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Population of Arbuscular Mycorrhizal Fungi (AMF) by Different Land Use in Sumatra, Indonesia —Comparison of AMF Spore Numbers in Primary Forest, Secondary Forest, Fields Growing Coffee and Native Grass—

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This study was carried out to clarify the properties of Arbuscular Mycorrhizal Fungi(AMF) from different types of land used at Sunbarjaya in Southern Sumatra, Indonesia.

The soil samples were collected from primary forest (P.F.: *Hopea mengarawan, H.sangal*, and *Dryobalanops* spp.), secondary forest (S.F.: *Daemonorops oblongata, H. ficus* and *H. calamus*), coffee field (C.F.: *Coffea robusta*) and native grassland (N.G.: *Imperata cylindrica*) where were about 1,200, 100, 800 and 800 m above sea level, respectively. Sampling sites for C.F. were selected from three locations along the hillslope.

There were four genera and ten species of AM fungal spores identified from the P.F., S.F., C.F., and bush soils of the Sunbarjaya areas.

Glomus etunicatum, G. constrictum and *G. aggregatum* were found in all land uses. The total spore numbers of *G. constrictum* and *G. aggregatum* were much greater in the C.F. soils compared to other soils. But, the number of genera and species in the C.F. soils was low compared to the P.F. and S.F. soils.

G. constrictum predominated in all soils and *G. aggregatum* only predominated in the C.F. soils.

Key words: Arbuscular Mycorrhizal Fungi, Sumatra, Primary Forest, Secondary Forest, Coffee

Introduction

In a hilly area of West Lampung (Southern Sumatra, Indonesia) intensive deforestation gave rise to a severe reduction in primary forest area of up to 13% in 1990[11]. During the last 20 years, most of the primary forest was into monocultural plantations (mostly coffee plantations). The area taken up by coffee plantations was accounted for about 41% in the hilly area of West Lampung[11]. For most farmers in Southern Sumatra, the coffee tree is the most economically important perennial crop.

Tropical soils are characterized by low soil phosphate content. When such soils are cultivated, the growth of several tropical crops, such as cassava, cowpea, coffee, avocado, and mango is quite dependent on the AMF activity of in the rhizosphere of these plants. It is well known that AMF is closely related to crop production systems in tropical

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regions[1].

The dominant soils in Southern Sumatra are Acrisols and Ferralsols (Ultisols). According to the FAO terminology, these soils are called Red Acid Soils. They are infertile and susceptible to soil erosion under careless land management[2].

As a consequence of long-term conversion of primary forest, soil degradation occurred by decreasing soil fertility, especially in the coffee plantation area of West Lampung[4]. In order to sustain and increase soil fertility status in the cultivated areas, it is very important to manage AMF in crop production systems.

The main purpose of this study was to survey the properties of AMF from different types of land uses at Sunbarjaya in Southern Sumatra, Indonesia. This report was described the identified species and the species composition of AM fungal spores in different land uses in Southern Sumatra.

Materials and Methods

Locations of the sampling areas

All soil samples were collected from the Sunbarjaya area which is located in West Lampung, Lampung Province, Southern Sumatra (Fig. 1). The altitudes of the primary forest (P.F.), secondary forest (S.F.), coffee field (C.F.) and native grass (N.G.) were about 1,200, 1,000, 800 and 800 m above sea level, respectively. Dominant plants at the P.F. site were *H. mengarawan, H. sangal, Dryobalanops* spp., and *Shorea* spp. Dominant plants at the S.F. site were *Daemonorops oblongata, H. ficus*, and *H. calamus*. Sampling sites for C.F. (*Coffea robusta*) were selected from three locations along the hillslope, at the top (plain area), the medium slope (10°), and the sharp slope (30°). The N.G. (*Imperata cylindrica*) was selected from the same site as C.F. but from a different slope orientation (sharp slope of 30°). The sampling site for field growing bushes was taken from a depression situated at the bottom of the C.F. site.

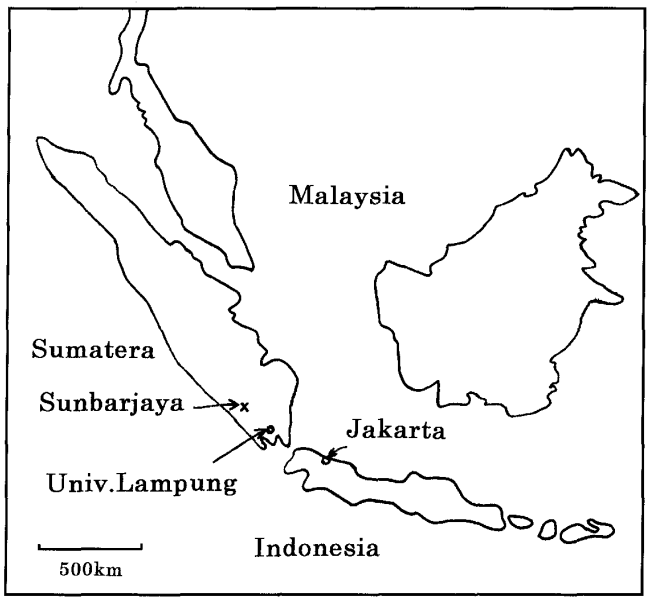

Fig. 1. Location in sampling site

Collection of soil and plant samples

In October, 1995 and July, 1996, samples were collected from each rhizosphere soil of the dominant plants at each site. The selected plants were *H. mengarawan* in the P.F. site, *D. oblongata* in the S.F. site, *C. robusta* in the C.F. site and *I. cylindrica* in the N.G. site. Each soil sample (20 kg wet basis) was collected at random using a zigzag method, from 10 plots at 0–20 cm depth. In 1995, plant seedlings from *H. mengarawan, D. oblongata* and *I. cylindrica* were collected from the P.F, the S.F., and the N.G., respectively.

Reproduction of indigenous AMF populations

A reproduction experiment on the indigenous AM fungal population was carried out in trap pots on trap plants from 1995 to 1996. After the soil sampling in October, 1995, the moist soils were passed through a 5 mm sieve and homogenized. The trap pots (2 Kg) of each transplanted host plant seedling were incubated in a greenhouse for five repeats over 120 days. The trap plant seedlings selected were *H. mengarawan* for P.F., *D. oblongata* for S.F., *C. robusta* for C.F. and *I. Cylindrica* for N.G..

Identification and count of AMF spores (sporecarps)

In 1996, AM fungal spores were extracted using wet-sieving and decanting techniques[6]. AM fungal spores in natural and reproduction pot soils were identified according to the method of Schenck and

Perez[9]. Samples used were made up of three hundred grams of wet soil (five repeats) in each treatment. Each AMF species was counted. *Sclerosystis* spp. was counted by the number of sporocarps.

AM fungal colonization in host plants

In 1996, AM fungal colonization (AM fungal infection) in the host plants was measured using a sullied method[5] from reproduction soils. Roots colonization by the AMF was stained by a trypan blue method[5].

Root, each 1 cm long, were selected at random from 20 stained root samples and mounted on microscopic slides. AM fungal colonization was determined as follows.

Root colonization ranges were scored into the corresponding figures as follows.

0%=0, 1~25%=1, 26~50%=2,

51~75%=3, 76%~=4

Then, the root colonization percentage was calculated according to the following formula.

Root colonization (%)

$=(13\times n_1+38\times n_2+63\times n_3+88\times n_4)/20$

n_1=number of 1 in stained root,

n_2=number of 2 in stained root,

n_3=number of 3 in stained root,

n_4=number of 4 in stained root,

Soil chemical analysis

During 1996, soil chemical properties such as pH (H_2O) , EC, available phosphate(Truog method), phosphate absorption coefficient, ammonium nitrogen, nitrate nitrogen and potassium content were determined[7].

Results and Discussion

Soil chemical properties

Soil chemical properties were shown in Table 1. Soil pH (H_2O) was from 4.62 (P.F. soil) to 5.87 (S.F. soil). Soil available phosphate contents (mg P_2O_5/100 g soil) were 1.28 and 1.31 in the P.F. and the S.F. soils, respectively. The highest soil available phosphate (4.24) was found in the top hill soil of the C.F. sites. Recent application of phosphate fertilizer by farmers in the coffee soil may explain the highest soil phosphate content occurring at the top of the C.F. soil. The sharp slope of the C.F. soil, medium slope of the N.G. soil and the bottom of the hill (bushes) clearly indicated low available phosphate contents. The highest phosphate absorption coefficient was found in the P.F. soil, while the lowest was found on the sharp hill slope in the C.F. soil. Inorganic nitrogen (ammonium and nitrate nitrogen) contents did not vary over regular checks at each site. Ammonium and nitrate nitrogen contents varied among the sampling sites. The highest ammonium content was observed in the C.F. soils. The highest nitrate contents were found in the top and medium hill slopes in the C.F. soils.

Overall these results suggest that soil chemical fertility in the Sunbarjaya area decreased on the sharp slope of the hill in the C.F. soil.

Table 1. Soil chemical properties

Sites	pH	EC	Avai.P.	P.A.C.	NH_4-N	NO_3-N	K
P.F.	4.66	94	1.28	1,720	2.60	0.87	33
S.F.	5.87	44	1.31	1,070	1.55	0.68	45
C.F.1	4.78	79	4.24	1,380	2.10	1.28	10
C.F.2	5.42	53	2.29	1,190	2.29	1.21	14
C.F.3	5.00	27	0.55	685	2.45	0.65	19
N.G.	5.06	58	0.23	1,520	0.92	1.09	11
Bush	4.98	66	0.49	899	2.42	2.00	14

pH: H_2O; EC: μS/cm; Avai.P: Troug Method; P_2O_5 mg/100 g dry soil; P.A.C.: Phosphate Absorption Coefficient P_2O_5 mg/100 g dry soil; NH_4-N, NO_3-N: Nmg/100 g dry soil; K: K_2O mg/100 g dry soil; P.F.: Primary Forest; S.F.: Secondary Forest; C.F.: Coffee Field; C.F.1: top of hill; C.F.2: medium hill slope; C.F.3: sharp hill slope

Identification of AM fungal species

Four genera and ten species were identified based on the morphology of the AM fungal spores from the P.F., S.F., C.F. N.G. and bush soils in the Sunbaljaya area.

Glomus etunicatum (Fig. 2, 3) forms chlamydospores. The spore color is yellow to brown in water. The average spore diameter is 117 μm and the width of the spore is 113 μm. *G. etunicatum* forms a single spore either in soil or dead roots. The spore has an attachment and a subtending hypha. There is one wall group in the spore wall. The spore wall consists of two layers and the thickness of each layer ranges from 5 to 6.5 μm. The type of wall within each group is laminated. The outer layer of the wall group was stained pink to reddish by Melzer's reagent, but the inner layer was not stained by Melzer's reagent.

G. constrictum (Fig. 4, 5) forms chlamydospores. The spore color is brown to black in water. *G. constrictum* forms spores which are single or in clusters and subglobose to globose. The average spore diameter is 121 μm and the spore width is 111 μm. The cluster diameter ranges from 912 to 1,037 μm and the cluster width diameter ranges from 336 to 384 μm. The spore has an attachment , a subtending hyphae. There is one wall group in the spore wall. The type of wall within each group is laminated. The width of a

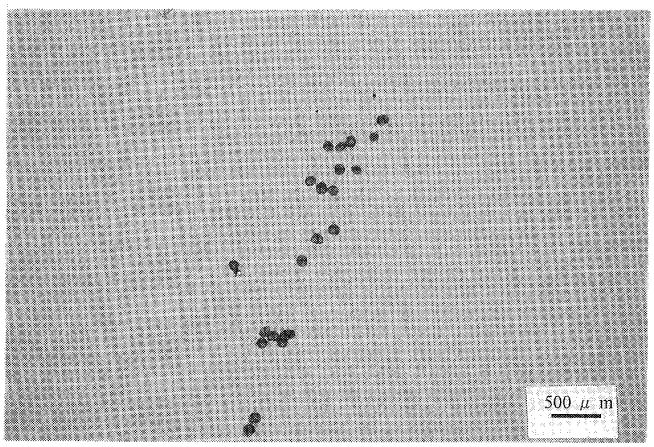

Fig. 2. Spores of *Glomus etunicatum*
The spore color was brown in water. The average spore diameter was 117 μm.

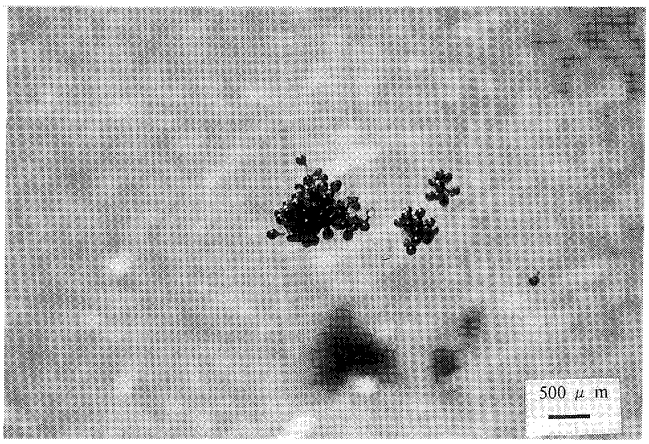

Fig. 4. Spores and clusters of *Glomus constrictum*
The spore color was black in water. The cluster diameter ranged from 912 to 1,037 μm.

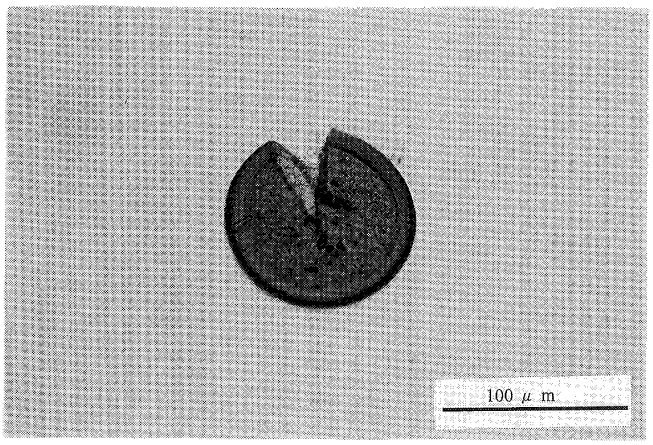

Fig. 3. Spores and clusters of *G. etunicatum*
There is one wall group in the spore wall and thickness ranged from 10 to 13 μm.

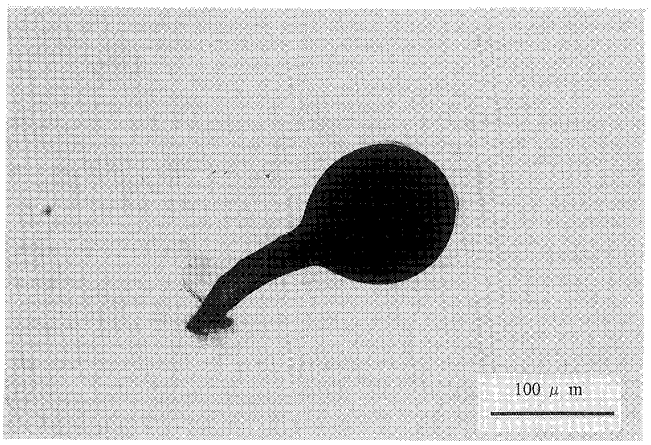

Fig. 5. Spore of *G. constrictum*
The average spore diameter was 121 μm and the spore width diameter was 111 μm.

wall group ranges from 5.2 to 7.8 μm. The wall reaction to Melzer's reagent is negative.

G. aggregatum (Fig. 6, 7) is a chlamydospore. The spore color is brown to yellow-brown in water. The spore shape is irregular. It forms in sporocarps, either single or in cluster in soils and is globose. The spore diameter ranges from 67 to 106 μm. The spore has an attachment; a subtending hyphae. The number of wall groups in the spore wall is one or two for mature spores. The type of wall within each group is amorphous. The width of each wall group ranges from 1 to 5 μm. The wall reaction to Melzer's reagent is negative.

Sclerocystis rubiformis (Fig. 8, 9) forms chlamydospores. The spore color is brown or red brown in water. It forms in sporocarps and is globose. The range of sporocarp diameters is 180–310 × 200–600 μm. It shape is irregular. The range of spore diameters is between 20–25 × 22–30 μm. There is one wall group in the spore. The type of wall within each group is laminated. The wall reaction to Melter's reagent is negative.

S. pachycaulis (Fig. 10, 11) forms chlamydospores. The spore color is yellow to yellow brown in water. The spore is formed in the sporocarps, clusters in soil and is subglobose. The range of sporocarp diameters is between 170–190 × 210–310 μm. The average spore diameter is 50 μm and the spore width is 25 μm.

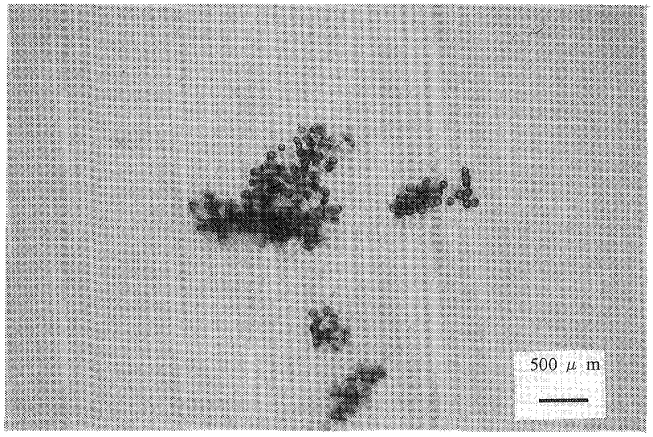

Fig. 6. Spore of *Glomus aggregatum*
The spore color was brown in water.

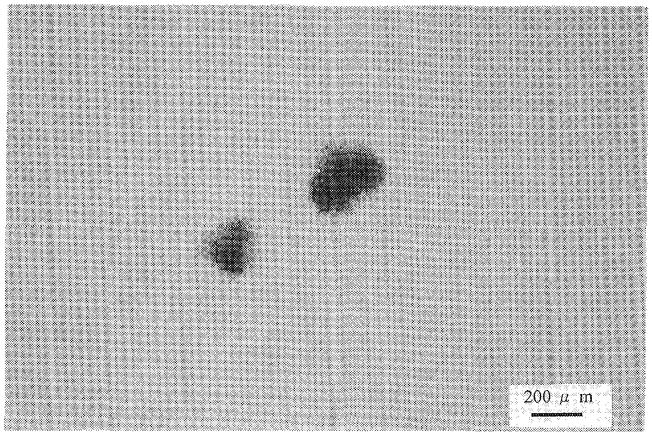

Fig. 8. Sporocarps of *Sclerocystis rubiformis*
The range of sporocarp diameters was 180 × 200 to 310 × 600 μm.

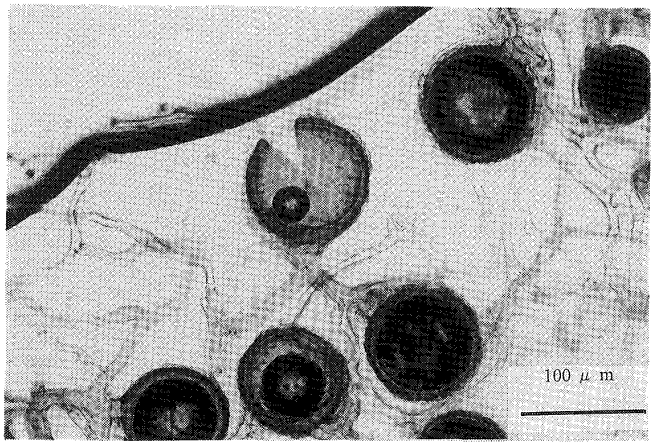

Fig. 7. Spore of *G. aggregatum*
The spore diameter ranged from 67 to 106 μm. The spore has an attachment; a hyphal terminus and a subtending hypha.

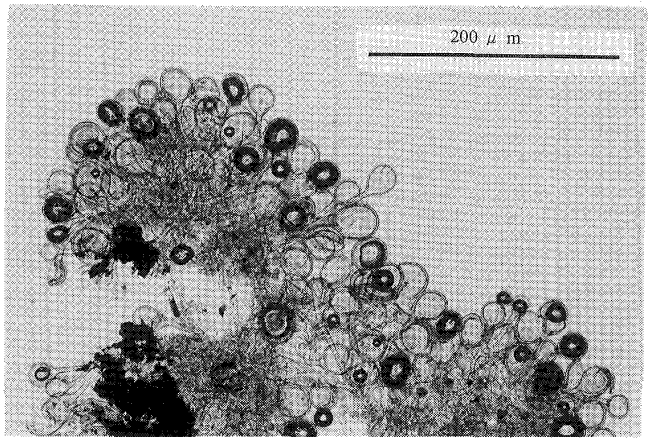

Fig. 9. Spores and sporocarps of *S. rubiformis*
The spore color was brown in water. The range of spore diameters was 20 × 22 to 25 × 30 μm.

There is one wall group in the spore wall. The type of wall within each group is laminated. The wall reaction to Melzer's reagent is negative.

Acaulospora tuberculata (Fig. 12, 13) forms azygospores. The spore color is yellow brown to honey brown for young spores and dark honey brown for mature spores. The average spore diameter is 245 μm and the spore width is 212 μm. The spore wall consists of three layers . The outer layer (A) is clear yellow, 3 to 7 μm thick and the wall reaction to Melzer's reagent is negative. A middle layer (B) is 0.5 to 1.0 μm thick and stains red purple with Melzer's reagent. The inner layer (C) is 1.0 μm thick and stains purple with Melzer's reagent.

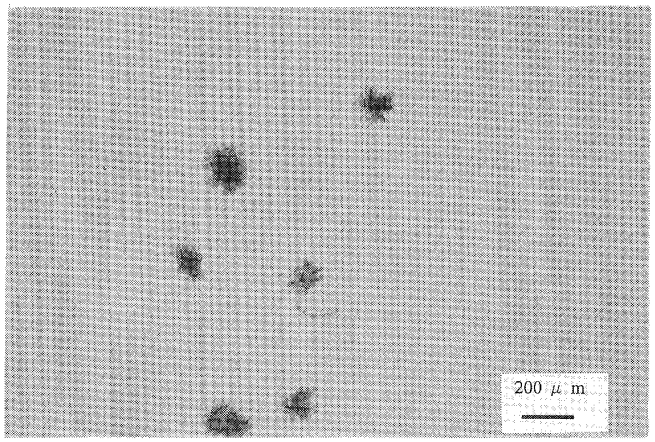

Fig. 10. Sporocarps of *S. pachycaulis*
The range of sporocarp diameter was 170×210 to 190×310 μm.

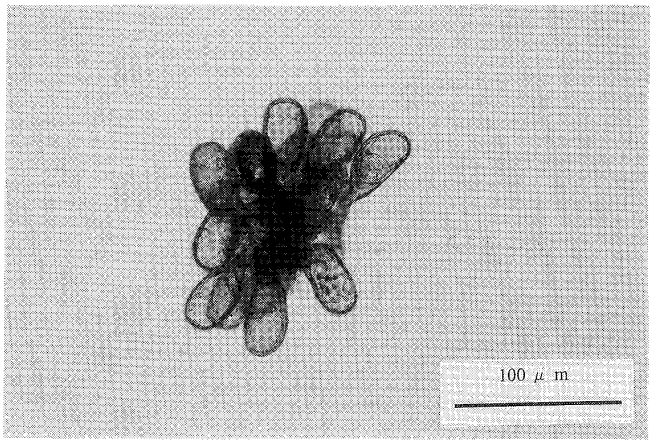

Fig. 11. Sporocarp and spores of *S. pachycaulis*
The spore color was brown in water. The average spore diameter was 50 μm and the spore width was 25 μm.

Some other species remained unidentified using the standard manual of AMF spore identification;two *Glomus* spp. and two *Scutellospora* spp. These AMF could not be classified as a species because the number of these spores was low in the Sunbaljaya areas.

Properties of AMF from different land use

The number of genera and species of AMF from different land use were shown in Table 2. The total spore numbers of each species in natural and reproduction soils from different land use was shown in Table 3. AM fungal colonization on the repro-

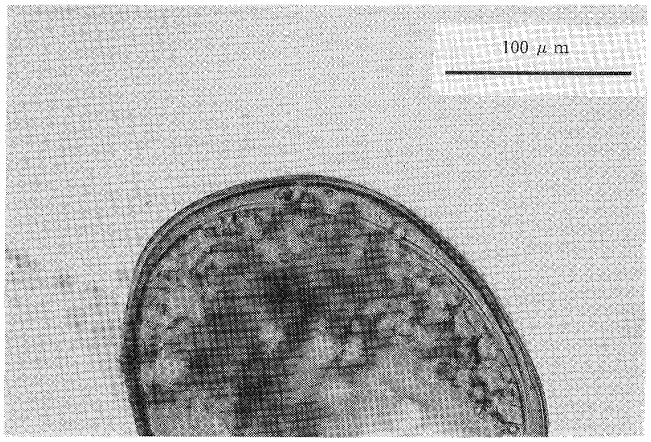

Fig. 12. Spore of *Acaulospora tuberculata*
The spore color was yellow brown. The average spore diameter was 245 μm and the spore width of was 212 μm. The spore wall consists of three layers.

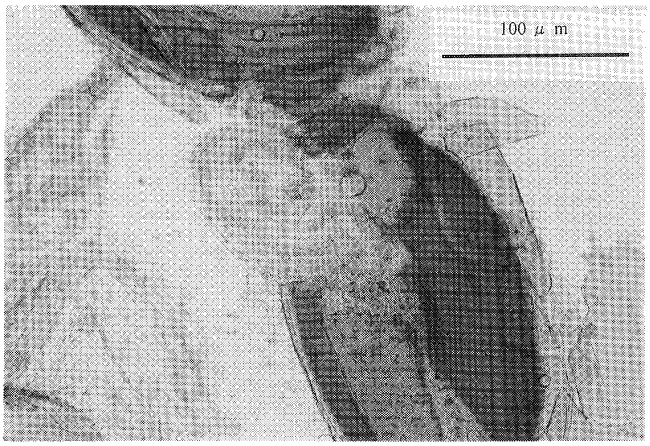

Fig. 13. Spore wall of *A. tuberculata*
The outer layer was clear yellow and Melzer's reagent was negative. The middle layer stained into red-purple. The inner layer stained purple.

Table 2. The number of genera and species of AMF

Sites	Genera and species
P.F.	3 genera and 6 species
S.F.	3 genera and 7 species
C.F.1	2 genera and 4 species
C.F.2	2 genera and 6 species
C.F.3	1 genus and 3 species
N.G.	3 genera and 7 species
Bush	3 genera and 7 species

P.F.: Primary Forest; S.F.: Secondary Forest; C.F.: Coffee Field; C.F.1: top of hill; C.F.2: medium hill slope; C.F.3: sharp hill slope; N.G.: Native Grass

duction host plant was shown in Table 4.

Three genera and six species were found in the P.F. soil. Three genera and seven species were found in the S.F. soil. Two genera and four species were found at the top of the hill, two genera and six species on the gentle hill slope and one genera and three species on the sharp hill slope in the C.F. soils, respectively.

The number of genera and species in the C.F. soil was low compared to the P.F. and the S.F. soils. The number of genera and species of AMF in the C.F. soil decreased in order from the gentle hill slope ,to the top of the hill, to the sharp hill slope, respectively.

Three genera and seven species were identified in the N.G. (gentle hill slope) soil and in the bush (bottom of the hill) soils ,respectively (Table 2). The diversity of AMF found in the N.G. (*Imperata cylindrica*) soil was higher compared to the C.F. soil (Table 2). The latter two land uses had different species of plants, i.e. the N.G. with native grass and the C.F. with coffee, although both land uses were found on the same gentle hill slope. The number of genera and species of AMF in the bush (bottom of the hill) soil was also higher than that of the C.F. soil (Table 2). From the view point of diversity of AM fungal species at those sites, it is suggested that the variety of AMF in the C.F. soil was rather low.

G. etunicatum, G. constrictum and *G. aggregatum* were found in all land uses (Table 3). However, the species composition of AMF differed considerably from all sites. *Scutellospora* sp. 1 and 2 were isolated only from the P.F. soil. *A. tuberculata* was isolated from the S.F., C.F. (sharp slope) and bush soil.

Table 4. AM fungal colonization(%)

Plants	Colonization
Hopea mengarawan	26.7
Daemonorops oblongata	51.7
Coffea robusta	81.7
Imperata cylindrica	78.3

Table 3. The total spore numbers in natural and reproduction soil

Species of AMF	P.F.		S.F.		C.F.1	C.F.2		C.F.3	N.G.		Bush
	N.	R.	N.	R.	N.	N.	R.	N.	N.	R.	N
Gl. etunicatum	25	25	4	88	29	56	132	18	10	31	11
Gl. constrictum	43	60	54	60	75	139	309	30	10	171	32
Gl. aggregatum	15	36	1	45	123	189	144	12	22	0	51
Glomus sp. 1	0	0	1	38	0	0	0	0	0	0	15
Glomus sp. 2	0	0	0	0	0	4	6	0	7	28	8
Sc. rubiformis*	2	5	2	4	4	10	13	0	8	22	4
Sc. pachycaulis*	0	0	0	1	0	1	2	0	5	0	0
A. tuberculata	0	0	31	0	0	0	0	0	1	2	2
Scutellospora sp. 1	11	10	0	0	0	0	0	0	0	0	0
Scutellospora sp. 2	9	20	0	0	0	0	0	0	0	0	0

Gl.: Glomus; Sc.: Sclerosystis; A.: Acaulospora; *: Sporocarp number; Spore numbers are averages of each treatment. Spores/300 g wet soil

P.F.: Primary Forest; S.F.: Secondary Forest; C.F.: Coffee Field; C.F.1: top of hill; C.F.2: medium hill slope; C.F.3: sharp hill slope; N: Natural soil; R: Reproduction pot soil

Glomus sp. *1* was isolated from the S.F. and bush soils. *G.* sp. *2* was isolated from the C.F. (gentle slope), N.G. (gentle slope) and bush soils. *S. pachycaulis* was isolated in the C.F. (gentle slope) and N.G. (gentle slope) soils. *S. rubiformis* was isolated from all sites except the C.F. (sharp slope) soil. It is not clear whether *S. rubiformis* is a predominant species, because the number of its sporcarps was very low in these sites.

The total spore (sporocarp) number of each species varied between the sites (Table 3). The total spore numbers of the *Glomus* species increased markedly in the C.F. soils compared to the P.F, S.F., N.G. and bush soils (Table 3). In paticular, the total spore numbers of *G. constrictum* and *G. aggregatum* were found to be higher in the C.F. soils (Table 3).

The results suggest that *G. constrictum* and *G. aggregatum* are the predominant species in the C.F. soil. Other species than *G. etunicatum*, *G. constrictum* and *G. aggregatum* are probably not be dominant in the C.F. sites. The total spore number of *G. constrictum* and *G. aggregatum* decreased strongly in the sharp slope of the C.F. soils (table 3). The lower total spore number, variety of genera, and species of AMF found in the C.F. soil on sharp slope may be due to the intensive soil erosion.

The spore numbers of *G. constrictum, G. aggregatum* and *Scutellospora* sp. *2* in the trap pots on the trap plant (Hopea mengarawan) from the P.F. site increased slightly during the reproduction experiment (Table 3). The spore numbers of *G. etunicatum, G. aggregatum* and *G.* sp. *1* in the trap pots on the trap plant (*D. oblongata*) from the S.F. site also increased (Table 3). Spores of *Aculospora tuberculata* dissappeared during the reproduction experiment in the trap pots on the trap plant (*D. oblongata*) from the S.F. site. The spore numbers of *G. etunicatum* and *G. constrictum* increased markedly in the trap pot on the trap plant(*C. robusta*). However, *G. aggregatum* decreased.

There is little reported data on the species composition of AMF in P.F., S.F., C.F. and N.G. soils in Indonesia[3]. There is also little reported data about the survival of AMF in C.F. soil where cultivated in P.F. and S.F. soil.

The P.F. site in Sunbaljaya was dominated by tree belonging to the Dipterocarpaceae family. It is well known that the family Dipterocarpaceae is associated with ectomycorrhizal fungi. Inaddition to Dipterocarpaceae, there are trees from other families, i.e. Euphorbiaceae, Cuttiferae, and Myristicaceae, which are associated with AMF in the P.F. soils[10]. Results from field surveys revealed that plants with mycorrhizal associations predominate in most natural ecocystems in the world[8]. One of the important aspect of mycorrhizal associations is to improve fitness between symbionts (AMF and host plants) in natural ecosystems[8].

In this survey, AMF in the P.F. and S.F. soils can be associated with the previously mentioned forest trees, except the family Dipterocarpaceae, because its AMF spore number did not increase in the trap pots on the trap plant (Dipterocarpaceae).

Growth of some mycorrhizal or non-mycorrhizal tropical crops and forest trees, when cultivated in tropical soils, are clearly different[1]. Coffee ,avocado, mango, cassava and cowpea are obligate mycotrophic plants when the soil phosphate contents are between 20 to 100 μg/g soil[1].

When indigenous AM fungi were isolated from coffee soils in Colombia, *G. manihotis, G. occultum, A. myriocarpa* and *Entrophospora colombiana* were the dominant species[1]. Coffee seedlings grew more vigorously when these species were inocculated in the seedbeds, and the survival rate for coffee plant after transplanting increased[1].

In this survey, *G. constrictum* predominated in each site and *G. aggregatum* only predominated in the C.F. soils (C.F. 1 and 2). The number of AM fungal species in the C.F. soil decreased , but the spore numbers of dominant species increased. Taking the overall results of this experiment, *G. constrictum* and *G. aggregatum* are probably effective AMF for coffee plants in these sites.

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