Report of a mission to Sabah Malaysia

24 to 29 june 1998

Marc DUCOUSSO
LSTM/CIRAD-Forêt

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First of all, I wish to greatly acknowledge **Dr Antoine Galiana** for his help in the organization of this mission and above all during its schedule.

This mission was indirectly linked to the framework of the Biorize project. Indeed, the mission undertaken in Singapore just before help greatly to decide to go to Sabah. Anyway, the technic of dual rhizobial and mycorrhizal inoculation developed with *Acacia mangium* growing under aeroponic conditions at NTU/NIE is susceptible to be set up on large scale and outplanted in Sabah.

The main goal of this mission was to make first observations on the importance of mycorrhizae and mycorrhization in ICSB plantations in Sabah. My work has been focused on major plantation species used in Sabah. Consequently, I make diagnostics on Teak, Acacia, Rattans and Dipterocaps mycorrhization under nursery and/or field conditions. I also present a paper on mycorrhizae in Luasong and meet **Dr Chan Hing Hon** in Kota Kinabalu to present the interest of CIRAD in Mycorrhizae research.

Chronology and schedule of the mission

June 23, Tuesday :	Arrival in Tawau at 5:00 pm, meeting with Dr Antoine Galiana, putting up at Marco Polo hotel.
June 24, Wednesday :	Meeting in Tawau with **Dr Doreen Goh** and **Ms Hannah Moo**. Visits to Taliwas (Teak nursery and plantations) meeting with **Mr Jikos Gidiman**, Face Foundation (Dipterocarp nursery) meeting with a technician in charge to represent **Dr Francis Goh** and Danum Valley (primary Dipterocarp forest).
June 25, Thursday :	Visit to Luasong (Rattans, Acacia, Teak and Dipterocarps). Meeting with **Mr David Alloysius, Dr Roberto Bacilieri, Mr Wilfried Schueller and Mr Kevin Pouët.**
June 26, Friday :	Visit to Brumas, Sabah Softwood plantations (mainly *Acacia mangium* and hybrids). Meeting with the technical staff present.
June 27, Saturday :	Departure from Tawau at 7:00 am, arrival in Kota Kinabalu at 9:00 am and meeting with Dr Chan Hing Hon. Putting up at Promenade hotel.
June 28, Sunday :	Departure from Kota Kinabalu at 11:00 am.
June 29, Monday :	Arrival in Montpellier at 1:00 pm.

List of met persons

Mr David Alloysius : Co-leader of the PISP project, ICSB.
Dr Roberto Bacilieri : CIRAD-Forêt researcher, genetician working for PISP project.
Dr Chan Hing Hon : Senior manager, Forestry upstream division, ICSB.
Dr Antoine Galiana : CIRAD-Forêt researcher, specialist in plant *vitro* culture and *Rhizobium* microbiology.
Mr Jikos Gidiman : Official in forest regeneration and research, ICSB.
Dr Doreen Goh : ICSB researcher, plant propagation and biotechnology specialist.
Dr Francis Goh : Face foundation manager.
Ms Hannah Moo : PBL Forest officer.
Mr Kevin Pouët : Trainee working in collaboration with Dr Roberto Bacilieri in the field of genetic.
Mr Walter Schueller : CSN working in collaboration with Dr Roberto Bacilieri in the field of genetic.

Description of some aspects of visiting sites

Taliwas

On the site of Taliwas, a now entierly devoted to Teak nursery is available for research activities (photo 1). *Vitro* cultured plantlet acclimatization is there possible (photo 2). It is also possible to make plantations round Taliwas.

Photo 1 : General view of Taliwas nursery.

Photo 2 : setting up of teak vitroplants for its acclimatization.

Face Foundation

A nursery able to produce 6 millions plants per year (photo 3). This nursery now produced mainly Dipterocarp plants by both seedlings and wildings.

Photo 3 : General view of an unit of Face foundation nursery.

Danum valley

The place to study primary Dipterocarp forest in Sabah (photo 4). It certainly worth to work there on mycorrhizae.

Photo 4 : Experimental plot for the study of Dipterocarps primary forest regenaration near Danum valley.

Luasong

The PISP project is localized at Luasong forestry center and Sylvicultural trials. An important part of the activities of this center is focused on rattans (photo 5). Now new activities are developed on Teak, *Acacia* and Dipterocarps.

Photo 5 : Production of rattan plants at the nursery of Luasong forestry center.

Brumas

The holy of holies of *Acacia mangium*. Sabah softwood plantations round Brumas cover more than 30,000 ha mainly with *Acacia mangium* (photo 6). A research nursery (photo 7), in the vicinity of the production nursery (able to produce 6 millions plants per year) provides facilities for cutting (photo 8), *vitro* plantlet acclimatization (photo 9), and any other research activities.

Photo 6 : exploitation of Sabah Softwood plantation (*Acacia mangium*) round Brumas.

Photo 7 : General view of Brumas research nursery.

Photo 8 : setting up of *Acacia* hybrid cuttings.

Photo 9 : Acclimatization of *Acacia* hybrid vitroplants.

Tawau : the PBL

The PBL localized in Tawau provides all facilities for *in vitro* culture of tree species. The essential part of the production of the laboratory involves two species : *Acacia mangium* and *Tectona grandis*. It is also a place where some other species such as rattans are worked.

Observations and comments on mycorrhizae in Sabah

Methods of study

Roots of different species have been collected under various conditions : plantation, nursery, natural forest and kept in GEE. Localization, species name and age of the tree as well as any other remarks have been quoted for each sample. Fixed roots have been stainned at the laboratory with Trypan blue according to Philips and Hayman (1970) methods. Stainned roots are then mounted in PVLG for microscopic observations. For each sample, 100 root fragments of approximatly 1 cm long have been examined. To quantify the infection by AMF, a mark between 0 and 5 is given for each root fragments. 0 means : no fungi present ; 1 means : discrete presence of a fungi without formal evidence of AMF infection ; 2 means : evidence of one or two very localized AMF infection ; 3 means : good evidences of AMF infection ; 4 : well developed and distributed AMF infection ; 5 means : heavily infected by AMF. For each sample, two values are calculated to characterize the AMF infection : the frequency (F) and the intensity (I) of infection. F is the ratio of the number of root fragments having a mark from 2 to 5 divided by the total number of root fragments examined (generally 100). I is the average mark calculated on all fragments (generally 100).

For ectomycorrhizae, we observe the presence of fungal mantle and Hartig net. In the case of *Acacia* species, we also calculate the frequency of the presence of a fungal mantle covering root tip (FM).

Teak

Roots of teak vitroplants (two different clones and two different ages), seedlings and cuttings have been examined for AMF infections under Taliwas nursery conditions. Teak AMF infections have also been recorded under three different plantations. All F and I datas of teak AMF infections are reported in table 1

Table 1 : Frequence and Intensity of AMF infections on teak roots under different conditions.

Ref. N°	Type of material	Origine	Site	Age*	F (%)	I
MD0205	Vitroplant	IC	TN	4.5	90	4.0±1.47
MD0206	Vitroplant	IC	TN	3	92	3.9±1.42
MD0207	Vitroplant	MBO	TN	7	97	4.8±0.72
MD0208	Vitroplant	MBO	TN	3.5	100	4.2±0.77
MD0209	Vitroplant	SIB	TN	0.5	0	0
MD0210	Seedling	?	TN	12	92	4.2±1.42
MD0211	Cutting	?	TN	3	100	4.3±0.64
MD0224	Seedling	?	FT	72	73	2.7±1.74
MD0225	Vitroplant	IC	FT	8	95	4.3±1.04
MD0226	Vitroplant	IC	FL	18	100	4.8±0.58

Origine : IC : Ivory coast ; SIB : Solomon Island bulk ; ? : unknown.
Site : TN : Taliwas nursery ; FT : field round Taliwas ; FL : field round Luasong.
* : age in months

These results clearly demonstrate the importance of AM for teak. The frequency as well as the intensity of AMF infection are generally very high. Under nursery conditions, we can remark that two week after transplantation, clone SIB is non mycorrhizal (photo 10). Indeed, the observation under the light microscope let see the total lack of cortical parenchyma round the center cylinder of fine roots. It is well known that only cells from the cortical parenchyma are receptive to AMF infection. In such conditions, the absence of AMF infection could be considered as normal. Nevertheless, 3-month-old vitroplants present well developed AMF infections (photo 11). The potential of AMF propagules in the substrate used in Taliwas nursery is certainly very high. Under Taliwas nursery conditions, we can also remark a slight difference between clones IC and MBO. F and I are higher in clone MBO. Such differences are already known in other species like *Ordeum* meanwhile, this first observation have to be confirmed in teak. We can also note a rooting typical of vitroplant (photo 12). Such a rooting may cause problem on a long term period.

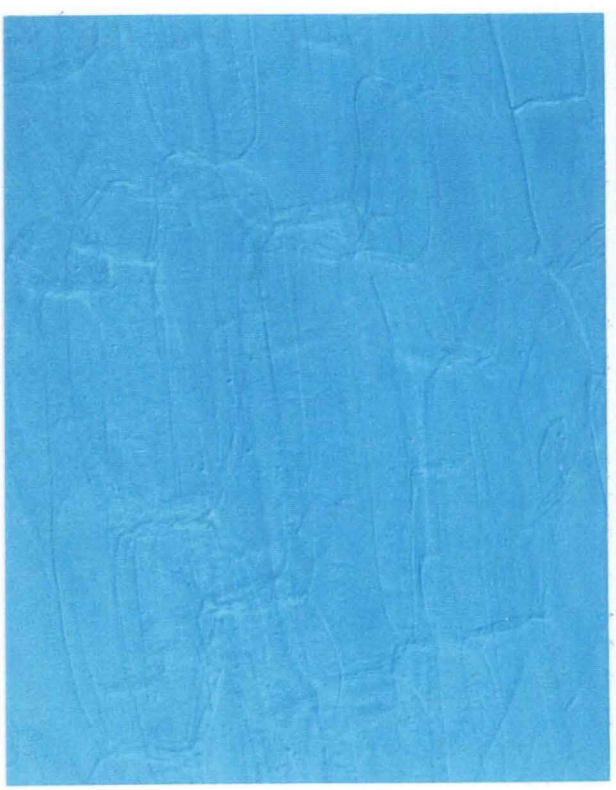

Photo 10 : Non mycorrhizal roots of a 2-week-old teak plant, clone SIB (sample MD0209).

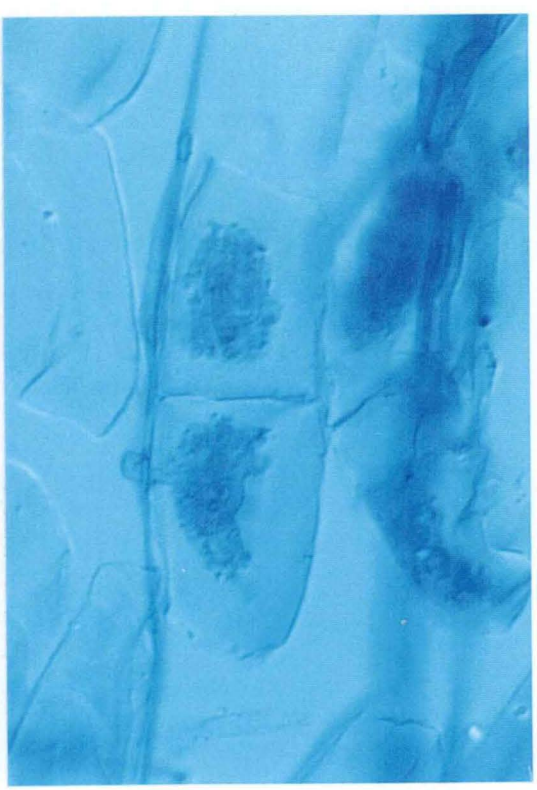

Photo 11 : AMF infection in teak vitroplant root (sample. MD0206)

Photo 12 : Typical rooting of a teak vitroplant (clone SIB ; sample reference N° MD0209).

Under field conditions, in the oldest plantation examined (72 months, photo 13) we note a relatively low F and I. It might du to the presence of a higher rate of dead roots in the 72-month-old plantation rather than in the 8 and 18-month-old plantations (photo 14).

Photo 13 : 72-month-old plantation of teak seedling round Taliwas.

Photo 14 : 8-month-old plantation of teak, clone IC, round Taliwas.

Acacia mangium and others

Four species of Australian *Acacia* seem very promising for humid tropic forestry. Among them, we found the well known *Acacia mangium* (photo 15) and three other related species : *Acacia aulacocarpa, Acacia auriculiformis* and *Acacia crassicarpa* (photo 16). Interspecific breedings seems a very promising source of new material for forest plantations. Consequently, breeding program succed in the obtention of putative hybrid between *Acacia mangium* and *Acacia auriculiformis* called *Acacia* hybrid who share morphological characters of its both parents (photo 17). The mycorrhizal infections of these species are reported in 9 plantations (table 2).

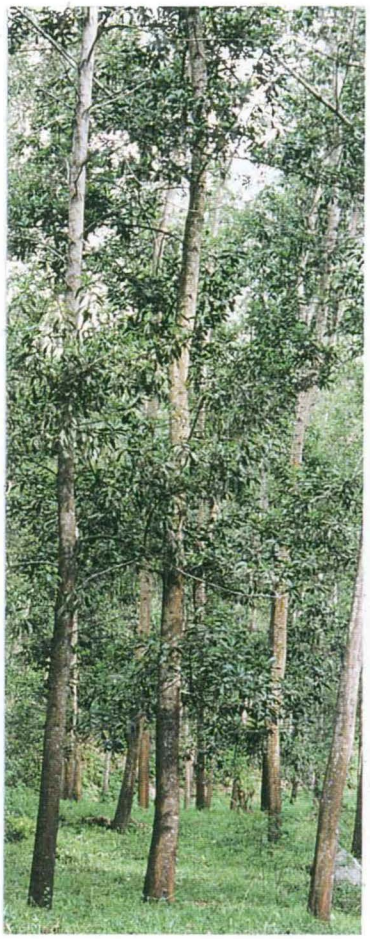

Photo 15 : 7-year-old *Acacia mangium* round Luasong.

Photo 16 : 7-year-old *Acacia crassicarpa* round Luasong.

Photo 17 : 3-year-old *Acacia* hybride round Brumas.

Table 2 : F and I of AMF infections and MF on *Acacia mangium* and related species roots under different conditions.

Ref. N°	Species	Site	Age*	F (%)	I	FM (%)
MD0201	*Acacia aulacocarpa*	FB	3	50	1.3±1.24	0
MD0204	*Acacia auriculiformis*	FB	3	95	3.3±1.35	5
MD0200	*Acacia crassicarpa*	FB	3	54	1.8±1.47	4
MD0229	*Acacia crassicarpa*	FL	7	65	2.1±1.66	30
MD0202	*Acacia mangium*	FB	3	83	3.2±1.71	52
MD0228	*Acacia mangium*	FL	7	78	2.4±1.46	14
MD0227	*Acacia mangium*	FL	1.5	48	1.6±1.52	34
MD0203	*Acacia* hybride	FB	3	3	0.2±0.56	100
MD0196	*Acacia* hybride	FL	1.5	67	1.9±1.14	19

Origine : FB : field round Brumas ; FL : field round Luasong.
* : age in years

Compared to teak, AM infections of *Acacia* are low. Nevertheless, the frequency of infection observed in Sabah look very similar to AMF infections of these species in other places such as Ivory coast or Senegal. The intensity of AMF infection seems generally low. This observation required further studies ; indeed, it probably means that the receptiveness of these *Acacia* species to local AMF is not satisfying.
We observed also the presence of fungal mantle in many samples (photo 18). This presence is very original and needs further studies. Indeed, these fungal mantles are probably du to an ectomycorrhizal fungus which now remain unknown. Nevertheless, we were not able to assess the presence of an Hartig net. It look very similar to an observation made the week before in Singapore where Dr Tham Fong Yee identify a species of *Ramaria*, as a putative ectomycorrhizal fungus of *Acacia mangium* (photo 19). It seems that this *Ramaria* sp. is able to form a fungal mantle and not a Hartig net with *Acacia mangium*. During the time of the mission I observe some sporophores of *Ramaria* sp. under *Pinus caribaea* but not under *Acacia* spp.
In a first assumption, I can make the hypothesis that *Ramaria* sp. is not a fully compatible ectomycorrhizal fungus of *Acacia* spp. In addition, the fungal mantle forming fungus is probably a competitor of AMF.

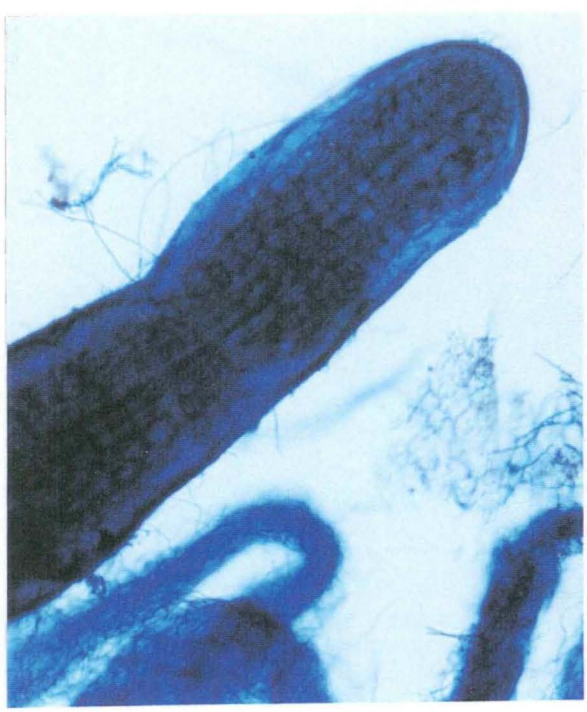

Photo 18 : Fungal mantle on *Acacia* hybrid root (sample MD0203)

Photo 19 : *Ramaria* sp. under *Acacia mangium* in Singapore.

In conclusion for *Acacia* spp., the compatibility of AMF as well as ectomycorrhizal fungi is not proven. The lack of fully compatible mycorrhizal fungi for *Acacia* spp. may cause sustainability problem in *Acacia* spp. plantation in Sabah.

We were able to observe nitrogen fixing nodules in each plantations (photo 20). We also remark the presence of parasitic nematods (photo 21).

Photo 20 : Nitrogen fixing nodules of *Acacia mangium* (sample MD0227).

Photo 21 : Nematod scurfs in *Acacia mangium* roots (sample MD0227).

Rattans

Four species of *Calamus* have been examined for their mycorrhizal status at Luasong. Three of them were growing under nursery conditions and one was planted in the vicinity of the nursery. Datas are reported in table 3.

Table 3 : F and I of AMF infections of four *Calamus* species at Luasong.

Ref. N°	Species	Site	Age*	F (%)	I
MD0190	*Calamus manan*	P	3	100	4,7±0,52
MD0191	*Calamus ornatus*	N	2	0	0
MD0192	*Calamus subinermis*	N	2	72	2,6±1,62
MD0193	*Calamus merrillii*	N	2	97	4,1±1,07

* : age in years

As many other Palmaceae, *Calamus* species are well known for their abilities to form AM. Moreover, *Calamus* species seem to depend largely upon AM. Under field conditions, *Calamus manan* is very heavily infected by AMF (photo 22 and 23). Nevertheless, we can take alarm at some of our observations under nursery conditions. No AMF infection have been seen on *Calamus ornatus* and F and I of *Calamus subinermis* remain probably too low. Only *Calamus merrillii* shows a well developed AMF infection. The lack of AM on 2-year-old *Calamus* species under nursery conditions may cause problem at transplanting time. I suggest to improve nursery conditions in order to obtain plants heavily infected by AMF.

Photo 22 : 3-year-old *Calamus manan* planted in the vicinity of Luasong nursery.

Photo 23 : AMF infection of *Calamus manan* roots (sample MD0190).

Dipterocarps

Dipterocarps are the most important source of tropical timber. This family is also well known for its ability to form ectomycorrhizae (photo 24). The main goal of our observations was to assess the (normal) presence of ectomycorrhizae under local nursery conditions (table 4 and photo 25). Among important differences between the two studied nurseries, Face Foundation nursery uses seeds for its production and Luasong nursery uses wildings.

Photo 24 : Ectomycorrhizae of *Dryobalanops lanceolata* : field observation at Danum valley.

Photo 25 : Ectomycorrhizae of *Shorea oleosa* at FFN.

Table 4 : Mycorrhizae record of some Dipterocarps species under two different nursery conditions and in the field at Danum valley.

Ref N°	Species	Site	Observations
MD0221*	*Dipterocarpus acutangulus*	FFN	Few EM with HN & few AM
MD0223	*Dryobalanops lanceolata*	DV	EM with HN & localized AM
MD0197	*Dryobalanops lanceolata*	LN	EM rares
MD0215	*Dryobalanops lanceolata*	FFN	EM with HN & PF
MD0216	*Hopea ssp.*	FFN	EM with HN
MD0217*	*Parashorea malaanonan*	FFN	EM with HN , typical FM
MD0213*	*Shorea guiso*	FFN	EM with HN (on all root tips)
MD0219	*Shorea johorensis*	FFN	EM with HN, few AM
MD0214	*Shorea leprosula*	FFN	EM abundant & few AM
MD0198	*Shorea leprosula*	LN	EM rares
MD0199	*Shorea parvifolia*	LN	EM with HN
MD0212*	*Shorea oleosa*	FFN	EM & AM (same root)
MD0220*	*Shorea symingtonii*	FFN	Few EM with HN & few AM
MD0222	*Aquilaria malaccensis#*	FFN	Localized AM.
MD0218	*Koompassia excelsa#*	FFN	few AM, presence of tanins.

FFN : Face foundation nursery ; DV : primary forest regeneration demonstration plot at Danum valley ; LN : Luasong nursery (IKEA project).
EM : Ectomycorrhizae ; AM : Arbuscular mycorrhizae ; HN : Hartig net ; FM : Fungal mantle ; PF : Parasitic fungus.
* : not already described as ectomycorrhizal in an international paper.
# : do not belong to the Dipterocarpaceae family.

We were able to see ectomycorrhizae on all Dipterocarp species studied. In 6 cases, among 13, we observed also the presence of AM. Nevertheless, ectomycorrhizae was never very abundant. Meanwhile, we can consider that the substrate used in both nurseries contained enough ectomycorrhizal propagules.

It is here important to make a remark about the substrate used in both nurseries. Indeed, in my opinion, it might be better to used this substrate as hardware for house building rather than to grow plants. As a result, the shoot root ratio of plant is very very high (photo 26 and 27) ; too much high to obtain physiologicaly well developed plants. Fortunately people in charge of filling bags do not take enough care to shake down properly the substrate into the bags. Thus, broken zones appear where fine ectomycorrhizal roots are able to develop. In order to obtain plants with a well developed root system, I suggest to lighten the substrate used by the adjunction of something like vermiculite or polystyren beads or compost or still coconut fibers. I do not recommend sawdust. I suggest also to used anticoil containers like WM to avoid the formation of root coils and also containers providing a bigger volume of substrate than polyethylene bags nos used.

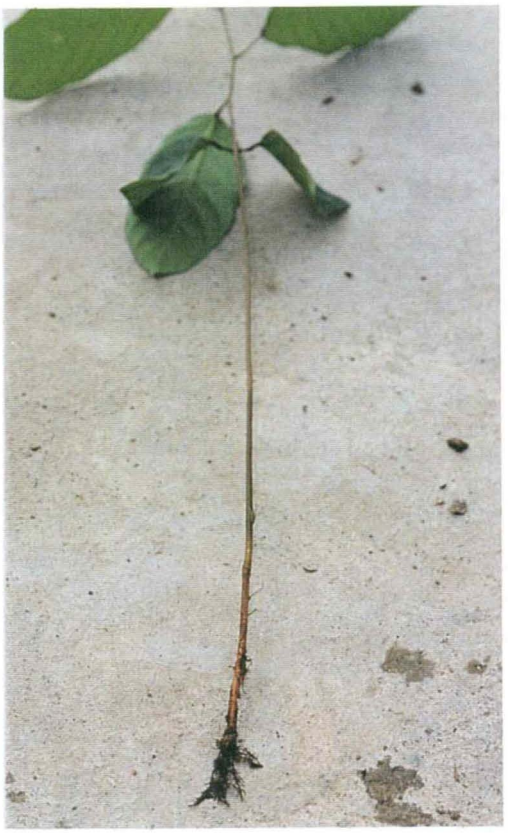

Photo 26 : *Shorea leprosula* plant from FFN (sample N° MD0214).

Photo 27 : Detail of the root system (photo 26).

Trap cultures for the isolation of AMF

We set up at LSTM trap cultures with teak, *Acacia mangium*, *Acacia crassicarpa* and *Acacia* hybride. The only goal of these trap cultures is to obtain specific AMF strains of the four species cited.
For that, we collect about 20 to 50 g fresh weight of roots of each species under field conditions (table 5).
Two methods of trap culture have been tested. In the first method (OPPAs), cuttings of *Plectranthus australis* inoculated with 5 to 10 g of fresh roots are cultivated in the greenhouse in 2 l open pot full with attapulgite. Pots are watered weekly to field capacity.
In the second method (SB), 0.25 l pots full with attapulgite are watered to field capacity, placed in a sunbag and autoclaved (120°C during 20 min.). In each pot, we transfere asepticaly one young seedling of *Trifolium subterraneum* (TS) or *Acacia mangium* (AM) inoculated with about 5 g of fresh surface desinfected (S) or not desinfected (s) roots. Roots have been surface desinfected with a 40 $g.l^{-1}$ calcium hypochlorite solution (5 min.) and then carefully rinced with sterile water.
For each sample, 5 trap cultures have been undertaken under the following conditions : OPPAs, SBTSs, SBTSS, SBAMs and SBAMS.

Table 5 : List and description of root samples collected in Sabah.

Cf (Ref. N°)	Species	Site
MD0224	*Tectona grandis*	FT
MD0229	*Acacia crassicarpa*	FL
MD0200	*Acacia crassicarpa*	FB
MD0228	*Acacia mangium*	FL
MD0202	*Acacia mangium*	FB
MD0203	*Acacia* hybride	FB

Site : FB : field round Brumas ; FL : field round Luasong ; FT : Field round Taliwas.

Isolation and purification of AMF strains may take at least one year. Thus, no results are now available. We attend to obtain two types of results : first of all, the creation of a collection of specific strains from teak and Acacia and secondly the characterization of these AMF.

Meeting with Dr Chan Hing Hon

The meeting with Dr Chan Hing Hon in Kota Kinabalu give me the opportunity to present the interest and potentialities of CIRAD-Forêt in mycorrhizae research and to make a first oral report of my mission in Sabah. I say that general working conditions in Sabah seem very good, specially in the transfert of results from the lab to the field. I make a remark on the quality of peoples and equipments to work their in forestry research. I also present the details of my visit and some impressions about two particular points. These two points concerne first the very heavy and clayey substrate used in nurseries ; specially at FFN and second the special rooting of vitroplants. I mention that it is now too early for me to speak about mycorrhizae in Sabah and that all collected samples required further observations in the lab. To develop a collaboration in Sabah in the field of mycorrhizae, Dr Chan Hing Hon suggest to take also contacts with FRIM researchers involved in mycorrhizae research.

First conclusions on mycorrhizae in Sabah

It is now an evidence that the importance of mycorrhizae on major species planted in Sabah cannot be forgoten. Some important points concerning major species are here after developed. Possibilities to develop collaborations and to set up field trials are also presented.

Teak

A very well mycorrhized specied, probably very dependent on AMF. Differences in AMF infections between clones and the effects of a controlled AMF infection worth to be studied. The late AMF infections of vitroplant have to be studied.

Acacia
It is well known that generally, indigenous microorganisms act as strong competitors against introduced symbiotic microorganisms. In the case of *Acacia* plantations in Brumas and Luasong, it seems that no fully compatible mycorrhizal fungi are present. Consequently, the development of a program of controlled inoculation is very promising. Ectomycorrhizal strains fully compatible with *Acacia mangium* and *Acacia auriculiformis* are already available at LSTM ; it is possible to plan one or two field trials of *Acacia* inoculation.

Rattans
A program of controlled inoculation under nursery conditions can be carry out very quickly to assess the importance of AMF for *Calamus* species, specially after transferring into the field.

Dipterocarps
A lot remain to do on Dipterocarp mycorrhizae. It is certainly the subject to study in close relation with FRIM researchers.

List of abbreviations

AM : Arbuscular Mycorrhizae.
AMF : Arbuscular Mycorrhizal Fungus.
CIRAD-Forêt : Forestry department of the Centre de coopération International de Recherches Agronomiques pour le Developpement.
CSN : Coopérant du Service National.
FRIM : Forest Research Institue of Malaysia.
GEE : A preservative mixture made of a third of water, a third of ethanol and a last third of glycerol.
ICSB : Innoprise Corporation Sdb Bdh.
LSTM : Laboratoire des Symbioses Tropicales et Méditerranéennes ; CIRAD/ORSTOM/INRA/ENSA-M joint laboratory localized in Montpellier.
PBL : Plant Biotechnology Laboratory.
PISP : Plant Improvement and Seed Production.