30, 2003. Plants were watered daily with two types of water: waste water from a former small station of purification installed on the campus, and tap water.

- *The mycorrhizal inocula were sent by our colleagues from INERA of Burkina Faso*
- *The specific strains of rhizobia by our colleagues from IRD Dakar.*

2.6. Measurement of plant growth in the nursery

The following measurements were carried out on twelve (12) plants / species / treatment at weekly intervals: total plant height, collar diameter of the plant and number of plant leaves.

On March 10, 2004, 4 months after planting, twelve (12) plants per species and per treatment were harvested. Each individual harvested plant was measured and separated into different organs: shoot, roots and nodules. Plant organs were oven dried at 75°C for 48h then weighed.

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

2.7. Sap flow measurement

The sap flow of different species was measured using Dynagauges and flow 32 software at 3 different periods of plant growth :

1. from December 29th, 2004 to January 7th, 2005;
2. from January 16th to 25th, 2005;
3. from January 27th to February 5th, 2005;

Plants were fed with treated waste water for one week before the beginning of the sap flow measurements. For the measurement, plants were selected according to their diameter in order to install the sensors. After installation on the plants, the thermocouple was connected to the datalogger. The data on sap flow was recorded each 15 mn and thereafter collected with a laptop using Flow 32 software.

Plant height and diameter were measured at the beginning and end of each sap flow measurements. At the end of the measurement, fresh weight of leaves of each tree was determined. Leaf area was calculated using regressions based on leaf scans and leaf fresh weight.

The equipment for sap flow measurement and software for data collection and treatment were provided by CEH of Edinburgh.

- *CEH determined the leaf area using the scans and leaf weights.*

2.8. Plant growth in field conditions

In a second experiment conducted in 2005, five (5) tree species were selected for their fuelwood and fodder production. These species were: *Acacia angustissima*, *A crassicarpa*, *Gliricidia sepium*, *Leucaena leucocephala* and *A. seyal*.

After inoculation with microsymbionts and cultivation in nursery (as described previously), trees were transferred to field on August 13, 2005. From August to February, trees were watered with treated waste water through a drip system at two days interval with 6 litres of treated water/tree. After March, with the high temperatures, the frequency of irrigation was increased. The density of planting was 1m x 1m corresponding to 10,000 trees/ha.

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

2.9. Coppicing experiment

On July 11, 2007, trees planted in field conditions (see, 2.8) were coppiced at 0,5 m height in order to estimate their fodder and fuelwood production. Thereafter trees were coppiced at three month intervals in order to determine their capacity for regeneration.

After coppicing, the following parameters were measured: fresh and dry weight of leaves and wood, and volume of wood.

2.10. Socio-economic studies of the actual system of fuelwood and fodder production

In order to assess the actual system of fuelwood production, 4 questionnaires were elaborated. These questionnaires are related to: i) production and commercialization of wood, ii) wood energy timber :questionnaire to wood cutters, wood sellers, and wood dealers, iii) utilization of energy source by householders, iv) description of actual system of fuel wood production.

3. RESULTS

3.1. Composition of the soil at the experimental site

The soil of the experimental site was mainly sandy at level of around 93% .The proportions of loam and clay were around 2% and 3% respectively. In all profiles, the level of organic carbon was very low (under 0.6%) and mainly concentrated in a shallow surface horizon (around 13 cm). Thereafter the organic carbon decreased with depth. According to the content of organic carbon the classification of the three profiles is below : $P3 \geq P2 > P1$.

Similar to organic carbon, the level of total N was very low in all profiles ($< 0.05\%$) and concentrated in a small layer in the surface horizon. However according to the pH of surface horizon, which about 6.6, the surface horizon of profiles P2 and P3 had a middle nitrogen content indicating good conditions for mineralization. The value of the ratio C/N is under 11 for all horizons. This indicated mineralized soil with a low organic matter. Therefore the soil of the site is exhausted. For the total P, all profiles indicated a level of deficiency, between 200 and 600mg kg⁻¹. The lowest values of total P content ($< 200 \text{ mg.kg}^{-1}$) were registered for the surface horizon of P1. All profiles showed a neutral pH (H₂O) on the surface. For P1 and P2 profiles, the pH become slightly to highly acid with depth before becoming again slightly acid to neutral in the deepest horizons. The CEC was very low ($< 5 \text{ cmol}^+/\text{kg}$) in all horizons of the 3 studied profiles except in the deepest horizon (141-178 cm) of P1 where a value of 6.76 was recorded. This indicated a soil with a low absorbent complex and which is therefore poor.

3.2. Chemical composition of water used for plant irrigation

There were significant differences in nutrient composition between the two types of water. The treated waste water was richer in nutrients than tap water. Differences were greatest for Mn (127,74 mg/l and 18,82 mg/l respectively), Na, NH₄-N and K. However, the composition of Fe was the same for the two types of water. The pH was also identical. But, the electric conductivity of treated waste water (0,31 mS/cm) was significantly higher compared to that of tap water (0,05 mS/cm).

The content of heavy metals was very low for both types of water. There were no significant differences in Al, Ba, Ni and V composition between waste water before and after treatment and tap water. The Cu composition of waste water before

purification was higher than that of tap water. In addition, the tap water level of Co and As was lower.

The treated waste water composition in heavy metals remained steady along time.

3.3. Growth of plants in the nursery

3.3.1. Effect of type of water on plant growth

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

For *B. rufescens*, waste water decreased the plant height and number of leaves at early stage of growth compared to tap water. At later stage of plant growth (4 months after planting) the effect of the type of water varied according to the species and the plant organs. Waste water decreased significantly root dry weight for *A. auriculiformis*, *A. nilotica*, *P. reticulatum* and *Bauhinia rufescens*. For remaining species, the effect of the type of water for irrigation was not significant. On the other hand, the irrigation with waste water decreased dramatically the nodule dry weight for all nodulating species. The effect of type of water on shoot dry weight varied according to the species. Waste water decreased significantly the shoot dry weight of *A. nilotica* and *A. seyal*, but increased shoot dry weight for *A. mangium*, *L. leucocephala*, *G. sepium* and *A. raddiana*. For remaining species, there was no significant difference between the shoot dry weight of plant watered with tap or waste water.

The increase of plant development by waste water was attributed to its high nutrient content compared with tap water.

3.3.2. Effect of type of inoculation on plant growth

For most of the species, inoculation affected plant growth even at early stages. Moreover, the combined inoculation with rhizobia and mycorrhiza tended to increase significantly plant development compared with single inoculation with rhizobia or mycorrhiza. Therefore the double inoculation with rhizobia and mycorrhiza contributed more nutrients to plant for its growth.

3.4. Sap flow measurement

The maximum of sap flow was reached around 12 to 2.00 p.m. for all the species. The results indicated a great variation in the maximum of sap flow between the species. The local species such as *B. rufescens*, *A. nilotica*, *P. reticulatum* and *A. raddiana* had lower values of sap flow compared to exotic species such as *A. angustissima* and *A. auriculiformis*.

3.5. Plant growth in field conditions

The results indicated a difference of tree height between the species. The mean tree height varied from 150 cm (*Gliricidia sepium*) to 270 cm (*Leucaena leucocephala*). Remaining species had an intermediate height. No significant effect of inoculation

was detected on tree diameter for *A. crassicarpa*. But the tree inoculations, mainly by rhizobium and combination of rhizobium and mycorrhiza have increased the total tree height for *A. seyal* and *Leucaena leucocephala*. The mean collar diameter varied also greatly among tree from 28.12 cm for *Gliricidia sepium* to 40 cm (*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 *Acacia angustissima*, *Acacia crassicarpa* and *Acacia seyal*. But the inoculation, mainly by rhizobium and combination of rhizobium and mycorrhiza has increased significantly the diameter of the tree for *Leucaena leucocephala*.

3.6. Coppicing experiment

The results of coppicing experiment indicated that after two years of growth, the production of fodder (leaves) varied greatly between the species, from 4,130 kg of dry matter (d.m.) for *Gliricidia sepium* to 18,400 kg of d.m. for *Acacia crassicarpa*. But this production is influenced by the microsymbiont inoculation mainly with mycorrhiza. Therefore, the highest production of fodder was recorded with mycorrhiza inoculation for all species.

Three months of growth after coppicing, the capacity of regeneration of the different tree species in terms of fodder and fuelwood production was significant, but varied according to species. For the control, the fodder production was about 2,031 kg for A. angustissima, 6,621 kg for A. crassicarpa and 4,914 kg for G. sepium. The corresponding production for plants inoculated with mycorrhiza was 4,826 kg; 9,048 and 8,784 kg. Thus production was higher for plants inoculated with mycorrhiza.

4. PROBLEMS ENCOUNTERED

The following main problems were encountered during the project:

- the build of the station for waste water treatment took a long time because of delay in the delivery of the prefabricated tarpaulin ordered in France;
- The delay, sometimes very long, in the UBENEFIT annual payment which slowed the conduct of activities.

5. Publication and paper

2008 : Valorisation des eaux usées pour l'irrigation des espèces forestières destinées à la production de bois de chauffe et du fourrage au Niger. Paper submitted to *Sécheresse*

6. Dissemination of results

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

7. Training

- **November 29 to December 3, 2005:** training of 3 persons on waste water techniques of treatment. The 3 trained persons were:

*Mr Seydou ISSAKA Faculté des Sciences, Département de Biologie

*Mrs Dahiratou MARAFA : ENS, Département de Sciences de la Vie et de la Terre

*Mr Halilou OUMAROU : ENS, Département de Sciences de la Vie et de la Terre

Mrs Françoise BOUROULET, from Société Canal de Province, which is partner of the UBENEFIT project gave this course.

- **April, 2004:** training of 3 persons sap flow and soil moisture measurements by the CEH (J.D. Deans and J. Wilson). The 3 trained persons were:

*Mr Seydou ISSAKA : Faculté des Sciences, Département de Biologie

*Mr Abdoulaye Harouna : Faculté des Sciences, Département de Biologie

*Sanoussi ATTA : ENS, Faculté des Sciences, Département de Biologie

- **June 2004:** Training of 2 persons on softwares (word, excel, powerpoint). The 2 trained persons were:

*Mr Seydou ISSAKA : Faculté des Sciences, Département de Biologie

*Mr Abdoulaye Harouna : Faculté des Sciences, Département de Biologie

- **2007 :** Mr Abdoulaye Harouna was trained for Master of Sciences on Botany and Phytoecology at University of Ouagadougou (Burkina Faso)

8. Conclusions

The results of UBENEFIT indicated a new way for production of fodder and fuelwood in the peri-urban zone such as Niamey by valorization of waste water. Therefore treated waste water to WHO standards can be recycled for agricultural or forestry purposes thus sustainably increasing efficiency of water use. In addition, the inoculation with microsymbionts (rhizobium and mycorrhiza) enhanced plant growth both in the nursery and in field conditions and minimised the need for fertiliser application.

Partner 6 IRD

Marc Neyra and Tatiana Krasova-Wade

Sub-Contractor

Société du Canal de Provence, BP 100, 13603 Aix-en-Provence Cédex 1, France.

I. Objectives

The objectives for IRD in this project were to characterise rhizobia using standard and molecular techniques, and to assist partners from Burkina Faso, Mali and Niger in the study of persistence of rhizobial strains in nursery trials and in the field under wastewater treatment, to train researchers from these countries in molecular techniques, and to provide consultancy to the partners on microsymbiont topics and exotic tree species selection. Through a sub-contract to European specialists, the aim was to design the most suitable and low cost industrial and domestic wastewater purification systems, appropriate to local site conditions in peri-urban environments in Mali, Burkina Faso and Niger, and adapted to WHO standards, to specify the necessary equipment, supervise the installation of the systems, commission the equipment and train researchers in its use and safety aspects.

II. Activities

Water treatment and irrigation (Work package number 1)

Two members of the subcontractor joined the “Start-up” Co-ordination meeting held in Niamey in January 2003, and continued their journey to Ouagadougou in Burkina Faso, and to Bamako and Niono in Mali, in order :

- to visit different sources of effluents and to select the most appropriate one that would be treated and used for irrigation,
- to visit several experimental sites in order to choose the best one for establishing a one hectare field trial in the following year,
- to assess climatic, soil and topography conditions,
- to identify local social problems and environmental risks caused by wastewater use in agronomics.

Thereafter, they calculated the ideal dimensions of the wastewater purification station and of the irrigation system for the field trial. Technical schedule of conditions was established.

Tree growth and Management (Work package number 2)

A choice of woody species of legumes for screening in the nursery was made. The selected hosts were identified and grouped into two groups: common species, to be tested in each country, and species that were of particular interest in specific countries

(see "Minutes of the start meeting of the EU Project Contract Number ICA4-2001-10007"). The plants were inoculated in nursery with rhizobia strains selected in WP4.

Tree water use and soil water status (Work package number 3)

We gave access to already established plantations and technical assistance to Dr. J. D. Deans from CEH (Partner 1) at our experimental site in Bel Air Research Station in Dakar, Senegal. Measures of sap flow were done on five of the six common species: *Acacia mangium*, *Acacia auriculiformis*, *Acacia crassicarpa*, *Leucaena leucocephala* and *Calliandra calothyrsus*. It was not possible to do this type of measures on *Gliricidia sepium* because at this period of the year, all the leaves were down.

An irrigated field trial was set up in Bel Air Research station. Two blocks of 16 plants of each common species were planted in November 2003. All these plants were inoculated in the nursery with effective rhizobial strains. Planting was done at a spacing of 2 metres between each tree of the same species, with irrigation done each day with normal water. The aim of this trial was to offer the possibility to determine on the same place the water requirement of these six species in order to optimize their irrigation in Mali, Niger and Burkina Faso.

Microsymbionts and Nitrogen-Fixation (Work package number 4)

A large part of our activities has been to receive partners from Burkina Faso, Mali and Niger in our laboratories in Dakar and Montpellier to train and assist them on microsymbionts and nitrogen-fixing micro-organisms, especially for their molecular characterisation. At the same time we selected microsymbionts, multiplied and distributed them for inoculation in nursery. Indigenous strain diversity was studied by PCR-RFLP of 16S-23S rDNA IGS. We developed a rapid and safe technique for DNA isolation from nodules, and in order to allow the development of a rapid and low-cost technique for identifying inoculated strains in the large number 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.

III. Results achieved

Water treatment and irrigation (Work package number 1)

1) Niger

The experimental site located in Niamey University, was examined for the installation of the experiment. Soil is sandy and silty, with good drainage capacity. There is a severe water deficit during all the year, except in August, and average evapotranspiration is about 6 mm per day. Effluent, coming from students' campus, consisted of "classical" domestic wastewater. Because of high temperatures, the lagooning method appeared to be well adapted to this wastewater with high organic

matters. Losses by evaporation have also been taken into account. Because of granule structure of the sand in the soil, it was proposed to establish a wastewater treatment based on a pre-treatment by anaerobic lagooning for suspension matters decantation, followed by an infiltration/percolation system, which consists in secondary and tertiary purification . Infiltration/percolation system is based on the principle of aerobic biological filtration through thin granule substrate. A very large specific area is colonised by bacterial purifying film. Percolation velocity must be very slow and substrate has not to be waterlogged. Quality of treated effluent will thus correspond to A-type constraints (according to WHO standards): number of intestine nematode eggs < 1 per litre and thermo-tolerant pathogens < 10^4 / l.

Gravity-fed irrigation seemed not to be well adapted, because sand showed rough texture and losses per percolation should be great, retention capability for sandy soils is very low and the terrain slope was slight (2%). Drip irrigation is the most suitable irrigation system. Irrigation supply should be short but frequent. Intensive irrigation in arid conditions often leads to a rise of the subsoil water table and of soil salinity. Because high soil drainage capability and because wastewater presents low conductivity and low salt content, the risk of salt accumulation seemed rather low, thus the project wouldn't need to apply extra water to leach the salts out. Water supply for one hectare of ligneous production irrigation was estimated to 50 m^3 / day. Because of evaporation losses and according to the very high irrigation efficiency of micro-irrigation (90%), the total volume of treated effluent necessary was estimated about 60 m^3 /day.

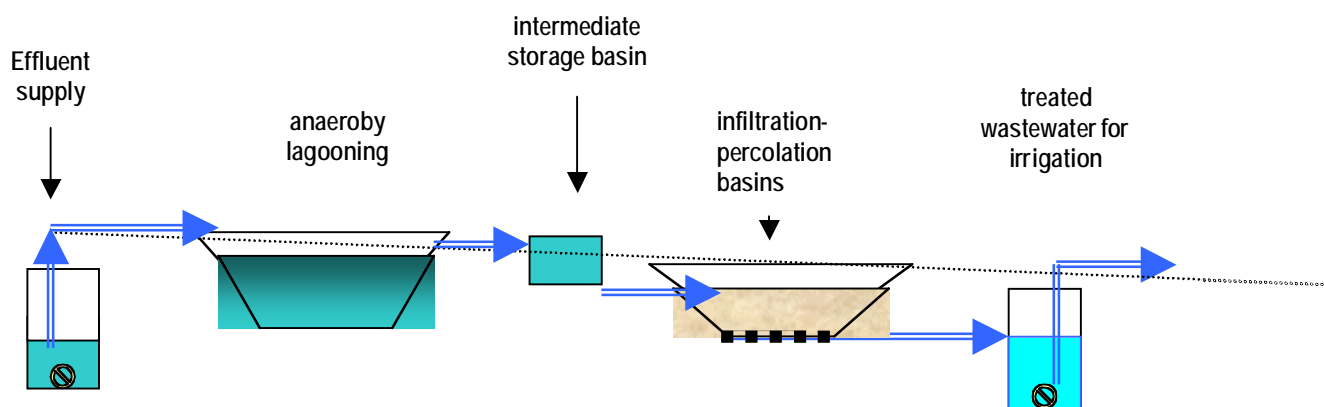


Figure 26 Schematic of waste-water treatment plant in Niger

2) Mali

The Minimana 4 hectares experimental site, near Siribala city on the Segou-Niono road, corresponded to a dead delta of Niger River. Effluent was drainage water from Minimana drain that collects irrigated agricultural plots runoff waters. It was supposed that effluent should be rather dilute with moderate organic matters. Soils

were brown wet hydromorphic, with a silt-clay-sandy texture, and ferric inclusions. Because of textural discontinuity near to 60 cm, it was foreseeable that plant roots will have some difficulties to explore the soil more deeply. There was a strong lack of drainage in the soil. Water deficit was high during all the year except during the rainy season. Mean evapotranspiration was estimated about 6 mm per day. Tree water needs were estimated 50 m³/ha /day.

Irrigation system proposed was gravity-fed irrigation with 50 m long lines (maximum efficiency 60%), thus water supply should be about 90 m³/day for one hectare of irrigated tree plantation. The effluent treatment proposed was purification – improvement directly by tree plantation irrigation, the soil / plant complex playing a role of natural biological living-filter. This system highlights the high soil capability for wastewater purification. Because of very low terrain slope (less than 1%) and because low soil drainage, some earthworks needed to be done, with the building of high point (“ados”) and bottom points (drainage canals in order to recover the filtered water).

Practically, it was proposed to:

- Implement a small dam in the Minimana drain in order to collect wastewater,
- Install a mobile pump (50 m³/h),
- Implement distribution pipes with micro floodgates,
- Make mechanical earthworks,
- Install a storage basin to collect ultimate drainage waters, after irrigation and filtration in the soil.

Soil drainage appeared to be the most important thing to follow, as well as salts concentration in the root zone due to water accumulation and lack of water evacuation.

3) Burkina Faso

In Ouagadougou, project was a little bit delayed, as the initial experimental site (tannery) was finally not retained and as tannery’s effluents were finally not available for the project. The experimental site that has been finally selected on the Ouagadougou University campus, showed plain topography, with a very slight slope and a rather homogeneous soil, made of sandy or silty clays (10 to 45% of sand and 55 to 90% of silty clay). Lagooning basins water tightness with a membrane appeared to be not mandatory. Effluent was composed of domestic wastewaters with high level suspension matters, coming from Faculty campus, restaurant and some offices. The BDO mean value considered for calculations was 430 mg/l.

It was assumed that lagooning will fit well with climatic data (high temperatures). Water deficit occurs all the year, except in summer time (July and August). In agreement with INERA and EIER partners, wastewaters treatment system proposed was composed of:

- pre-treatment by screening/grit removal in a concrete sewer lift station, equipped with one electrical pumps,
- a pipe leading the effluent to the lagooning basins (about 400 linear metres)
- primary treatment with anaerobic lagooning (one 150 m³ basin, 3 m depth basin, wastewaters retention time 2 days),

- secondary treatment with facultative aerobic lagooning (one 800 m³ basin, 1,5 m depth basin, wastewaters retention time 9 days),
- tertiary treatment with maturation lagooning (one 500 m³ basin, 1 m depth basin, wastewaters retention time 5 days),
- a concrete storage basin (reservoir) (one 100 m³ basin, 1 m depth, storage 1 day).

All basins being fed by gravity, it was decided that a homogenisation basin would be implemented before the anaerobic lagooning basin, in order to reduce effluent speed. Thus, and as asked by Burkina Faso Authorities, quality of treated effluent would correspond to A-type constraints (according to WHO standards): number of intestine nematode eggs < 1 per litre and thermo-tolerant pathogens < 10⁴ / l.

Tree water supply was estimated 50 m³/ha/day (as average daily evaporation is about 5 mm), and because losses due to gravity-fed irrigation (estimated 50%), water requirement was finally estimated 100 m³ / ha / day, applied by gravity-fed irrigation with 50 m long lines

The experimental site was divided into two 5000-m² parts, each divided into four sub-groups of 5 irrigation lines. Each sub-group could be irrigated in an independent manner. Mechanical earthworks was needed to build 1 m high, 50 m long and 5 m large earth boards between the lines, that would each be planted of two rows of trees. Irrigation lines would be about 20 x 40 cm, with a slope of 2 mm/m, with 5 m space between them. It appeared that soil drainage will be important to follow in the project, as well as salts concentration in the root zone due to water accumulation. Salinity would have to be controlled regularly.

Microsymbionts and Nitrogen-Fixation (Work package number 4)

1. Microsymbionts selected, multiplied, distributed and inoculated in nursery

The N-fixing microsymbiont strains used for inoculation studies are presented in Table 13. These strains belong to different phylogenetic groups and have been selected because of information previously obtained for some of them in other research projects on their nodulation and fixation capacities, and competitive ability against native rhizobium strains (André *et al.*, 2003; de Lajudie *et al.*, 1994; Dresler-Nurmi *et al.*, 2000; Galiana *et al.*, 1994; Lemkine and Lesueur, 1998; Lesueur *et al.*, 2001; Lesueur, 2000; Odee and Machua, 2000; Sarr *et al.*, 2003; Thiao *et al.*, 2004).

Table 13 Strains used for inoculation

Strains	Taxonomical position	Host plants / origin	Geographical origin	Reference / source
11C	<i>Bradyrhizobium</i> sp.	<i>A. mangium</i>	Australia	Galiana <i>et al.</i> , 1994
13C	<i>Bradyrhizobium</i> sp.	<i>A. mangium</i>	Australia	Galiana <i>et al.</i> , 1994
LDK4	nd	<i>Leucaena</i>	Kenya	Lemkine and Lesueur, 1998
GSK4	nd	<i>Gliricidia sepium</i>	Kenya	Thiao <i>et al.</i> , 2004.
KWN35	<i>Rhizobium</i> sp.	<i>Calliandra calothyrsus</i>	Kenya	Lesueur <i>et al.</i> , 2001
CIRADF 300	<i>Sinorhizobium</i> sp.	<i>A. senegal</i>	Senegal	Sarr <i>et al.</i> , 2004
CIRADF 301	nd	<i>A. senegal</i>	Senegal	Sarr <i>et al.</i> , 2004
CIRADF 302	<i>Sinorhizobium</i> sp.	<i>A. senegal</i>	Senegal	Sarr <i>et al.</i> , 2004
CIRADF 304	nd	<i>A. nilotica</i>	Senegal	Sarr <i>et al.</i> , 2004
CIRADF 305	<i>Sinorhizobium</i> sp.	<i>A. nilotica</i>	Senegal	Sarr <i>et al.</i> , 2004
CIRADF 306	<i>Sinorhizobium</i> sp.	<i>A. nilotica</i>	Senegal	Sarr <i>et al.</i> , 2004
CIRADF 308	nd	<i>A. nilotica</i>	Senegal	Sarr <i>et al.</i> , 2004
ORS 1096	<i>M. plurifarium</i>	<i>A. tortilis</i> subsp. <i>raddiana</i>	Senegal	de Lajudie <i>et al.</i> , 1998
ORS 1032 ^T	<i>M. plurifarium</i>	<i>A. senegal</i>	Senegal	de Lajudie <i>et al.</i> , 1998
ORS 3324	<i>Mesorhizobium</i> sp.	<i>A. seyal</i>	Senegal	Diouf <i>et al.</i> , 200.
ORS 3327	<i>Mesorhizobium</i> sp.	<i>A. seyal</i>	Senegal	Diouf, pers. com.

nd, not determined

The selected strains were sent to the project partners for inoculation according to each corresponding selected host plant (Table 14). Plants of five tree species (*Acacia mangium*, *Acacia auriculiformis*, *Acacia crassicarpa*, *Leucaena leucocephala* and *Acacia senegal*) chosen for planting in Mali, Burkina Faso and Niger, were inoculated with the corresponding strains (respectively, Aust 13 C, Aust 11 C, Aust 13 C, LdK4, CIRAD 300, 301, 302) in nursery conditions in order to have a sufficient number of

nodules to be used for molecular tests in the laboratory (DNA extraction and hybridization).

Table 14 Tree species and corresponding strains used for inoculation tests

Host plants	Strains	Niger	Mali	Burkina Faso
<i>A. crassicarpa</i> , <i>A. mangium</i> , <i>A. auriculiformis</i> , <i>A. lebbek</i>	13C, 11C	•	•	•
<i>Leucaena</i>	LDK4	•	•	•
<i>G. sepium</i>	GSK4	•	•	•
<i>C. calothyrsus</i>	KWN35	•	•	•
<i>A. senegal</i>	CIRADF 300, 301, 302		•	
<i>A. nilotica</i>	CIRADF 304, 305, 306, 308	•		
<i>A. raddiana</i>	ORS 1096, 1032 ^T	•		
<i>A. seyal</i>	ORS 3324, 3327	•	•	

2. Optimization of DNA isolation from legume nodules

In order to improve protein denaturation and DNA yield, different treatments involving guanidine thiocyanate, a strong protein detergent (Chomczynski *et al.*, 1997, *Biotechniques* 22:550-553), were tested initially on cowpea nodules. The data were compared to those obtained by CTAB/PVPP (Hexadecyltrimethylammonium Bromide / Polyvinylpolypyrrolidon) extraction method associated to Phenol : Chloroform : Isoamyl Alcohol purification, routinely used in laboratory.

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

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

ready for PCR-RFLP without any additional purification. It allows an easy transfer of DNA extraction technique to developing country laboratories.

Table 15 Comparison of treatment effects on DNA yield obtained on cowpea nodules

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

^aMean weight of one dry nodule determined as a ratio between the weight of all of nodules in crushed mixture and nodule number. ^bResults are given as the mean of three replicates.

The method was tested on nodules collected from the woody legumes grown in nursery. The results were compared with those obtained by CTAB/PVPP protocol (Table 16). For each tree species, a mixture of six crushed nodules was divided into six samples to allow three replicates per treatment (GES protocol or CTAB/PVPP protocol).

Table 16 DNA yield obtained on woody tree nodules by GES protocol

Tree species	Nodule dry weight (g) ^a	Protein purity A260/A280 ^b		DNA quantity (mg g ⁻¹ dry matter) ^c	
		GES	CTAB/PVPP	GES	CTAB/PVPP
<i>Acacia mangium</i>	0.0025	1.5	1.67	1.67	0.39
<i>A. auriculiformis</i>	0.005	1.3	1.55	2.06	0.28
<i>A. crassicarpa</i>	0.005	1.6	1.63	0.76	0.20
<i>A. senegal</i>	0.005	1.4	1.53	0.83	0.21
<i>Leucaena leucocephala</i>	0.005	1.7	1.45	0.87	0.26
<i>Gliricidia septum</i>	0,0025	1.4	nd	0.93	nd

^aThe mean dry weight of one nodule determined as a ratio between the weight of all of crushed nodules in mixture and nodule number. ^bResults are given as the mean of three replicates for both DNA extraction protocols with GES or with CTAB/PVPP. nd, not determined

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

3. DNA probe design for inoculants

The rDNA operon (*rrn*) was selected to define the specific DNA probes. It is a particularly useful target for the development of nucleic acid hybridization- and PCR-based assays, since it provides all prerequisite marker molecules, i.e. universal distribution, structural and functional conservation, and sufficient size. There are available sequences of 16S rRNA molecules of many rhizobia, but because of their highly conserved structure, only genera- species- and sometimes subspecies specific, but rarely strain-specific probes can be designed. The 16S-23S rDNA IGS region has received attention as a target in molecular detection and identification (Jensen *et al.*, 1993; Gürtler *et al.*, 1996; Leblond-Bourget *et al.*, 1996; Normand *et al.*, 1996). The high IGS sequence variation allows intraspecies strain differentiation. We prioritized the nonradioactive labelling because of it is safer and more easy to transfer to DC Partners laboratories.

Some DNA probes were defined for strains nodulating cowpea, because their diversity was well studied, and because cowpea is nodulated by the same rhizobia as many tree legume species, such as *A. albida*, for example (Krasova-Wade *et al.*, 2003). They belong to bradyrhizobium genera and represent a heterogeneous group of slow-growing rhizobia nodulating promiscuous tropical and subtropical legume species known as ‘cowpea cross-inoculation group’. Table 17 shows the selected oligonucleotide probe sequences, their corresponding target DNA and hybridization temperatures. The specificity of each probe was tested by slot blot hybridization into total DNA extract of target and non-target DNA. It was ensured by optimization of experimental hybridization conditions. Non-target DNAs served as negative controls for the tests.

Table 17. Sequences and properties of oligonucleotide probes IgS1, 2, 3 & 4

Probes	Sequence 5' - 3'	Target strain	Length (bases)	% (G+C)	T_d hybrid.
IgS1	CAACGGCAGGATCCAGGGTCCTTAACG	ORS 3257	27	57	55
IgS2	TGCGCTGGTCGCCGATGCATGCA	ORS 3259	23	65	55
IgS3	TGGCTGAAGCCAGCTACGGCTAGGCG	ORS 3260	26	65	50
IgS4	TGAAGGCTTCGCGTGGCGCGGCCA	ORS 3262	24	71	66
Aust13C	CGCTTGTTTCATCGCGGCTCATCG	Aust 13C	23	61	52
Aust11C	GGTGAGCGGGTTGTAAATGATCCC	Aust 11C	24	54	50

$T_d = 81.5 + 16.6 \times (\log_{10} [\text{Na}^+]) + 0.41 \times (\% \text{ G+C}) - (600/\text{N})$ (Sambrook *et al.*, 1989), where N is the length of the oligonucleotide and $[\text{Na}^+]$ is the concentration of sodium ions in the final stringent solution. The formula is suitable for oligonucleotides from 14 to 70 nucleotides complementary to the target sequence.

The first hybridization tests applied on total strain DNA were carried out with the non-radioactive oligonucleotide probes labelling by the addition of a longer tail (ROCHE) of digoxigenin-tailing-dUTP. However, weak signal intensities and non-repeatable results were obtained, and it is in spite of lessening of stringency. Consequently, we used probes labelled with three DIG-molecules at 5', 3' and inside positions. Results obtained after probe hybridizations show a good signal intensity and quick appearance, 20 or 30 min after exposure. We chose the detection by colour reaction because of the easiness to follow-up relation of hybridization signal and background colour development.

The probes (IgS1, IgS2, IgS3 and IgS4) were tested on nodules formed by target strains. Results showed that the denaturation treatment (enzyme and hot temperature) applied to crushed nodules was not strong enough.

DNA of selected rhizobia strains (Aust 11 C, Aust 13 C, GsK4, LdK4, CIRAD 300, CIRAD 301, CIRAD 302) corresponding to the different trees used in inoculation trials were amplified and purified for sequencing of 16S-23S rDNA IGS region. 16S-23S rDNA IGS region of strains Aust 11 C and Aust 13 C were entirely sequenced. The sequences of the strains GsK4 and LdK4 were partially obtained. The sequences of Aust 11C and 13C were used for development of strain-specific DNA probes. The specificity of the probes was checked by alignments of their sequences with those available in laboratory and in international GenBank database by using the algorithm BLAST (Altschul *et al.*, 1997). The designed specific probes are presented in Table 17.

The protocol for probe hybridization has been made available for partners from Burkina Faso, Mali and Niger.

4. Nodule analysis

Analyses of nodules collected in field studies, which has been done in collaboration with partners 3, 4 and 5 are detailed in the respective reports of these partners.

5. Establishment of partnership with farmers' organisations

We developed a partnership with representative farmers' organizations regrouped under the umbrella of ROPPA (Réseau des Organisations Paysannes et des Producteurs Agricoles d'Afrique de l'Ouest, who federates millions of farmers through twelve west African countries): CPF (Confédération Paysanne du Faso) in Burkina Faso, CNOP (Coordination Nationale des Organisations Paysannes) in Mali and PFP (Plate Forme Paysanne) in Niger, and, through a complementary funding from the French foreign Ministry, with FUPRO (Fédération des Unions de Producteurs) in Benin, and CNCR (Conseil National de Concertation et de Coopération des Ruraux).in Sénégal. Workshops have been held, associating farmers, scientists and extension services. Some very positive participative field inoculation trials have been set up and the feasibility of production of mycorrhizal fungi in small units supervised by farmer's organizations is being studied.

IV. Problems encountered

The major problem encountered has been due to the delay that occurred at different levels on all the three sites. Implementation of wastewaters treatment plants and irrigation systems has delayed for different reasons (late irrigation materials supplying in Niger, delay for the choice of final experimental site in Burkina Faso...).

Perspectives

Although beneficial effect of inoculation of trees and crops with selected micro-organisms is known since many years and currently used in different parts of the world, inoculation technology is not developed in Africa. An increasing adoption by local users should hopefully be induced by actual promoting initiatives including networks such as ROPPA, who federates millions of farmers through twelve west African countries. Nitrogen fixing symbiotic bacteria inoculants can be either distributed by international manufacturers or produced by local units. Mycorrhizal fungi are multiplied on plants and initiatives exist to test small production units supervised by farmer's organizations.

In all cases, inoculants distributed to users must be controlled. To assess the quality of microbial inoculants applied in tropical agriculture and forestry, we propose the development of simple tools using molecular hybridization with specific non-radioactive probes for microbial inoculant characterization, coupled with a simplified procedure to isolate DNA from nodules of tropical legumes. The proposed tools are reproducible, safe and inexpensive, and adapted to basically equipped laboratories. New probes will be designed and tested for their specificity according to the selection of new efficient strains. Training will be provided for technicians and scientists of different laboratories and of potential quality control entities.

The primary end-users of these quality control tools will be:

- Laboratories selecting appropriate microbial strains, to follow their behaviour and efficiency in field experiments.
- Quality-control services, to assess the identity of the strains in the inoculants.

More largely, the farmers and foresters will at term benefit of high quality inoculants.

The insufficiency of rules for the distribution and use of biofertilizers in Africa implies that, together with technical developments, a co-construction approach needs to be developed with all stakeholders (farmers organizations, scientists, policy makers, extension services) to define the legal frameworks for the use of inoculants, including technical options for quality control. A reflection has also to be led on the opportunity of creating specific quality control independent services (that could be start-up companies). The role, the mode of functioning and the position of these potential services in the global system of production and use of inoculants will need to be clarified.

VI. Publications and papers

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

A manuscript on the results on hybridization probes is in preparation.

Results on field studies will be published in collaboration with partners from Burkina Faso, Mali and Niger.

Catalogue page

Summary

We aim to improve efficiency in water use and create novel high yielding urban-urban sources of fuel and fodder. Low-cost irrigation systems that recycle wastewater, reduce health risks and downstream pollution will be set up by specialists and used to irrigate fast growing trees that are managed to maximise either leaf (fodder) or wood (fuel) production. Good growth and establishment will be ensured by inoculation with effective micro symbionts whose persistence will be assessed using molecular tools. Irrigation will be based on measurements of soil water and tree water use thus avoiding salinization/pollution. Training in water management, molecular biology and use of ecological instrumentation will be provided to DC partners. Results will include reusable wastewater, novel urban-urban sources of fuel and fodder, an economic analysis, species selection and plantation management prescriptions and trained DC researchers.

Results achieved

Water treatment plants were successfully set up in Burkina Faso, Mali and Niger. The growth of a range of tree species, and the effects of inoculation with mycorrhizas and rhizobium were tested. Many tree species grew quickly under irrigated conditions and positive effects of inoculation upon tree growth were found, although molecular tering indicated poor persistence of specific rhizobial inoculants. Most tree species regrew well after coppicing. Fuelwood use in the capital cities was quantified and sources identified. The large quantities of fuel imported into Ouagadougou highlight the pressure on fuel resources. This is further indicated by the observations in Niger and Mali. Dissemination to stakeholders has been conducted and is continuing. The irrigation sites are continuing to be run.

**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

Totals (cumulative)

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1

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3

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2

1

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4

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Yes	No ✓
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Yes	No ✓
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Yes	No ✓
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Yes ✓	No
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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)

¹ Less than 500 employees