Report of a mission to Singapore and Malaysia
14 to 23 november 2000

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This mission was undertaken in the framework of the TQS (Total Quality Sapling) project developped jointly by NTU-NIE, the forestry department of CIRAD and the French Embassy in Singapore.

The main goals of this mission were :

1- to provide technical assistance to Jean Weber (French PhD student) and to make an overview of his activities in the TQS project,
2- to present to our partners the first results obtained
3- to prepare experimental field tests in Malaysia,

Chronology and schedule of the mission

November 14, Tuesday :	Departure from Montpellier (06:30 am)
November 15, Wednesday :	Arrival in Singapore (2:30 pm). Meeting at NTU/NIE Jurong new campus with **Jean Weber**.
November 16, Thursday :	Working session at NTU/NIE (Bukit Timah) with **Jean Weber.** Visit of experiments at the aeroponic greenhouse and in the campus field.
November 17, Friday :	Working session at NTU/NIE (Bukit Timah) with **Jean Weber.** Meeting at the French Embassy with **Mrs Laure Bourdarot** and **Mr Aymer de la Chevalerie**.
November 18, Saturday :	Morning working session at NTU/NIE (Bukit Timah) with **Jean Weber,** meeting with **Dr Tham Foong Yee.** Afternoon, meeting with **Eric Seah** at aerogreen farm in Singapore.
November 19, Sunday :	Visit of the NTU/NIE (Bukit Timah) Botanical garden. Participation with **Jean Weber** at the opening ceremony of the « Conference on Vegetale biotechnology and Agri-biotechnology in Asia and Pacific ». Meeting with **Pr Leo Tan Wee Hin** and **Pr Lee Sing Kong**.
November 20, Monday :	Departure from Singapore to Kuala Lumpur (07h50). Meeting at the FRIM (Kepong center) with **Dr Lee Su See** (in the morning). Afternoon meeting with **Dr Gan Lian Tiong** (Sime Darby) at Kuala Lumpur Aerogreen farm in Seremban ; visit of a field trial (Australian *Acacia* spp.). Departure from Kuala Lumpur to Kuching, Sarawak (23h30).
November 21, Tuesday :	Morning field trip in some *Acacia mangium* plantations. Collection of biological sample for an expertise of sylvicultural problems occuring in Sarawak. Afternoon: meeting at STA (**Dr Barney Chan**), presentation of CIRAD and its activities conducted in the framework of the TQS project.
November 22, Wednesday :	Visit of the Forest tree seed bank and of experimental plantations of some Dipterocarps (e.g.: *Shorea macrophylla* planted in 1936). Departure from Kuching to Paris via Singapore (18h00)
June 23, Tuesday :	Arrival in Paris (07:00 am). End of the mission.

List of persons met during the mission

Pr Leo Tan Wee Hin : Rector of NTU/NIE, President of the Singapore National Academy of Science, Singapore.

Dr Lee Sing Kong : Head of the Division of Biology at NTU/NIE, Dean of NTU/NIE, Singapore.

Dr Tham Foong Yee : Lecturer in the Division of Biology at NTU/NIE, Singapore.

Mr Eric Seah : Maintenance foreman, Aero-Green Technology Pte Ltd, Singapore.

Mr Jean Weber : PhD student working jointly at NTU/NIE and Nancy university in France. He works on the production in aeroponic conditions of inoculated (rhizobium and mycorrhizae) *Acacia mangium* in the hart of the TQS project, Singapore.

Mr Dominique Aymer de la Chevalerie : Attaché for Science and Technology, French Embassy, Singapore.

Mrs Laure Bourdarot : Consellor for Co-operation and Cultural Action, French Embassy, Singapore.

Dr Lee Su See : Research officer, Forest Research Institute Malaysia, Kepong, Kuala Lumpur, Malaysia.

Dr Gan Lian Tiong : General manager, Sime Darby plantations, Selangor, Malaysia.

Mr Lee Cheng Yee : Farm Manager, Sime Aerogreen technology sdn bhd, Seremban, Malaysia.

Dr Barney Chan : General manager, Sarawak Timber Association, Kuching, Sarawak, Malaysia.

Dr Peter C. S. Kho : Manager, Technical and Research, Sarawak Timber Association, Kuching, Sarawak, Malaysia.

Mr John Keen Chubo : Research officier, Sarawak Timber Association, Kuching, Sarawak, Malaysia.

Technical assistance to Jean Weber (a total of three full days)

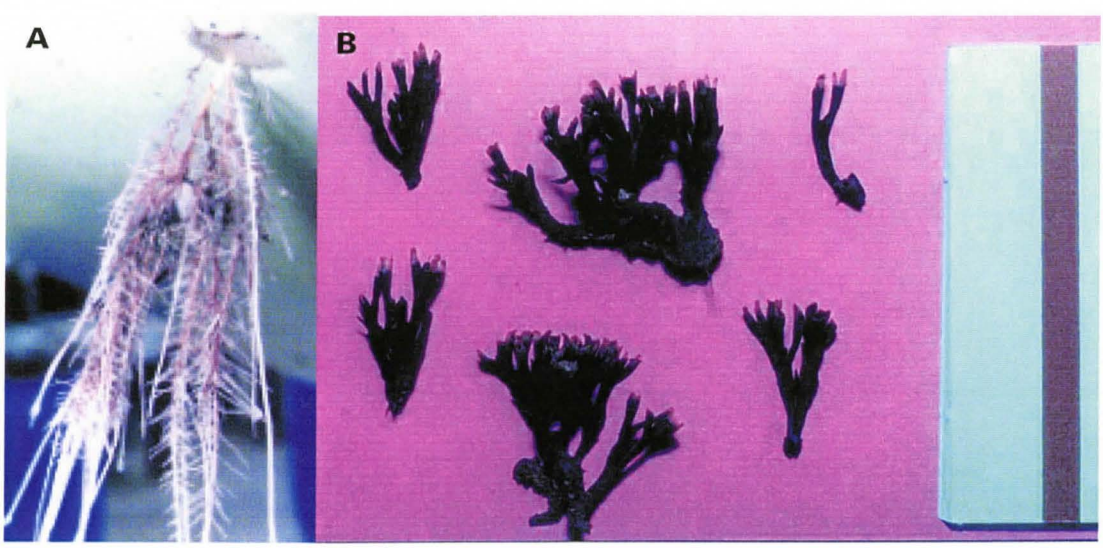
The technical assistance provided to Jean Weber has been focused on a synthesis of the work already done in Singapore and on the establishment of a work plan for, at least, the six next months. Some administrative problems have also been evoked.

Evaluation of results obtained

1- Mineral balance of the nutrient solution. It seems that the composition of the nutrient solution have to be optimized. In a first analysis, the presence of high amount of Ca^{++} in the solution affect iron nutrition and consequently nitrogen metabolism, leading to the yellowing of plants particularly at the end of experiments. That had to be corrected as soon as possible.

2- State of the art of controlled inoculations in aeroponic. Inoculation procedures using *Bradyrhizobium* are well managed. The strain used: Aust13c gives very satisfactory results. New methods of arbuscular mycorrhizae inoculation developped by Jean Weber with one isolate of *Glomus intraradices* are much satisfactory. Indeed, the use of alginate as sticking agent for AM spores or crushed roots is innovative and efficient. Individual inoculation with *Bradyrhizobium* and *Glomus intraradices* are well mastered in aeroponics. The dual inoculation *Bradyrhizobium* and *Glomus intraradices* may not cause much problems. Inoculation with ectomycorrhizal fungi cause some problems in aeroponics. Strains of *Pisolithus* sp. already known for their compatibility with *Acacia mangium* (*e.g.*: COI.007 isolated in Senegal from *Acacia mangium* and successfully inoculated on *Acacia mangium* in Madagascar) failed to infect and to develop in aeroponic. In each experiments, there were problems in mycelial growth in aeroponics. The use of *Telephora ramarioides* sporophores (crushed or as a whole) failed also (figure 1).

Figure 1: A:Piece of *Telephora ramarioides* sporophore attached to *Acacia mangium* roots in aeroponic; B: Sporophores of *Telephora ramarioides* collected under *Acacia mangium* in Singapore during the mission.



3- Transfer to the field of bare root *Acacia mangium*. The aim of this experiment was to evaluate possibilities of direct transfer of bare root *Acacia mangium* in field plantation and also to evaluate effect of a preservation time simulating an aircraft transportation. The plantation is a success (figure 2). The different tested conditions allowed, 2 months after transplantation, survival rates ranging from 90 to 100% without significant differences among treatments. The ability of direct transfer from aeroponic to the field of bare root *Acacia mangium* is demonstrated. Possibilities of sapling preservation allowing a long distance aircraft transportation are also well mastered. The method of sapling preservation is new and original. Thus Jean Weber has written a proposal of patent. This proposal will be submitted either to CIRAD and NTU/NIE.

Figure 2: view of the field trial two month after transplantation.

Some elements for the 2001 work plan

1- Nutrient solution. Composition of solution used at NTU/NIE and at LSTM in Montpellier are different and have to be compared. As a matter of fact, the solution used in Montpellier gives very satisfactory results with *Acacia mangium* and the solution used in Singapore is used extensively for salads. Rapid progress in the improvement of the nutrient solution are expected.

2- Inoculation. Effects (physiological and anatomical) of Infection by *Rhizobium* and or *Glomus intraradices* can be studied in aeroponic. Experiments have to be pursued that way. Inoculation with ectomycorrhizal fungi failled in aeroponic. Basic work on the compatibility of ectomycorrhizal strains have to be confirmed in the laboratory before new trials in aeroponics.

3- Field trials. Possibilities offered in Singapore for field trials are too far from true field conditions. Consequently, contacts have been developped with Sime Darby in peninsular Malaysia and STA in Sarawak to set up field trials. An experimental design enabling to set out differences between TQS and classical nursery methods have to be proposed in the first half-year 2001. Sapling have to be ready for a field transfer before the end of 2001.
4- Infection process. Infection process (*Rhizobium* and mycorrhizas) in *Acacia mangium* is poorly documented. Interaction between symbiotic partners can be studied in that point of view. Important progress can be obtained in this domain. Jean Weber will propose experimental design enabling these progress in the knowledge of infection processes with one or more symbiotic partners.

Administrative constraints

1- Move from the old campus (Bukit Timah) to the new campus (Jurong): a highly time consuming operation. It is foreseeable that the beginning of year 2001 will be a transition phase, thus technical problems might occur much frequently than before.
2- Availability of a budget to purchase locally consumables and small equipments. The problem has to be traeted as soon as possible.
3- Possibilities for Jean Weber to come at LSTM are subject to his participation in PhD courses. From that, will depend the dates of his mission in France.
4- A planning of PhD thesis committee meeting has to be established. A first meeting could be organized in France in january.

Meeting at the French embassy

Participants: **Laure Bourdarot, Dominique Aymer de la Chevalerie, Jean Weber, Marc Ducousso**

The meeting started by the presentation of the TQS project progress. The first six month of Jean Weber's work in Singapore are very satisfactory and the project fullfil all its objectives. I pursued by a presentation of the schedule of my mission and more particularly the importance of contacts taken with Sime Darby and STA to set up field trials. Mrs Bourdarot asked a question about the French private partner (Robin S.A.). A first contact has been established and we have now to plane a visit of Mr Robin in Montpellier for a presentation of the TQS project and of our partnership with NTU/NIE and Sime Darby. Mister Aymer de la Chevalerie evoked the possibility to create a joint-venture between Robin S.A. and Sime Darby.
The possibility to deposit a patent concerning *Acacia mangium* sapling packaging and preservation as bear roots is presented. This possibility have to be considered regarding the wait-and-see policy of Dr Lee Sing Kong concerning patent.
For year 2001, 130 KF will be granted. An official advice will be transmitted to CIRAD in December. Mrs Bourdarot indicates that founding from the French Embassy cannot be considered as automatic for next year. They care a lot about scientific results and quality of the partnership developped.
A visit of the new NTU/NIE campus at Jurong have to be organized soon. Jean Weber will transmit this demand to Dr Lee Sing Kong.

Contact with NTU/NIE staff (synthesis of four meetings)

Participants: **Pr Leo Tan Wee Hin, Pr Lee Sing Kong, Dr Tam Foong Yee, Jean Weber, Marc Ducousso**

Professor Leo Tan Wee Hin confirmed his interest in the development of our collaboration. He insisted on the importance of the partnership already established with Sime Darby. Then, I presented the schedule of my mission in Malaysia : 1- Visit to the FRIM, 2- Meeting with Dr Gan Lian Tiong (Sime Darby) and 3- Visit to STA (prospection of new possible collaborations and expertise of some problems occuring in an *Acacia mangium* plantation. Doctor Lee Sing Kong made a remark about the tediousness of administrative procedures in FRIM and confirmed Sime Darby interests for field trials. Jean Weber will participate in this mission he will thus make a debriefing as soon as he will be back in Singapore. Dr Lee Sing Kong found Jean Weber's work fulfill objectives assigned in the timing of the TQS project.

Dr Tam Foong Yee made some remarks about the scientific content of Jean Weber PhD work. She proposed to develop some fundamental aspects of symbioses establishment in laboratory conditions to complement the work already done on *Acacia mangium* production in aeroponic. Studies in the field of infection processes may be accurate.

Meeting with Dr Lee Su See (FRIM), Malaysia

This meeting had three goals: 1- to prospect the possibilities of collaboration and scientific exchanges between CIRAD and FRIM in the field of mycorrhizae research, 2- to present CIRAD activities, especially the TQS project and 3- to examine herbarium specimens of *Pisolithus* spp. found under *Acacia mangium* and some Dipterocarps in peninsular Malaysia.

The main field of interest of FRIM is forest health and sustainability. Though, they are interested in mycorrhizae research mainly for land reclamation and reabilitation. Teak and Dipterocarps are importants in peninsular Malaysia forestry. The use of *Acacia mangium* is restricted to land reabilitation. In Borneo, the use of *Acacia mangium* is more important but, currently, FRIM has no research programme in this domain in Sarawak or Sabah.

Possibilities of scientific exchanges and sample collections in Malaysia have to be considered in the framework of a scientific project joining CIRAD and FRIM. Other possibilities exist but are subject to tedious administrative formalities. Lastly, she informs us that a Malaysian company is now marketing arbuscular mycorrhizae (Malaysian Agri Care, Block Stroma, Pusat Teknologi Pintar UKM-MTDC, 43600 UKM Bangi, Selangor Darul Ehsan, Malaysia)

After an overview of CIRAD organization and activities, we made a quite detailled presentation of LSTM and the TQS project. Jean Weber presented his first results concerning the establishment of root symbiosis in aeroponic. He also presented the possibilities of direct field transfer from aeroponic to the field after a period of storage in polybags.

Ectomycorrhizal partner of Acacia mangium in peninsular Malaysia

Acacia mangium and *A. auriculiformis* are found in symbiosis with *Pisolithus albidum* (figure 3) and some species of the genus *Riessiella* and *Riessa*. *Pisolithus albidum* is quite common particularly along roads. The *Pisolithus* found under *Acacia* differs from the *Pisolithus* found under Dipterocarps which have been named as *Pisolithus aurantioscabrosus* (figure 3). Three slides of two specimens of *Pisolithus aurantioscabrosus* and three specimens of *Pisolithus albidum* have been prepared for microscopic observation of spores. Spore diameters are presented in table 1 (figure 3).

Figure 3: A: Herbarium specimens of *Pisolithus aurantioscabrosus*, B: Herbarium specimens of *Pisolithus albidum*; C: Spores of *Pisolithus aurantioscabrosus*, D: Spores of *Pisolithus albidum*.

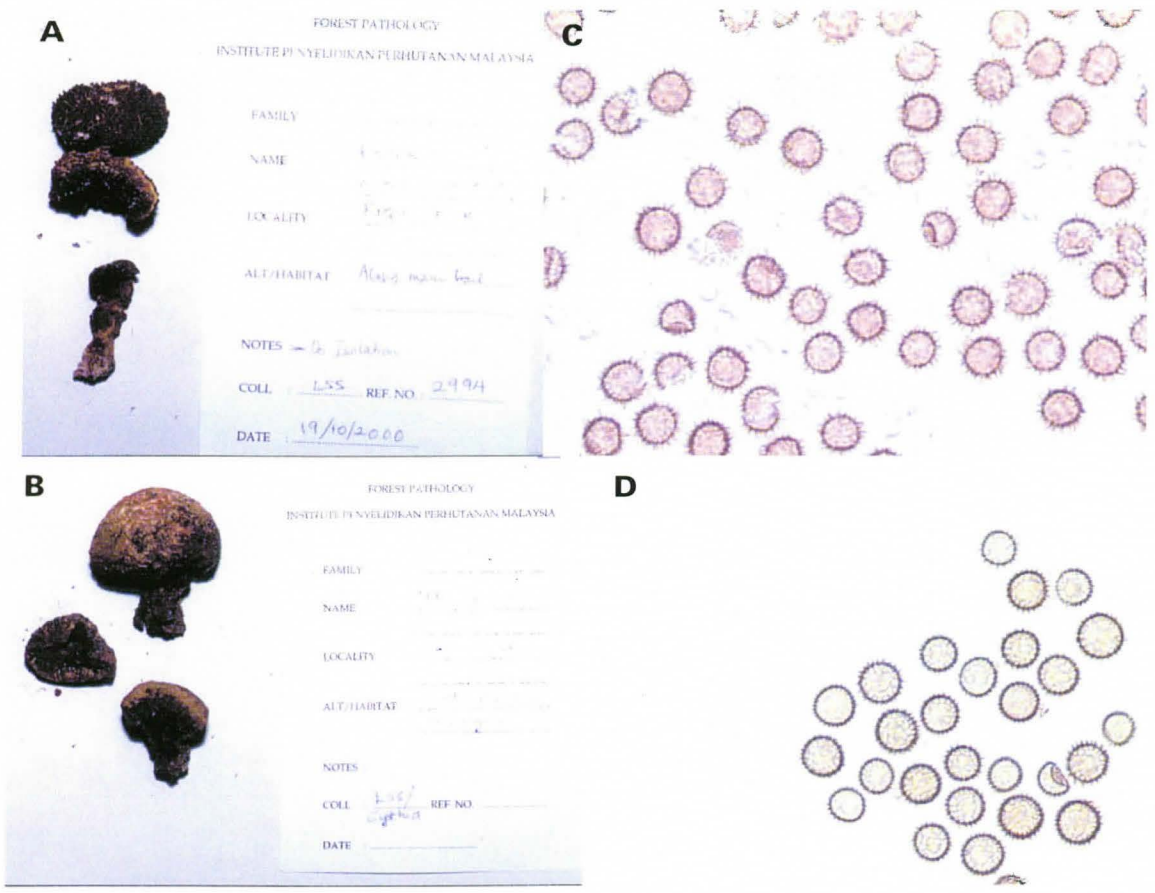


Table 1: Diameter, minimum, maximum and average of at least 300 spores of two samples of *Pisolithus aurantioscabrosus* and three samples of *Pisolithus albidum*.

Species/Reference	Spores nb	Av. diameter	Min. av diam	Max. av diam
P. auranthioscabrosus ET170	386	6.94	5.94	7.88
P. auranthioscabrosus 2994	312	6.85	5.52	8.13
P. albidum Kepang Nathan	398	8.44	7.42	9.31
P. albidum Gemas LSS	311	8.22	7.43	9.31
P. albidum Chong B.G.	303	8.52	7.39	9.25

Remark : many spores of *Pisolithus aurantioscabrosus* are damaged.

Meeting with Dr Gan Lian Tiong and Mr Lee Cheng Yee (Sime Darby) at the Aerogreen farm in Seremban, Malaysia

This meeting had two goals: 1- to present the results obtained in the framework of the TQS project and 2- to prepare the establishment of an experimental plantation of *Acacia mangium*.

Summary of presented results

The production of aeroponically grown *Acacia mangium* sapling is now well mastered. Notwithstanding, the nutrient solution used can be improved. Inoculation with selected Bradyrhizobium sp. is also well mastered and the obtention of a very satisfactory level of nodulation do not raise any problem. A new technic of arbuscular mycorrhizae inoculation have been developped. This technic using spores and/or crushed AM infected roots sticked with alginate allow to obtain the successful development of AM infection in aeroponic. Further research have to be pursued to master ectomycorrhizal infection in aeroponics. Indeed, first results are promising but new strains have to be tested and aeroponics conditions have also to be adapted. The possibilities of sapling preservation for transport (at least one week) and of direct transfer of bare root saplings to the field have also been presented.

Field experiments

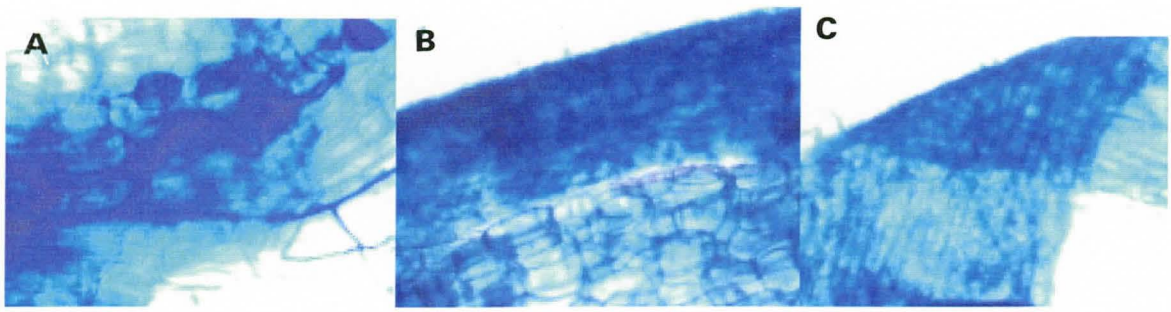
Doctor Gan Lian Tiong wanted, as possible, to simplify administrative procedure. Thus, he proposeed to replace the proposal of a MOU by an exchange of letters allowing to set out all conditions of the experimental plantation. As this proposal seemed acceptable to me, I proposed to submitted it as soon as possible to NTU/NIE and CIRAD staff. The required surface for the trial will range from 2 to 3 hectares. Trees will be planted during the next rainy season (november to december 2001). A more detailed experimental design will be established jointly by CIRAD and NTU/NIE and presented to Sime Darby. Then, a visit to the field took place. This visit was an opportunity to see some places where a field trial can be established and to visit a plantation of Australian *Acacia* species (*Acacia mangium* and some related species) (figure 4); samples have been collected to assess the mycorrhizal status of the plantation under local conditions. Results are summarised in table 2 (figure 5).

Figure 4: Plantation of Australian *Acacia* species near Seremban. A : general view of the trial ; B : view from inside the *Acacia cicinnata* plot.

Table 2: Mycorrhizal status of *Acacia* spp. in a field trial near Seremban (Malaysia)

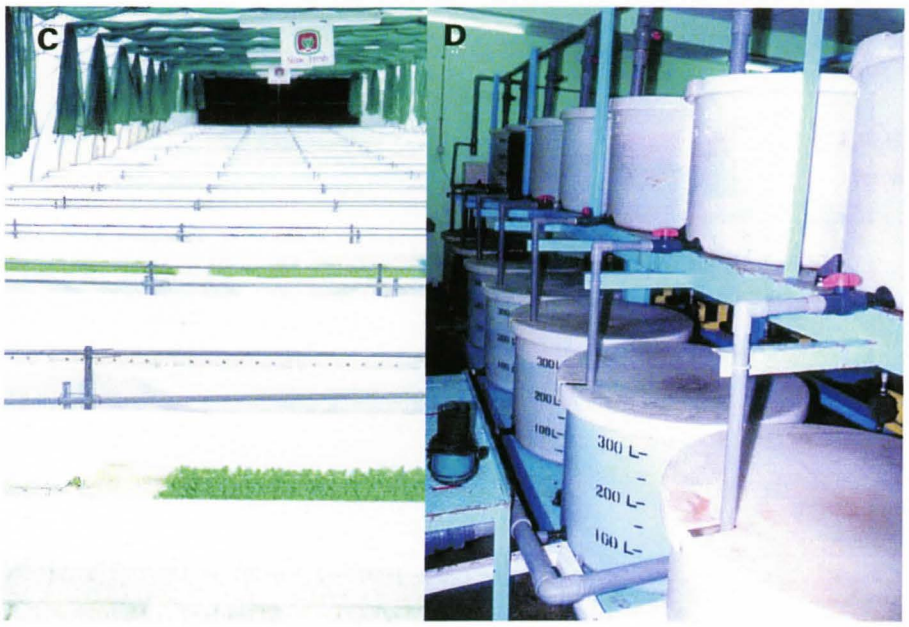
	Arbuscular mycorrhizas		Ectomycorrhizas
	%	Intensity (max=5)	%
Acacia cicinnata	100	3.8	0
Acacia mangium	25	3.9	85

Figure 5: A : Arbuscular mycorrhizal infection of *Acacia cicinnata* in a field trial near Seremban (Malaysia) ; B : Ectomycorrhizas of Acacia mangium with a well developped mantle (B1) and a discrete Hartig net (B2).



This visit has also been the opportunity to visit the aeroponic farm (figure 6) which technology have been considerably improved regarding the Singapore farm.

Figure 6: A: partial view of a greenhouse of Sime Darby aeroponic farm in Seremban (from outside A1 and inside A2) ; B: seeding greenhouse, C: building for fully automatic nutrient solution preparing.



Meeting at STA, Kuching, Sarawak, Malaysia

The mission was an opportunity to organize a first contact in Sarawak with STA. Three main points have been treated during this visit: 1- a general presentation of CIRAD and a more

detailed presentation of the TQS project developped jointly between Singapore and France, 2- an expertise of some sylvicultural problems now occuring in 14-years-old *Acacia mangium* plantations and 3- the opportunity to develop futher collaboration and among them, to set up a field trial using aeroponically grown *Acacia mangium*.

General presentation

Sarawak Timber Association is what we call in France an "union patronale" grouping as free members all companies working in Sarawak in the field of forestry from forest management to exported wood products such as furnitures. The general presentation of CIRAD and of the TQS project have been presented as part of a conference in the presence of : Barney Chan, Peter Kho, John Keen Chubo, Marini Husin (Sarawak Timber Association), Semilan Ripot, Latifah Teh, Salina Hj Hamdi, Salleh Mit, Nixon, Girang, Mang (Forestry Department HQ), Lucy Chong, John Sabang (Forest Research Center), Glen Mac Nair (Borneo Pulp and Paper Sdn Bhd), Willi Wunder, Ting Sing Chuon (Goodmatch Sdn Bhd), Yii Puan Ching, Philip Ho (Rimbunan Hijau Sdn Bhd) Ngieng Ping Wei (KTS Forest Plantation Sdn Bhd), Fred Liew (Samling Reforestation (Btu) Sdn Bhd).

Acacia mangium plantation health.

In three sites, we have the opportunity to find problems within *Acacia mangium* survival. In each cases, dead individuals stand dry and almost intact (figure 7). On some individuals, lower branches were still alive; the upper part being definitly dead. Dead individuals were not isolated but grouped in almost circular spots. In one spot more than one hundred dead trees can be counted. Root samples have been collected on healthy and obviously non healthy, but not dead individuals. A first examination reveals an important infection by nematodes (Probably *Meloidogyne* sp.) of unhealthy individuals (figure 8). Healthy individuals do not exhibit any sign of nematods infection and are poorly nodulated by *Rhizobium*. Sporophores of *Telephora ramarioides* have been observed under some trees along the road.

Figure 7 ; A : Decaying plot of *Acacia mangium* ; B : Healthy plot of *Acacia mangium*.

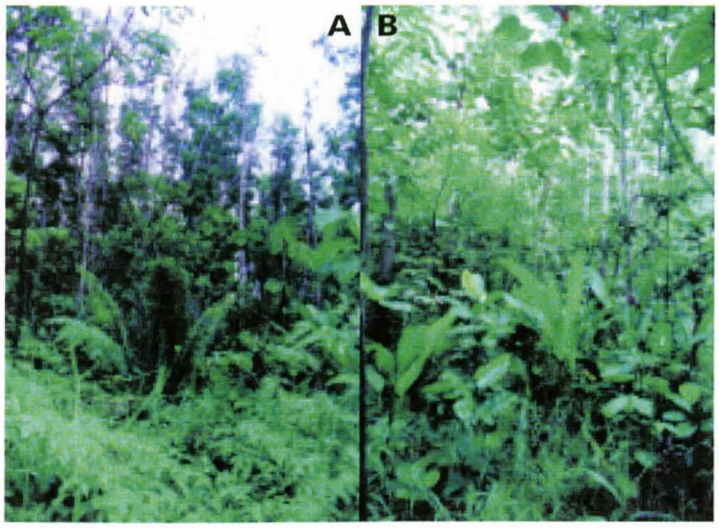
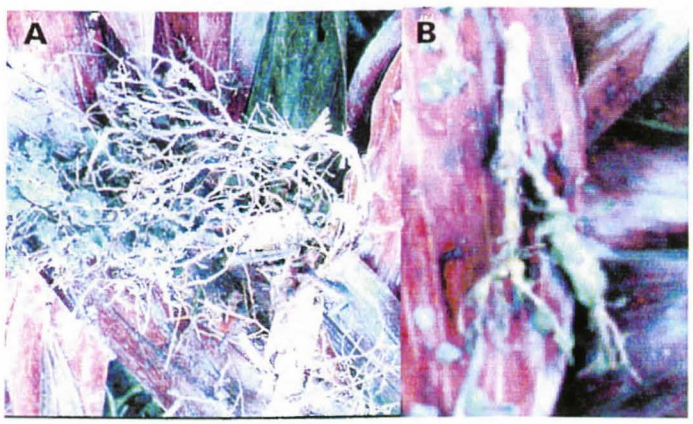


Figure 8: A: Healthy root system of *Acacia mangium*; B: Roots of *Acacia mangium* infected by nematodes.



Examination of root infection levels by mycorrhizas revealed : 1- the almost absence of any mycorrhizal infection in unhealthy individuals ; 2- the extremely high level of arbuscular mycorrhizal infection (90 to 100 % with an average intensity of 4.2) in healthy individuals and 3- the presence of ectomycorrhizas (10 to 20 %) also in healthy individuals (table 3).

Table 3: Percentage and intensity of arbuscular mycorrhizal fungi infection of healthy and decaying *Acacia mangium* in Sarawak.

	Arbuscular mycorrhizas		Ectomycorrhizas
	%	Intensity (max=5)	%
Site 1 : Healthy individual	100	4.2	10
Site 1 : Decaying individual	10	1.5	0
Site 2 : Healthy individual	90	4.2	5
Site 2 : Decaying individual	20	1.8	0

Possibilities to develop new collaborations

STA is ready to act as a mediator between CIRAD and its members to facilitate the development of cooperation. Major fields of interest are on the Dipterocarp forest and on *Acacia mangium* plantations.

Ectomycorrhizal fungi associated with Dipterocarps

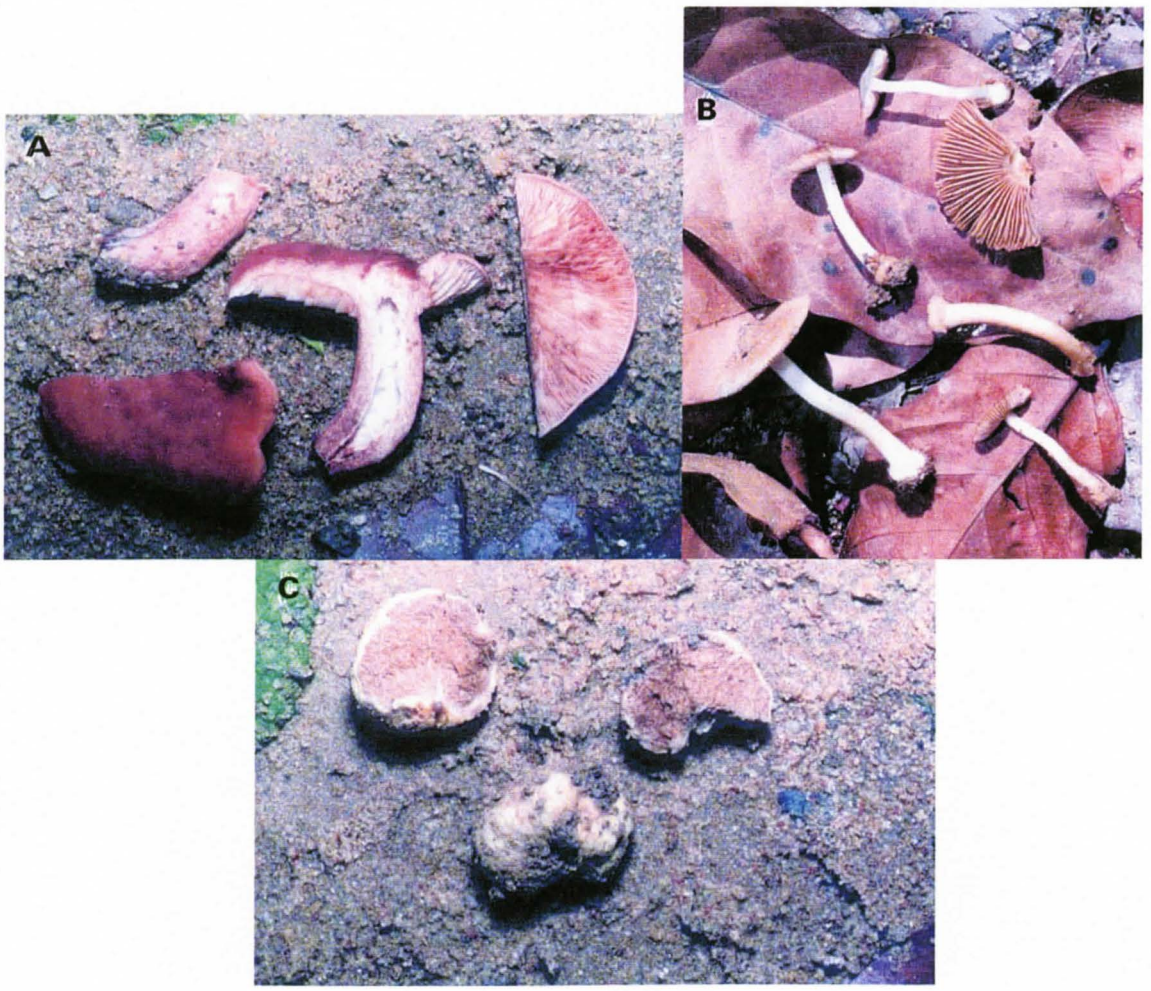
In Singapore botanical garden

Four species of ectomycorrhizal fungi have been found under two species of Dipterocarps (table 4 and figure 8).

Table 4: Presence and relative abundance of four ectomycorrhizal fungi under two species of Dipterocarps.

	Hopea odorata	***Vatica sp.***
Lactarius sp.	Abundant	No
Inocybe sp1	Abundant	No
Inocybe sp2	1 sporophore	Abundant
Hypogeous ind.	2 sporophores	No

Figure 8: A: Lactarius sp.; B: Inocybe sp1; C: Hypogeous ind.



In Kuching, Sarawak

Plantations of *Shorea macrophylla* (figure 9) have been visited to prospect ectomycorrhizal fungus fruitbodies. Four species of ectomycorrhizal fungi have been observed: *Phylloporus* sp., *Colostoma* sp. (called locally "pig eye"), *Russula ciliata* and *Telephora* sp. (figure 10). Pig eye is considered locally as a valuable edible mushroom.

Figure 9: Plantation (1.62 ha) of *Shorea macrophylla* established in 1936.

Figure 10: A: *Phylloporus* sp.; B: *Colostoma* sp.; C: *Russula ciliata*; D: *Telephora* sp.

Concluding remarks

First results obtained in the framework of the TQS project have to be published. At present, two proposals are identified: 1- Using alginate as sticking agent for production of AM mycorrhized seedlings of *Acacia mangium* (Willd.) in aeroponic culture and 2- Production, storage and transfer of bare root *Acacia mangium* (Willd.) saplings from aeroponic culture to the field.

The first proposal relates the use of sodium alginate as sticking agent for direct inoculation of AM spores, as well as crushed root inoculum, of *Glomus* sp. in aeroponic. Six weeks after inoculation (AM spores or crushed root), the roots were mycorrhized up to 18 cm deep, showing typical arbuscules and vesicles. No spores was observed during a later month of aeroponic culture. One single inoculated sapling generated enough crushed-root inoculum to inoculate 50 new trees. Aeroponic culture is an easy way to produce in one step, mycorrhized saplings of *Acacia mangium* and the necessary AM inoculum.

The second proposal relates the ability of bare-root seedlings of *Acacia mangium* produced in aeroponics to be used in plantation programmes. Two months after transfer to the field the survival rate was up to 96% with no significant differences between treatments ($P=0.05$). The production of *Acacia mangium* in aeroponic appeared to be well adapted to an easy transport and a few days storage before field use.

Inoculation with *Rhizobium* and AM fungi are well mastered. Physiological studies on their effect can be now developped.

Progress have to be done to control inoculation with ectomycorrhizal fungi. Researches will be developped jointly in Montpellier and Singapore to study *Acacia mangium* ectomycorrhizal symbiosis. This phase is essential before trials in aeroponics.

The setting up of at least one field trial during 2001 is essential. The experimental design have to be ready before june 2001, saplings have to be ready for field transfer in november or december 2001.

Mid november is a fruiting period of ectomycorrhizal fungi associated with Dipterocarps in Singapore and Sarawak.