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AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE: 10/19/87

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18
FROM: AID/SCI, Victoria Ose *VO*
SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 6, 575
Annual PR rec'd 9/30/87

Attachment

2 ep

6.575

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ANNUAL PROGRESS REPORT

TITLE: The Effects of Mycorrhizal Inoculation and other Nursery Pre-conditioning Treatments on Field Survival and Growth of Bare-Root Planted Eucalyptus and Dipterocarps.

GRANTEE: University of the Philippines at Los Baños
College of Forestry, College, Laguna
Philippines

RESEARCH STAFF:

Reynaldo E. dela Cruz	Project Leader
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Rec'd in SCI: SEP 30 1987

I. OVER-ALL AIM AND SPECIFIC OBJECTIVES

The over-all goals are to determine the feasibility of using mycorrhizal fungi in promoting the survival and growth of Eucalyptus and Dipterocarps and to test the feasibility of using mycorrhizal fungi in combination with various silvicultural pre-conditioning treatments in the development of a bare-root planting technology for Eucalyptus and Dipterocarp seedlings.

II. PROGRESS REPORT

A. METHODOLOGIES

Study 1. Collection, Isolation and Identification of Ectomycorrhizal and Endomycorrhizal Fungi Associated with Eucalyptus and Dipterocarps species.

ECTOMYCORRHIZA

Collection trips were conducted in Carranglan and Pantabangan, Nueva Ecija; Tarlac; Quezon; Surigao and Makiling Forest, where there are plantations on natural forests of Eucalyptus deglupta, E. camaldulensis, Dipterocarpus grandiflorus and Anisoptera thurifera. Fruiting bodies of suspected mycorrhizal fungi were collected around the base of these species.

Close-up pictures of basidiocarps of ectomycorrhizal fungi were taken. Important macroscopic characteristics in the field were recorded, including those fruiting bodies that change in color when bruised and those that exude sap. Some of the sporocarps were oven-dried at 70°C for herbarium collection. Ecto-

mycorrhizal fungi were isolated by transferring the inner tissues of stipe and pileus to test tube slants containing Modified Melin Norkrans (MMN) medium. After a few days, tissues were sub-cultured in another fresh MMN slants for further purification.

To isolate from mycorrhizal roots, the roots were first washed with tap water then surface sterilized with 1% $HgCl_2$ for 5 to 10 minutes. After sterilization, the roots were rinsed 10 times with sterile distilled water and then placed into test tube slants containing MMN medium. Growth was observed after a few days. Contaminated tissues were disregarded and uncontaminated ones were subcultured in another fresh MMN slant for further purification.

The mass production of vegetative mycelia in MMN broth is also going on. Test tube cultures were transferred aseptically to sterilized dextrose bottles containing 100 ml of MMN broth (see Fig. 1a & 1b). The fungi were allowed to multiply in the broth for a month. These were kept in an improvised aseptic cabinet.

For further purification of cultures, isolates from test tube slants were re-isolated in Petri plates containing MMN medium with or without coir dust (Fig. 2a & 2b). A simple experiment using MMN with agar and coir dust was performed to determine if coir dust inhibits the growth of ectomycorrhizal

species. The pH of the substrate was between 4.5 to 5.5 after sterilization.

Mycorrhizal synthesis study

To prove that the isolated fungi are true mycorrhizal symbionts, mycorrhizal synthesis study was performed. Palosaplis seedlings were planted in a 1-liter jar using 3:1 sterilized perlite and quarry sand. The seedlings were supplemented with varying levels of MMN minus the carbohydrate source (i.e., 100, 200, 300 and 400 ml) and inoculated with the fungus coded as AID-D2. Each treatment was replicated 3x. Uninoculated seedlings served as controls. The set-up was placed in a growth room at 28°C and illuminated with artificial lights. The seedlings were given enough time to synthesize mycorrhizal roots (Fig. 3).

ENDOMYCORRHIZA

The field collected rhizosphere soil (about 20 to 30 grams) containing VA mycorrhizal fungi, from the same source and host plant as with the ectomycorrhiza was mixed in 500 ml aluminum beaker containing tap water. The solution was thoroughly agitated to separate spores from soil clods and poured through a series of sieves (18, 60, 250, 325 um) to make sure that VAM spores were collected in the sieves. The latter were then transferred in centrifuge bottles,

with the aid of wash bottles containing tap water, and centrifuged for 3 min. The supernatant liquid in the centrifuge bottle was poured off, leaving spores plus soil particles at the bottom. These were then further centrifuged using 40% sucrose solution to allow spores to float. Immediately after centrifuging for 1 minute, the liquid content was poured-off to a 325 μ m sieve and thoroughly washed with tap and dionized water. Collected VAM spores were transferred to small plastic Petri dishes and observed under microscope for classification into genera, based on important microscopic features, such as presence of bulbous base, color, surface appearance, presence of hyphal attachment, presence or absence of mother hypha, etc.

Initially, isolated endomycorrhizal fungi were mass produced singly into plastic tubes and/or plastic cups containing mixtures in 50-50 proportion of fumigated forest soil and quarry sand, with Bahia grass as host plant and alfalfa as nitrogen fixer to provide nitrogen for the host (Fig. 4). The VAM was allowed to infect the host's roots for 5 months before evaluation.

A second attempt to mass produce inoculants was conducted using different media proportion, i.e., forest soil, quarry sand and Mayondon sand. A separate experiment using complete fertilizer plus $\frac{1}{2}$ strength

Hoagland's solution applied to pure medium, i.e., Mayondon sand alone and quarry sand alone was also conducted. This experiment will be useful to determine the best potting media for VAM production, which in turn will be needed in succeeding studies. VA mycorrhizal fungi inoculated were starter cultures obtained from International Culture collection of VA Mycorrhizal Fungi University of Florida, Gainesville, Florida, U.S.A.

Synthesis study

Using the pure isolated VAM from the field, a synthesis study was initiated to determine whether these VAM fungi forms mycorrhizal association with the host species. Palosapis seedlings were inoculated with isolate AID-1. The plants were grown in a 1 liter jar containing a mixture of 1:3 sterilized perlite and quarry sand (see Fig. 3). The plant was supplemented with varying levels of MMN minus the carbohydrate source, i.e., 50, 100, 150, 200, 300, 400, 525 and 650 ml. Each of these were replicated three times, including uninoculated control. The seedlings will be given enough time to synthesize mycorrhizal roots.

Study 2. Screening for Effectiveness among
Mycorrhizal Fungi

Sub-Study 2a - Screening for effectiveness among
ectomycorrhizal fungi using
vegetative mycelia.

Pure cultures of mycorrhizal fungi associated
with Dipterocarps and Eucalyptus were grown in
MMN broth. This will be inoculated later to
palosapis and Eucalyptus seedlings.

Sub-Study 2b - Screening for effectiveness among
ectomycorrhizal fungi using
basidiospores.

Spores were extracted from matured sporocarps
of Pisolithus and Rhizopogon and dried in an oven
at 30°C. Pre-germination of palosapis and Eucalyptus
seeds are going on. The seedlings will be
inoculated after a month.

Sub-Study 2c - Screening for effectiveness using
endomycorrhizal fungi

Five VAM fungi plus the starter cultures will
be used for the study. The medium 1:3 forest soil
and quarry sand, was already prepared and fumigated
with Methyl Bromide. This experiment will be set-up
once the seeds of Eucalyptus species and Dipterocarps
are available.

B. RESULTS

Study 1. Collection, Isolation and Identification of Ectomycorrhizal and Endomycorrhizal Fungi Associated with Eucalyptus and Dipterocarps species.

ECTOMYCORRHIZA

The ectomycorrhizal fungi associated with Dipterocarps stand totalled about 32 species (see Fig. 7-20). Some belong to the genera Russula, Pisolithus, Thelephora, Lactarius and Rhizopogon. The others still need to be identified. Purification and mass production in test tubes slants, Petri plates and MMN Broth are on-going.

Results of synthesis study are not available yet. Seedlings will be given enough time to synthesize mycorrhizal roots.

ENDOMYCORRHIZA

Based on important microscopic features the following genera were identified and inoculated singly into plastic tubes or plastic cups.

Under Dipterocarps Plantation

- 1) Glomus species - 5 species
- 2) Acaulospora sp. - 5 species
- 3) Gigaspora sp. - 1 species

Under Eucalyptus Plantation

- 1) Glomus species - 9 species
- 2) Gigaspora species - 1 species
- 3) Acaulospora sp. - 8 species
- 4) Sclerocystis sp. - 1 species

Five months after inoculation, the set-up was assessed. Only 5 VAM species produced many and pure cultures.

The following are the data for 5 field collected VAM which had successfully sporulated in culture (pictures will be available after identification).

<u>CODE</u>	<u>REMARKS</u>
AID - 1	collected under <u>Eucalyptus declupta</u> in PICOP, Bislig, Surigao del Sur on October 23, 1986.
AID - 22	VAM isolated from Burgos, Carranglan, Nueva Ecija under <u>Eucalyptus camaldulensis</u> plantation.
AID - 79	collected from Makiling Forest, UPLB, College of Forestry around the rhizosphere of <u>Amisoptera thurifera</u> and <u>Dipterocarpus grandiflora</u> plantation on October 22, 1986.
AID - 119	also originated from Makiling Forest under Apitong plantation.
AID - 95	same provenance as in AID-79.

The pure VAM cultures were stored in plastic containers and refrigerated at 5°C while some were mass produced in pots containing only Bahia grass.

Some of the inoculated fungi did not sporulate well while others were contaminated. At this point the project needs to have a glasshouse solely for mass production of inoculants to prevent the entry of foreign bodies (contaminants). It was observed that

other VAM species need a longer time to sporulate, hence the experiment was extended. Moreover, the best medium for better sporulation given a particular genera was also conducted to know the best way of producing inoculants.

Synthesis study

There was no result yet, since this study need more time for the VAM to infect the roots of palosapis species.

III. OTHER ACCOMPLISHMENTS/ACHIEVEMENTS:

1. An existing greenhouse was expanded to accomodate experimental set-ups.
2. An airconditioned room for the growth chamber was already constructed in the headhouse.
3. USAID through WINROCK International invited Dr. dela Cruz to two workshops on Multipurpose Tree Species (MPTS), first, in Bangkok, Thailand (September 22-28, 1986) and second in Kuala Lumpur, Malaysia (Dec. 14-18, 1986). During the workshops, mycorrhiza was given emphasis as a major priority IN MPTS establishment in Asia-Pacific countries. Dr. R.E. dela Cruz was selected as member of the Research Committee.
4. The ASEAN-New Zealand Afforestation Project (ANZAP) had wrote their invitation for this project to conduct their nursery and field trial experiments

in ANZAP in Mayantoc, Tarlac. ANZAP is very much interested on the bare-root technology for both Eucalyptus and Dipterocarps.

5. Dr. R.E. dela Cruz had undergone a training on Endomycorrhiza Taxonomy last August, 1986 at the University of Florida. He also attended a Taxonomy Workshop in Florida (April-May, 1987) and the 7th North American Conference on Mycorrhiza in Florida (May 3-8, 1987).
6. A glasshouse for VAM inoculants sporulations was constructed with funds coming from Dr. Norman Schenck, University of Florida.
7. Starter VAM cultures were obtained from the International Culture Collection of VA Mycorrhizal Fungi (INVAM) through Dr. Schenck.

IV. PROBLEMS ENCOUNTERED:

1. Collection of fruiting bodies was temporarily shelved during the dry season.
2. Availability of seed of both Eucalyptus and Dipterocarps but most especially palosapis and apitong hindered activities of the project.
3. The best potting media for mass producing VAM inoculants are still being studied, although Glomus species were found to be suited in a 1:1 proportion of fumigated forest soil and quarry sand. Other species however, required different media. Moreover, some has to take longer period

of time to sporulate.

4. Processing of papers at USAID office was delayed because of transfer of personnel.
5. Set-up of mycorrhizal synthesis was delayed because of some technical problems with the growth chamber. Also the space of the growth chamber is not enough for the synthesis experiment.

V. PLAN OF ACTIVITIES FOR CY JULY 1987 - JUNE 1988

<u>ACTIVITIES</u>	<u>DURATION</u>
STUDY 1	
- Continue collection, isolation, mass production and purification of VAM and Ectomycorrhizal fungi	JULY 1987-JUNE 1988
- Training of staff on taxonomy for endomycorrhiza by Dr. R.E. dela Cruz	JULY
- Synthesis study and Micro-technique work	SEPTEMBER
- Training of staff on taxonomy of Ectomycorrhiza by Dr. R.E. dela Cruz	OCTOBER
- Report Writing (semi-annual)	DECEMBER
STUDY 2	
- Inoculant Preparation	JAN. '87 - JUNE 1988
- Screening Tests	JULY - DECEMBER
- Report Writing (semi annual)	DECEMBER
STUDY 3	
- Nursery bed preparation	AUGUST
- Pre-conditioning Treatments	SEPT. - JUNE
- Report Writing (annual)	JULY

VI. FINANCIAL STATEMENT (YEAR 1)

	<u>1986</u>	<u>1987</u>	<u>TOTAL</u>
	(July-Dec.)	(Jan.-June)	
PERSONAL SERVICES			
1) Salaries			
a. (2) Research Assistants			
1 at P1802/mo	P10,812		P10,812
1 at P1715/mo	10,290		10,290
(1) Research Aide at P1337/mo	8,022		8,022
(2) Laborers at P992/mo	11,904		11,904
b. (2) Research Assistants			
1 at P1991/mo		11,946	11,946
1 at P1894/mo		11,364	11,364
(1) Research Aide at P1447/mo		8,862	8,862
(2) Laborers at P1096/mo		13,152	13,152
	SUB-TOTAL		<u>86,352</u>
2) Incentives (Bonus: P1,000 + 40%)			
(2) Research Assistants			
1 at P1991/mo(40%) + P1,000			1,746.40
1 at P1894/mo(40%) + P1,000			1,757.60
(1) Research Aide at P1447/mo(40%) + P1,000			1,590.80
(2) Laborers at P1096/mo(40%) + P1,000			2,876.80
	SUB-TOTAL		<u>8,021.60</u>
	TOTAL		P94,373.60

	<u>1986</u>	<u>1987</u>	<u>TOTAL</u>
	(July-Dec.)	(Jan.-June)	
E. 1) COLA			
a. (2) Research Assistants at P350/mo	4,200		4,200
(1) Research Aide at P400/mo	2,400		2,400
(2) Laborers at P400/mo	4,800		4,800
b. (2) Research Assistants at P450/mo		5,400	5,400
(1) Research Aide at P500/mo		3,000	3,000
(2) Laborers at P500/mo		6,000	6,000
			<hr/>
	SUB-TOTAL		25,800.00
C. HONORARIUM			
(1) Project Leader at P1,250/mo			15,000.00
D. MEDICARE			1,176.00
F. MAINTENANCE & OPERATING EXPENSES			
Supplies			142,510.66
Sundry			7,945.20
Travel			31,315.30
F. EQUIPMENT			4,850.00
G. ADMINISTRATIVE SUPPORT			32,409.00
			<hr/>
	GRAND TOTAL		P355,379.76 XXXXXXXXXXXX

BUDGETARY REQUIREMENTS (YEAR 2)

A. SALARIES		
(2) Research Assistants		
1 at P2190.10	26,281.20	
1 at P2083.40	25,000.80	
(1) Research Aide at P1624.70	19,496.40	
(2) Laborers at P1205.60	<u>28,934.40</u>	P99,712.80
B. COLA		
(2) Research Assistants @ P450	10,800.00	
(1) Research Aide @ P500	6,000.00	
(2) Laborers at P500	<u>12,000.00</u>	28,800.00
BENEFIT: (Bonus) 1 month salary		
(2) Research Assistants		
1 at P2,190.10	2,190.10	
1 at P2,083.40	2,083.40	
(1) Research Aide @ P1,624.70	1,624.70	
(2) Laborers at P1,205	<u>2,410.00</u>	8,307.00
C. HONORARIUM		
(1) Project Leader P1,250/mo		15,000.00
D. MEDICARE		
		3,600.00
D. MAINTENANCE & OPERATING EXPENSES		
Supplies	118,000.00	
Sundry	15,000.00	
Travel	<u>23,520.00</u>	156,520.00
E. ADMINISTRATIVE COST		
		31,638.00
F. EQUIPMENT		
Light banks & Timer	6,000.00	
1 unit screenhouse	26,000.00	
1 unit back sprayer	1,200.00	
1 unit distilling apparatus all glass	<u>40,000.00</u>	<u>73,200.00</u>
GRAND TOTAL		<u>P416,777.80</u> XXXXXXXXXXXXX

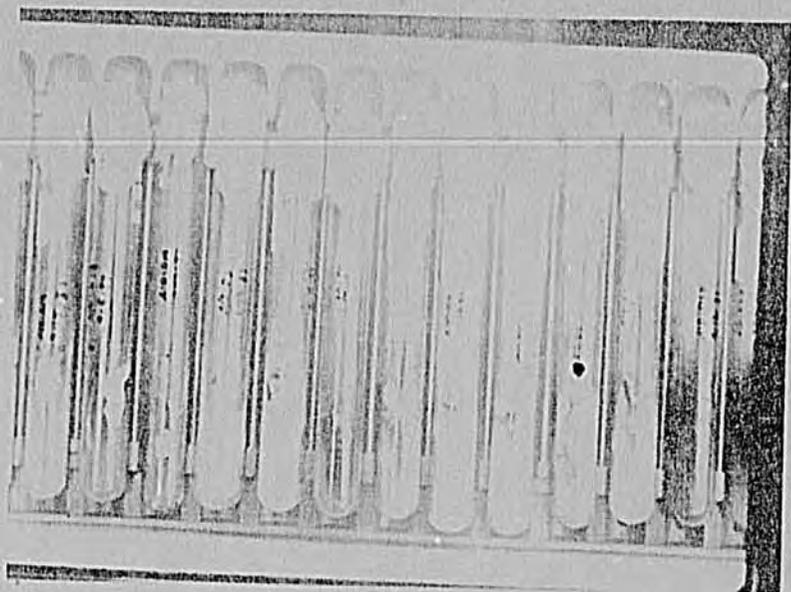


Fig. 1a. Isolation and purification of mycorrhizal fungi in test tube slants with Modified Melin Norkran's Agar, as medium.



Fig. 1b. Mass production of vegetative mycelia in MMN broth.

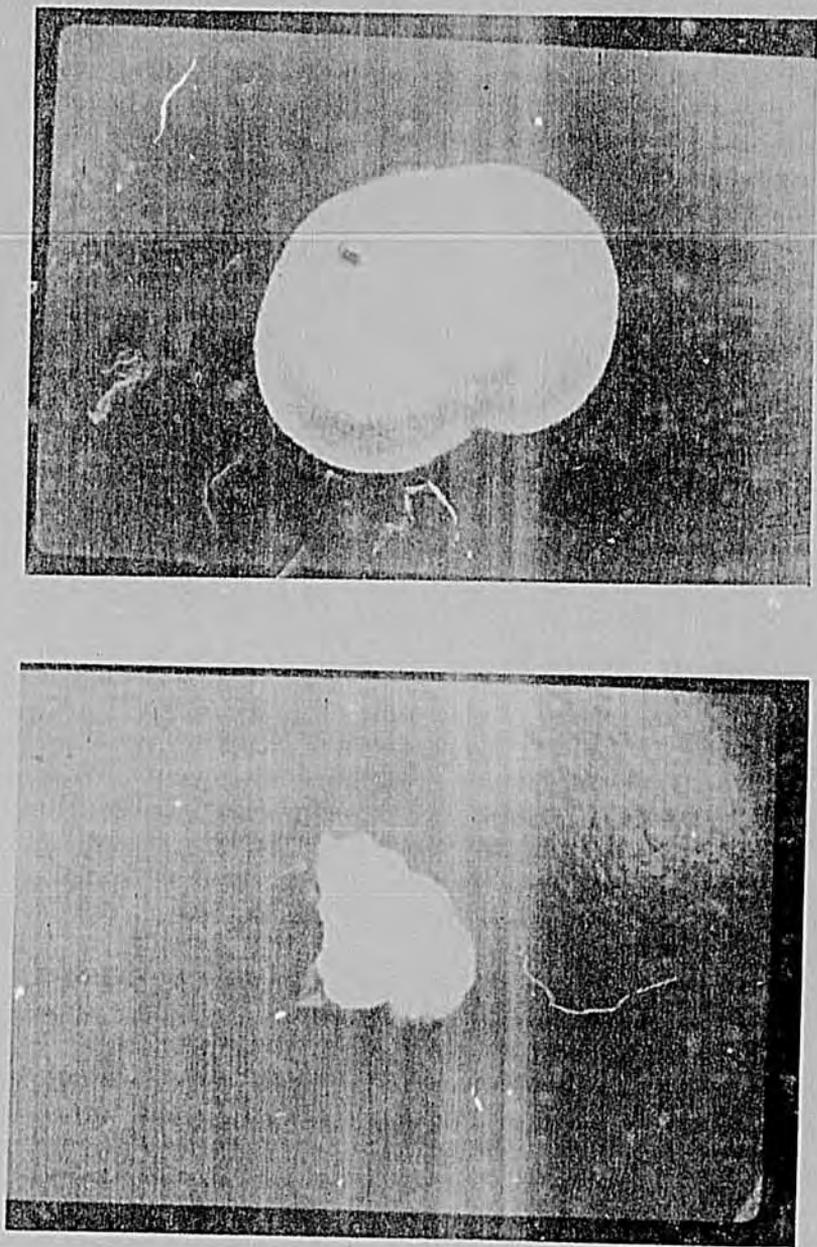


Fig. 2a. Mass production of mycorrhizal fungi in petri plates with MMN medium and without coir dust.

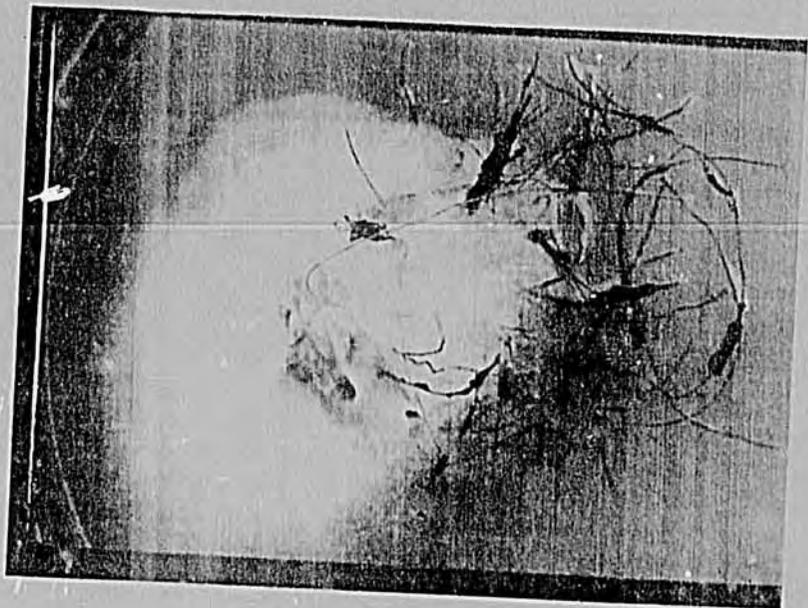


Fig. 2b. Mass in situ production of mycorrhizal fungi
in petri dishes with MN broth and with
coir dust.

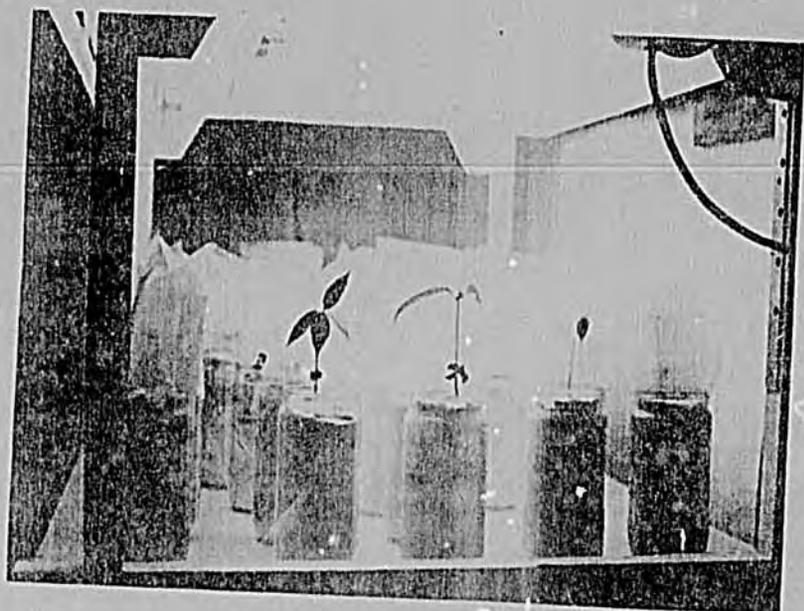


Fig. 3. Mycorrhizal synthesis study. A set-up of mycorrhizal synthesis using *Anisoptera thurifera* grown in a 3:1 fumigated perlite and sand media, placed in an improvised growth chamber.

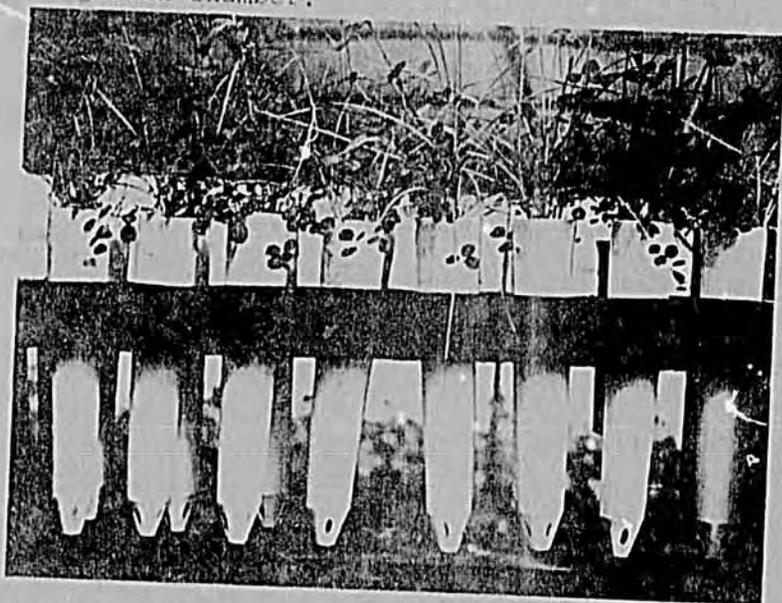


Fig. 4. Mass inocula production technique of VAM inoculated in plastic tubes using a mixture of fumigated forest soil and fine sand with Pensacola bahia grass as host plant and alfalfa as nitrogen fixer.

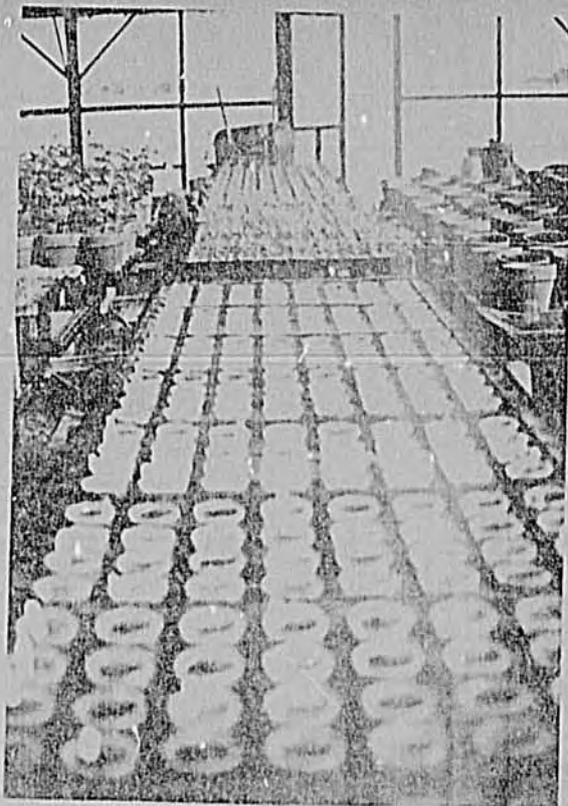


Fig. 5. Single-spore inoculation set-up of field collected VAM under Dipterocarps and Eucalyptus plantation.

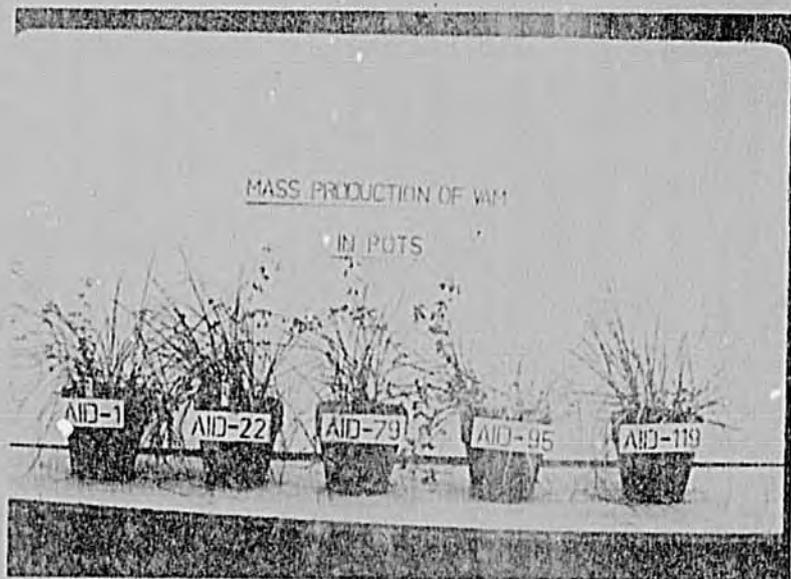


Fig. 6. Mass production of 5 VAM species found to sporulate well and pure in single spore-inoculation experiments as seen in Fig. 4.

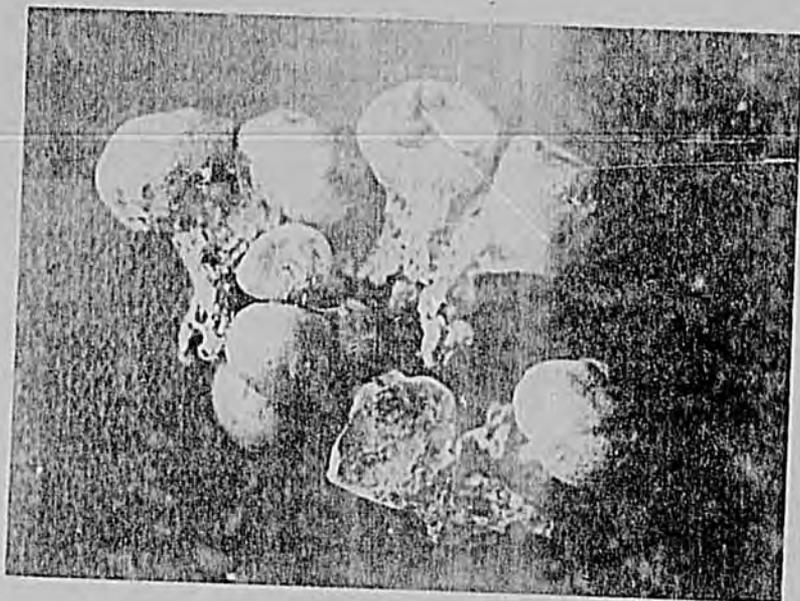


Fig. 7. Sporocarps of Pisolitus species collected under Apitong plantation at Sakiling Forest.

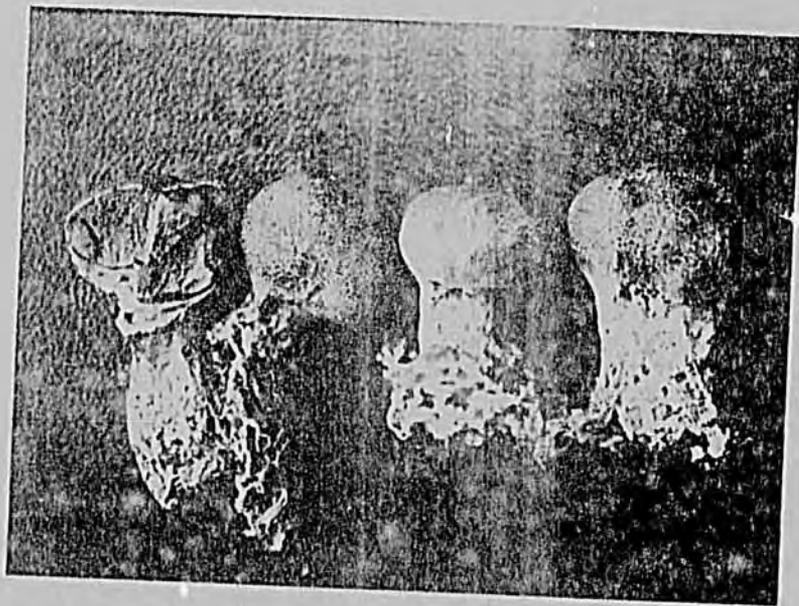


Fig. 8. Sporocarps of Pisolitus species collected under Dipterocarp at FOR1, Bislig, Surigao del Sur.

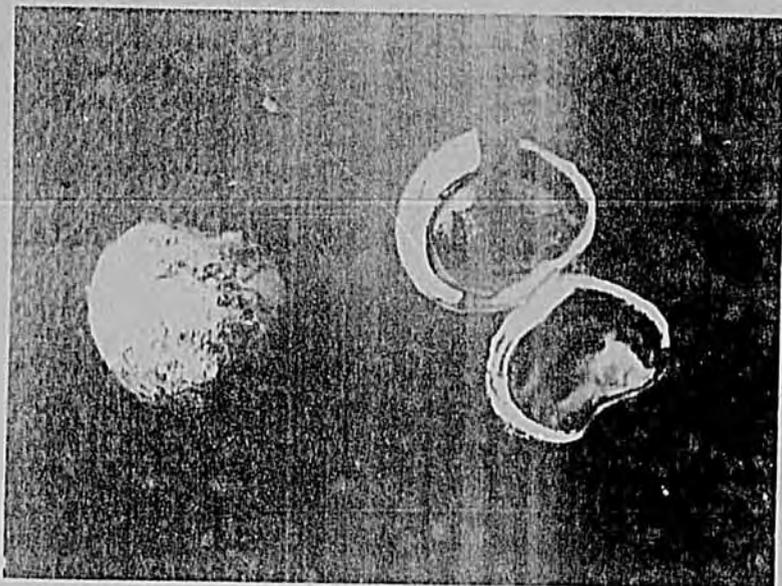


Fig. 9. Fruiting bodies of *Rhizopogon* collected under Dipterocarp plantation at Makiling Forest.

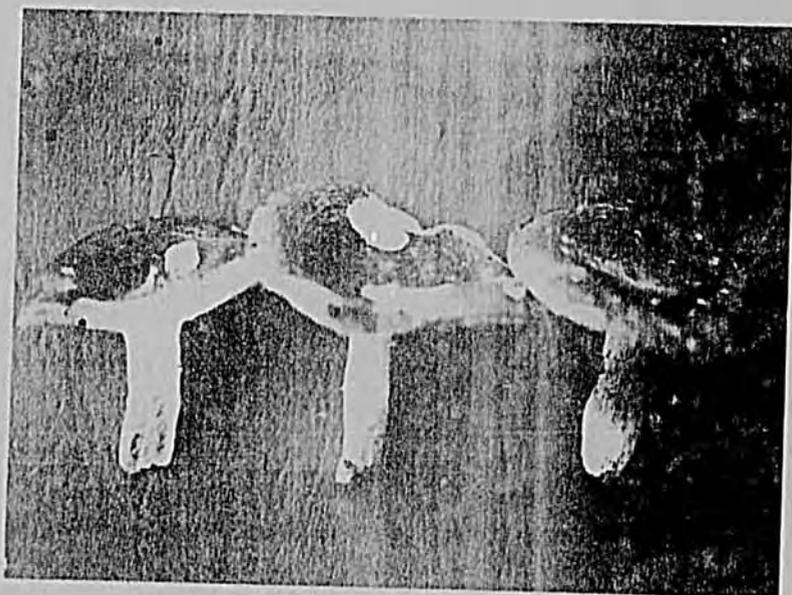


Fig. 10. *Russula* species found associated with Dipterocarp species.

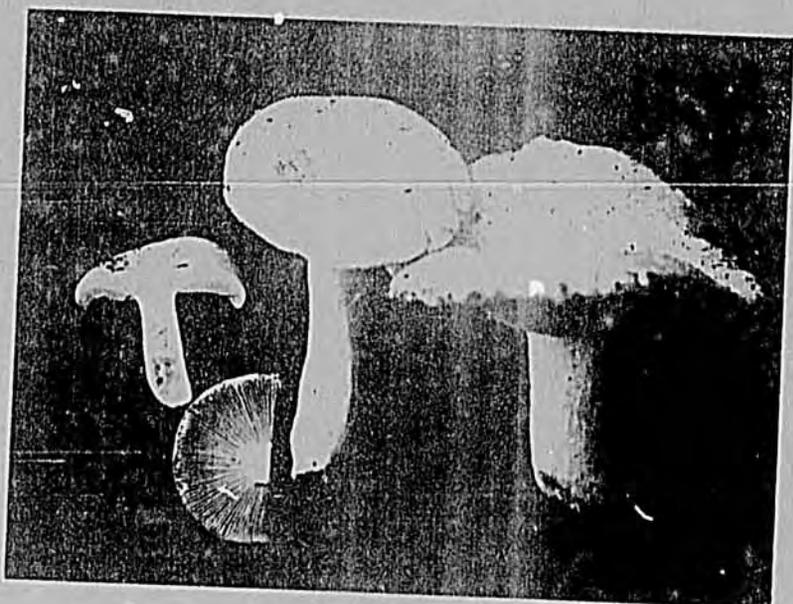


Fig. 11. Russula species found associated with Dipterocarp species.

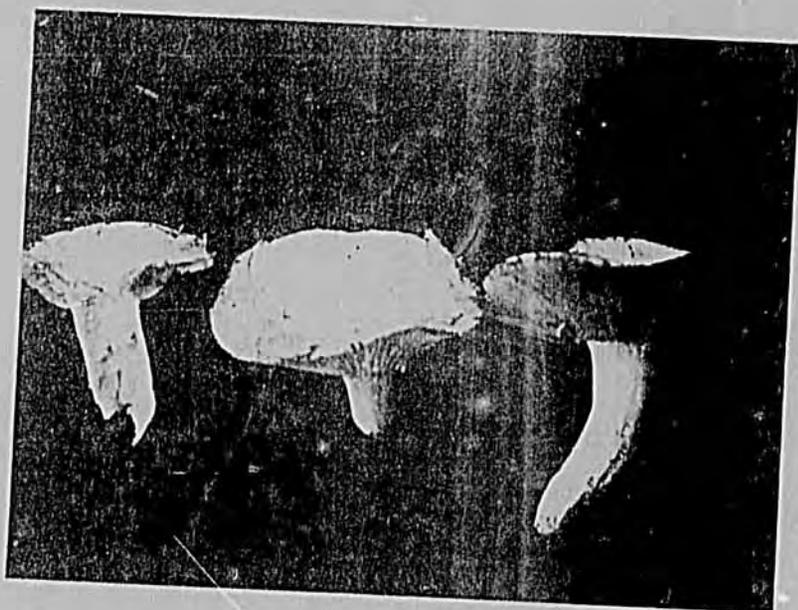


Fig. 12. Another kind of Russula species found associated with Dipterocarp species.

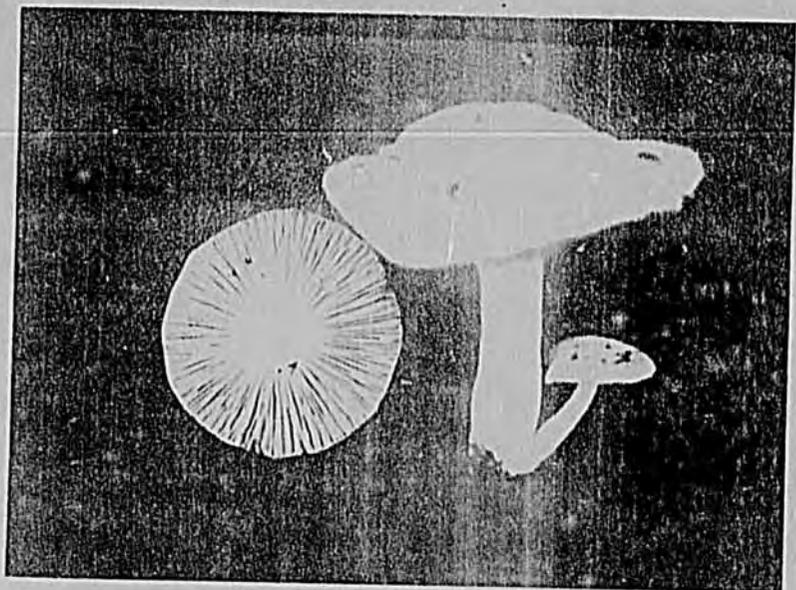


Fig. 13. Russula species found associated with Dipterocarp plantation.

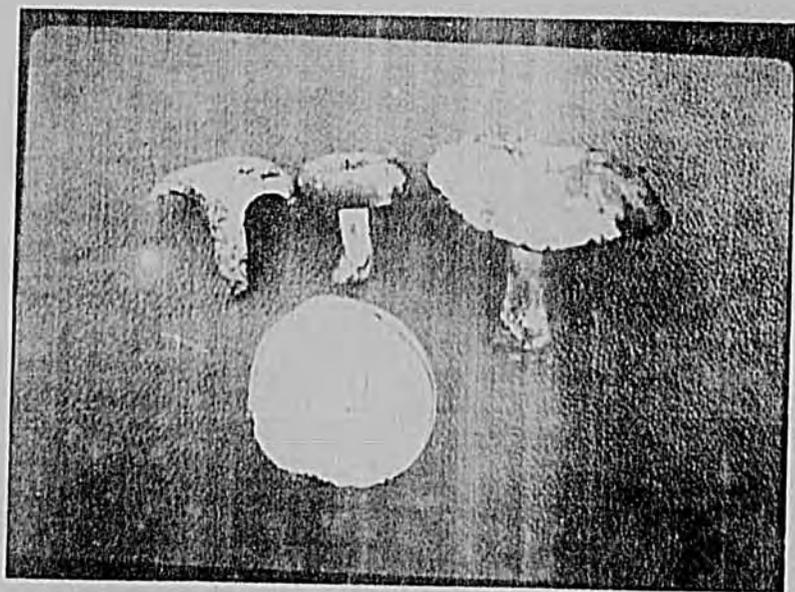


Fig. 14. Another kind of Russula species found associated with Dipterocarp plantation.

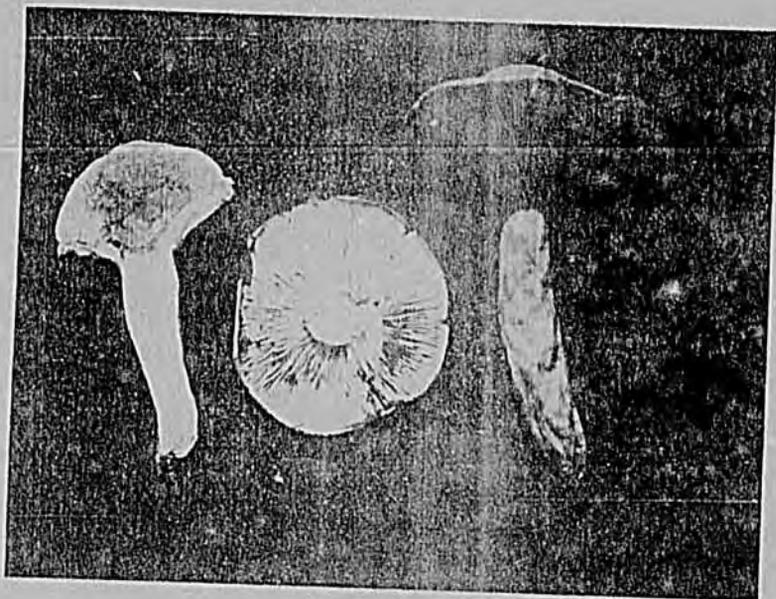


Fig. 15. *Lactarius* species collected under Dipterocarp plantation.

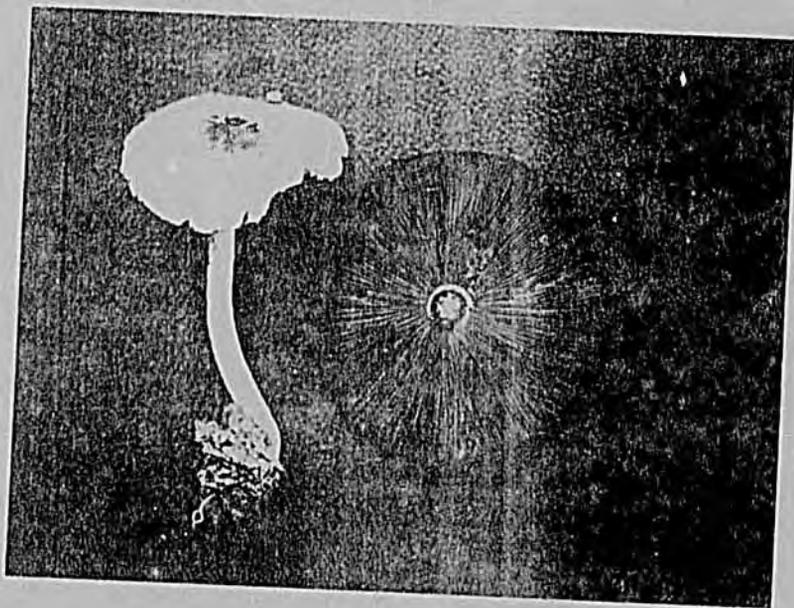


Fig. 16. *Volvaria* species collected under Dipterocarp plantation.

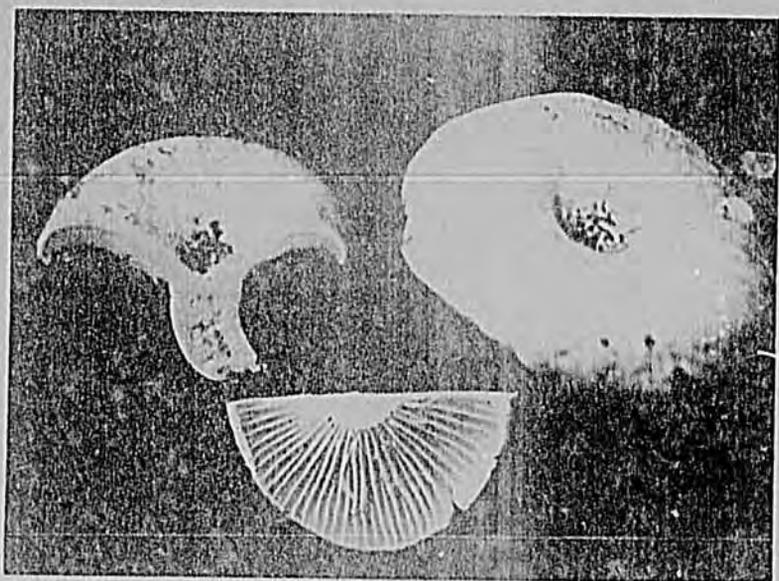


Fig. 17. Lactarius species collected under Dipterocarp plantation.

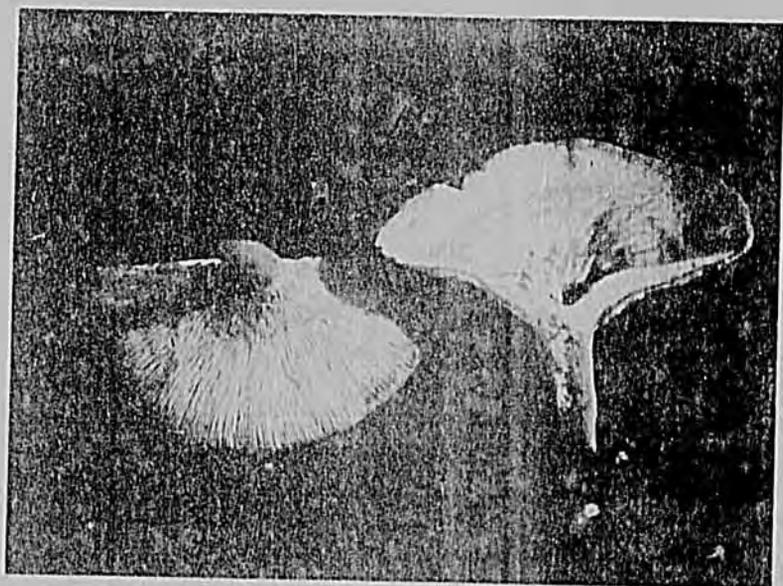


Fig. 18. Lactarius species collected under Dipterocarp plantation.

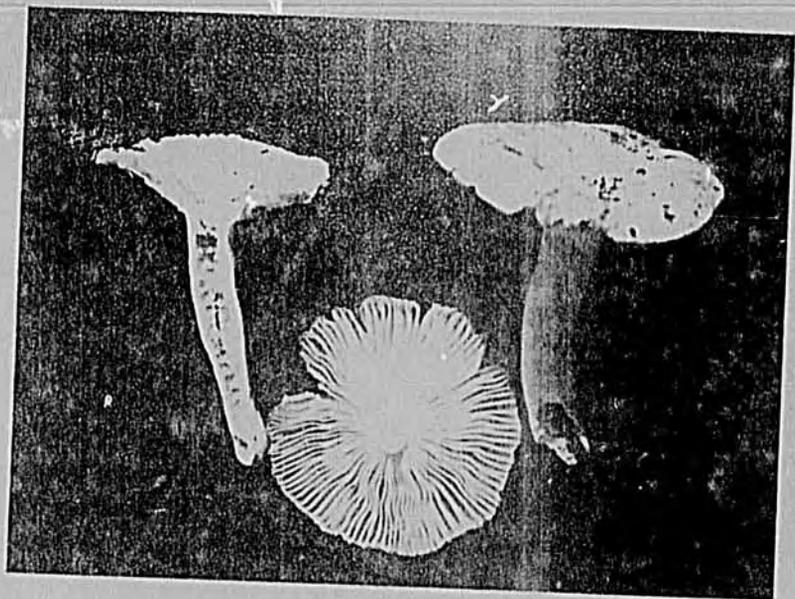


Fig. 19. Lactarius species collected under Dipterocarp species.



Fig. 20. Sporocarp of Thelephora species found associated with Apitong at Makiling forest.

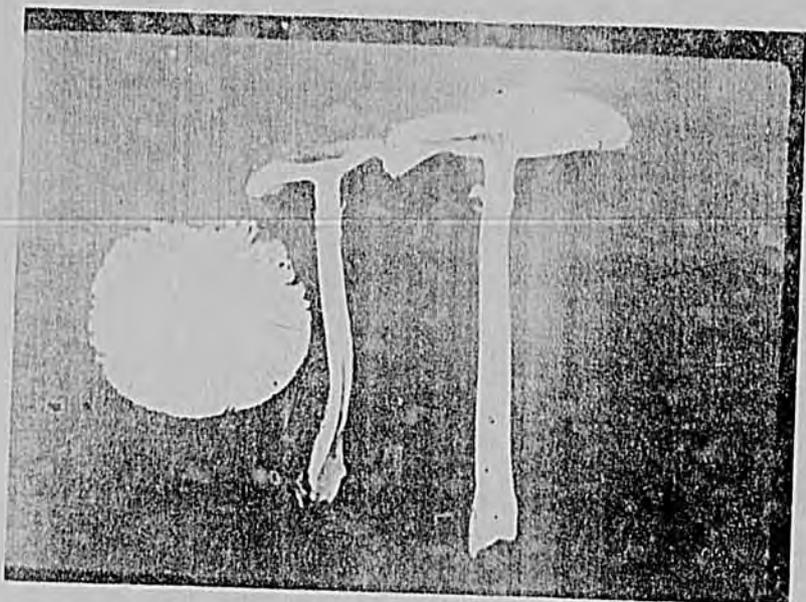


Fig. 21. Sporocarps of *Amanita* sp. collected under Dipterocarp plantation.

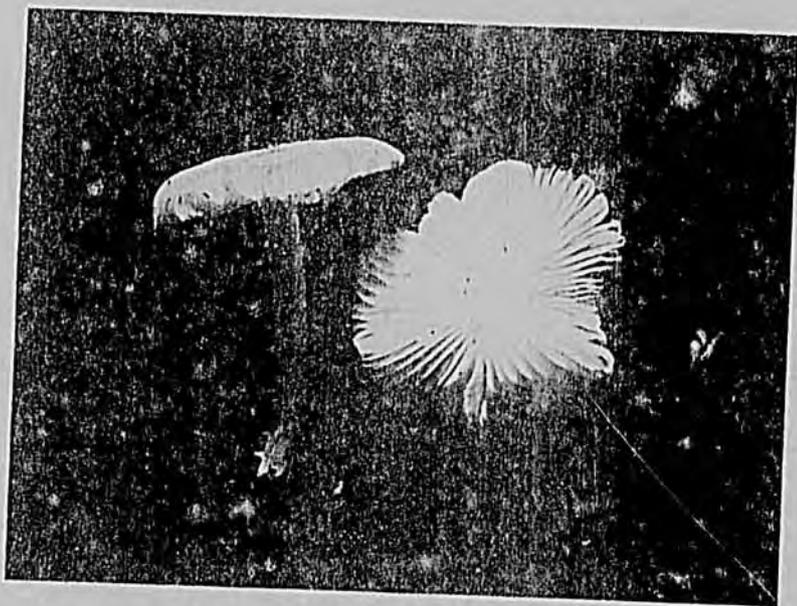


Fig. 22. Unidentified ectomycorrhizal fungi found associated with Dipterocarp species.