โครงสร้างสังคมของราเอคโตไมคอร์ไรซาและการประยุกต์เพื่อการปลูกป่าไม้วงศ์ไม้ยาง

นางสาวสุนัดดา โยมญาติ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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COMMUNITY STRUCTURE OF ECTOMYCORRHIZAL FUNGI AND REFORESTATION APPLICATION IN DIPTEROCARPACEAE

Miss Sunadda Yomyart

A Dissertation Submitted in Partial Fulfillment of the Requirements
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Thesis Title	COMMUNITY STRUCTURE OF ECTOMYCORRHIZAL FUNGI AND REFORESTATION APPLICATION IN DIPTEROCARPACEAE
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สุนัดดา โยมญาติ : โครงสร้างสังคมของราเอคโตไมคอร์ไรซาและการประยุกต์เพื่อการปลูกป่าไม้วงศ์ไม้ยาง. (COMMUNITY STRUCTURE OF ECTOMYCORRHIZAL FUNGI AND REFORESTATION APPLICATION IN DIPTEROCARPACEAE).
อ.ที่ปรึกษาวิทยานิพนธ์หลัก : รศ.ดร.ประกิตติ์สิน สีหนนทน์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ผศ.ดร.จิตรตรา เพียภูเขียว, Professor Roy Watling, Ph.D, 160 หน้า.

การสำรวจโครงสร้างสังคมของราเอคโตไมคอร์ไรซาในระดับบนดินและใต้ดินได้ทำการศึกษาในป่าเต็งรัง จ. น่านและป่าปลูกไม้ยางนา จ.ฉะเชิงเทรา ระหว่างปี 2548 และ 2549 ในระดับบนดินพบดอกเห็ดจำนวน 30 ชนิดในป่าเต็งรัง ชนิดดอกเห็ดที่พบเด่นคือ *Russula* spp.และในป่าปลูกไม้ยางนาพบดอกเห็ด 9 ชนิด ชนิดดอกเห็ดที่พบเด่นคือ *Scleroderma columnare* และ *Russula siamensis* ใช้ลักษณะสัณฐานวิทยาและวิธีทางอณูพันธุศาสตร์ในการจำแนกชนิดรากเอคโตไมคอร์ไรซาที่พบในระดับใต้ดิน ในป่าเต็งรังพบรากเอคโตไมคอร์ไรซาจำนวน 12 แบบมากกว่าในป่าปลูกไม้ยางนาซึ่งพบ 4 แบบ *Tomentella* spp. และ *Cenococcum geophilum* เป็นชนิดรากเอคโตไมคอร์ไรซาที่พบเด่นในป่าเต็งรัง ขณะที่ *S. columnare* และ *R. siamensis* เป็นชนิดที่พบเด่นในป่าปลูกไม้ยางนา การศึกษานี้ชี้ให้เห็นว่าไม่มีความสอดคล้องระหว่างดอกเห็ดเอคโตไมคอร์ไรซาและรากเอคโตไมคอร์ไรซาในป่าเต็งรัง ขณะที่พบความสอดคล้องระหว่างดอกเห็ดเอคโตไมคอร์ไรซาและรากเอคโตไมคอร์ไรซาในป่าปลูกไม้ยางนา การศึกษาสมบัติของดินได้แก่ความชื้นในดิน อินทรียสารในดินและความเป็นกรด-ด่างของดิน พบว่าการเปลี่ยนแปลงโครงสร้างสังคมของราเอดโตไมคอร์ไรซาขึ้นอยู่กับความชื้นในดินอย่างมีนัยสำคัญทางสถิติ

การศึกษาอิทธิพลของราเอคโตไมคอร์ไรซา *Astraues asiaticus*, *A. odoratus* และ *Pisolithus abditus* ต่อการการเจริญของกล้าไม้วงศ์ไม้ยาง 7 ชนิด คือ *Shorea siamensis*, *S. roxburghii* , *S. farinose*, *Dipterocarpus intricatus*, *D. obtusifolius*, *D. alatus* และ *Hopea odorata* พบว่าสายพันธุ์ราเอคโตไมคอร์ไรซาที่คัดเลือกทำให้เกิดการติดเชื้อในกล้าไม้ระหว่าง 26.27% ถึง 74.13% และทำให้การเจริญของกล้าไม้ที่มีการเพาะหัวเชื้อเติบโตได้ดีกว่ากล้าไม้ที่ไม่มีการเพาะหัวเชื้อ และพบว่าการเจริญทางความสูงของลำต้น เส้นผ่านศูนย์กลางที่ระดับคอรากและน้ำหนักแห้งในส่วนของลำต้นเพิ่มขึ้นสูงสุด 51.94%, 50.11% และ 64.38% ตามลำดับ การศึกษานี้เป็นส่วนสำคัญที่ทำให้เข้าใจลักษณะโครงสร้างสังคมของราเอคโตไมคอร์ไรซาในป่าเต็งรังและป่าปลูกไม้ยางนาและคัดเลือกสายพันธุ์ราเอคโตไมคอร์ไรซาไปใช้สำหรับปลูกป่าในประเทศไทย

สาขาวิชา..เทคโนโลยีชีวภาพ.. ลายมือชื่อนิสิต .....
ปีการศึกษา .....2551..... ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์หลัก .....
ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....
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SUNADDA YOMYART : COMMUNITY STRUCTURE OF ECTOMYCORRHIZAL FUNGI AND REFORESTATION APPLICATION IN DIPTEROCARPACEAE. THESIS PRINCIPAL ADVISOR : ASSOCIATE PROFESSOR PRAKITSIN SIHANONTH,Ph.D., THESIS COADVISOR : ASSISTANT PROFESSOR JITTRA PIAPUKIEW,Ph.D. AND PROFESSOR ROY WATLING, 160 pp.

Ectomycorrhizal (ECM) community structure was investigated in a dry dipterocarp forest in Nan province and *Dipterocarpus alatus* plantation in Chachoengsao province between 2005 and 2006. Aboveground, 30 ECM species in the natural dipterocarp forest and 9 ECM species in the *D. alatus* plantation were observed in both years. The dominant ECM species were *Russula* spp. in the forest and *Scleroderma columnare* and *Russula siamensis* in plantation. Underground, ECM morphotypes were identified by morphological and molecular analysis. Twelve ECM morphotypes found in the forest was higher than 4 morphotypes in the plantation. *Tomentella* spp. and *Cenococcum geophilum* were dominant in the forest whereas *S. columnare* and *R. siamensis* were dominant in the plantation. This indicated that species composition of the underground ECM community did not correlate with aboveground sporocarps community in the native dipterocarp forest whereas correlate in the *D. alatus* plantation. The effect of soil properties including moisture content, organic matter and pH on ECM community were studies. The ECM community structure significantly varied depending on soil moist content.

An experiment was conducted to determine the effect of 3 selected ectomycorrhizal fungi including *Astraues asiaticus*, *A. odoratus* and *Pisolithus abditus* on the growth of 7 dipterocarp species; *Shorea siamensis*, *S. roxburghii*, *S. farinose*, *Dipterocarpus intricatus*, *D. obtusifolius*, *D. alatus* and *Hopea odorata*. The selected ECM species colonized seedling of all dipterocarp species ranging from 26.27 to 74.13% and stimulated growth of the seedlings more than non inoculated seedlings with increasing shoot height, stem diameter and shoot dry weight up to 51.94%, 50.11% and 64.38%, respectively. This study would be an important step towards understand fundamental characteristics of the ECM community in dipterocarp forest and the use of selected ECM fungi for reforestation and afforestation in Thailand.

Field of Study : Biotechnology
Academic Year : 2008

Student's Signature : Sunadda Yomyart
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Co-advisor's Signature : Jittra Piapukiew
Co-advisor's Signature :

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CONTENTS

Page

Page

Page

LIST OF TABLES

LIST OF FIGURES

Figure Page

Figure Page

LIST OF ABBREVIATIONS

°C	Degree Celsius
cm	Centrimeter
g	Gram
l	Liter
µl	Microliter\
mg	Milligram
ml	Milliliter
M	Molar
mM	Millimolar
s	Second
h	Hour

CHAPTER I

INTRODUCTION

Forest cover in Thailand has diminished rapidly. There are a number of reasons for these losses including logging and over harvesting of forest products, slash and burn agriculture and by encroachment into forest land by industrial agriculture. Reforestation of many degraded areas is difficult because the soils are also very infertile and very few native species are able to tolerate these conditions and factors such as summer drought, reduction natural in regeneration and increase the risk of erosion in deforested zones. Field trials have been undertaken to screen ectomycorrhizal fungi suitable for introduction into plantations such regions to improve tree survival and productivity. Key criteria used in the selection of fungi included the capacity of the inoculant fungus to increase tree productivity and its ease of management in the nursery (Dell, 2000). Another important factor to consider in any mycorrhizal inoculation program is whether the selected inoculant fungus can survive and persist on roots as an effective symbiont for the host tree (O'Dell et al., 1992; Thomson et al., 1996).

Dipterocarps are commercial timber species in southeast asia and they form ECM with multiple species of basidiomycetes and some ascomycete fungi. Indeed, dipterocarp forests predominate on low-nutrient soils but grow least well on dry, nutrient-deficient, laterites but some research has shown that ECM can increase biomass production on such soils.

However, information on host specificity and factors that maintain or change the ECM community remain incompletely known. A number of biotic and abiotic factors such as ECM succession, interspecific interaction of ECM, host species, climate, fire and soil properties have been identified as influencing their community structure. All these studies were carried out in temperate and boreal areas and little is known about the ECM community structure and dynamics in natural forests and plantations in the tropics where ECM fungi are also found. Because the ECM community affects host productivity and seedling establishment they are neccessary to maintain and rehabilitate

dipterocarp forests and also to establish sustainable timber production. However, before this can be achieved, it is necessary to understand fundamental characteristics of the ECM community of these forests. ECM fungi will need to be identified, cultured and used to produce selected indigenous ECM inocula for local conditions of reforestation where ECM hosts are present. For this to be effective, investigations into the host range and growth stimulation are required.

Therefore, the main objectives of this study are as follow:

1. To observe dynamic changes in ECM root community composition in a dipterocarp forest and plantation during the dry and wet seasons to provide a unique view into the dynamics of these fungi on the root.
2. To select strains of the ectomycorrhizal fungi, *Astraeus odoratus*, *Astraeus asiaticus* and *Pisolithus abditus*, which have good properties for producing inocula and stimulate growth of Dipterocarpaceae seedlings.

CHAPTER II

LITERATURE REVIEW

2.1 Ectomycorrhizal fungi

Ectomycorrhizas are symbiotic associations that form between the roots of most woody plant species and fungi. These symbiosis are characterized by bi-directional movement of nutrients where carbon flows to the fungus and inorganic nutrients move to the plant. In infertile soils, nutrients taken up by ectomycorrhizal (ECM) fungi can lead to improved plant growth and reproduction. As a result, mycorrhizal plants are often more competitive and better able to tolerate environmental stresses than are nonmycorrhizal plants (Harley and Smith, 1983). Then, ECM fungi are also viewed as biofertilizers, the alternative to chemical fertilizers for infertile tropical soils where reforestation is being carried out (de la Cruz, 1990).

2.1.1 Morphological and anatomical features of ectomycorrhizas

The diagnostic feature of the ectomycorrhiza is the presence of a modified lateral root branching pattern. ECM fungi typically change the morphology of the colonized plant root tips, often inducing much greater rates of branching that result in high numbers of tips. This pattern, which is called heterorhizy, consists of short mycorrhizal lateral roots (Fig.2.1) supported by a network of long roots and ECM fungal hyphae between root epidermal cells in angiosperms and cortical cells in gymnosperm producing a netlike structure called the Hartig net (Fig.2.2). Many ECM also have a sheath or mantle of fungal tissue that may completely cover the absorbing root (usually the fine feeder roots). The mantle can vary widely in thickness, color and texture depending on the particular plant-fungus combination. The mantle increases the surface area of absorbing roots and often affects fine-root morphology, resulting in root bifurcation and clustering. Contiguous with the mantle are hyphal strands that extend

into the soil. Often the hyphal strands will aggregate to form rhizomorphs that may be visible to the unaided eye. The modulla of rhizomorphs can differentiate into tubelike structures specialized for long-distance transport of nutrients and water.

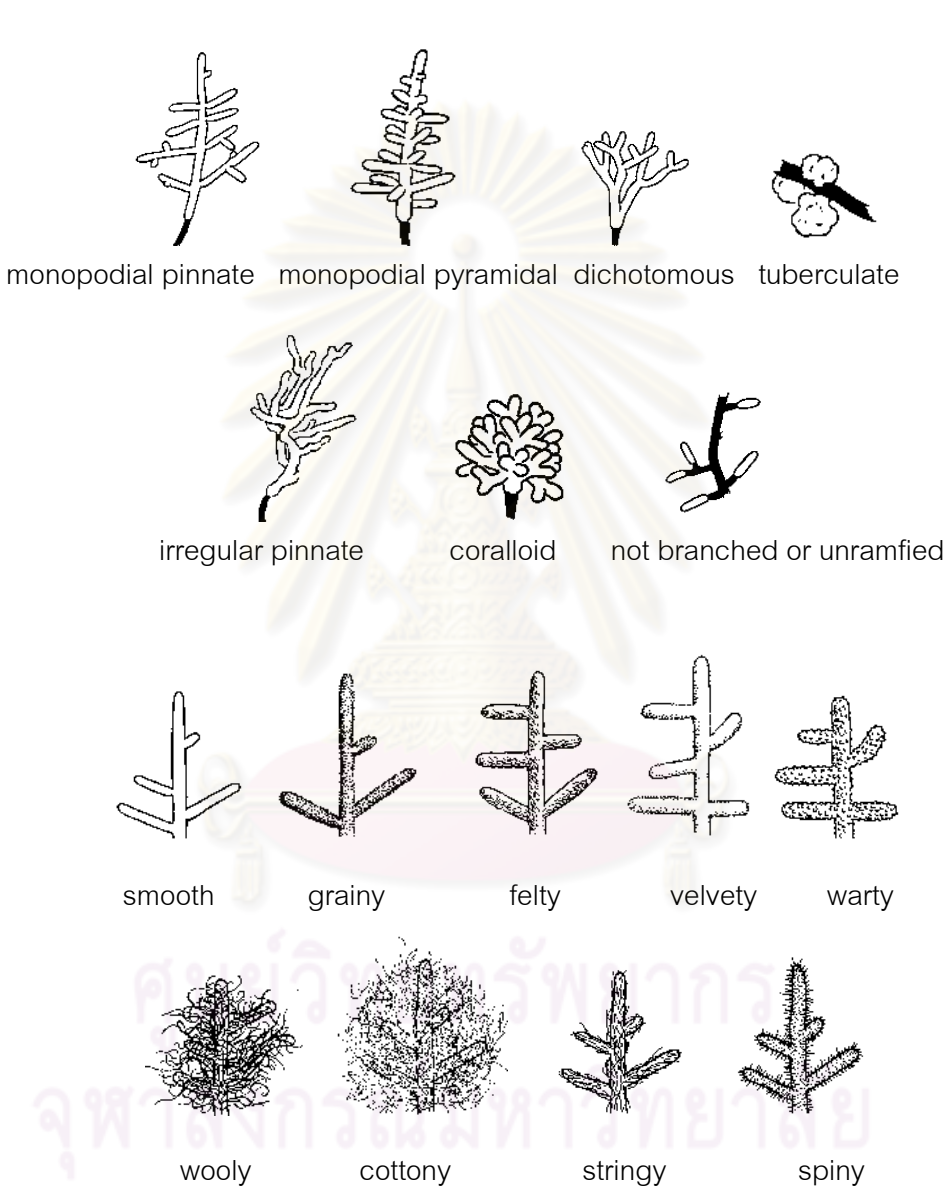


Fig. 2.1 Morphology of an ectomycorrhizal system (A). Texure of ectomycorrhizas (B) from Durall et al. (1996).

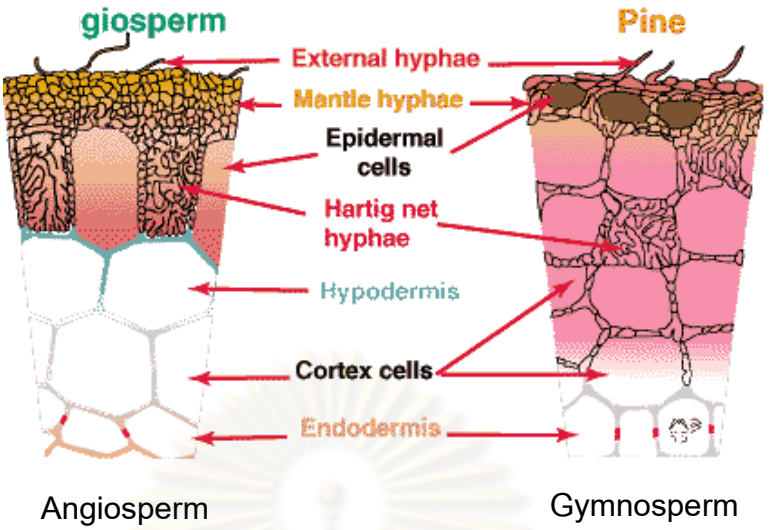


Fig. 2.2 Transverse of ECM angiosperm and gymnosperm (pine) (Brundrett et al., 1996)

2.1.2 Role of ectomycorrhizal symbiosis

ECM fungi play important roles in ecosystems, both in natural forests and plantations. They provide plants with increase productivity in plantation forestry or plant establishment during ecosystem recovery after severe disturbance. Extramatrical hyphae take up nutrients from the soil solution and transport them to the plant root. By this mechanism, mycorrhizas increase the effective absorptive surface area of the plant. In nutrient-poor or moisture-deficient soils, nutrients such as nitrogen and phosphorus are taken up from soil by the extramatrical hyphae (Smith and Read, 1997). Moreover, not only mycorrhizal plants are often more competitive and better able to tolerate environmental stresses such as high or low pH and heavy metal contaminations than are nonmycorrhizal plants (Leyval et al., 1997) but also root colonization by ECM can provide protection from parasitic fungi and nematodes (Duchesne et al., 1989; Morin et al., 1999).

2.1.3 Mycorrhizal associations of different tree species

While a relatively small number of plants, *c.* 8000, form ectomycorrhizas. Trees with ECM associations are dominant in coniferous forests, in cold boreal or alpine regions and many of the broad-leaved forests in temperate or mediterranean regions,

but they also occur in some tropical or subtropical savanna or rain forest habitats (Meyer, 1973; Alexander and Hogberg 1986 ; Brundrett, 1991). The majority of ECM hosts are trees or shrubs belonging to the families Pinaceae, Fagaceae, Betulaceae, Myrtaceae, Salicaceae and Dipterocarpaceae (Smith and Read, 1997) but associations are formed by a few herbaceous plants, including *Kobresia* (Cyperaceae), *Polygonum* (polygonaceae) and *Cassiope* (Ericaceae) species found in alpine/arctic regions (Kohn and Stasovski, 1990 ; Massicotte et al., 1998).

2.2 Taxonomically the ectomycorrhizas forming fungi

Majority of fungi that form ECM associations are Basidiomycota, with some Ascomycota and a few Zygomycota (species of *Endogone*) (Molina et al. 1992). Reproductive structures of ECM fungi are consequently very diverse, include epigeous fungi in various forms such as mushrooms, puffballs, coral fungi, resupinate or crust fungi etc. and subterranean structures such as hypogeal fungi which are called truffles or truffle-like fungi. It has been estimated that 6,000 or more species of fungi form ECM associations with approximately 10% of the Angiosperms and many Gymnosperms (Trappe, 1987). The total number of ECM fungi is likely to be considerable underestimated. Since the early 1990s, the use of molecular markers to identified mycobionts directly from ectomycorrhizas has greatly increased the number of known taxa. In addition, a number of fungal groups previously considered to be saprotrophic have been found to be ECM fungi. In summary, an accurate estimate of the size of the global community of ectomycorrhiza is likely to be *ca.* 7,000 – 10,000 species (Taylor and Alexander, 2005).

2.2.1 Diversity and Community of ECM fungi

Diversity is commonly expressed simply as the total number of species present in a community (species richness) or as an index which includes two components (species richness and species evenness). Species evenness refers to the proportional abundance of each species within a community (Wiensczyk et al., 2002).

A community is the set of all populations that inhabit a certain area. Communities are not static, they gradually change overtime because the environment changes and the species themselves tend to also change their habitats.

An ecosystem is a higher level of organization the community plus its physical environment. Ecosystems include both the biological and physical components affecting the community/ecosystem. Ecosystems were studied from a structural view of population distribution or from a functional view of energy flow and other processes. Populations of a few species are dominant within a community which provides the community with its name.

Community ecology is a subdiscipline of ecology which studies the distribution, abundance, demography and interactions between coexisting populations. Interactions between populations determined by specific genotypic and phenotypic characteristics, is the primary focus of community ecology.

Species composition is a list of the species present in a community, along with a measure of their relative abundance.

Community diversity is usually reported as two components, species richness, the number of species in the community and community eveness, a measure of the abundance of each species in the community (Magurran, 1988). Community eveness is usually reported as the number or percentage of root tips colonized by an individual species.

2.3 Community structure of ECM fungi

Most research on ectomycorrhizal fungal communities has analysed above-ground sporocarps because they are easy to study (Jonsson et al., 1999 ; Izzo et al., 2005). However, sporocarp production is strongly influenced by annual variation in weather conditions (Eveling et al., 1990) and non-mushroom species such as *Cenococcum geophilum* Fr. and species of *Tomentella*, often dominate the underground ECM community and cause a discrepancy between the sporocarps and underground ECM communities (Gardes and Bruns, 1996; Dahlberg et al., 1997; Peter et al., 2001).

Studies from North America and Europe unanimously confirm that fungi that do not form obvious fruiting structures form the major mycorrhizal abundance. Fruiting species merely constitute 20-30% of the mycorrhizas (Jonsson et al., 1999). Investigations based upon collecting only sporocarps may lead to a rough underestimation of the diversity, abundance and ecological role of ECM fungi.

Within days of emergence, almost every fine root of an ECM plant is colonized by ECM fungi (Smith and Read, 1997; Taylor et al., 2000). The number of the ECM tips, commonly a few mm in length, is often very high. Ectomycorrhizas are short-lived structures, being regenerated on a yearly basis. Regeneration may occur throughout the growing season, but peaks at certain periods. Ectomycorrhizas are vertically distributed to depths occupied by the host tree roots. However, the majority are located in surface soil layers where mineralization processes are most active. In a dry forest ecosystem, ectomycorrhizas are located deeper in the mineral soil (Visser, 1995 ; Taylor and Bruns, 1999).

Therefore, when investigating ECM fungal community structure, direct analysis of ectomycorrhizas in soil samples is needed. However, it is difficult to investigate morpho-anatomy alone. Morphotyping is useful for analyzing ECM communities but is labor intensive and is confounded by natural gradations in morphological characteristic (Izzo et al., 2005). These gradations result from differences in environmental conditions, root tip age and phenotyping expression. As a result, morphotyping tends to overestimate or underestimate ECM richness (Burke et al., 2005).

Much of our knowledge about ECM community dynamics is based on the observations of fungal sporocarps (Nantel and Neumann, 1992; Matsuda and Hijii, 1998 ; Jumpponen et al., 1999) and it remains unclear if variation reflects underground fluctuations. Molecular methods are necessary to balance this tendency with accurate identification of ECM fungal species colonizing roots (Horton and Bruns, 2001).

2.3.1 Molecular methodologies for analyzing ECM fungal communities

A wide range of primers is available for preferential amplification of particular regions. A selection commonly used with ECM Basidiomycota and Ascomycota is given

by Henrion *et al*. (1992). Since many of these code specifically for fungal internal transcribed spacer (ITS) region (Fig. 2.3), they can be used with total DNA extracted from green plants associated in some way with one or more fungi. Such direct inspection of underground ECM fungal community becames possible by the technical development of molecular identification of the fungal species (Horton and Bruns, 2001). Various molecular identification methods are currently in use for characterization of ECM fungal species colonizing roots. Restriction fragment length polymorphism (RFLP) analysis, using the ITS region in nuclear rDNA, has been used the most widely (Horton and Bruns, 2001). Recently, Terminal restriction fragment length polymorphism (T-RFLP) appears to be more direct, easy to use and offers better resolution. T-RFLP targeting rDNA genes has effectively been used to characterize fungal communities in soil including mycorrhizal communities (Dickie et al., 2002; Klamer *et al*., 2002; Vandenkoornhuyse et al., 2003; Edwards et al., 2004; Edel-Hermann et al., 2004; Dickie et al., 2002; Klamer et al., 2002; Ishida et al., 2006; Koide et al., 2006).

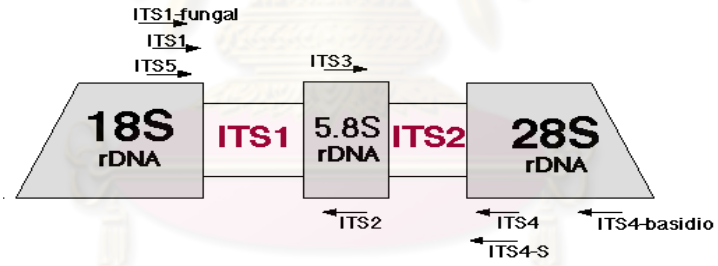


Fig. 2.3 Diagram of primer locations in the ribosomal cassette, primers are positioned above (forward primers) or below (reverse primers) their sequence positions in the ribosomal cassette (http://plantbio.berkeley.edu/~bruns/picts/results/its-map.GIF).

Despite this increase in molecular research, some limitations of the understanding of ectomycorrhizal community ecology still remains. For example, when sporocarps cannot be matched to ECM roots using restriction fragment length polymorphism (RFLP) or TRFLP of the internal transcribed spacer (ITS) region, ectomycorrhizal fungi on roots remain taxonomically unknown (Horton and Bruns, 2001). Although ITS sequencing is now commonly used for identification, this region provides

poor resolution for fungal groups underrepresented in public databases such as hypogeous fungi (Izzo et al., 2005).

However, many studies using molecular technique have demonstrated poor correspondence between species composition of sporocarps and underground ECM community (Mehmann et al., 1995; Nylund et al., 1995; Gardes and Bruns, 1996; Dahlberg et al., 1997; Jonsson et al., 2000 and Peter et al., 2001; Zhou and Hogetsu, 2002) as exemplified by the works of Gardes and Bruns (1996) who examined the ECM community in a *Pinus muricata* forest, *Suillus pungens* ECM root tips were rare and *Russula amoenolens* root tips abundant, whereas *S. pungens* sporocarps were abundant and *R. amoenolens* sporocarps rare.

Zhou and Hogetsu (2002) detected 30 ECM fungal species in narrow areas under *Suillus grevillei* sporocarps and 8 ECM fungal species that developed sporocarps in *Larix kaempferi* forest. Compositions of underground ECM fungal species were much more abundant than above-ground ones.

2.3.2 Effect of biotic and abiotic factors to ECM community structure

Information on host specificity and factors that maintain or change underground ECM communities remain incompletely known. A number of biotic and abiotic factors such as ECM succession, interspecific interaction of ECM, host species, climate, fire, soil pH, nitrogen level, soil parent material, soil moisture content, soil organic matter content, litter quality, soil fertility and as a consequence of distinct forest floor layers or soil horizons have been identified as influencing their community structure. There have been a number of reports as exemplified by the works of Shi et al. (2002) who showed that the number of types of ectomycorrhizas identified was no affected by drought treatment, however, the abundance of single mycorrhizal types was affected. Under drought conditions, mycorrhizas formed with *Xerocomus chrysenteron* increased in abundance from 23% to 46%, indicating an increased drought resistance/survival rate in comparison to the other types of ectomycorrhizas.

Buée et al. (2005) reported that species structure and metabolic activity of each morphotype change depended on the season, temperature, soil moisture content and silviculture treatment (strong thinning). In addition, a number of morphotypes are more abundant and active in winter than in summer, *Clavulina cristata* formed ECM mostly in winter and was more metabolically active during this season, while *Cenococcum geophilum* populations built up and expressed maximal activity in summer.

Ishida et al. (2005) examined ECM root tips of eight coexisting species belonging to six genera (three famiries): *Abies* and *Tsuga* (Pinaceae), *Betula* and *Carpinus* (Betulaceae) and *Fagus* and *Quercus* (Fagaceae). In total, 205 ECM species were detected. Of the 55 ECM species occurring three or more times, eight showed significant bias towards host preference. A Mantel test showed a significantly negative correlation between ECM community similarity and host taxonomic distance. Detrended correspondence analysis separated ECM communities mainly by host taxonomic and successional status. Thus, ECM communities are similar on hosts with similar taxonomic and successional status. A significant proportion of ECM exhibited host specificity, which may contribute to the extremely diverse ECM community in conifer-broadleaf forests.

Edwards et al. (2006) investigated effect of an optimal nitrogen nutrition strategy designed to maximize loblolly pine (*Pinus taeda*) growth on rank abundance structure and diversity of associated basidiomycete communities. They used TRFLP of selectively PCR- amplified nrDNA ITS to determine distribution and abundance of ECM species in 200 soil samples co-selected from optimally fertilized and unfertilized treatments. Their results indicated an increased relative abundance of *Tylopilus* and *Thelephora* spp. on optimally fertilized stands. In addition, these result suggest a trend toward reduced basidiomycete diversity and that large-scale application of optimal nutrition may need to be sensitive to increased nitrate availability.

Korkama et al. (2006) compared ECM communities of eight Norway spruce (*Picea abies*) clones planted in a clear-cut area. The ECM fungi were identified from randomly sampled root tips using denaturing gradient gel electrophoresis (DGGE) and rDNA ITS sequence similarity. ECM diversity varied among clone groups, showing two

fold growth differences. Moreover, according to detrended correspondence analysis (DCA), ECM community structure varied not only among but also within slow-growing or fast-growing clones. These results suggest that ECM diversity and community structure are related to the growth rate or size of the host in boreal forests.

Gebhardt et al. (2007) investigated effect of host age on ECM communities of red oak (*Quercus rubra* L.) in the Lusatian lignite mining district, East Germany. Above-ground, sporocarp surveys were carried out during the fruiting season. Underground, ECM morphotypes were identified by comparing sequences of ITS regions from nuclear rDNA with sequences from the Genbank data base. Fifteen ECM fungi species were identified as sporocarp and 61 ECM fungi species as determined by morphological/anatomical and molecular analysis of their ECM root tips. The number of ECM morphotypes increased with stand age along the chronosequence. However, the number of morphotypes was lower in stands with disturbed soil than with undisturbed soil. Dominant ECM species in all stands was *Cenococcum geophilum*, which reached an abundance of 80% in 21-year-old stand.

Scattolin et al. (2008) suggested that ECM species composition does not change significantly either on the same tree or among trees growing in the same stand, whereas, they differ greatly with bedrock pH and exposure, even if no spatial or temporal trends were found. The ECM species composition revealed instead a significant connection with two environmental features, with a few species significantly associated to them. Thus pH and exposure patterns play a primary role in adaptive selection of ECM species.

2.4 Observation of ECM fungi in tropical ecosystem

Almost all studies have been carried out in temperate and boreal areas and little is known about the ECM community structure and dynamics in natural forests and plantations of tropical ecosystems where ECM fungi are also found. In lowland tropical regions, only Dipterocapaceae are dominant among the tree species associated with ectomycorrhizal fungi. Trees of the family Dipterocarpaceae dominate forests across

much of south and south-east Asia (Ashton, 1982). Dipterocarps are typically canopy trees or emergents and reach considerable dimensions throughout forests of the region. Consequently, they are a source of most tropical hardwood timber, comprising about 80% of the region's timber exports (Ashton, 1988) and supply half of the world's demand for hardwood (Newman et al., 1995, Aminah et al., 2002). Therefore they are both ecologically and economically importance.

Members of the Dipterocarpaceae so far examined are ectomycorrhizal (Singh, 1966; Bakshi, 1974; Hong, 1979; de Alwis and Abeyanake, 1980; Ashton, 1982; Becker, 1983; Alexander and Högberg, 1986; Smits, 1994; Chalermpongse, 1987; Hadi and Santoso, 1988; Hadi et al., 1991). Normally, dipterocarp roots have no root hairs. Uninfected roots are thin, light to slightly dark brown with very young roots pale to hyaline and ultimate branches are usually > 6 mm in length whereas ECM roots of dipterocarps are variously branched and generally much shorter than non-mycorrhizal roots. They are characterized by the presence of a well-developed mantle of varying thickness and hyphal arrangement, and one layer of distinctly radially elongated epidermal cells between which are located the hyphae of the Hartig net (Fig.4). This is followed by 1–2 layers of cortical cells compared to the numerous layers found in non-mycorrhizal roots. The paraepidermal Hartig net does not penetrate beyond the epidermal cells (Godbout and Fortin, 1985).

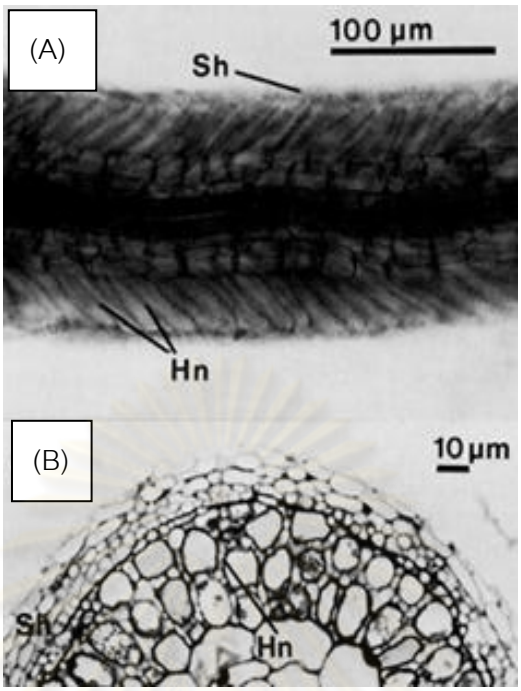


Fig. 2.4 *Hopea parvifolia*. whole root cleared and stained (Phillips and Hayman, 1970) to show oblique elongation of epidermal cells (A) appearance of (A) in transverse section (B) Abbreviations : Sh, Sheath; Hn, Hartig net .

Many researchers reported putative ECM fungal species collected in dipterocarp forests and plantations. Moreover, fruiting body recording of the large ECM fungi present in dipterocarp forests has focused increasingly on ectomycorrhizal species. Chalermpongse (1987) surveyed ECM sporocarps in a dry-deciduous dipterocarp forest and a semi-evergreen dipterocarp forest in Amphur Pak Thong Chai and reported 35 species belonging to the genera *Amanita*, *Boletus*, *Lactarius*, *Pisolithus*, *Russula* and *Tricholoma* fruiting over three years in the wet season.

Smits (1994) collected over 80 species of putative ECM fungi in wet dipterocarp forests in East-Kalimantan and suggested the diversity of ECM fungi in dipterocarp forests is lower than in temperate forests.

Watling et al. (2002) surveyed logged and unlogged lowland dipterocarp forest at Pasoh Forest Research, Negri Sembilan, Malaysia. Three hundred and twenty-four distinct collections of sporocarps were collected between 1995 and 1997 in five 1 ha plots. The records were distributed among 95 different taxa in 14 families of basidiomycetes and there were 218 sporocarps of the Russulaceae. Members of

Amanitaceae and Boletaceae were ranked a distant second and third with 26 and 22 collections of sporocarps, respectively.

Ogawa (2006) reported 34 species of ectomycorrhizal fungi from dipterocarp forest in Bukit Suharto Experiment Forest and Lempake Experimental forest, Malasia in the rainy season, from 1989-1991, among the mycorrhizal fungi the genera *Scleroderma*, *Laccaria*, *Russula*, *Amanita* and *Boletus* were dominant. Generally, most sporocarps were produced only in the rainy season and disappeared after being attacked by insects or microbes, soon after their appearance. The number of sporocarps collected from a stand in one season was smaller than those in temperate forests. Many sporocarps of *Scleroderma columnare* were collected from the nursery and young stand in the campus of Mulawarman University. *Laccaria* sp., which was similar to *Laccaria vinaceovellanea* and *Inocybe* sp. were found in nursery and around the seedlings of *Shorea* and *Hopea* in the arboretum. These three ECM species seemed to be dominant on the seedlings and also as the precursors of succession among mycorrhizal fungi of dipterocarps. The growth of seedlings that were infected with *S. columnare* was apparently better than those infected by *Laccaria* sp. or *inocybe* sp. in the nursery. *Scleroderma columnare* formed a white mycorrhiza with 11 species of Dipterocarpaceae. Whereas, *Laccaria* sp. formed mycorrhiza with only 2 host species. It seems from these facts that *S. columnare* has a wide-host range.

Sanmee et al. (2004) reported that deciduous dipterocarp forests in the north of Thailand had greater diversity of fruiting ectomycorrhizal fungi than evergreen dipterocarp forests (11 families, 21 genera and 52 spp.; 8 families, 15 genera and 24 spp., respectively). The dominant genera in the dry dipterocarp forests were *Russula*, *Boletus*, whereas in the wet dipterocarp forests, *Amanita* was the main genus followed by *Lactarius* and *Russula*.

In recent years, new species of dipterocarp ECM fungi have been described (Sanmee et al., 2003, Kanchanaprayudh et al., 2004, Phosri et al., 2004, Yomyart et al., 2006; Sudhakara Reddy et al., 2005). Such information on the range and potential host preferences of ECM fungi has contributed to an improved ability on the part of mycorrhizal researchers to separate and make tentative identifications of ECM roots. In

this way, accurate field assessments of ECM populations are being made and baseline information is accumulating on the ecological roles of different fungi (Becker, 1983; Lee and Alexander, 1996).

The diversity of ECM fungi on dipterocarps has been observed ley recording sporocarps and ley stidying ectomycorrhizas. Twenty-eight ECM species were found on *Shorea leprosula* (Lee et al., 1997) and 26 ectomycorrhizal morphotypes were found on *Shorea pavifolia* (Ingleby et al., 1998). Yuwa-Amornpitak et al. (2006) reported diversity of ECM fungi on Dipterocarpaceae in Thailand. Eight study sites were conducted in 4 sections of Thailand during the rainy season of 2002 and 2003. The results revealed that over 40 ECM morphotypes of 33 ECM taxa in 8 families were observed including Thelephoraceae, Russulaceae, Amanitaceae, Cortinariaceae, Sclerodermataceae, Agaricaceae, Pisolitaceae and Boletaceae. The host speciation showed that these fungi could associate with more than one host plant species.

2.5 Application of ECM fungi

2.5.1 Reforestation and afforestation of degraded areas in tropical forests

Forests in many parts of the world have decreased rapidly. There are a number of reasons for these losses including logging and over harvesting of forest products, slash and burn agriculture and by encroachment into forest land by industrial agricultural such as oil palm estates and rubber plantations. Furthermore, it has become clear that forest depletion has strongly influenced the global environment. Consequently, the recovery of destroyed forests is a very important issue. However, reforestation of many degraded areas is difficult because their soils are also very infertile and very few native species are able to tolerate conditions such as summer drought, and this reduces natural regeneration and increases the risk of erosion in deforested zones. Seedlings of some fast-growing exotic species such as *Eucalyptus* and *Acacia* were monocultures established in many countries of south-east Asia such as Vietnam, Indonesia, the Philippines and Thailand. These species are able to tolerate infertile soils with limited

phosphorus and nitrogen (Specht, 1996) and quickly produce a marketable product such as timber or wood pulp. But watershed protection or biodiversity conservation which may not be favoured by monoculture plantations of fast-growing exotic species. Valuable long-rotation species forest or indigenous-species forests such as teak (*Tectona grandis*) or dipterocarps should be established, where their economic value and ability to conserve environment may be higher than for the fast-growing species forest. Dipterocarpaceae in Thailand can be grouped into evergreen and deciduous species consisting of 8 genera with 65 species (Pherngklia and Niyomdhum, 1999). The majority are evergreen species which are scattered all over the country, while the deciduous ones are represented by only five xerophytic species which are *Dipterocarpus intricatus*, *Dipterocarpus tuberculatus*, *Dipterocarpus turbinatus*, *Shorea obtusa* and *S. siamensis*. However, semi-evergreen trees can be recognised in some species such as *Shorea farinosa*, *Shorea roxburghii* and *Anisoptera costata*, and several evergreen dipterocarps such as *Dipterocarpus alatus*, *Dipterocarpus turbinatus*, *Dipterocarpus retusus* and *Dipterocarpus costatus* can be briefly deciduous during exceptionally dry years. Most of the evergreen species have a very large and emergent canopies in lowland evergreen dipterocarp forest, while deciduous species can reach only medium-sized trees as a results of their response to the environment.

2.5.2 Effect of ECM fungi associated with dipterocarps

The large degree of dependence by the trees on their fungal partners is now also widely accepted; the survival and growth of seedlings being particularly reliant on the nutrient acquiring abilities of their fungal partner (Smits, 1994). Lee (1998), in a review of Dipterocarpaceae root symbiosis and nutrition, mentioned that the role of mycorrhizas in increasing the absorptive efficiency of roots is well known, and forms the basis for understanding the relationship between mycorrhizal fungi and forest function. Indeed, dipterocarp forests predominate on low-nutrient soils and Connell and Lowman (1989) hypothesized that their ability to maintain dominance was linked to the ability of newly germinated seedlings to form mycorrhizas via mycelial links with "mother" trees

and thus connect into the nutrient acquisition network of the canopy trees. Alexander et al. (1992) have shown the importance of these connections for the early infection of dipterocarp seedlings and the mycelial transfer of nutrients and carbohydrates from canopy trees to shaded seedlings has been demonstrated by Read et al. (1985) for temperate tree species. Severe forest disturbance that results in the elimination of ectomycorrhizal mycelial networks from the soil could therefore have serious implications for the regeneration of dipterocarp forest.

Dipterocarp ectomycorrhiza could play an important role in establishment of seedlings by increasing plant uptake of nutrients and water. There are many parameters that can be measured to describe relative growth differences between plants inoculated with and without ECM fungi. Their selection will depend on type of plants and its physiology as well as the specific objective and duration of the experiment. Parameters required to evaluate plant response to inoculation must be chosen by

2.5.2.1 Tissue weight weight of shoots and/or roots can be taken as fresh weights but more accurately as dry weight. Taking these two measurements separately will allow the calculation of a root/shoot ratio which is of particular importance in evaluation quality of such plants as forest trees or woody ornamental plants.

2.5.2.2 Plant height linear extension of main shoot upward of many plants is a valuable measure of response to ECM association. Measurements should be from the base of the plant, at soil line, to the tip of the terminal shoot bud.

2.5.2.3 Stem diameter stem diameter (stem caliper or root collar diameter) is useful for evaluating response of many single stem plants. This is often the most important parameter for evaluation of forest tree seedlings.

2.5.2.4 Shoot volume this parameter is useful as a nondestructive estimate of shoot size or weight. The formula height multiplied by the square of stem diameter has been used as an estimate of shoot size of trees.

2.5.2.5 Leaf area and number determining the number of leaves and leaf area on a plant can be done nondestructively, but with many plants this method would be extremely time consuming or impossible.

2.5.2.6 Root colonization roots colonized by ECM fungi exhibit morphological change compared to non-ECM roots. Surface area increases as a results of branching habit of most ectomycorrhizas and extensive vegetative growth of hyphae of fungal symbionts from the ectomycorrhizas in to soil for nutrient and water absorption (Lei *et al*., 1990).

2.5.2.7 Transplant survival some plants normally are grown as seedlings. ECM may affect survival of transplants. This response may be recorded as either a plus or a minus to enable calculation of percentage of survival between ECM and non-ECM seedlings.

2.5.3 Use of ectomycorrhizal symbiosis for plantation forestry

Pisolithus, a gastromycete genus, has a worldwide distribution and forms ectomycorrhizal associations with a wide range of woody plants (Marx, 1977) including members of the Pinaceae, Myrtaceae, Fagaceae, Mimosaceae, Dipterocarpaceae and Cistaceae. *Pisolithus* is a commonly used ectomycorrhizal fungus in forestry inoculation programs and shown some success at improving post-transplant seedling growth and survival rates of many tree species (Marx and Bryan, 1970). Many studies have shown that positive host plant growth responses have been observed for seedlings inoculated with *Pisolithus tinctorius* (abbrev.: Pt) (Marx and Bryan, 1970; Burgess et al., 1994 and Dixon et al., 1984). However, isolate specificity may play an important role in the success of Pt inoculation programmes. Studies have shown that isolates obtained from one geographical region or genus may be less effective at colonizing plants from other genera or regions. Burgess et al. (1994) used 20 Pt isolates to inoculate *Eucalyptus grandis* W. Hill ex Maiden. They showed significant differences in colonization rates and level of colonization of Pt on the host tree. These studies suggest that although Pt is an effective colonizer of a wide array of higher plants, other factors can influence the effectiveness of Pt as a mycobiont. Dry sites with high soil temperatures seem to illicit the greatest host growth benefit from Pt inoculation. Cline et al. (1986) showed that Pt exhibited the greatest growth compared to three other species of mycorrhizal fungi, with

the highest mycelial growth rates occurring at temperatures between 21 and 32 ^{o}C. Field studies support Pt as an effective colonizer in warmer, drier regions.

There has been successful synthesis of ectomycorrhizas between various fungal species such as *Pisolithus* and dipterocarps in the nursery in Indonesia (Hadi et al., 1991; Turjaman et al., 2005), Thailand (Sangwanit and Santhien, 1991) and Malaysia (Yazid et al., 1994, 1996 and Lee et al., 1995). Colonization of dipterocarp roots by ECM fungi resulted in increased growth of seedlings, as exemplified by the works of Yazid et al. (1994 and 1996) who showed that a selected strain of *P. tinctorius* was able to stimulate growth of *Hopea odorata* and *Hopea helferi* under control conditions. Whereas Hadi et al. (1991) found that *P. tinctorius* had no significant effect on the growth of *H. odorata* and *Shorea pinanga*.

Chang et al. (2006) isolated Ptmsn from a sporocarp collected in a pine forest, in Thailand and inoculated roots of *D. alatus* , *S. glauca* and *H. odorata*. They reported that this ECM fungus persisted well on roots of *D. alatus* and *S. glauca* but not on *H. odorata*. The Pt441 was isolated from a sporocarp collected in Brazil under a eucalypt, also persisted well on roots of *S. glauca* but did not persist well on roots of *H. odorata* in the nursery in Malaysia. These results suggest that ECM fungal can react differently with different host plants. While exotics *P. tinctorius* can colonizes selected dipterocarp species in the nursery but in field, both Ptmsn and Pt441 showed poor persistence on the roots of *S. glauca* after outplanting in logged-over forests. It virtually disappeared after 6 months in the field and was outcompeted by indigenous ectomycorrhizal fungi suggesting that they may not have been able to fully adapt to wet dipterocarp forest of Malaysia.

However, Martin et al. (2002) have revealed that *P. tinctorius* comprises many phylogenetically different species. Thus, different host specificities among *Pisolithus* isolates may be derived from different *Pisolithus* species. In addition, based on morphology of sporocarps, spores, isolated culture mycelia and ITS sequences, Kanchanaprayudh et al. (2003a) reported various *Pisolithus* species associated with pine, eucalyptus and dipterocarp trees in Thailand. Moreover, *Pisolithus* species under dipterocarp trees whose ITS sequence and morphology did not match any previously

reported species was found and described as a new species, *P. abditus* (Kanchanaprayudh et al., 2003b). *Pisolithus* have been found not only in Thai dipterocarp but also from dipterocarp forests in other countries, *P. aurantioscabrosus* has been reported from Malaysia (Watling et al. 1995) and *P. indicus* were collected under dipterocarp plantation of *Vateria indica* native forest in Uppangala, Karnataka, India (**Sudhakara Reddy** et al., 2005).

Regeneration and recovery of forests depend on survival and establishment rate of young plants. However, failure in reforestation or afforestation have previously been attributed to absence of suitable mycorrhizal fungi because survival and growth of seedlings are particularly reliant on nutrient-acquiring abilities of their fungal partner. Smits (1983) suggests that low survival of dipterocarp seedlings in logged-over forests may partly be due to death of mycorrhizal fungi and that inoculation of plant stocks with proper ECM fungus may offer better prospects for regeneration. Ectomycorrhiza can play an important role in protection of plants against environmental stresses after transplant such as drought, high soil temperatures, organic and inorganic toxins and extreme soil acidity (Lee, 1998). Therefore, the controlled ECM inoculation of seedlings in nursery usually promotes the establishment of forest plantations, mainly by improving initial seedling growth. Moreover, many of these fungi produce mushrooms and puffballs on the forest floor, not only ECM may simultaneously stimulate host tree growth but also present an additional income for forestry farms through potential production of edible sporocarps.

The method of inoculation is an important consideration when using Pt .Research has shown that Pt can effectively be applied with spore inoculations and also by the use of mycelium. Marx et al. (1989) showed that vegetative inoculum was more effective at colonizing *Pinus* than Pt spores. Although Pt is an effective colonizer of many tree species, it may be ineffective at promoting growth and survivability of host trees at outplanting due to an inability to compete with indigenous fungi. Castellano and Trappe (1991) showed that Pt was ineffective at improving growth in southwest Oregon when compared to trees unintentionally colonized with indigenous mycobionts. They suggest

that the moisture levels prevalent in Oregon make it more difficult for Pt to compete successfully with other fungi.

In Thailand, one of the most abundant ECM fungal genera is *Pisolithus*, Many sporocarps of *Pisolithus albus* were observed every year in *Eucalyptus camaldulensis* plantations that are distributed over the country (Kanchanaprayudh et al., 2003a). Not only *P. albus*, but also Kanchanaprayudh et al. (2003a) reported *P. abditus* and *Pisolithus* sp.5 associated with *Dipterocarpus alatus* and *Pinus kesiya*, respectively. Additionally, *Pisolithus* has the ability to promote growth of *E. camaldulensis* and *Pinus kesiya* (Phosri, 1998 and Yomyart, 2004).

Pisolithus has a worldwide distribution and forms ectomycorrhizal associations with a wide range of woody plants and is commonly used as the ectomycorrhizal fungus in forestry inoculation programs. In addition, these species have advantage over many other ECM fungi as their spores can be collected from mature fruiting bodies. Preparation of ECM inocula from spores is comparatively easy with field-collected materials because these fungi produce a lot of sporocarp in fruiting season. Nevertheless, *Pisolithus* has not been intensive marketed in the world, whereas, many edible ECM species have such as *Amanita, Boletus, Lactarius, Russula, Chantarellus* and *Astraeus*. In Thailand, *Astraeus* is the most popular ECM fungus consumed by people in northern and north-eastern. It commands a high price because it is available early in the fungal fruiting season.

Astraeus is widespread in temperate and tropical regions (Lloyd, 1902 ; Zeller, 1948 cited in Phosri, 2004) and has a wide variety of host plants including members of the Pinaceae and Dipterocarpaceae. In addition, *Astraeus* and *Pisolithus* are closely related, both belonging to the Sclerodermataceae. *Astraeus*, like *Pisolithus*, is common in the hot, dry regions and is adapted to dry and infertile sites. However, there is far less research addressing the effectiveness of *Astraeus* as a mycobiont than research studying *Pisolithus*. In Thailand, *Astraeus* is primarily found in dipterocarps forests in northern and northeasthern regions and common names is earth star, Hed Phor or Hed Thop. It can be found growing around the bases of single dipterocarp trees although it is more likely to be found in forests. Fruiting occurs in the early rainy season during a hot

spell of weather. Generally, the fungi collected are consumed fresh but large quantities of this mushroom are processed in cans for domestic consumption and export. Like almost all symbiotic edible mushrooms, fruiting bodies of *Astraeus* have never been cultivated. Consequently, the commercialization of this mushroom is dependent on its collection from natural forests. The large quantities of this mushroom which are picked over several years from natural forest lead to decrease in mushroom production in future.

Currently, the production of edible ECM fungi from plants inoculated under control conditions has been especially developed for truffles (*Tuber* spp.) and matsutake (*Tricholoma matsutake*). As demand increases and cultivation technology improves, other species are likely will be cultivated in plantations or forests may be managed to enhance mushroom production. Because forest trees provide sufficient carbohydrates for abundant fruitings, wild harvests will probably also remain cost effective.

2.5.4 Production of ectomycorrhizal fungi inoculums

The need of many species of forest trees to form ectomycorrhizas was initially observed when attempts to establish plantations of exotic pines in parts of the world deficient in the fungus partners routinely failed until essential fungi were introduced (Mark and Kenney, 1991). The rapid expansion of economic tree plantations in the world has lead to increased research into the practical use of ECM fungus technology to promote tree growth. However, lack of practical and efficacious forms of inocula has been a limiting factor. Inocula of ECM fungi are usually comprised of biomass and carrier material. The biomass should be of an appropriate genotype, be produced via axenic culture and have a consistent physiological status appropriate for the initiation of mycorrhiza. The carrier material, if used, should be associated with a consistent amount of biomass and protect the biomass against physical and chemical stress during production and handling procedures. The inoculum should be in a form which is practical for large scale production and use, allow control of production parameters, is

low in volume and cost effective. Several types of natural and laboratory-produced ECM inocula and variety methods for inoculation of nursery-seedlings have been investigated and reviewed as 3 types

2.5.4.1 Soil inoculums The most widely used natural inoculum, especially in developing countries, is soil or humus collected from forests or plantations. The soil inoculum is either mixed into rooting medium (usually a 5 to 10 percent volume), broadcast 0.5-1 cm deep onto soil and watered into the soil around seedlings or suspended in water (1 kg soil into 20 L of water) and poured onto seedlings. Better results are obtained with freshly collected soil than with soil collected and stockpiled for several months. A major drawback with these inoculum type is that the species of ECM fungi in the inoculum can not be controlled.

2.5.4.2 Spore inoculums Spores of various ECM fungi have been used as inoculum to form specific ectomycorrhizas on seedlings. Whole or chopped sporocarps are dried before use. Gasteromycetes, such as puffball-producing genera *Rhizopogon*, *Scleroderma* and *Pisolithus*, produce numberous basidiospores that are easier to collect in large quantities than those of mushroom-producing ECM fungi. Inoculum composed of spores mixed with a moistened carrier such as vermiculite, kaolin or sand can be mixed into nursery soil or mixed with water and poured onto seedlings (Theodorou and Bowen, 1973) or can be added as tablets (de la Cruz, 1990). A major advantage is that spores require no extended growth phase under aseptic conditions like vegetative mycelium Moreover, spore inoculum is very light that convenient to transport, easy application and tolerates long storage periods.

One of major disadvantages of spore inoculum obtained from ECM fungi is lack of standard laboratory tests to determine spore viability (Brundett et al., 1996) and it is not possible to inoculate with a specific fungal isolate due to inherent variability among spores (Marx et al., 1984; Cordell et al., 1987). Another disadvantage is that sufficient sporocarps of many ECM fungi may not be available all time. Sporocarps should be collected and stored large numbers of fruiting bodies when they are abundance only in fruiting season (Cordell et al., 1988; Marx et al., 1991). In addition,

formation of ectomycorrhiza by basidiospores usually takes longer than vegetative inoculum of the same fungi (Marx et al., 1976)

2.4.5.3 Vegetative inoculums Mycelia obtained from pure cultures of ECM fungi, also called vegetative inoculum, has proven to be the most suitable method since harmful organisms are excluded. Vegetative inoculum can be prepared from any fungus which are able to be cultivated in pure culture, allowing the use of selected isolates that have been previously tested in terms of their efficiency in promoting plant growth (Marx, 1980). However, some ECM fungi are difficult to grow in laboratory. Many species have never been isolated and grown in pure culture. Moreover, some species grow slowly, others often die after a few months in culture.

This type of inoculum production offers the greatest potential for large scale application, for which the techniques have been now developed and improved so the inoculum remains viable during storage and transport, maintaining its infectivity for several months after its production. Furthermore, the formulated inocula must be easy to apply and must also be free of contamination by plant pathogens. Production of the vegetative ECM fungal inocula could be cultivate in 2 conditions

2.4.5.3.1 Cultivation of ECM fungi in solid substrate Vermiculite and peat moss moistened with a modified of Melin-Norgrans (MMN) medium was found to be an excellent substrate for the production of mycelial inoculum of various ECM fungi. The carriers are produced via solid substrate culture. The mycelium remains protected inside the vermiculite particles, where it can survive until receptive roots are produced by host plants. However, this method of culture is subject to low culture uniformity, and difficulties in control of culture conditions and scale up. Moreover, the inocula produced often contain undesirable levels of residual carbohydrates which can adversely affect the success of inoculation (Marx, 1980).

2.4.5.3.2 Submerged cultivation of ECM fungi Submerge fermentation requires less space and time since in liquid medium the contact between the phases is maximized and nutrients are more efficiently utilized. Mycelium produced by submerged fermentation is pure and concentrated and has to be protected from physical and biological factors before its application. Various forms of inocula are currently available.

After cultivation by statistic-flask, shake-flask or airlift bioreactor, mycelia may be processed in a sterilized blender to produce mycelial slurries for inoculation (Boyle et al., 1988; Gagnon et al., 1988; Brundrett et al., 2005) or immobilized in calcium alginate gel (Parladé et al., 2004) or other types of polymeric matrices such as polyacrilamide and carrageenan (Le Tacon et al., 1985). Inocula beads can remain viable for several months under refrigeration, although the results vary between fungal species (Mauperin et al., 1987; Kuek et al., 1992).

2.5.5 Selection of effective ECM fungi inocula

Some of the ECM fungi are host specific, others have a wide host range and at the same time a single tree may have several different ECM partners on its roots (Yuwa-Amornpitak et al., 2006). Controlled inoculation techniques are useful as an additional nursery culture method to increase field performance of out-planted seedlings. The pre-selection of ECM fungi is a critical step for establishing nursery inoculation programmes (Trappe, 1997). The selection criteria are based on physiological and ecological differences between different fungi and even between fungal strains (Wong et al., 1990; Ricón et al., 2001). These criteria include the symbiotic compatibility of fungus and host, the ecological adaptability of the ECM fungus to the site of transplantation, the ability of the fungus to compete against native fungi and the each of inoculum production. Once compatibility of plant and fungus is established, the development of suitable methods for inoculum production and application is necessary. Vegetative inocula of selected fungal strains have been frequency recommended as the method of choice (Marx 1980; Brundrett et al., 1996). However, spore inoculum is commonly used because of its easy application and the availability of large quantities of spores from few sporocarps. Moreover, growth in pure culture is not required and spores can tolerate long storage periods. The main disadvantage of spore inoculum is the large genetic variability, the lack of reliable laboratory method to determine spore viability and the delay in mycorrhization compared with vegetative inoculum (Brundrett et al., 1996).

Key criteria used in the selection of fungi included the capacity of the inoculant fungus to increase tree productivity, the narrow host range of the fungus and its ease of management in the nursery (Dell et al., 2001). Another important factor to consider in any mycorrhizal inoculation program is whether the selected inoculant fungus can survive and persist on roots as an effective symbiont for the host tree (O'Dell et al., 1992; Thomson et al., 1996).

Thus, use of mycorrhizal fungi for inoculation experiments should be conducted with indigenous fungal species that are better adapted to local environmental conditions. Wu et al. (1999) showed that while Pt grew faster than *Suillus luteus* in a rhizobox, at the same time, an unidentified ECM (indigenous species) overgrew the Pt mycelia and mycorrhizas and inhibited Pt development over the course of 63 days. However, Lee et al. (1995) reported *P. aurantioacabrosus* which was discovered in a lowland dipterocarp forest in Malaysia (Watling et al., 1995) failed to form ectomycorrhizas with dipterocarps.

Dell et al. (2001) inoculated seedlings of *Eucalyptus camaldulensis* and *Eucalyptus urophylla* with pure cultures of selected Australian ectomycorrhizal fungi, including three isolates of *Pisolithus albus* and one isolate each of two unnamed *Pisolithus* spp.; ectomycorrhizal seedlings were out-planted at two sites in Guangzhou Province, China. Sequences of the internal transcribed spacers (ITSs) of ribosomal DNA (rDNA) from inoculant fungi and fungi re-isolated from sporocarps in the field were compared. Sequencing confirmed that an Australian isolate (H4111) of one unnamed *Pisolithus* spp. had persisted in competition with an indigenous Chinese *Pisolithus* spp. (host-*Pinus* or fagaceous). Selected isolates of Australian *Pisolithus* formed morphologically typical ectomycorrhizas in pure culture syntheses and in the field. In comparison, the indigenous Chinese *Pisolithus* formed an incomplete association with a poorly developed mantle. Because *Pisolithus* isolate H4111 produced sporocarps in south China under eucalypts and promoted tree growth, this fungus would be useful in spore orchards to provide inoculum for eucalypt nurseries. The local Chinese *Pisolithus* is not recommended for inoculation programs because it is ineffective in forming mycorrhizas with eucalypts.

In addition, Lee et al., (2002) reported that ECM fungus inoculants of *Hopea odorata* seedlings may be replaced by indigenous contaminant ECM fungus in nursery such as *Tomentella* and the seedlings was not stimulated in growth by the latter fungi.

It is necessary to understand fundamental characteristics of ECM communities in of dipterocarp forests and produce quality ECM seedlings for better plant growth and survival in plantations and reforestation efforts. For this to be effective, investigations into the selection of the most suitable inoculation method for a particular ECM fungus, host range and growth stimulation in nursery are required to maintain and rehabilitate these forests and also to establish sustainable timber production.

CHAPTER III

MATERIALS AND METHODS

3.1 Chemicals used in this study as following;

- Peptone (Difco Laboratories, U.S.A.)
- Sodium hydroxide (NaOH) (Merck KGaA, Germany)
- Ammonium hydrogen phosphate $(NH_4)_2HPO_4$ (Merck KGaA, Germany)
- Potassium dihydrogen phosphate (KH_2PO_4) (Merck KGaA, Germany)
- Trizma base, minimum 99.9% titration (Sigma-Aldrich Co., Inc., Singapore)
- Ethylenediaminetetraacetic Acid (EDTA) (Sigma-Aldrich Co., Inc., Singapore)
- Hydrogen peroxide 30% (Merck KGaA, Germany)
- Ethanol absolute, Analytical grade, ACS. (Scharlau Chemie S.A., Spain)
- Xylene (Mallinckodt Chemical, U.S.A.)
- 100 bp Ladder Sharp DNA Marker (Fermentas International Inc., Canada)

3.2 Instruments used in this study as following:

- Incubator (Model 800, Memmert GmbH and Co. KG., Western Germany)
- Incubator shaker (Model SK-737, Amerex Instruments, Inc., USA)
- Autoclave (Model Autoclave ES-315, Tomy Seiko Co., Ltd., Tokyo, Japan)
- Hot air oven (Model UC 30, Memmert GmbH and Co. KG., Western Germany)
- Spectrophotometer (Genesys 20 Model 4001/4, ThermoSpectronic, Rochester., New York, USA)
- Laminar flow 'clean' (Model V6, Lab Service Ltd., Part)
- Cold room (Model Compakt 880(B)H, Foster Refrigerator (U.K) Ltd., U.K)

- 4-Digit precision weighting balance (Model AG 204, Mettler Toledo, Switzerland)
- Microwave (Model 000502174, Thai Cityelectric Co. Ltd.,Thailand)
- Hot plate stirrer (Model C-MAG HS 10, Becthai Bangkok Equipment & Chemical Co., Ltd., Thailand)
- Vortex mixer (Model G-560E, Scientific Industries, Inc., Bohemia. N.Y., 11716, USA)
- Water bath (Model WB14, Becthai Bangkok Equipment & Chemical Co., Ltd., Thailand)
- pH meter (Mettler-Toledo International Inc., New York, U.S.A.)
- Gel Documentation system (Bio-Rad Laboratories Gel Doc ^{TM} XR, California, U.S.A.)
- Electrophoresis chamber set (Mupid-ex, Bruker BioSpin Inc., Fällanden, Switzerland)
- High Speed Refrigerated Centrifuge (Beckman Coulter $_{TM}$ Avanti J-30I, Palo Alto, California, U.S.A.)
- Microscope (Model CH 30RF200, Olympus Optical Co., Ltd., Japan)
- Kubota Refrigerated Microcentrifuge 6500 (Kubota Corporation, Tokyo, Japan)
- UV-VIS spectrometer model V-530 (PC) (PerkinElmer instruments Lambda 25, Massachusetts, U.S.A.)
- DNA thermo cycler TP 600 (TaKaRa Bio Inc., Otsu, Shiga, Japan)

3.3. Description of study sites

Study site A was located in a natural dry dipterocarp forest on a low hill of tambon Lhainan at Veingsa district in Nan province, northern Thailand (18° 34' N, 100° 46'E). The forest has an overstorey dominated by *Dipterocarpus intricatus* Dyer, *Dipterocarpus obtusifolius* Teijsm ex Miq., *Dipterocarpus tuberculatus* Roxb, *Shorea obtusa* Wall.ex Blume and *Shorea siamensis* Miq,. Grasses, *Vietnamosasa* spp., members of Zingiberaceae and terrestrial orchids are also common understorey components in the forest. Soils at the site are infertile fine sand mix with laterite. This type of forest has an open canopy and is composed of small to medium-size xeric species. Most of the tree species shed their leaves during November and April which is dry season. Vegetative ground were grasses became dried and died (Fig.3.1A) However, dipterocarps young leaves sprout at the beginning of the rainy season between May and October. The forest turned to be green and under story covered with green grasses (Fig. 3.1B). Forest fire occurred in the study site 4 weeks before sampling date in dry season of 2006. In burned forest areas most of the canopy were changed drastically by drying out branches and whole crown of seedlings. The understory species were completely destroyed (Fig. 3.1C). However, forest fires have been of low intensity, with a considerable all of the dipterocarp trees in experimental site which survived and formed new leaves and produced sprouts in rainy season of 2006. The understory covered with green grasses and portion of seedlings survived (Fig. 3.1D). The forest fires occured naturally in dipterocarp forest during December to March. The site receives annual precipitation of approximately 5-10mm in dry season and 150-200mm in rainy season with annual temperature that 30-34°C in dry season and 28-31 in rainy season.

Fig. 3.1 A natural dry dipterocarp forest on a low hill of tambon Lhainan at Veingsa district in Nan Province, Thailand. Study site in dry (A) and rainy season (B) of 2005 and study site in dry (C) and rainy season (D) of 2006.

Study site B was a 12-year-old *Dipterocarpus alatus* Roxb. plantation located at Khao Hin Sorn Royal Development Center in Chachoengsao province, eastern Thailand (13º44', 101º30' E). The plantation understorey consists mostly of Malasian grasses, growing on infertile sandy soil (Fig. 3.2).

Fig. 3.2 A *Dipterocarpus alatus* plantation at Khao Hin Sorn Royal Development Center, Chachoengsao province. Study site in dry (A) and rainy season of 2005 (B) and study site in dry (C) and rainy season (D) of 2006.

3.4 Sampling plots and sampling of soil blocks

Eight square plots (1x1 m) were randomly assigned in area about 6,300 m^2 and 1,200 m^2 at the study site A and B, respectively (Fig 3.3). In the both study site, four plots were firstly made during dry season (January) and remaining four plots were made during wet season (September) in 2005. The plots in the both site were divided into 16 blocks (25x25 cm). Within each plot, four soil blocks (10x10x10 cm) were randomly taken and placed in plastic bags (Fig. 3.4-3.5). All soil samples were stored at 4 ^{O}C for further study.

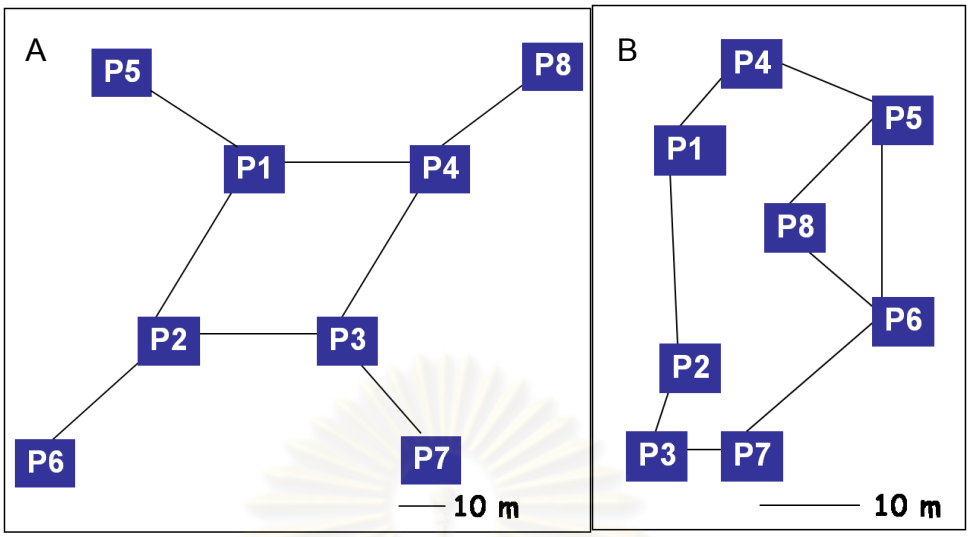


Fig. 3.3 The aspect of eight plots at the study site A (A) and study site B (B).

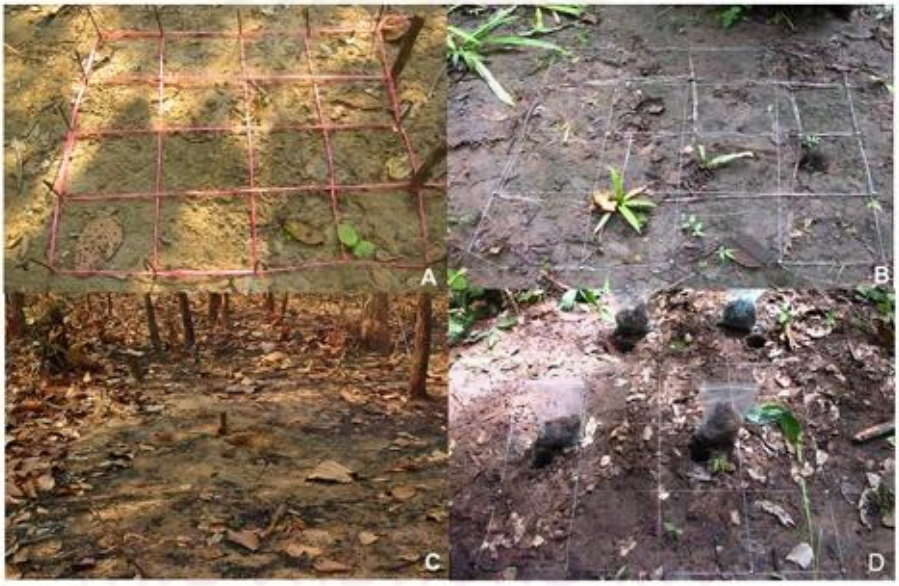


Fig. 3.4 Plots were constructed at a dry dipterocarp forest. A and B showing plot in dry and rainy season of 2005. C and D showing plot in dry and rainy season of 2006.

Fig. 3.5 Plots were constructed at a *Dipterocarpus alatus* plantation (A) and soil samples were randomly collected (B).

3.4.1 Analysis of physical soil properties

All soil samples collected from the both study sites in wet season during May and October and dry season during November and April between 2005 and 2006 were determined soil properties including moisture content, organic matter and soil pH as described in Pepper et al., 1995 (Appendix B).

3.5 Sampling of sporocarps and ectomycorrhizal root tips from soil blocks

Sporocarps of putative ectomycorrhizal fungi were collected from each study site. Fresh sporocarps of each fungus were gently brushed free of soil, photographed and characterized macroscopic and microscopic morphology. All sporocarps were divided into two portions. One portion of collected sporocarps were dried in silica gel for voucher specimens and deposited in herbarium at Department of Microbiology, Faculty of Science, Chulalongkorn Universitty. For remaining portion of sporocarps, several tissue pieces of stipe or central part of the cap were excised from each sporocarp and dried by a sealed polyethylene bag with silica gel. The collected sporocarps were used as reference patterns for molecular identification to accurately identify the underground ectomycorrhizal fungal species at the study sites by molecular analysis using ITS regions.

All roots contained in each soil block were carefully washed with running tap water on a set of sequential sieves, the finest of which has a 0.5 mm mesh and pooled

in a water-filled container. Roots of non target plants (i.e. grass) were excluded. The root tips were excised from the main roots as each tip and separated from the others. They were spread on a transparent plastic box under which a 5-mm mesh was placed. The individual root tips were selected up to 100 root tips from randomized grids on the mesh. The root tips were observed under a stereo dissecting microscope (Olympus SZ-PT) to determine the percentage of ectomycorrhizal root tips. Ectomycorrhizal root tips were sorted into morphotypes on the basis of surface colour, texture, emanating hyphae, rhizomorphs and ectomycorrhizal branching pattern. Ectomycorrhizal root tips of each morphotype were photographed and randomly picked 2-10 replicates from each morphotype in each sampling date. Individual root tips were placed in 2-ml tubes containing five zirconia ball (2 mm in diameter) for and dried by closing the loosely-capped microtubes in polyethylene bags with silica gel. Ectomycorhizal root tips were further used for the DNA extraction.

The ECM morphotype composition was expressed in terms of relative frequency and relative abundance. Relative frequency was defined as absolute frequency of individual species divided by the sum of absolute frequencies for all species. The absolute frequency was the number of samples in which a species occurs devided by the total number of samples (Gardes and bruns, 1996; Horton and bruns, 2001). The relative abundance was defined in the same way as the relative frequency.

3.6 Molecular identification of ectomycorrhizal fungi

3.6.1 DNA preparation and ITS-TRFLP analysis

Genomic DNA was prepared from dry small pieces of sporocarp tissue and dry ectomycorrhizal root tips with cetyltrimetylammonium bromide (CTAB) described in Nara *et al.* (2003). Briefly, each sample was pulverized in a 2.0-ml tube containing zirconia balls using a homogenizer (FastPrep, Funakoshi Co., Tokyo, Japan) for 30 s. After that, 500 µl of 2X CTAB solution [2% cetyltrimethylammonium bromide, 100 mM Tris-HCl (pH 8.0), 20 mM EDTA (pH 8.0), 1.4 M NaCl, 0.5% b-mercaptoethanol] was added to the tube and homogenized it again for 30 s. After incubation in a block heater at 65 ^{o}C for 1

h, 500 µl chloroform: isoamyl alcohol mixture (24 : 1) was added to the tube. After the tube had been vortexed and centrifuged (20,000 ***g***, 7 min, room temperature), the supernatant was transferred to another 1.5-ml tube. The DNA was precipitated by adding an equal volume of isopropyl alcohol and keeping the tube at - 20 °C for 15 min. After centrifugation (3,300 ***g***, 10 min, 4 °C), the DNA pellet was washed with ethanol (80%) and dried. The DNA pellet was dissolved in 100 µl TE buffer (10 mM Tris-HCl (pH 8.0), 1 mMEDTA), and stored at -20 °C until use.

PCR amplification was conducted using BioTaq™ DNA polymerase (Bioline, London, UK) following the manufacturer's instructions. For the ITS fragment length analysis (ITS 3–4), template DNA was amplified by PCR using primers ITS3 and ITS4 (White *et al* ., 1990; Gardes & Bruns, 1993) (Ta = 51 °C); primer ITS4 was labeled with Texas Red. PCR products were used for fragment size analysis. For a second ITS fragment length analysis (ITS 1F-*Hin*fI), template DNA was amplified by PCR using primers ITS1F and ITS4 (Ta = 51 °C); primer ITS1F was labeled with Texas Red. PCR products were checked on a 1.5% agarose gel run at 100V. Successful PCR products (2 µl) were digested with 8 µl *Hin*fI solution (Takara Shuzo Co., Shiga, Japan) at 37 °C for 12 h following the manufacturer's instructions. The fragments of ITS3-4 and ITS fragment digested with *Hin*fI were used for fragment length analysis using a DNA sequencer (SQ-5500E, Hitachi Electronics Engineering Co., Tokyo, Japan) after denaturation (95 °C, 5 min) with the loading dye (Zhou & Hogetsu, 2002). DNA size standards (Amersham Pharmacia Biotech UK Ltd, Buckinghamshire, UK) were loaded every ten lane on the gel. The length of each fragment was estimated from the size standards using FRAGLYS3.0 software (Hitachi Electronics Engineering Co., Japan). Those samples that shared the same pattern were considered the same TRFLP pattern.

3.6.2 Identification of underground ECM fungi

To identify the underground ECM fungal species, the length of ITS3-4 and ITS fragment digests with the enzyme *Hin*fI were used to differentiate morphotypes into different TRFLP patterns. TRFLP patterns of two to ten samples of each morphotype and

two or three individual sporocarps were determined. The TRFLP patterns were used to identify underground ECM fungi by matching TRFLP patterns produced from mycorrhizal root tips with those of identified ECM sporocarps collected from my study sites. If the lengths of ITS3-4 and ITS1F-*Hin*fI from a single ECM root tip were within the range of intraspecific variation of a given sporocarp species, I concluded that the fungal constituent on the ECM root tip belonged to that species. If each morphotype had different TRFLP patterns or no TRFLP match between a morphotype and sporocarp from the study sites was found, representative of each TRFLP pattern was used for ITS sequencing.

3.6.3 ITS sequence analysis

The sporocarps and the ectomycorrhizae could then be sequenced to confirm their conspecificity as well as their taxonomic affiliation. One or two root tips from each of the matched and unmatched ECM types were used for sequencing. The PCR products amplified using primers ITS1F and ITS4 were subcloned with Takara Cloning Kits (Takara Bio Inc., Japan) following the manufacturer's instructions. Plasmid DNA was extracted from transformed cells suspended in 50 µl of sterile water in a 1.5-ml tube that was incubated in boiling water for 5 min. The supernatant was PCR amplified with primers Texas Red M13F and M13R (Amersham International plc., Buckinghamshire, England). After confirming fragment insertion on agarose gels, we sequenced the insert using Thermo Sequenase Pre-mixed Cycle Sequencing Kits (Amersham International plc. Buckinghamshire, England) using primers Texas Red M13F and T7 (Amersham International plc.) following the manufacturer's instructions. The sequences obtained, including the complete ITS regions , which contains the 3' end of the 18S gene, the ITS1 spacer, the 5.8S gene, the ITS2 spacer and the 5' end of the 28S gene, were deposited in DNA Data Bank of Japan (DDBJ) and compared with the sequences of known species in the UNITE database (http://www.unite.zbi.ee/; Kõljalg *et al.*, 2005) using blastn. When close matches were unavailable, a blast homology search was conducted in DDBJ/ EMBL/GenBank database (http://www.ncbi.nlm.nih.gov/).

3.4.6 Statistical analysis

The abundance of the ECM fungal taxon in the sites were performed in PC-ORD, version 5.10 (McCune and Mefford, 2006). I measured the rate at which species accumulated in our sampling by constructed species-area curve. Estimates of the potential number of ECM species on the site were gained by obtaining first- and second-order jackknife estimates of species richness. The effect of soil parameters on underground ECM species composition was examined using Nonmetric multidimensional scaling (NMS) and Canonical correspondence analysis (CCA). Species abundances per sample were standardized by total abundance per sample. For NMS, Sorenson's (Bray-Curtis) distance measure was used to compare all samples using a maximum number of iterations of 400, an instability criterion of 0.00001, a starting number of 6 axes, 40 real runs and 50 randomized run. For CCA, soil data including pH, organic matter and moisture content were analyzed and Monte Carlo permutation tests (n=999) were performed to test the significance of the relationship between community data and the soil parameters.

3.7 Selection of ectomycorrhizal fungi on growth stimulation of seedlings

3.7.1 Pure culture isolation and inoculum production

To obtain pure culture isolates, sporocarps of putative ectomycorrhizal fungi associated with dipterocarp trees including *Astraeus odoratus* Phosri et al., *Astraeus asiaticus* Phosri et al. and *Pisolithus abditus* Kanch. et al. were collected from various locations in Thailand. Voucher specimens had been deposited at the Microbiology Herbarium, Chulalongkorn University, Thailand. Fresh and young sporocarps were excised and small pieces of inner tissue were placed on Petri dishes containing with

malt extract agar (MEA) for *Astraeus* spp. and modified Melin-Norkrans agar medium (MMN) for *P. abditus* and incubated at room temperature (30-35°C). The cultures obtained were subcultured every 2 months to fresh medium (Molina & Palmer 1982).

Fungal inocula of *A. ordoratus*, *A. asiaticus* and *P. abditus* were produced in 100 ml sterilized liquid MEA or MMN medium in 250 ml Erlenmeyer flasks. Each flask was inoculated with five mycelial plugs (8 mm diameter) of each ECM species from perimeter of an actively growing fungal culture (20 day old) and incubated at room temperature for 1 month without shaking. After 1 month, prior to inoculation, the mycelial culture were filtered through an 85 µm net and gently washed three times of sterile distilled water in order to eliminate nutrients and to reduce the growth of saprophytic contaminants in the seedling root systems. Afterwards, *ca*. 40 g of fresh mycelia were fragmented in a stirring blender in 500 ml sterile distilled water for approximately 10-15 s and the resulting suspensions (*ca*. 80 g mycelium L^{-1} were kept at 4 °C and used within 3 days. The mycelium suspension were tested for inoculum viability by placing 100 µl of them on solid MEA or MMN medium in Petri dish and incubating at room temperature for 7 days.

3.7.2 Ectomycorrhizal formation test in selected dipterocarp species and experimental design

Seeds of 7 dipterocarp species including *Shorea siamensis* Miq*, Shorea roxburghii* G.Don, *Shorea farinose* C.E.C. Fischer*, Dipterocarpus intricatus* Dyer, *Dipterocarpus obtusifolius* Teijsm ex Miq., *Dipterocarpus alatus* Roxb. ex G.Don and *Hopea odorata* Roxb. were obtained from natural forests in Khon Khan province, Thailand. The seeds were surface sterilized by 5% Sodium hypochlorite for 15-30 min (vary with dipterocarp species) and washed 3 times of sterile distilled water. Disinfected seeds were sown in plastic trays filled with wet autoclaved sand and placed in a greenhouse under ambient temperature for 1 month.

In 2005, 3 fungal isolates were tested in pure culture with 2 dipterocarp species seedlings and checked compatibility and inocula viability. From them, *Astraeus* spp. previously reported as mycorrhizal with *S. siamensis, D. intricatus* , *D. obtusifolius*

(Phosri, 2004) and *P. abditus* previously reported as mycorrhizal with *D. alatus* and *H. odorata* (Karnchanaprayudh, 2004). A total of five replicates were prepared for each host-fungus combination. The synthesis plastic boxes were maintained for 3 months. At the end of the growing period, seedlings were carefully removed from the boxes and the roots gently washed in tap water to clean off the substrate. Root systems were examined by stereomicroscopy to evaluate ectomycorrhizal short roots and checked microscopically for hartig net formation.

In 2006, the experiments were conducted in Chanthaburi province, Thailand. Seeds of 7 dipterocarp species were surface disinfected and sown in plastic trays filled with wet autoclaved sand and placed in a greenhouse under ambient temperature for 1 month. Then, dipterocarp seedlings were transplanted into 0.5-L polyethylene bags previously filled with non-sterile sand and garden soil (1:1, v/v). Each seedling was transplanted into a bag. After that, the three-month-old seedlings were transplanted into 1-L polyethylene pots and inoculation was performed during the sowing, using 10 ml ectomycorrhizal mycelial suspension (liquid inoculum) per pot. The viability of the mycelium in liquid inoculum was previously confirmed in solid MEA or MMN at room temperature. Control seedlings were not inoculated with any mycelial suspension. The seedlings were watered to field capacity daily with tap water and grown under 50% shade with a day-light of about 12 h. Low P fertilizer were supply every month (Appendix A4). Three months after inoculation, shades were removed. The seedlings exposed to sun light.

The experimental design is completely randomized block design with 4 treatments per one dipterocarp species (control, *A. asiaticus*, *A. odoratus* and *P. abditus*). There were three replicates (10 seedlings per a replicate) in each treatment.

3.7.3 Plant measurements and harvest

Eighteen months old seedlings were harvested. Height of 30 seedlings per treatment was measured from base of the seedlings at soil line to tip of terminal shoot bud. Diameter of all seedlings also measured. After that, 15 seedlings were randomly selected per treatment. Shoots then were cut at soil level. Before removing the roots,

each soil sample was soaked in tap water for 1 h. Subsequently, the roots were carefully washed free of soil under running tap water over a 85 μm mesh sieve. Roots were divided into fine and coarse > 0.5 mm diameter root fractions. A subsample 1.0-1.5 g of the fine root was taken by cutting the root tips from the main roots as each tip was separated from the others for the determination of ectomycorrhizal percentage infection by using gridline intersection method (Brundrett et al., 1996). ECM root tip were photographed under dissection microscope (Olympus XZ60) and mounted on glass slides stained in trypan blue for microscopic observation in detail. Morphology of morphotypes based on its surface colour, luster, branching, texture, rhizomorphs and emanating hyphae were investigated. Anatomy of morphotypes was studied on freshly prepared hand sections of hyphal mantle tissue and observed Hartig net using microscope (Olympys BX51). The remaining fractions of fine roots, coarse roots and shoots were oven-dried at 70° C for 72 h to obtain weight.

3.7.4 Statistics analysis

Statistical analysis was conducted with SPSS 11.5 for Windows. One-way ANOVA followed by Duncan's Multiple Range Test (DMRT) were used to compare the soil properties and percentage of ectomycorrhizal colonization among rainy and dry season with $P < 0.05$ as the level of significance. Using the same method, I also test selected of ectomycorrhizal fungi on growth stimulation of dipterocarp seedlings.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Community structure of ECM fungi on a dipterocarp forest and a *D. alatus* plantation in Thailand

The pattern correlation in ECM aboveground and underground communities and dynamic change in ECM root community compositions were observed in 2 study sites using morphological and molecular techniques between dry and rainy seasons of 2005 and 2006. The first site was a natural dry dipterocarp forest, Nan province and the second site was a *D. alatus* plantation, Chachoengsao province.

4.1.1 Analysis of soil properties

The soil organic matter, soil moisture content and soil pH were analyzed. In natural dipterocarp forest, organic matter at 2.45% and 2.30% in the rainy reason was significantly higher than 2.08% and 1.58% in the dry season of 2005 and 2006, respectively. Similarly, soil moisture content at 8.71% and 8.53% in the rainy season was significantly higher than 5.38% and 2.64% in the dry season of 2005 and 2006, respectively. Soil pH at 6.60 in the rainy season 2005 was significantly higher than 5.62 in the dry season 2005. However, soil pH was 6.68 and 6.14 in the rainy and dry season 2006, which was not significantly (Fig. 4.1A and Appendix E).

In *D. alatus* plantation, organic matter at 2.90% and 2.82% in the rainy reason was significantly higher than 2.38% and 2.46% in the dry season of 2005 and 2006, respectively. Similarly, soil moisture content at 10.67% and 10.02% in the rainy season was significantly higher than 6.60% and 6.48% in the dry season of 2005 and 2006, respectively. Soil pH at 6.84 and 6.89 in the rainy season were significantly higher than 6.22 and 6.23 in the dry season 2005 and 2006, respectively (Fig. 4.1B and Appendix E).

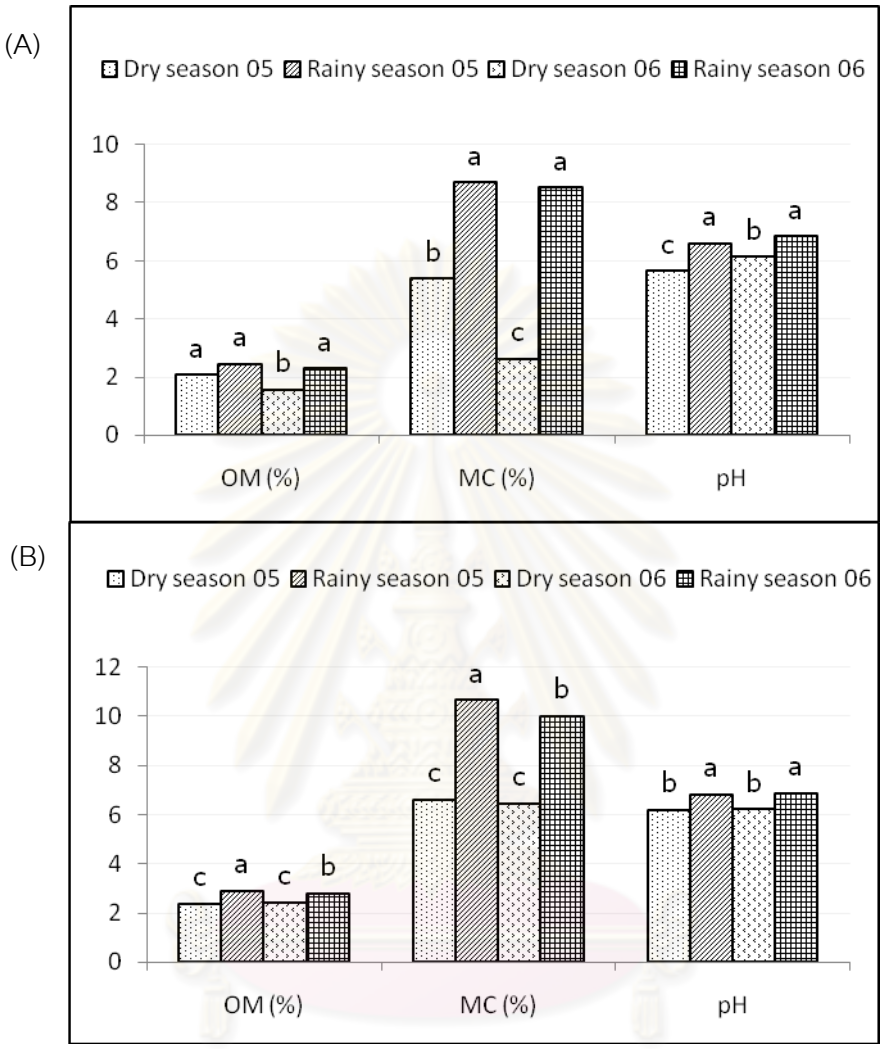


Fig. 4.1 Comparing soil organic matter (OM), soil moisture content (MC) and soil pH of dipterocarp forest (A) and *Dipterocarpus alatus* plantation (B) during dry and rainy seasons of 2005 and 2006. Different letters within a column indicate significant effect of season on soil property (Duncan test, $P<0.05$).

4.1.2 Analysis of aboverground and underground of ECM community

Aboveground sporocarps and underground ectomycorrhizas were collected and identified using morphological and molecular techniques. The sporocarps were

used as reference ITS T-RFLP patterns for ectomycorrhizas which were collected from the same study site.

4.1.2.1 Identification of the aboveground ECM sporocarps

In dipterocarp forest, a total of 30 putative ECM species were collected aboveground (Fig. 4.2) and T-RFLP analysis based on rDNA ITS region are shown in Table 4.1. Only 3 ECM species, *Astraeus asiaticus* Phosri et al., *Mycoamaranthus cambodgensis* (Pat) Trappe et al., and *Cantharellus atratus* Corner occurred in the plots. The ITS region of 30 ECM species were amplified and sequenced. Only *Cantharellus* sp. P1 was not successfully sequenced. The ITS sequences were submitted to DDBJ and compared with the sequences of known species in the UNITE/ DDBJ/ EMBL/GenBank database as shown in Table 4.1.

The putative ECM taxa were distributed among 12 families of basidiomycetes and were mostly belong to 11 species of Russulaceae, especially members of genus *Russula*. Members 4 species of the Amanitaceae and 4 species of Cantharellaceae were ranked a distant second and third. The collections of sporocarps recorded were assigned to 30 ECM different taxa of which 22 were identified to species level.

Fig. 4.2 Sporocarps of 30 aboveground ECM fungi were collected from dipterocarp forest in September 2005 and July 2006. A; *Amanita hemibapha* subsp. *javanica* Corner and Bas, B; *Amanita hemibapha* subsp. *hemibapha* (Berk. & Broome) Sacc., C; *Amanita vaginata* (Bull.) Lam., D; *Amanita pseudoporphyria* Hongo, E; *Lactarius volemus* (Fr.) Fr., F; *Lactarius* sp. Nan MN15, G; *Lactarius sp.* Nan M4, H; *Lactarius* sp. Nan MN22, I; *Lactarius piperatus* (L.) Pers, J; *Russula* sp. Nan MN5, K; *Russula* sp.Nan MN7, L; *Russula cyanoxantha* (Schaeff.) Fr., M; *Russula virescens* (Schaeff.) Fr., N; *Russula* cf. *delica* Fr., O; *Russula nigricans* (Bull.) Fr., P; *Laccaria vinaceoavellanea* Hongo, Q; *Hygrocybe* cf. *psittacina* (Schaeff.) P.Kumm., R; *Cantharellus* sp. P1, S; *Cantharellus cibarius* Fr., T; *Pterygellus polymorphus* Corner, U; *Cantharellus atratus* Corner, V; *Boletus* cf. *rufoaureus* Mass., W; *Xerocomus* sp.Nan-MN4, X; *Clavulina* cf. *cristata* (Holmsk.) J.

Schröt., Y; *Thelephora psudoterrestris* Ehrh., Z; *Astraeus asiaticus* Phosri et al., AA; *Scleroderma* sp., AB; *Scleroderma sinnamariense* Mont., AC; *Mycoamaranthus cambodgensis* (Pat.) Trappe. et al., *Thelephora* sp.2 is not shown in this figure.

Table 4.1 Sequence affinity of ECM sporocarps and corresponding terminal fragment lengths for the ITS1regions

ECM Taxa	ITS3-4 (bp)	ITS1F-Hinf I (bp)	Sequence affinity and accession number (BLAST)						
			Accession no.	UNITE closest species match	Overlap (bp)	Identity (%)	GenBank/DDBJ/EMBL closest species match	Overlap (bp)	Identity (%)
Amanita hemibapha subsp. *javanica*	334	325	AB458887	NM	-	-	[AB015697] *Amanita hemibapha*	519/571	87%
Amanita hemibapha subsp. *hemibapha*	340	344	AB458888	NM	-	-	[AB015699] *Amanita hemibapha*	337/362	90%
Amanita vaginata	346	321	AB458889	*[UDB001111] *Amanita vaginata*	-	-	[AJ889925] *Amanita vaginata*	521/589	88%
Amanita pseudoporphyria	349	-	AB458890	NM	-	-	[AB015702] *Amanita pseudoporphyria*	599/614	97%
Lactarius volemus	398	372	AB458891	[UDB002503] *Lactarius volemus*	542/597	90%	[AY606959] *Lactarius volemus*	599/660	90%
Lactarius sp. Nan MN15	425	342	AB458892	[UDB002503] *Lactarius volemus*	627/679	92%	[DQ422008] *Lactarius volemus*	627/679	92%
Lactarius sp. Nan M4	402	347	NA	NA	NA	NA	NA	NA	NA
Lactarius piperatus	435	355	AB458893	[UDB002495] *Lactarius piperatus*	665/699	95%	[DQ422035] *Lactarius piperatus*	665/699	95%
Russula sp. MN5	457	362	-	NM	NM	NM	[AY061690] *Russula melliolens*	375/423	88%
Russula sp.Nan MN7	444	350	AB458894	[UDB000010] *Russula puellaris*	376/425	88%	[DQ422013] *Russula lepida*	605/713	84%
Russula cyanoxantha	391	356	AB458895	[UDB001483] *Russula cyanoxantha*	542/571	94%	[DQ777996] *Russula sp.* FH H.ES04.F1.SY	622/666	93%
Lactarius sp. Nan MN22	403	367	AB458896	[UDB002495] *Lactarius piperatus*	221/240	92%	[AF354455] *Lactarius sp.* L-C	352/409	86%
Russula virescens	428	360	AB453021	[UDB000893] *Russula mustelina*	629/696	90%	[AF350058] *Russula sp. R25*	654/664	98%
Russula cf. *delica*	352	119	AB458685	[UDB001483] *Russula cyanoxantha*	516/558	92%	[AF350061] *Russula* sp. R28	515/529	97%

A dashed line (–) in place of a TRF indicates that the ITS fragment is unrestricted. NM indicates no close species match. NA indicates no analysis which sequence was not obtained.

* sequence locked by A. Taylor

Table 4.1 (continued) Sequence affinity of ECM sporocarps and corresponding terminal fragment lengths for the ITS1regions

		ITS1F-	Sequence affinity and accession number (BLAST)						
ECM Taxa	ITS3-4 (bp)	Hinf I (bp)	Accession no.	UNITE closest species match	Overlap (bp)	Identity (%)	GenBank/DDBJ/EMBL closest species match	Overlap (bp)	Identity (%)
Russula nigricans	417	-	AB458686	[UDB000059] Russula nigricans	426/468	91%	[AY061695] Russula nigricans	560/634	87%
Cantharellus sp. P1	431	374	-	NA	-	-	NA	-	-
Hygrocybe sp.	475	351	AB453022	[UDB000579] *Hygrocybe persistens*	151/159	94%	[EU435151] *Hygrocybe occidentalis*	346/411	84%
Laccaria vinaceoavellanea	389	403	AB453023	*Laccaria amethystina*	650/695	93%	[EU819478] *Laccaria laccata*	658/697	94%
Cantharellus cibarius	399	268	AB453024	NM	-	-	[EF546767] *Cantharellus cibarius*	302/350	86%
Pterygellus polymorphus	NA	NA	AB445116	[UDB002389] *Cantharellus tubaeformis*	194/201	96%	[AY082606] *Craterellus lutescens*	272/298	91%
Cantharellus atratus	NA	NA	AB445115	[UDB000053] *Craterellus cornucopioides*	276/294	93%	[DQ205680] *Craterellus cornucopioides*	322/355	90%
Boletus cf. *rufoaureus*	560	-	AB453025	[UDB002371] *Xerocomus cisalpinus*	241/260	92%	[EU526005] *Boletus subtomentosus*	527/653	80%
Xerocomus sp.Nan-MN4	540	-	AB453026	[UDB002371] *Xerocomus cisalpinus*	241/260	92%	[DQ533981] *Xerocomus chrysenteron*	505/627	80%
Clavulina cf. *cristata*	421	388	AB458886	[UDB001121] *Clavulina cristata*	195/204	95%	[EF634121] Uncultured ectomycorrhiza (*Clavulina*)	560/672	83%
Thelephora psudoterrestris	408	175	AB453027	[UDB002429] *Tomentella stuposa*	534/602	88%	[EF218831] Uncultured ectomycorrhiza (*Tomentella*)	611/678	90%

A dashed line (–) in place of a TRF indicates that the ITS fragment is unrestricted. NM indicates no close species match. NA indicates no analysis which sequence was not obtained.

Table 4.1 (continued) Sequence affinity of ECM sporocarps and corresponding terminal fragment lengths for the ITS1regions

		ITS1F-	Sequence affinity and accession number (BLAST)						
ECM Taxa	ITS 3-4 (bp)	Hinf I (bp)	Accession no.	UNITE closest species match	Overlap (bp)	Identity (%)	DDBJ/EMBL/ GenBank closest species match	Overlap (bp)	Identity (%)
Astraeus asiaticus	444	403	AB453028	NM	-	-	[EU718089] *Astraeus asiaticus*	731/740	98%
Scleroderma sp.	431	239	AB453029	[UDB000044] *Scleroderma verrucosum*	397/446	89%	[EU819438] *Scleroderma areolatum*	684/786	87%
Scleroderma sinnamarians	405	367	AB453030	[UDB000044] *Scleroderma verrucosum*	307/353	86%	[EU718126] *Scleroderma xanthochroum*	650/675	96%
Mycoamaranthus cambodgensis	422	243	AB453031	NM	-	-	[AY185183] *Boletus dryophilus*		81%
Thelephora sp.2	406	-	AB453032	[UDB000119] *Thelephora caryophyllea*	612/672	91%	[AJ889980] Thelephora caryophyllea	621/678	91%

Column ITS3-4 gives the lengths of the PCR products amplified by primer ITS3 and ITS4. Colunm ITS1F-Hinf1f lists the terminal (ITS1F side) restriction fragment lengths of the PCR products amplified by primer ITS1F and ITS4. Sequences, including the complete ITS regions of the species found underground, are available in the UNIT/DDBJ/EMBL/GenBank database and the accession number are listed. Their similarities with known species in close matches are shown. A dashed line (–) in place of a TRF indicates that the ITS fragment is unrestricted. NA indicates not analysis. NM indicates no close species match.

In *D. alatus* plantation, 9 ectomycorrhizal sporocarps occurred aboveground (Fig. 4.3) and TRFLP analyses based on ITS region are shown in Table 4.2. Three ECM species, *Russula siamensis* Yomyart et al., *Lactarius piperatus* (L.) Pers. and *Scleroderma columnare* Berk. and Broome were occured in the plots. The ITS region of all were successfully amplified and sequenced. The ITS sequences were submitted to DDBJ and compared with the sequences of known species in the UNITE/ DDBJ/ EMBL/GenBank database as showed in Table 4.2. The putative ECM taxa were distributed among 6 families of basidiomycetes and were mostly of Russulaceae. The collections of sporocarps recorded were assigned to nine ECM different taxa of which 7 were identified to species level.

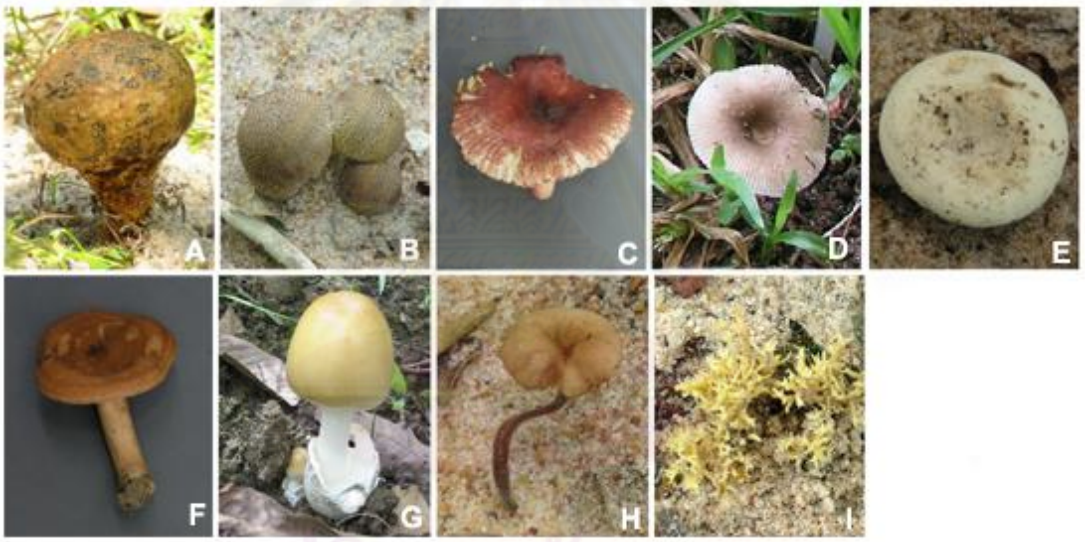


Fig. 4.3 Sporocarps of nine ectomycorrhizal fungi were collected aboveground in a *Dipterocarpus alatus* Roxb. plantation. A; *Pisolithus abditus* Karn. et al., B; *Scleroderma columnare* Berk. and Broome, C; *Russula* sp., D; *Russula siamensis* Yomyart et al, E; *Lactarius piperatus* (L.) Pers.; F; *Lactarius* sp., G; *Amanita hemibapha* (Berk. & Broome) Sacc., H; *Laccaria vinaceoavellanea* Hongo, I; *Clavulina* cf. *cristata* (Fr.) Schroet.

Table 4.2 Sequence affinity of ECM sporocarps and corresponding terminal fragment lengths for the ITS1regions

ECM Taxa	ITS3-4 (bp)	ITS1F-Hinf I (bp)	Sequence affinity and accession number (BLAST)						
			Accession no.	UNITE closest species match	Overlap (bp)	Identity (%)	GenBank/DDBJ/EMBL closest species match	Overlap (bp)	Identity (%)
Pisolithus abditus	405	-	AB459510	[UDB001206] *Pisolithus arhizus*	167/172	97	[AB099922] *Pisolithus abditus*	583/593	98
Scleroderma columnare	417	314	AB459512	[UDB001212] *Scleroderma areolatum*	231/249	92	[DQ146377] Uncultured ectomycorrhiza	627/630	99
Russula sp.	416	357	AB459514	[UDB000116] *Russula violeipes*	381/409	93	[EU019934] *Russula variispora*	468/501	93
Russula siamensis	429	376	AB459511	[UDB000343] *Russula amoenolens*	574/632	90	[AB206535] *Russula siamiense*	700/707	99
Lactarius piperatus	402	372	AB459515	[UDB002495] *Lactarius piperatus*	208/217	95	[DQ414726] Uncultured fungus EC2.4	732/740	98
Lactarius sp.	425	384	NA	NA	NA	NA	NA	NA	NA
Amanita hemibapha	334	325	-	NM	NM	NM	[AB015697] *Amanita hemibapha*	533/611	91
Laccaria vinaceoavellanea	390	396	AB459516	*Laccaria amethystina*	650/695	93	[DQ414726] Uncultured fungus JM600-01	695/739	94
Clavulina cf. *cristata*	420	380	AB459513	[UDB001121] *Clavulina cristata*	407/446	91	[DQ672317] Uncultured soil basidiomycete	650/724	87

Column ITS3-4 gives the lengths of the PCR products amplified by primer ITS3 and ITS4. Colunm ITS1F-Hinf1f lists the terminal (ITS1F side) restriction fragment lengths of the PCR products amplified by primer ITS1F and ITS4. Sequences, including the complete ITS regions of the species found underground, are available in the UNIT/DDBJ/EMBL/GenBank database and the accession number are listed. Their similarities with known species in close matches are shown. A dashed line (–) in place of a TRF indicates that the ITS fragment is unrestricted. NA indicates no analysis which sequence was not obtained.

During collecting trips to *D. alatus* plantations. One of ECM fungi was a species of *Russula* characterised by a distinctly annulate stipe. Its basic appearance was one of a thin-capped pileus with pectinate-striate margin, slender stature and isolated spines on the basidiospores (Fig. 4.4). These *Russula* was a new species of annulate *Russula siamensis* Yomyart et al. (Yomyart et al., 2006).

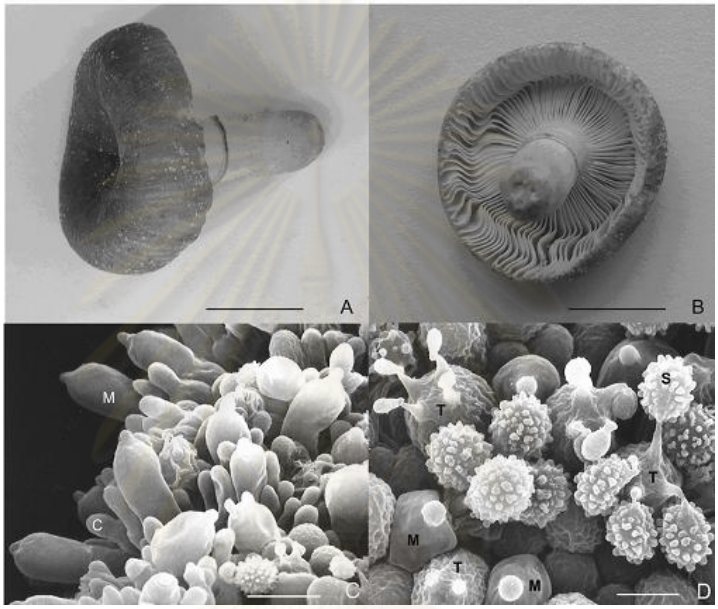


Fig. 4.4 *Russula siamensis* Yomyart et al., holotype. Fresh basidiome (A and B) . Scanning electron micrograph of cheilocystidia (c), macrocystidia (M) of *Russula siamensis* (C). High magnification of macrocystidia (M), basidiospore (S) and tetrasterigmatic basidia (T) with various stages of basidiospore formation of *Russula siamensis* (D). Note: smooth surface of papillate macrocystidia compared to wrinkled surface of basidia. Scale bars: A = 10 mm; B and C = 10 µm; D = 5 µm

The ECM fungi dominated in this study are similar to those of previous studies based on sporocarp surveys in dipterocarp forests and ECM taxa recorded. For instance, Chalermpongse (1987) surveyed ECM sporocarps in a dry-deciduous dipterocarp forest and a semi-evergreen dipterocarp forest in Thailand and reported 35 species belonging to the genera *Amanita*, *Boletus*, *Lactarius*, *Pisolithus*, *Russula* and *Tricholoma* fruiting over three years in the rainy season.

Watling et al. (2002) surveyed lowland dipterocarp forest in Malaysia. The records were distributed among 95 different taxa in 14 families of basidiomycetes and dominated with member of the Russulaceae. Members of Amanitaceae and Boletaceae were ranked a distant second and third, respectively. Sanmee et al. (2004) reported the dominant genera in the dry dipterocarp forests were *Russula*, *Boletus* whereas in the wet dipterocarp forests, *Amanita* was the main genus followed by *Lactarius* and *Russula*. Ogawa (2006) reported 34 species of ectomycorrhizal fungi from dipterocarp forest, among the mycorrhizal fungi the genera *Scleroderma*, *Laccaria*, *Russula*, *Amanita* and *Boletus* were dominant.

This suggests that Russulaceae and Amanitaceae dominate in the dipterocarp forest in southeast Asia region. A number of ECM species appeared in this study was quite low because sporocarp survey and sample collecting was not frequent. However, Smits (1994) suggested that the diversity of ECM fungi in dipterocarp forests is lower than in temperate forests dominated by Cortinariaceae and Russulaceae.

4.1.2.2 Identification of underground ectomycorrhiza

In dipterocarp forest, abundance and distribution of ECM fungi were determined by grouping the ECM root tips into 12 morphotypes (Fig.4.5).

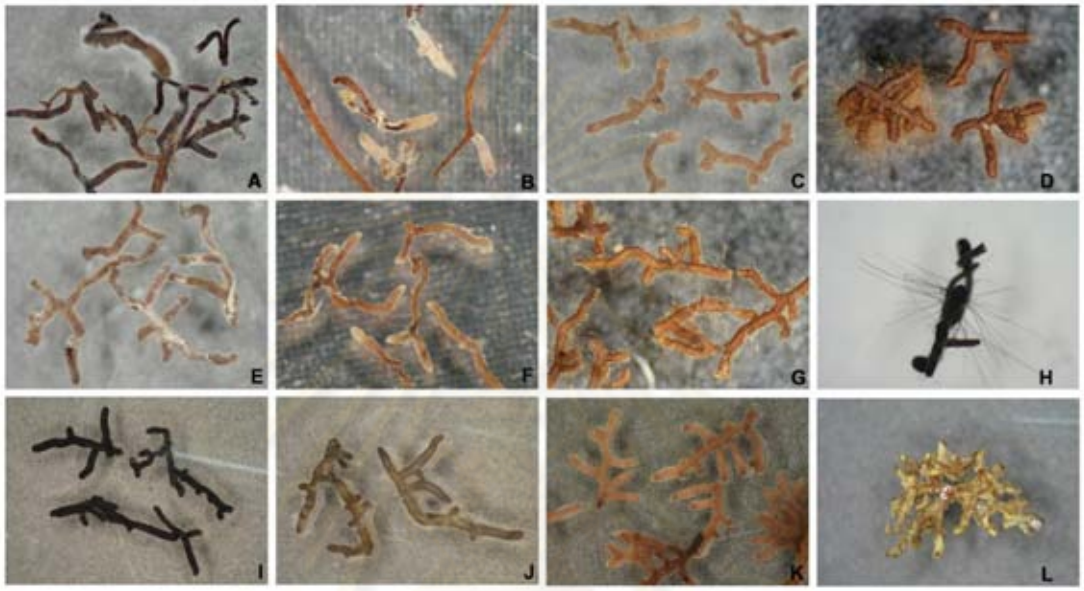


Fig. 4.5 Twelve morphotypes ectomycorrhizas formed on dipterocarps in September 2005 and July 2006. A; *Sebacina* sp., B; *Astraeus asiaticus* Phosri et al., C; *Russulaceae*, D; Thelephoraceae 1, E; *Lactarius* sp.1, F; *Tomentella* sp.1, G; *Tomentella* sp.2, H; *Cenococcum geophilum* Fr., I; Thelephoraceae 2, J; *Lactarius* sp.2, K; *Lactarius volemus* (Fr.) Fr., L; *Mycoamaranthus cambodgensis* (Pat.) Trappe et al..

Morpho-anatomy was described (Table 4.3). Of these,10 morphotypes were detected in the dry season whereas 12 morphotypes were detected in the rainy season (Fig. 4.5). The ECM colonization was 26.15% and 43.27% in dry season and 43.74% and 49.03% in the rainy season 2005 and 2006, respectively.

Table 4.3 Morpho-anatomy describtion of 12 ECM taxa.

ECM Taxa	Morpho-anatomy description
A; *Sebacina* sp.	Irregularly pinnate, dark brown and emanating woolly numerous hyphae
B; *Astraeus asiaticus* Phosri et al.	Simple, whitish and emanating cottony hyphal bundles
C; *Russulaceae*	Irregularly pinnate, beige, smooth, sparse single hyphae
D; Thelephoraceae 1	Monopodial-pinnate, golden brown and emanating woolly numerous hyphae
E; *Lactarius* sp.1	Irregularly pinnate, whitish light brown, smooth
F; *Tomentella* sp.1	Irregularly pinnate, brown, smooth, sparse single hyphae
G; *Tomentella* sp.2	Irregularly pinnate, light brown and emanating woolly numerous hyphae
H; *Cenococcum geophilum* Fr.	Simple, black, hairy, single thick dark hyphae
I; Thelephoraceae 2	Monopodial-pinnate, black and smooth mantle
J; *Lactarius* sp.2	Monopodial mycorrhizal system, green-brown mycorrhiza and smooth mantle
K; *Lactarius volemus* (Fr.) Fr.	Monopodial-pinnate, ochraceous to fleshy brown, smooth mantle
L; *Mycoamaranthus cambodgensis* (Pat.) Trappe et al	Closely packed mycorrhizal system, colloid mycorrhiza, yellow and emanating thin cottony hyphae

Table 4.4 Sequence affinity of ECM root tips and corresponding terminal fragment lengths for the ITS1 regions

ECM Taxa	ITS3-4 (bp)	ITS1F-Hinf I (bp)	Sequence affinity and accession number (BLAST)						
			Accession no.	UNITE closest species match	Overlap (bp)	Identity (%)	GenBank/DDBJ/EMBL closest species match	Overlap (bp)	Identity (%)
A; *Sebacina* sp.	386	327	AB453033	NM	-	-	[AF440664] *Sebacina* sp. MAS1	575/617	93%
B; *Astraeus asiaticus*†	444	403	AB453034	NM	-	-	[EU718089] *Astraeus asiaticus*	714/734	97%
C; *Russula foetens*	419	363*	AB453035	[UDB002424] *Russula foetens*	629/668	94%	[DQ422023] *Russula* cf. *foetens*	655/693	94%
D; Thelephoraceae 1	348	-	AB453036	[UDB000960] *Tomentella fuscocinerea*	517/585	88%	[AY940642] Uncultured ectomycorrhiza (Thelephoraceae)	538/611	88%
E; *Lacctarius* sp.1	385	363*	AB453037	[UDB002495] *Lactarius piperatus*	221/240	92%	[AY606985] *Lactarius rubroviolascens*	556/706	78%
F; *Tomentella* sp.1	405	354*	AB453038	[UDB000120] *Tomentella* sp.	491/532	92%	[EU668208] Uncultured *Tomentella*	610/673	90%
G; *Tomentella* sp.2	402	358	AB453039	[UDB001658 *Tomentella* sp.]	585/660	88%	[EU668208] Uncultured *Tomentella*	604/675	89%
H; *Cenococcum geophilum*	327	110*	AB453040	[UDB002301] *Cenococcum geophilum*	494/532	92%	[AM084698] *Cenococcum geophilum*	497/533	93%
I; Thelephoraceae 2	402	351	AB453041	[UDB000219] *Tomentella ellisii*	551/563	97%	[FJ013069] Uncultured Thelephoraceae	665/688	96%
J; *Lactarius* sp.2†	425	342	AB453042	[UDB002503] *Lactarius volemus*	627/679	92%	[DQ422008] *Lactarius volemus*	627/679	92%
K; *Lactarius volemus*	421	354*		[UDB000817] *Lactarius* sp.	440/487	90%	[AY606959] *Lactarius volemus*	607/696	87%
L; *Mycoamaranthus cambodgensis*†	423	243*	AB453043	[UDB001524] *Boletus pinophilus*	206/215	95%	[EU819456] *Boletus* cf. *chrysenteron*	502/620	80%

Column ITS3-4 gives the lengths of the PCR products amplified by primer ITS3 and ITS4. Colunm ITS1F-Hinf1f lists the terminal (ITS1F side) restriction fragment lengths of the PCR products amplified by primer ITS1F and ITS4. Sequences, including the complete ITS regions of the species found underground, are available in the UNIT/DDBJ/EMBL/GenBank database and the accession number are listed. Their similarities with known species in close matches are shown. A dashed line (–) in place of a TRF indicates that the ITS fragment is unrestricted. NM indicates no close species match. * indicates the other ITS fragment is unrestricted. † indicates species found in sporocarp

ECM of dipterocarp forest species –area curve relating the number of taxa observed to the number of soil samples (Fig. 4.6). A total of 12 ECM taxa were observed in dipterocarp roots. Estimates of the actual species richness were 12 for the first- and second jackknife estimate. Species –area curve showed that estimated 30 soil samples required to examine chance of finding all 12 morphotypes in ECM community. This suggested about 30 sampling soils enough to investigate ECM community in this study.

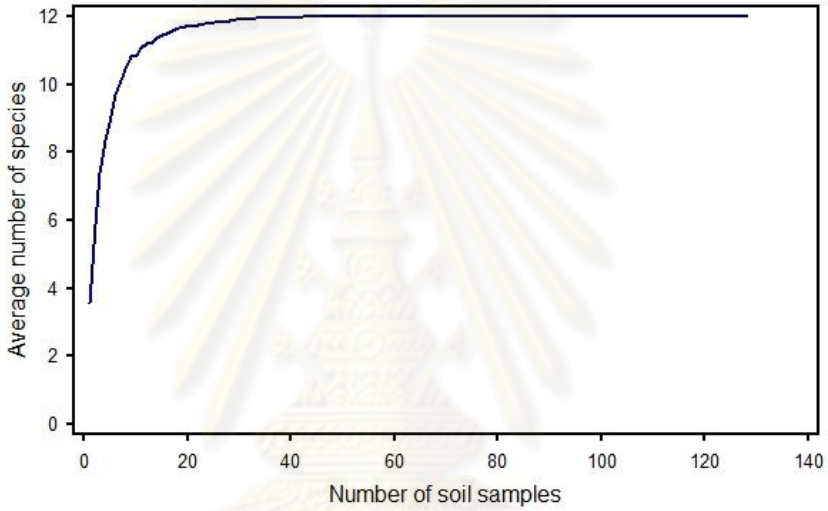


Fig. 4.6 Species –area curve showing increase in number of observed ECM fungal taxa with increased sampling effort in dipterocarp forest. Each soil sample consisted of 100 root tips.

Twelve morphotypes were described as resulting from a combination of TRFLP and ITS rDNA sequencing and morphotyping. Of these, 12 ECM taxa were observed and detected 18 distinct TRFLP patterns (Table 4.4); 3 of which had TRFLP patterns identical with those of aboveground ECM species, *A. asiaticus* designed as morphotype B, *Lactarius* sp. 2 designed as morphotype J and *M. cambodgensis* designed as morphotype M. Ectomycorrhizas were identified at the family, genus or species level after matching sequences either with those of sporocarps in the study site or with those available in UNITE/DDBJ/EMBL/GenBank DNA database using algorithm blastn. In all cases, sequence similarity was 78-97% between the sequences and their

closest known putative ECM sequences. Eleven ECM morphotypes were belonging to Basidiomycetes, four morphotypes were closely related to the family Thelephoraceae, four to the Russulaceae, two to the Sclerodermataceae, one to the Sebacinaceae and one to the Ascomycetes, *C. geophilum* (Table 4.4).

ITS T-RFLP may detected only one fragment terminal end labeled with Texas Red of one species. In this study , 18 distinct T-RFLP patterns were detected from 12 ECM morphotype. In general, only one species should detected in single root of a morphotype. In some case, two ITS fragments of different size were amplified or two ITS-T-RFLP fragments were detected, from DNA of individual ectomycorrhiza or sporocarps. Such intra-individual variation has also been described by Buscot et al. (2000). Because there are multiple rDNA copies in nuclei and two rDNA alleles in ECM fungal cells, variation at the individual level may reflect variation among rDNA copies or alleles. ITS-sequencing of 2 T-RFLP pattern in single root tip could be used to confirm species.

In *D. alatus* plantation, abundance and distribution of ECM fungi were determined by grouping the ECM root tips into morphotyping, in total 4 morphotypes were distinguished (Fig.4.7). Morpho-anatomy was described (Table 4.5). Of these, 4 morphotypes were detected in both dry and rainy season. Ectomycorrhizal colonization was 34.58% and 39.24 in dry season and 51.37% and 58.05% in the rainy season.

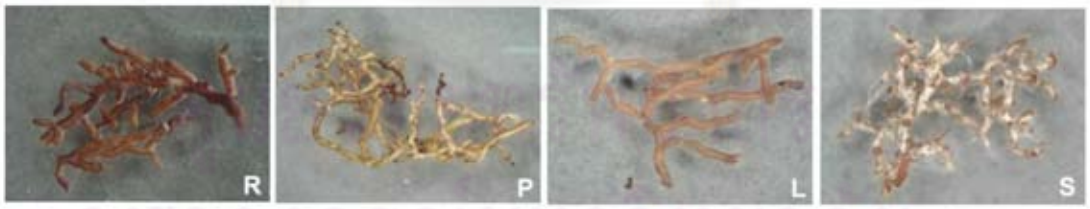


Fig. 4.7 Four morphotypes of ectomycorrhizas formed on *Dipterocarpus alatus* roots. R; *Russula siamensis* Yomyart et al , P; *Pisolithus abditus* Karn. et al., L; *Lactarius piperatus* (L.) Pers. and S; *Scleroderma columnare* Berk. and Broome.

Table 4.5 Morpho-anatomy describtion of 12 ECM taxa.

ECM Taxa	Morpho-anatomy description
R; *Russula siamensis* Yomyart et al.	Irregularly pinnate, brown and smooth
P; *Pisolithus abditus* Karn. et al.	Irregularly pinnate, yellow brown and emanating cottony hyphal bundles
L; *Lactarius piperatus* (L.) Pers.	Irregularly pinnate, beige, smooth
S; *Scleroderma columnare* Berk. and Broome	Irregularly pinnate, white and numerous emanating cottony hyphae

Ectomycorrhizas of *D. alatus* plantation species–area curve showed relating the number of taxa observed to the number of soil samples (Fig. 4.8). A total of 4 ECM taxa were observed on *D. alatus* roots. Estimates of the actual species richness were 4 for the first- and second jackknife estimate. Species –area curve showed that estimated 10 soil samples required to examined chance of finding all 4 morphotypes in ECM community. This suggested about 10 sampling soils enough to investigate ECM community in this study.

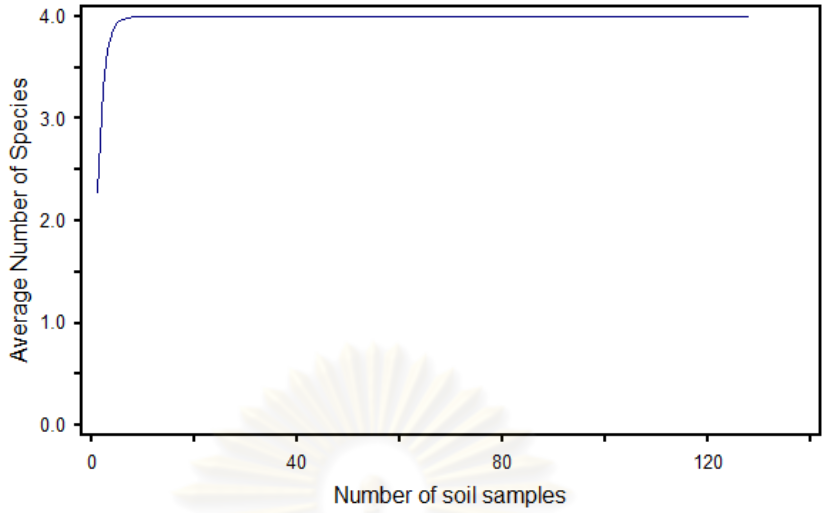


Fig. 4.8 Species –area curve showing increase in number of observed ECM fungal taxa with increased sampling effort in *Dipterocarpus alatus* plantation. Each soil sample consisted of 100 root tips.

Four ECM morphotypes of *D. alatus* roots were described resulting from a combination of T-RFLP patterns, ITS rDNA sequencing and morphotype. Of these, 4 ECM taxa were observed and detected 5 distinct T-RFLP pattern (Table 4.6); all of which had TRFLP patterns identical with those of aboveground ECM species, *R. siamensis* designed as morphotype R, *P. abditus* designed as morphotype P, *L. piperatus* designed as morphotype L and *S. columnare* designed as morphotype S. Ectomycorrhizal roots were also identified after matching sequences either with those available in UNITE/DDBJ/EMBL/GenBank DNA database using algorithm blastn. In all cases, sequence similarity was 79-99% between the sequences and their closest known putative ECM sequences.

ITS T-RFLP may detected only one fragment terminal end labeled with Texas Red of one species. In general, only one species was detected in single root of a morphotype. However, 3 of ten single root tip samples of morphotype S showed 2 TRFLP which were *S. columnare* and *R. siamensis.* Zhou and Hogetsu (2001) suggested that single root tip may be colonized by more than one ECM fungus.

Table 4.6 Sequence affinity of ECM root tips and corresponding terminal fragment lengths for the ITS1 regions

		ITS1F-	Sequence affinity and accession number (BLAST)						
Morphotype/ECM Taxa	ITS3-4 (bp)	Hinf I (bp)	Accession no.	UNITE closest species match	Overlap (bp)	Identity (%)	GenBank/DDBJ/EMBL closest species match	Overlap (bp)	Identity (%)
R; *Russula siamensis*	429	376	AB459518	[UDB000343] *Russula amoenolens*	574/632	90	[AB206535] *Russula siamiense*	616/632	97%
P; *Pisolithus abditus*	405	-	AB459517	[UDB001206] *Pisolithus arhizus*	167/172	97	[AB099922] *Pisolithus abditus*	648/650	99%
L; *Lactarius piperatus*	402	372	-	[UDB002495] *Lactarius piperatus*	208/217	95	[DQ414726] Uncultured fungus EC2.4	732/740	98%
S; *Scleroderma columnare*	417	314	AB459519	[UDB001212] *Scleroderma areolatum*	231/249	92	[DQ146377] Uncultured ectomycorrhiza	604/764	79%
Russula siamensis	429	376	-	[UDB000343] *Russula amoenolens*	574/632	90	[AB206535] *Russula siamiense*	616/632	97%

Column ITS3-4 gives the lengths of the PCR products amplified by primer ITS3 and ITS4. Colunm ITS1F-Hinf1f lists the terminal (ITS1F side) restriction fragment lengths of the PCR products amplified by primer ITS1F and ITS4. Sequences, including the complete ITS regions of the species found underground, are available in the UNIT/DDBJ/EMBL/GenBank database and the accession number are listed. Their similarities with known species in close matches are shown. A dashed line (–) in place of a TRF indicates that the ITS fragment is unrestricted.

This results indicated that members of family the Thelephoraceae and Russulaceae were abundance in underground late successional dipterocarp forest parallel to old-growth forests in other studies (Horton and Bruns ,2001; Lilleskov et al.,2002; Dickie et al. , 2002; Kennedy et al.,2003;Horton et al.,2005). The member of family Russulaceae were abundance in underground early successional *D. alatus* plantation. Nara et al. (2003) suggested underground community of vegetation development accompanied by the colonization of nonmushroom fungi. In contrast, species richness of underground ectomycorrhizas which formed sporocarp may be high in the early stages of primary succession.

However, the number of ECM fungal species in the underground ECM community in this study was less than previous studies. For instant, in temperate region, a total of 66 ECM taxa were distinguished on Scots pine (*Pinus sylvestris*), Norway spruce (*Pinus abies*) and birch (*Betula pubescens*). Twieg et al. (2007) reported 105 ECM fungal species in mixed temperate forests. Twenty three taxa were found only on Douglas-fir, 40 were found only on paper birch and 42 occurred on both hosts. Moreover, underground ECM community in this study was also less than previous studies in dipterocarp forests. For instance, 28 ECM species were found on *S. leprosula* roots (Lee et al., 1997) and 26 ectomycorrhizal morphotypes were found on *S. parvifolia* (Ingleby et al., 1998). By contrast, these result did not differ from previous study of ECM fungi on Dipterocarpaceae recorded in Thailand. Yuwa-Amornpitak et al. (2006) conducted 8 study sites in 4 sections of Thailand. They reported 1-10 ECM species from each site. In total, 33 ECM taxa from 8 study sites were observed.

Ectomycorrhizal species richness in this study which less than the others was discussed. First, the number of ECM fungal species in dipterocarp forest should be small compared with developed temperate forests as discussed in study of Smits (1994). Second, while soil sampling was only set up to test ECM community structure within plots. It is possible that their ectomycorrhizas where outside the plots in other part of the study site and that were not collected. Then, this samples were not representative of overall ECM diversity in the study site. Studies examining ECM communities of study site including additional soil core samples random direction

across the site and under ECM sporocarps were therefore necessary to assess the real impact of the ECM community.

4.1.2.3 Underground ECM fungi compared with ECM sporocarps

In dipterocarp forest, 6 species of non-mushroom such as *C. geophilum*, *Tomentella* (Thelephoraceae) and *Sebacina* sp. often dominated the underground ECM community whereas Russulaceae, Amanitaceae and Cantharellaceae dominated the aboveground. This result suggests poor correspondence between species composition of sporocarps and underground ECM community similar previous studies (Mehmann et al., 1995; Nylund et al., 1995; Gardes and bruns, 1996; Dahlberg et al., 1997; Jonsson et al., 2000 and Peter et al., 2001; Zhou and Hogetsu, 2002). Nara et al. (2003) suggested underground community of late successional forests accompanied by the colonization of nonmushroom fungi. Morover, I suggested that some ECM fungi that do not form obvious fruiting structures such as *Tomentella* or hypogeous fungi which were not collected and some ECM fungi may take longer than two years after initial colonization to produce sporocarps. In addition, given the small size or unhealthy nature condition of the dipterocarps, there may not have been sufficient carbon flow to mycorrhizas to sustain the formation of large sporocarps.

In *D. alathus* plantation, I found 9 aboveground sporocarps which more than 4 ECM fungal species in the underground ECM community. It was similar to the result of dipterocarp forest in this study. Moreover, this result contrast to previous studies that reported compositions of underground ECM fungal species were much more abundant than aboveground ones (Zhou and Hogetsu, 2002; Gebhardt et al., 2007).

All of ECM morphotypes had T-RFLP patterns identical with those of above-ground ECM species, *L. piperatus* designed as morphotype L*, R. siamensis* designed as morphotype R, *S. columnare* designed as morphotype S and *P. abditus* designed as morphotype P. The underground ECM fungal community at *D. alatus* plantation was dominated by the species whose sporocarps were recorded at this site. I recorded 9 ECM fungal species in sporocarp surveys in two years, and all the abundant

sporocarp species, including *R. siamensis, P. abditus, L. piperatus* and *S. columnare*, were also found in the underground community in this study. In contrast to 5 rare aboveground ECM species were not detected in the underground community. The correspondence between the sporocarp and underground ECM communities may also be explained by the favorable conditions for fruiting. The patterns in ECM abundance observed aboveground correlate significantly with patterns observed underground. In contrast with natural dry dipterocarp forest in this study, the patterns in ECM abundance observed aboveground did not correlate significantly with patterns observed underground which dominated by non-mushroom such as *Tomentela* spp., *Sebacina* sp. and *C. geophilum*. Nara et al. (2003) suggested that the correspondence between the sporocarp and ECM communities may be high in the early stages of primary succession and may decrease with late successional accompanied by the colonization of nonmushroom fungi.

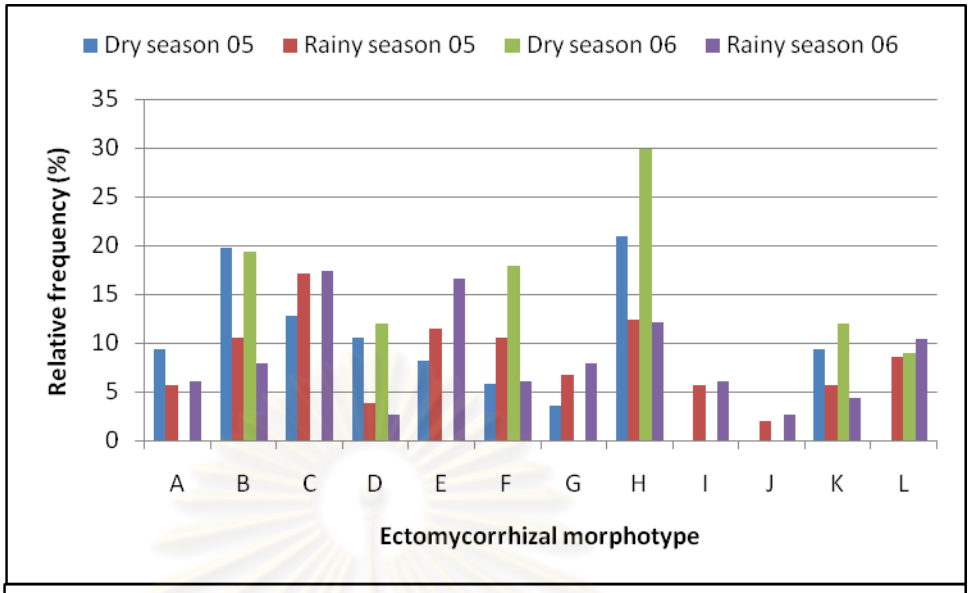
In both study sites, the number of ECM fungi aboveground was higher than the diversity of morphotypes underground. By contrast, previous studies reported compositions of underground ECM fungal species were much more abundant than aboveground ones. For instance, Zhou and Hogetsu (2002) detected 30 underground ECM fungal species and 8 ECM fungal species that developed sporocarps in *Larix kaempferi* forest. Gebhardt et al. (2007) reported 61 ECM fungi species by morphological/anatomical and molecular analysis of their ECM root tips and 15 ECM fungi species were identified as sporocarps.

It is suggested that that sampling strategies what were used in the present study were not ideal for assessing the overall structure of an underground ECM community as discussed in topic of underground species richness in this study that less than the others. However, this sampling strategies appropriated to analyze dynamic of ECM community.

4.1.2.4 Dynamic change of ECM community

In natural dry dipterocarp forest, the results of underground ECM relative frequency and relative abundance are shown in Fig. 4.9. The occurrence of individual morphotypes was related to seasons. Ten and 12 ECM taxa were observed on the roots of dipterocarps in dry and rainy season 2005 while 6 and 12 ECM taxa were observed on the roots of dipterocarps in dry and rainy seasons of 2006, respectively. *C. geophilum* was the most dominant species, with the highest relative frequency of 20.93% and 29.85% and the highest relative abundance of 22.05% and 23.82% in the dry season of 2005 and 2006, respectively but its relative frequency decreased to 12.38% and 12.17% and relative abundance decrease to 13.20 and 11.41% in the rainy season of 2005 and 2006, respectively. By contrast, in the rainy season, *Russulaceae* morphotype was the dominant, with the highest relative frequency of 17.15% and 17.39% and the highest relative abundance of 14.30 and 16.85% in the rainy season of 2005 and 2006, respectively. Its relative frequency and relative abundance decreased to 12.78 and 7.14%, respectively in the dry season of 2005. *Russulaceae* morphotype was not observed in the dry season of 2006.

(A)



(B)

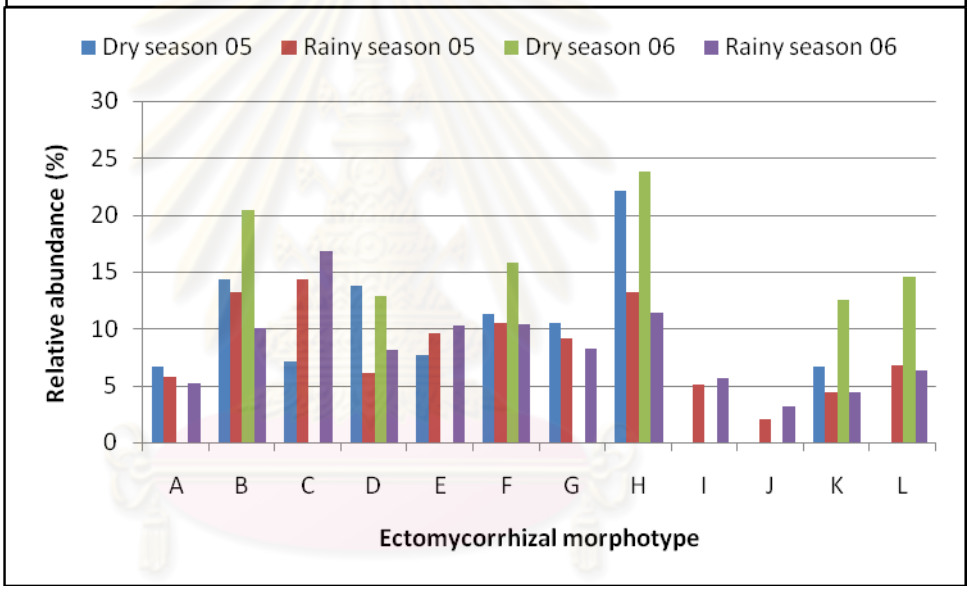


Fig. 4.9 Structure of ECM community expressed as species relative frequency (A) and relative abundance (B) in the dipterocarp forest in dry season and rainy season 2005 and 2006. A; *Sebacina* sp., B; *Astraeus asiaticus*, C; *Russulaceae*, D; Thelephoraceae 1, E; *Lacctarius* sp.1, F; *Tomentella* sp.1, G; *Tomentella* sp.2, H; *Cenococcum geophilum*, I; Thelephoraceae 2, J; *Lactarius* sp.2, K; *Lactarius volemus*, L; *Mycoamaranthus cambodgensis.*

The present studies showed that the distribution of the frequencies of the fungal species in this community was typical, with a few abundant species and

many species of lower abundance (Stendell et al., 1999; Grogan et al., 2000; Taylor, 2002). The ectomycorrhizas formed by *A. asiaticus* were the second in abundance and frequency after the *C. geophilum* morphotype and its distribution showed a clear seasonal pattern with dominant species in the dry season. *C. geophilum* was frequently one of few ECM fungal species in other communities (Karen and Nylund, 1996; Goodman and Trofymow, 1998; Baxter *et al*., 1999; Byrd *et al*., 2000; Dahlberg, 2001). Moreover, *C. geophilum* was found in many ecosystems during the dry period (Read and Haselwandter 1981; Vogt et al. 1981, Pigott 1982; Buee et al. 2005; Gebhardt *et al.* 2007). Its ECM remain metabolically active and even colonized new roots and formed mycorrhizas at low soil water potential, when other morphotypes stop functioning and begin to decline. In addition, *A. asiaticus* sporocarps were found early in the rainy season during a hot spell of weather. This result suggests *C. geophilum* and *A. asiaticus* were more drought resistant than those formed by other fungi and/or their formation was favoured by low water potentials.

In rainy season, ectomycorrhizas formed by *Russulaceae* was the most abundance and frequent. *C. geophilum* and *A. asiaticus* morphotype also were found abundantly. Moreover, Thelephoraceae 2 designed as morphotype I and *Lactarius* sp.2 designed as morphotype J were found only in the rainy season. The species structure of the ECM community of each morphotype change depending on the season and soil moisture. These results were similar to previous studies (Erland and Taylor, 2002; Shi et al., 2002). Buée et al. (2005) showed that season, temperature and soil moisture has been identified as influencing the ECM community and metabolic activity. A number of morphotypes were more abundant and active in winter than summer.

Unfortunately, forest fire occurred in the study site 4 weeks before sampling took place in the dry season of 2006. Therefore, it was found that fire affected the ECM community by reducing species richness and percentage colonization, inducing a shift in the relative abundance of each species and changing species composition. Several studies have also reported a decrease in the number of ECM tips and diversity after fire. For instance, Baar et al. (1999) found a shift in the species

composition of the ECM community of a *Pinus muricata* forest after fire. Dahlberg et al. (2001) reported not only a change in the species composition but also a decrease in the ECM diversity of several conifer forests affected by fire. In this study, 6 ECM species were observed in the dry season of 2006 whereas 10 ECM species were found in dry season, 2005. Indeed, 6 ECM species found were similar following fire to dry season, 2005.

Colonization of burned forest sites by ECM fungi has not been well studies and the main inocula source is still unknown. Colonization may occur from different sources of inocula such as resident mycelia that survived the fire or resident and dispersed spores (Visser, 1995). Horton et al. (1998) suggested that initial colonization of *P. muricata* by ECM fungi mainly occurred from resident mycelia and propagule. Mineral soil deeper than 5 cm probably did not experience excessive heating.

However, 6 months after the fire, the absent morphotypes in the dry season occurred again in the rainy season of 2006 and were similar to ECM taxa in 2005. It is likely that the former dominants *Russula* and *Lactarius* species, dominate again, maybe by new spore inoculum from outside burned plot or by mycelial expansion from surviving patches as above-mentioned in dry season. Many factors would influence the impact of fire on the ECM community, of which the intensity of fire could be important. After a low-intensity fire, many trees survive and organic matter remains intact. In this situation, the effects of the fire on the ECM community are probably small.

The result of the rainy season, 2006 suggests that the influence of fire on the ECM community in dipterocarp forest differs in several aspects from the effects reported in previous studies of ECM fungi in other forest ecosystems. Several studies based on sporocarp surveys and ectomycorrhizas taxa reported that fire significantly affected the ECM community by reducing populations and species richness and changing species composition by forest fires (Danielson, 1984; Parke et al., 1984; Visser, 1995; Baar et al., 1999; Grogan et al., 2000; Dahlberg et al., 2001). Fires in those studies were of high intensity and not only killed trees but also strongly altered ground vegetation and humus conditions.

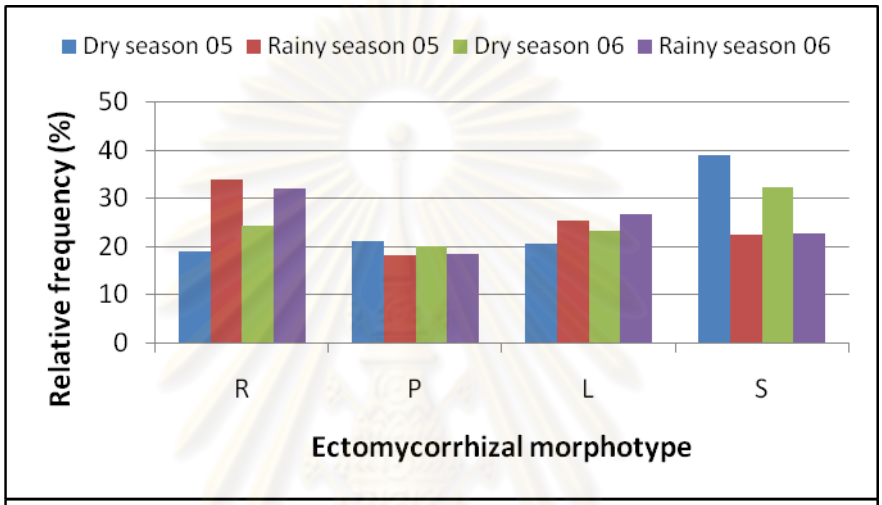
These results are similar to those of an ECM community in a boreal forest in Sweden. The number of ECM species recorded as ecyomycorrhizas /sporocarps were not changed following fire (Jonsson et al.,1999). Moreover, Román and Miguel (2005) reported that relative abundance and percentage of ECM root tips was significantly lower in burned than in control plots. Nevertheless, there were no significant differences in the diversity, species richness or species composition of the ECM community of Mediterranean ecosystems, where fire was a common hazard and low intensity as occurrence in dipterocarp forest. Perry et al. (1989) suggested that aboveground ecosystem recovery after fire was directly linked to the survival of ECM fungi. This results showed that the process of recovery of mycorrhizal fungi in dipterocarp forest much more rapid due to dipterocarps easily producing sprouts and no dead trees were found.

Moreover, sporocarps of ECM fungi were found within 6 months after the fire. By contrast, Baar et al. (1999) reported that no sporocarps of any mycorrhizal fungi was observed within the burned area until 15 months after the fire. They expected the former dominants species will dominate again and to be complete by 35 years.

In *D. alathus* plantation, relative frequency of underground ECM and relative abundance are shown in Fig. 4.10. The occurrence of individual morphotypes was related to the seasons. In the dry season, *S. columnare* was the most dominant species, with the highest relative frequency of 39.11% and 32.38% and the highest relative abundance of 39.65% and 40.07% in the dry season of 2005 and 2006, respectively but its relative frequency decreased to 22.43% and 22.85% and relative abundance decrease to 15.60 and 22.33% in the rainy season of 2005 and 2006, respectively. By contrast, in rainy season, *R. siamensis* morphotype was the dominant, with the highest relative frequency of 33.89% and 32.12% and the highest relative abundance of 39.18 and 35.27% in the rainy season of 2005 and 2006, respectively. Its relative frequency and relative abundance decreased to 19.02 and 24.36% and relative abundance decrease to 23.70 and 19.95% in the dry season of 2005 and 2006, respectively.

The species structure of the ECM community of each morphotype in the *D. alatus* plantation change depending on the season and soil moisture. This results were similarity to dipterocarp forest in this study and the other previous studies (Eeland and Taylor, 2002; Shi et al., 2002; Buée et al., 2005). However, a number of morphotypes was not different between dry and rainy season.

(A)



(B)

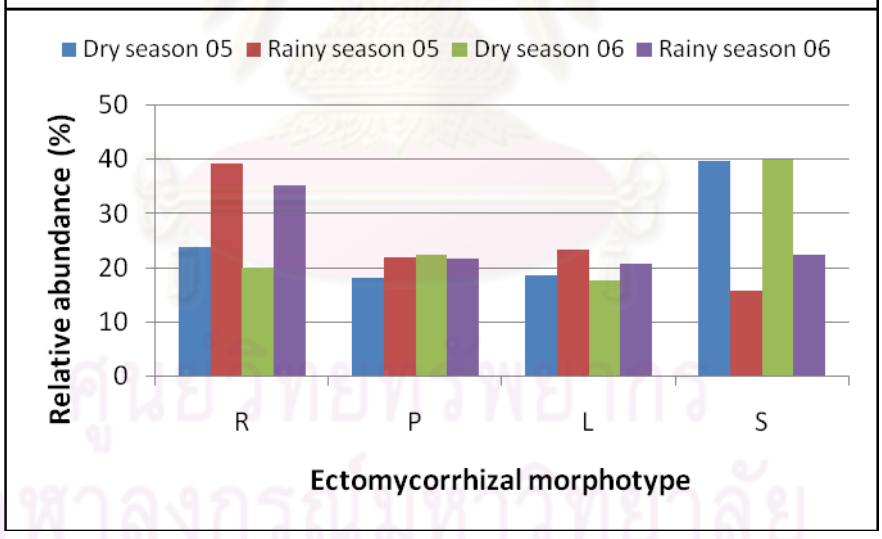


Fig. 4.10 Structure of ECM community expressed as species relative frequency (A) and relative abundance (B) in the dipterocarp forest in dry season and rainy season 2005 and 2006. R, *Russula siamensis*; P, *Pisolithus abditus*; L, *Lactarius piperatus*; S, *Scleroderma columnare*.

4.1.2.5 Statistical analysis of ECM communities

In dipterocarp forest, ordination of ECM communities were subjected to Nonmetric multidimentional scaling (NMS) and Canonical correspondence analysis (CCA) using PC-ORD software (McCune and Mefford, 2006). NMS ordination of the ECM fungi communities showed that soil parameters were highly significant correlated along the transect (Fig.4.11). In general, left cluster of ECM communities sampling from dry season segregate from those right cluster of rainy season along axis 1. Segregation patterns were also similar when data were ordinated separately.

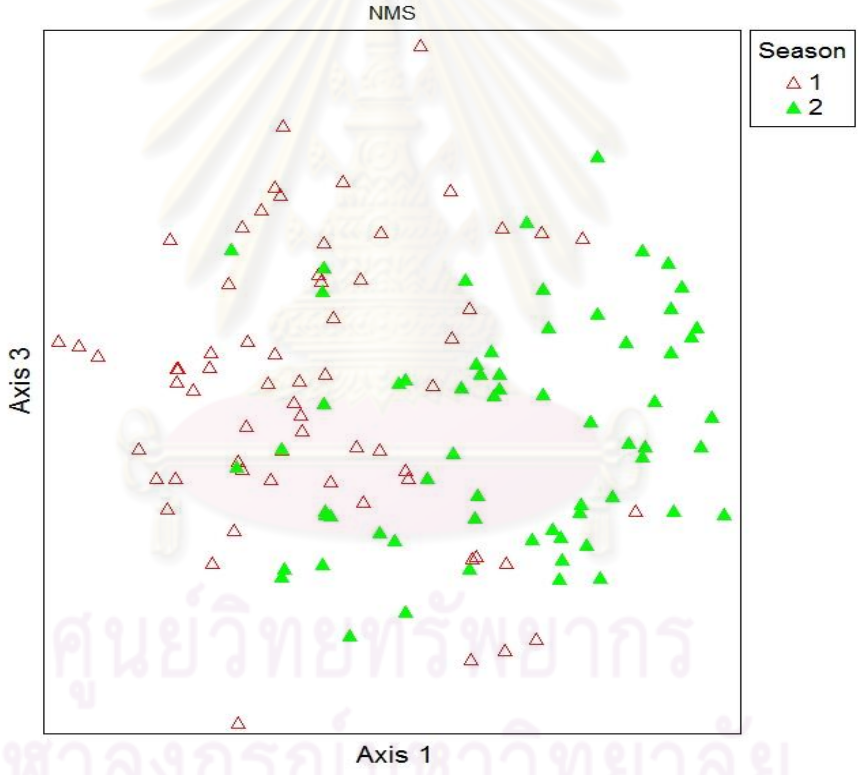


Fig. 4.11 Nonmetric multidimensional scaling (NMS) ordination of combined 2005 and 2006 ECM fungi colonizing roots in dipterocarp forest. The two axes with the highest R^2 values are shown. Open triangles, dry season; Closed triangles, rainy season. R^2 represent the proportion of variation in relative Sorensen distance among soil samples explained by the axes. Axis 1; $R^2 = 0.219$ and Axis 3; $R^2 = 0.226$.

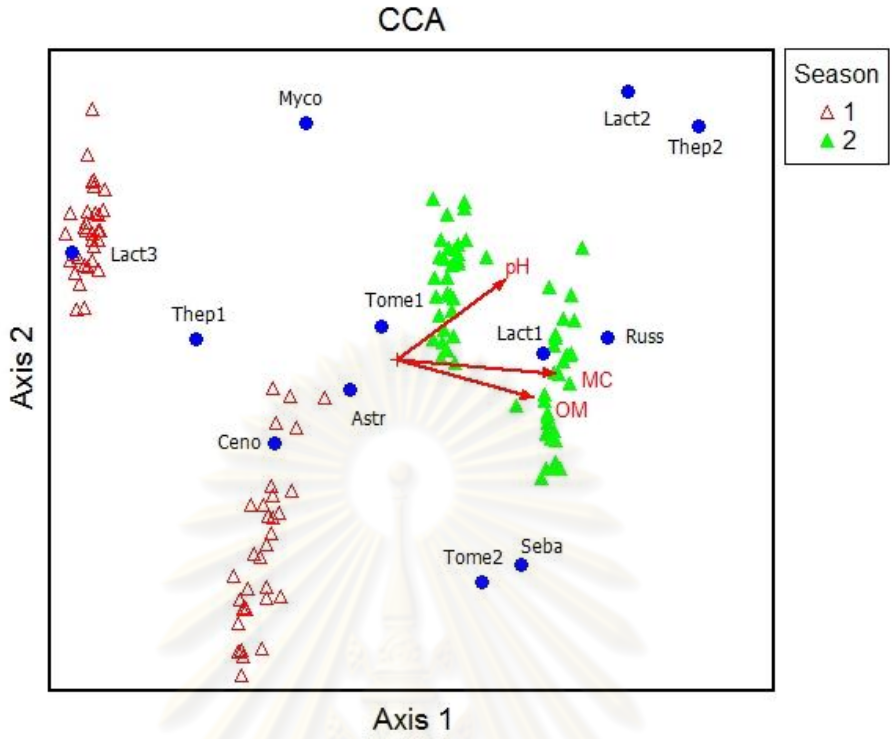


Fig.4.12 Canonical correspondence analysis (CCA) biplot of ECM community and soil parameters data. Lines represent important soil properties variables from those included and are labeled. Line length and direction from center indicate their relationship with ECM fungi. Seba: *Sebacina* sp., Astr: *Astraeus asiaticus*, Russ: *Russulaceae*, Thep1: Thelephoraceae 1, Lact1: *Lacctarius* sp.1, Tome1: *Tomentella* sp.1, Tome2: *Tomentella* sp.2, Ceno: *Cenococcum geophilum*, Thep2: Thelephoraceae 2, Lact: *Lactarius* sp.2, Lact3: *Lactarius volemus*, Myco: *Mycoamaranthus cambodgensis*.

In the CCA, the communities of ECM fungi in the 128 soil samples were successfully segregated into groups corresponding to the soil parameters distinguished on the transect (Fig.4.12). The CCA biplot graph showing moisture content explained the most variation and affected the ECM community. The total variance in the taxa data was 2.4829 and the eigenvalues of the first and second axes were 0.136 and 0.046, respectively. Using this data to determine variance explained by the first and second axes gave figures of 5.5% and 1.8%, respectively. The Monte Carlo permutation test showed that only the first axes significantly (P = 0.001) related to the soil parameters. However, strong autocorrelation among the soil parameters make it difficult

to determine the influence of the individual parameters in this combined analysis. Further CCA analysis were therefore carried out that examined the unique influence of each parameter on the community structure. These demonstrated that extractable moisture content individually explained the most variation of 5.4% followed by organic matter of 4.3% and pH of 3.4%. These parameters gave significant species-environment correlations (MC, r= 0.666, P=0.001; OM, r= 0.585,P=0.001; pH, r= 0.553, P=0.001) when analysis separated.

Analysis of the community data using CCA demonstrated that the composition and abundance of taxa were related to position on the transect (Fig. 4.12). The soil moisture content was the strongest determinant of the ECM. One group of species, including *C . geophilum*, *A. asiaticus*, *Tomentella* sp.1, Thelephoraceae 1, *M. cambodgensis* and *L. volemus*, showed a clear preference for the poor of moisture content of dry season. Whereas a second group, *Sebacina* sp., *Tomentella* sp.2, *Russulaceae*, *Lactarius* sp.1, Thelephoraceae 2 and *Lactarius* sp.2 were found in soil samples from higher moisture content of rainy season.

In *D. alatus* plantation, ordination of ECM communities were subjected to Nonmetric multidimentional scaling (NMS) and Canonical correspondence analysis (CCA) using PC-ORD software (McCune and Mefford, 2006). NMS ordination of the ECM fungi communities showed that soil parameters were strongly correlated along the transect (Fig.4.13). In general, lower cluster of ECM communities sampling from dry season segregate from those upper cluster of rainy season along axis 2. Segregation patterns were also similar when data were ordinated separately.

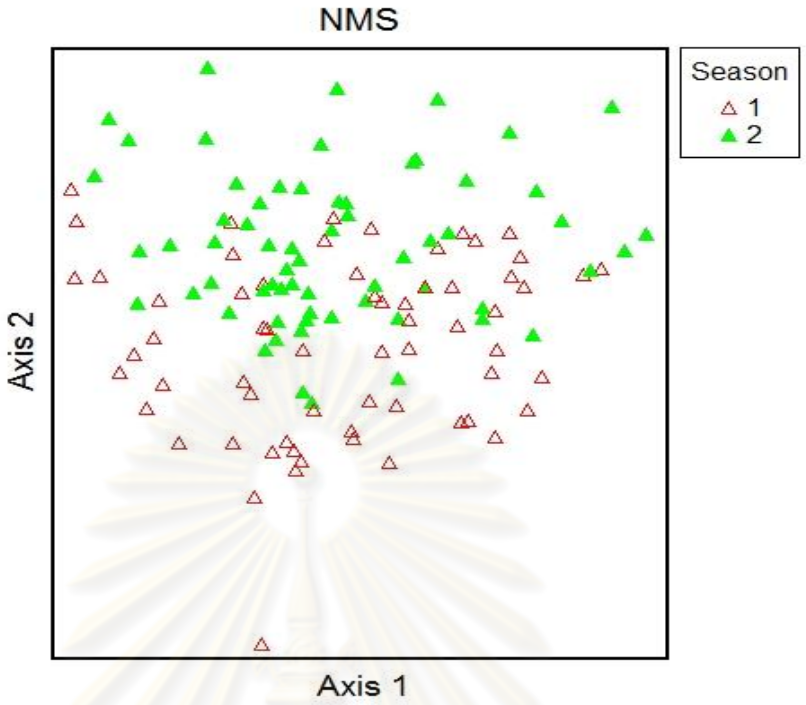


Fig. 4.13 Nonmetric multidimensional scaling (NMS) ordination of combined 2005 and 2006 ECM fungi colonizing roots in *Dipterocarpus alatus* plantation. The two axes with the highest R^2 values are shown. Open triangles 1; dry season and Closed triangles 2; rainy season. R^2 represent the proportion of variation in relative Sorensen distance among soil samples explained by the axes. Axis 1; $R^2 = 0.135$ and Axis 2; $R^2 = 0.199$.

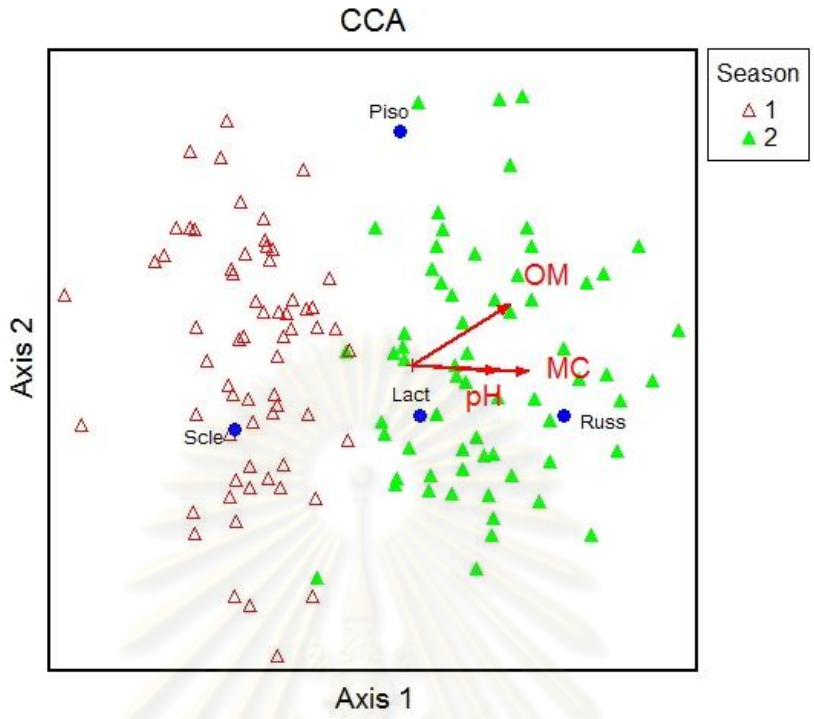


Fig.4.14 Canonical correspondence analysis (CCA) biplot of ECM community and soil parameters data. Lines represent important soil properties variables from those included and are labeled. Line length and direction from center indicate their relationship with ECM fungi. Open triangles 1; dry season and Closed triangles 2; rainy season. Piso; *Pisolithus abditus*, Scle; *Scleroderma columnare*, Lact; *Lactarius piperatus*, Russ; *Russula siamensis*.

In the CCA, the communities of ECM fungi in the 128 soil samples were successfully segregated into groups corresponding to the soil parameters distinguished on the transect (Fig.4.14). The CCA biplot graph showing moisture content explained the most variation and affected the ECM community. The total variance in the taxa data was 1.6 and the eigenvalues of the first and second axes were 0.022 and 0.014, respectively. Using this data to determine variance explained by the first and second axes gave figures of 1.6% and 1.0%, respectively. The Monte Carlo permutation test showed that only the first axes related to the soil parameters but not significantly (P = 0.6957). However, strong autocorrelation among the soil parameters make it difficult to determine the influence of the individual parameters in this combined analysis. Further

CCA analysis were therefore carried out that examined the unique influence of each parameter on the community structure. These demonstrated that extractable moisture content (MC) individually explained the most variation (1.4%) followed by organic matter (OM:1.2%) and pH (0.7%). These parameters gave significant species-environment correlations (MC, r= 0.191, P=0.2853; OM, r= 0.185,P=0.3403; pH, r= 0.140, P=0.5435) when analysis separately.

Analysis of the community data using CCA demonstrated that the composition and abundance of taxa were related to position on the transect (Fig. 4.14). *S. columnare* showed a clear preference for the poor of moisture content of the dry season. Whereas *R. siamensis* found in soil samples from higher moisture content of the rainy season.

Ordination analysis in dipterocarp forest and *D. alathus* plantation showed that community structure was significantly correlated with soil properties. This is similar to other ecosystems where abiotic factors such as soil moisture content, soil organic matter and soil pH effected ECM community structure. For instance, ECM community changed depending on season and soil moisture (Shi et al., 2002; Giachini et al., 2004; Izzo et al., 2004; Buée et al., 2005; Koide et al., 2006). Harvey et al. (1987) suggested that dynamic of ECM fungal community in pine forest may occur as a consequence of variation in soil organic matter content. Soil pH and calcium effected mycorrhizas of *Picea abies* (Lehto ,1994). Toljander et al. (2006) reported that NH_4, C/N ratio and pH effected ECM fungal community structure of a boreal forest. Scattolin et al. (2008) suggested that species composition of ECM fungal community in high mountain Norway spruce stands can be strongly influenced by pH.

4.1.3 Host effected on ECM fungal community: insight from natural dry mixed dipterocarps forest and *D. alatus* plantation

ECM diversity in natural dry dipterocarp forest is higher than in plantation both aboveground and underground. Four ECM fungi, *A. hemibapha*, *Clavulina* cf. *cristata*, *Laccaria vinaceoavellanea* and *L. piperatus* sporocarps were found in both sites with

different host. This result suggested that some ECM species were shared among coexisting host species and some ECM fungi were not shared. Such ECM fungal specificities to a certain host taxon may affect ECM fungal community structure. ECM species exhibit biased occurrence between host species belonging to different genera within the Pinaceae (Horton and Bruns,1998) and among host species from different families (Nara, 2006). The number of host species may affect the number of ECM species associated with an individual host species. The observed ECM richness was lower in the single host *D. alatus* plantation. By contrast, dipterocarp forest dominated with mixed dipterocarp species were associated with higher numbers of ECM species.

Another host effect on the ECM community is the time lapsed after the establishment of the host species. The ECM richness associated with young *D. alatus* plantation was lower than old-growth dipterocarp forest. The result is similar to previous studies by Ishida et al. (2006) who reported host effects on ECM fungal communities from eight host species in mixed conifer-broadleaf forests. Horton & Bruns (2001) showed that in Norway spruce (30 yr old), Bishop pine (35 yr old), Ponderosa pine (100 yr old), and Red fir (350–400 yr old) forests, 21, > 20, > 50, and 80 ECM fungal taxa were identified in underground ECM communities, respectively. They suggested the number of fungal species in early plantation sites should be small compared with developed forests.

4.2. Selection of ECM fungi on growth stimulation of dipterocarps seedlings

Seven dipterocarp species including *Shorea siamensis* Miq, *Shorea roxburghii* G.Don, *Shorea farinose* C.E.C. Fischer, *Dipterocarpus intricatus* Dyer, *Dipterocarpus obtusifolius* Teijsm ex Miq., *Dipterocarpus alatus* Roxb. ex G.Don and *Hopea odorata* Roxb. were inoculated with 3 ECM fungi, *Astraeus odoratus* Phosri et al., *Astraeus asiaticus* Phosri et al. and *Pisolithus abditus* Kanch. et al. and after 18 months under nursery condition, shoot height, shoot diameter, shoot dry weight and percentage of infection were measured.

4.2.1 Macroscopic examination of sporocarp of *A. odoratus*, *A. asiaticus* and *P. abditus*

Sporocarps of *A. odoratus*, *A. asiaticus* and *P. abditus* were examined (Fig. 4.15-4.27). Twelve isolates of *A. odoratus* and 2 isolates of *A. asiaticus* were collected from 8 dipterocarp forests and 5 isolates of *P. abditus* were collected from *D. alatus* plantations during April to June 2004-2006 (Table 4.7). On basis of macroscopic characters, *A. odoratus* sporocarps were globose or depressed globose at young stage, expanding to hard, smooth surface with few soil adhering particles and splitting to become star-shaped, basal mycelium consisting of brown vinaceous or bay rhizomorphs (Fig. 4.15). *A. asiaticus* sporocarps were globose or depressed globose, splitting to become star-shaped, covered with thin, white mycelial layer when unexpanded which tears away at maturity (Fig.4.16). *P. abditus* has yellowish-brown club-shaped sporocarp, peridium thin, smooth, often shiny, and slender brown stipe (Fig. 4.17).

Fig. 4.15 Sporocarps of *Astraeus odoratus* Phosri et al. were collected from Tak province. Unexpanded sporocarp develop under the ground (A). Young sporocarps of

Astraeus odoratus has smooth outer peridium (B). Unexpanded sporocarp of *Astraeus odoratus* showing purplish black gleba with smooth outer peridium (C). *Astraeus odoratus* at maturity stage showing expanded sporocarp (D). Scale bar = 1 cm.

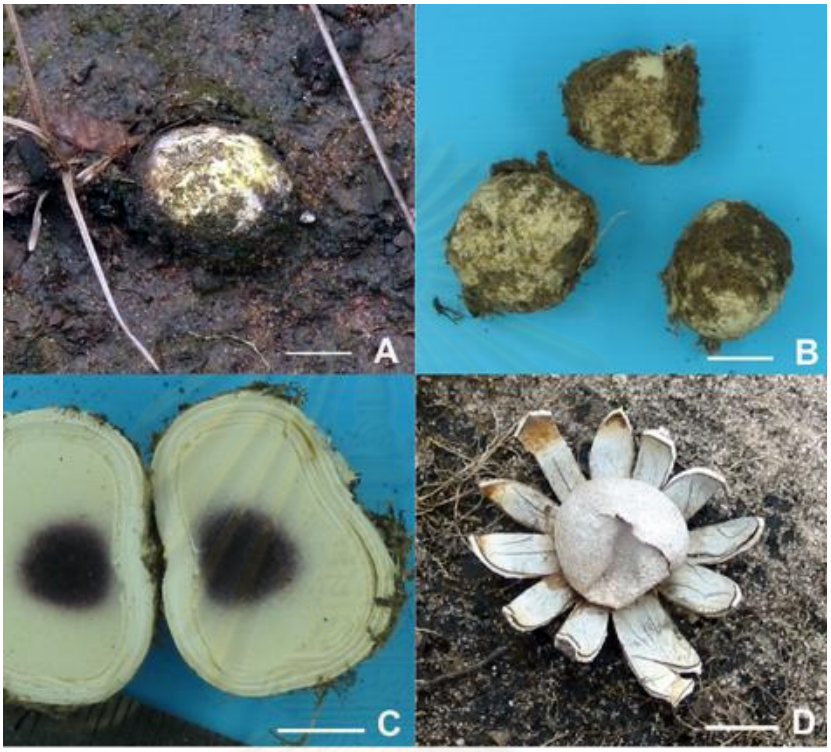


Fig. 4.16 Sporocarps of *Astraeus asiaticus* Phosri et al.. Unexpanded sporocarp develop under the ground (A). Young sporocarps of *Astraeus asiaticus* showing granulate outer peridium (B). Unexpanded sporocarp showing white and purplish chestnut gleba with granulate outer peridium (C). *Astraeus asiaticus* at maturity stage showing expanded sporocarp (D). Scale bar = 1 cm.

Fig. 4.17 Sporocarp of *Pisolithus abditus* Karn. et al.. Young sporocarp showing yellowish-brown club-shaped, peridium thin and smooth with slender brown stipe, deeply rooted base (A). Longitudinal section of basidiocarp showing dark-brown gleba, yellowish brown peridioles (arrow) developing in a black gelatinous matrix (B). Scale bar = 1 cm.

Table 4.7 Nineteen isolates of ectomycorrhizal fungi from various dipterocarp forest and *D. alatus* plantation

ECM fungal isolate	Habitat	Collection month
Astraeus odoratus Phosri et al. isolate 1	Natural dipterocarp forest, Chaiyaphum province	April 2004
Astraeus odoratus isolate 2	Natural dipterocarp forest, Lumpang provine	April 2004
Astraeus odoratus isolate 3	Natural dipterocarp forest, Chiang Mai province	May 2004
Astraeus odoratus isolate 4	Natural dipterocarp forest, Nan province	April 2004
Astraeus odoratus isolate 5	Natural dipterocarp forest, Tak province	April 2004
Astraeus odoratus isolate 6	Natural dipterocarp forest, Tak province	June 2005
Astraeus odoratus isolate 7	Natural dipterocarp forest, Karnchanaburi provine	May 2005
Astraeus odoratus isolate 8	Natural dipterocarp forest, Nan province	July 2005
Astraeus odoratus isolate 9	Natural dipterocarp forest, Lumphun provine	April 2006
Astraeus odoratus isolate 10	Natural dipterocarp forest, Unon Ratchathani provine	May 2006
Astraeus odoratus isolate 11	Natural dipterocarp forest, Tak province	June 2006
Astraeus odoratus isolate 12	Natural dipterocarp forest, Chaiyaphum province	June 2006
Astraeus asiaticus Phosri et al. isolate 1	Natural dipterocarp forest, Khon Khan provine	May 2005
Astraeus asiaticus isolate 2	Natural dipterocarp forest, Nan province	April 2006

Table 4.7 (continued) Nineteen isolates of ectomycorrhizal fungi from various dipterocarp forest and *D. alatus* plantation

ECM fungal isolate	Habitat	Collection month
Pisolithus abditus Karn. et al. isolate 1	*Dipterocarpus altus* plantation, Chachoengsao province	May 2004
Pisolithus abditus isolate 1	*Dipterocarpus altus* plantation, Chachoengsao province	July 2004
Pisolithus abditus isolate 2	*Dipterocarpus altus plantation,* Chachoengsao province	April 2005
Pisolithus abditus isolate 3	*Dipterocarpus altus plantation,* Chachoengsao province	May 2005
Pisolithus abditus isolate 4	*Dipterocarpus altus plantation,* Chachoengsao province	April 2006
Pisolithus abditus isolate 5	*Dipterocarpus altus plantation,* Chachoengsao province	June 2006

4.2.2 Scanning electron microscopy examination of basidiospores

Basidiospore ultrastructure of the *A. odoratus*, *A. asiaticus* and *P. abditus*. collected could be separated into three groups. Each group was defined by its type of basidiospore spine morphology and size of spores. *A. odoratus*, *A. asiaticus* and *P. abditus* was defined as “coalesced spines” had 3-4 spines joining at their tips to form a hollow triangle (Fig. 4.18).

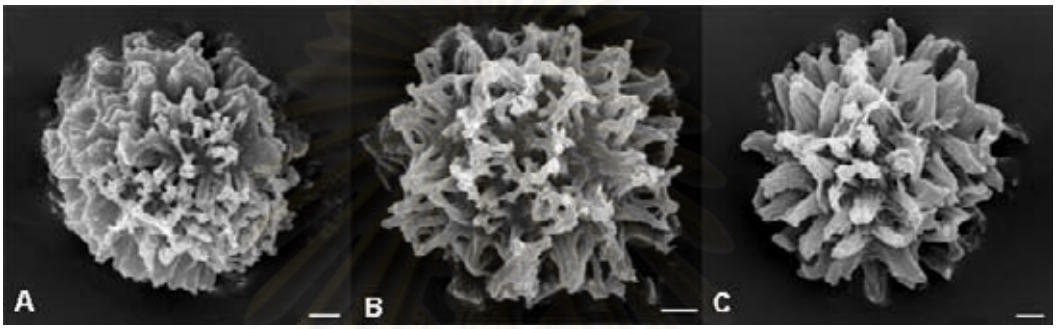


Fig 4.18 Scaning electron micrograph of spore with coalesced spines of *Astraeus asiaticus* Phosri et al. (A), *Astraeus odoratus* Phosri et al. (B) and *Pisolithus abditus* Karn. et al. (C). Scale bar = 1µm.

4.2.3 Pure culture isolation and inoculum production

Three species collected from 2 habitats of ECM fungal isolates used in experiments, were *Astraeus* spp. from dry mixed dipterocarp forest and *P. abditus* from evergreen *D. alatus* plantation. Twelve isolates of *A. odoratus* (Fig. 4.19 A-L), two isolates of *A. asiaticus* cultured on MEA and five isolates of *P. abditus* cultured on MMN agar (Fig. 4.19 O-S)showed different colour and rates of mycelial growth. Colour of MEA plates were changed to dark brown by *A. odoratus* and *A. asiaticus*. Colony of *A. odoratus* isolate No. 6 (Fig.4.19F), *A. asiaticus* isolate No. 2 (Fig.4.19N) and *P. abditus* isolate No. 2 (Fig.4.19P) had the most rapid growth on MEA and MMN media and were used for inocula production.

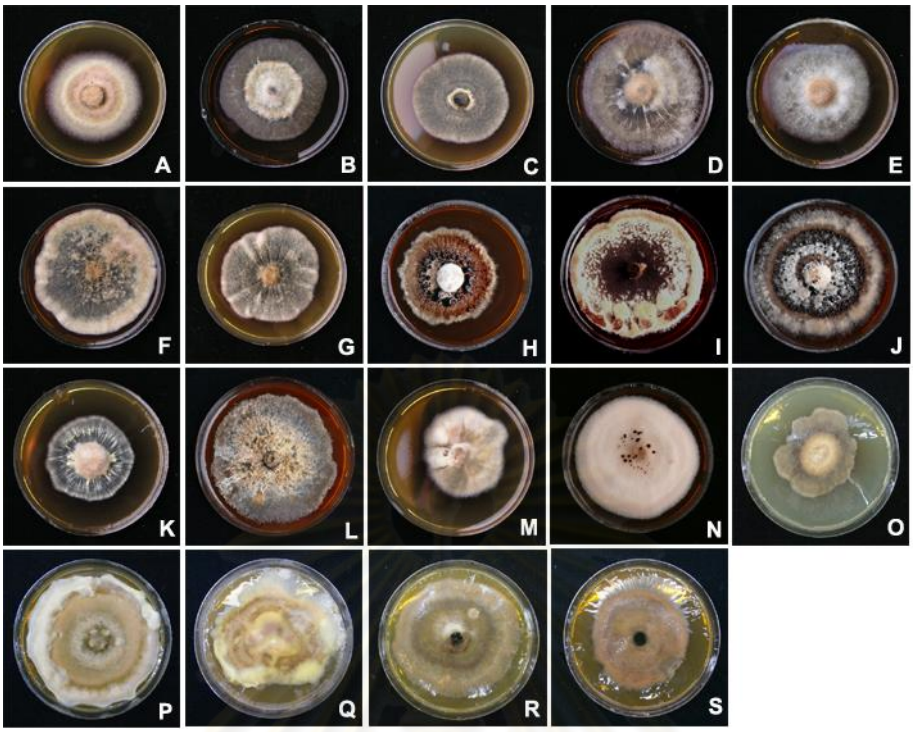


Fig. 4.17 Nineteen isolates of ectomycorrhizal fungi were cultivated for 30 days at room temperature. *Astraeus odoratus* Phosri et al. (A-L) and *Astraeus asiaticus* Phosri et al. (M,N), on MEA and *Pisolithus abditus* Karn. et al. (O-S) on MMN.

4.2.4 Ectomycorrhizal formation test in selected Dipterocarpaceae species and experimental design

4.2.4.1 Preliminary test of ectomycorrhiza formation

Three species of ECM fungi were tested in the rhizobox system for their abilities to initiate ectomycorrhiza with 2 dipterocarps species, *H. odorata and D. alatus*. Inoculation of mycelial slurries onto roots of *H. odorata* and *D. alatus* under greenhouse conditions, was effective for production of colonized seedlings with all three species of *A. odoratus*, *A. asiaticus* and *P. abditus* and tested within 3 months (Fig. 4.20 and Fig. 4.21).

Fig. 4.20 Root systems of *Hopea odorata* Roxb. associated with *Astraeus asiaticus* Phosri et al.. Three months old *Hopea odorata* seedling showing a number of ectomycorrhizas (arrow) (A). Enlarge portion of a root system showing well developed ectomycorrhizas (B). White brownish ectomycorrhizas and extraradical mycelia extending from root-fungi interface (C). Transverse section of *Hopea odorata* with a thick mantle (m) and Hartig net (hn) was detected (D).

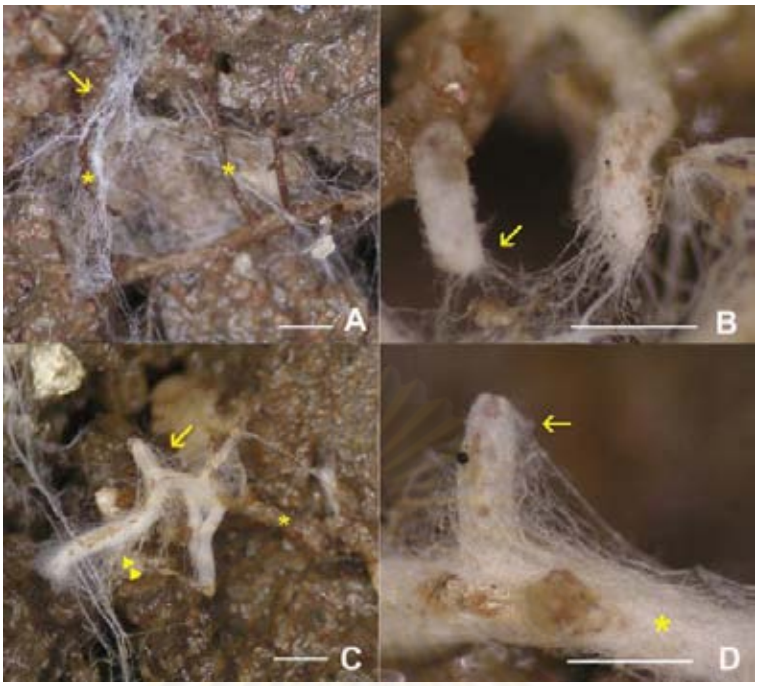


Fig. 4.21 *Dipterocarpus alatus* Roxb. was inoculated with *Astraeus odoratus* Phosri et al.. Fungal hyphae (arrow) attached lateral roots (*)(A). Root apex partially were covered with loose mantle (arrow) and extraradical mycelia were extending from root tips (B). Monopodial lateral of *Dipterocarpus alatus* showing mantle of fungal hyphae (arrow) and root (*) is free of hyphae (C). Well developed mantle (*) of lateral root and root apex (arrow) were cover with mycelium (D).

4.2.4.2 Testing for ectomycorrhizal colonization of dipterocarp seedling roots

In 2006, the experiments were conducted in Chanthaburi province. The 7 dipterocarp species including *D. obtusifolius, S. siamensis, D. intricatus, S. roxburghii, H. odorata, D. alatus* and *S. farinose* were inoculated with mycelial slurry inocula of 3 ECM fungi. The experimental design was a completely randomized block design with 4 treatments per each dipterocarp species (control, *A. asiaticus*, *A. odoratus* and *P. abditus*).

Seven dipterocarp seedlings were inoculated with mycelial slurriy inocula of *A. asiaticus* , *A. odoratus* and *P. abditus* in the nursery The mycorrhizal formation was observed in all inoculated dipterocarp seedling after six months.

However, ECM colonization was not observed in the root of control seedlings. Non-ectomycorrhizal dipterocarps root were easily distinguishable from ECM root after visual examination of the root system. The non-ectomycorrhizas were long, thin and pale brown to translucent (Fig. 4.21A) whereas the ectomycorrhizal roots were short, swollen coloured white creamish to darker shades of brown colour and bore emanating hyphae. Ectomycorrhizas of the *A. asiaticus* and *A. odoratus* associated with *D. alatus* were characterized by whitish brown mantle colour, emanating hyphae (Fig 4.21B) and presence of clamp connections at septa of the hyphae. The other host plants with those ECM fungi showed similar features. *A. odoratus* associated with *S. siamensis* mycorrhizal roots showed clamp connections at septa of the hyphae (Fig.4.22A). Transverse sections showed mantle sheath with a well-developed epidermal Hartig net (Fig. 22B). Ectomycorrhizas of the *P. abditus* associated with dipterocarps were characterized by light brown mantle colour, emanating hyphae, presence of clamp connections at septa of hyphae. In whole ECM root cleared and stained *P. abditus* associated with *H. odorata* seedling showed oblique elongation of epidermal cells (Fig. 4.23A). Transverse section through ECM tip showed mantle and Hartig net formation which usually surrounded the epidermal cells (Fig. 4.23B). In whole ECM root cleared and stained of *D. intricatus* associated with *A. asiaticus* showed mantle sheath and Hartig net (Fig. 4.24). SEM observation showed mantle sheath and Hartig net of *D. intricatus* associated with *A. asiaticus.*

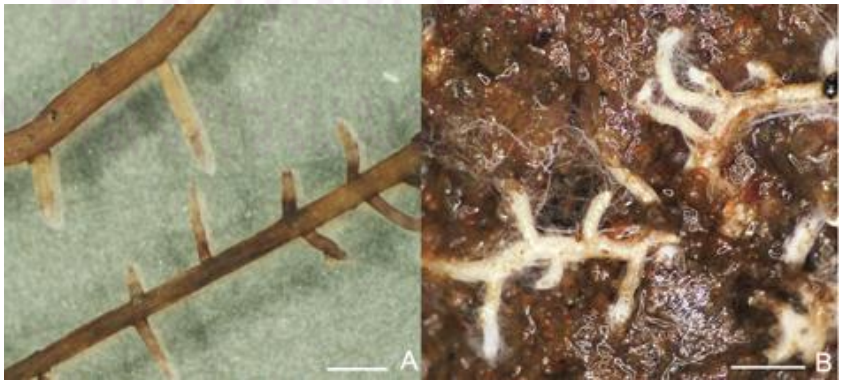


Fig. 4.21 Microscopic pictures of uninfected and infected *Dipterocarpus alatus* Roxb. roots. Uninfected root showing light brown colour and no root hair (A). Ectomycorrhizal root infected with *Astraeus odoratus* Phosri et al. having white brownish in colour (B).

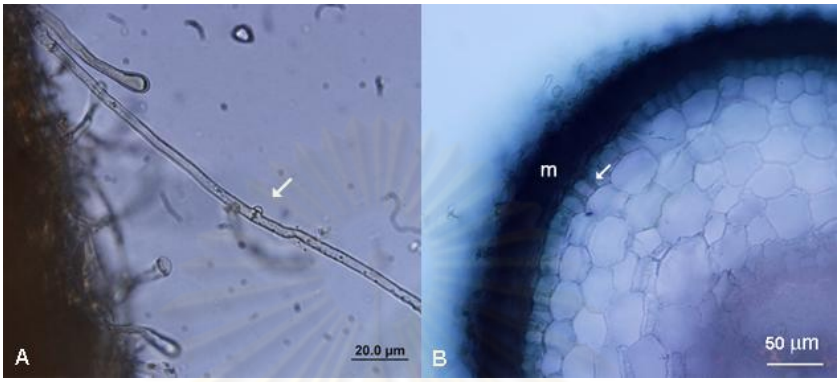


Fig. 4.22 Light micrograph of *Shorea siamensis* Miq. roots infected with *Astraeus odoratus* Phosri et al. showing surface view of mantle and emanating hyphae with clamp connections (arrow) at septa of hyphae (A). Transverse section showing mantle (m) with a well developed epidermal Hartig net (arrow) (B).

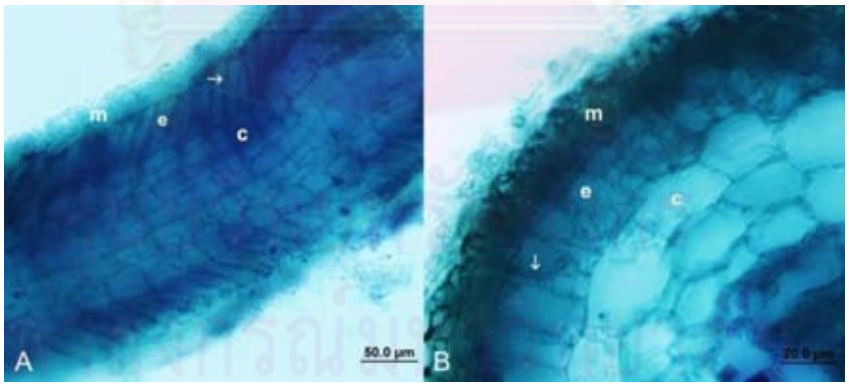


Fig. 4.23 Light micrograph of *Hopea odorata* roots associated with *Pisolithus abditus* showing *Hopea odorata* root stained with oblique elongation of epidermal cells (A). Transverse section through ECM tip showing mantle (m) and Hartig net formation (arrow) which usually surrounded the epidermal cells (e) and not prenetrated to cortical cell (c) (B).

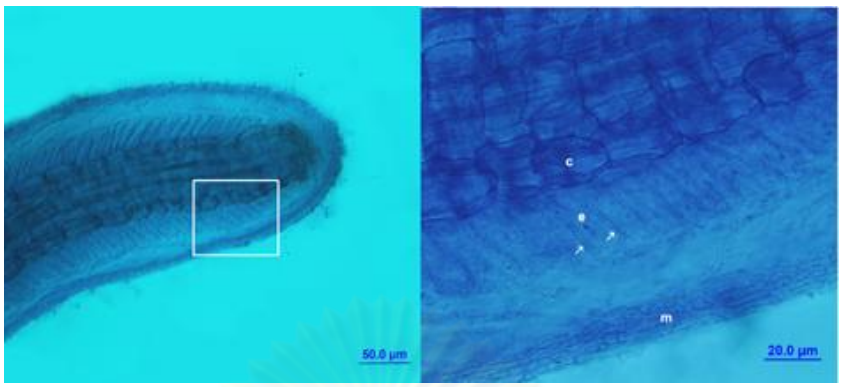


Fig. 4.24 Light micrograph of *Dipterocarpus intricatus* Dyer associated with *Astraeus asiaticus* Phosri et al. showing mantle sheath (m)and Hartig net (arrow) which usually surrounded the epidermal cells (e) and not prenetrated to cortical cell (c).

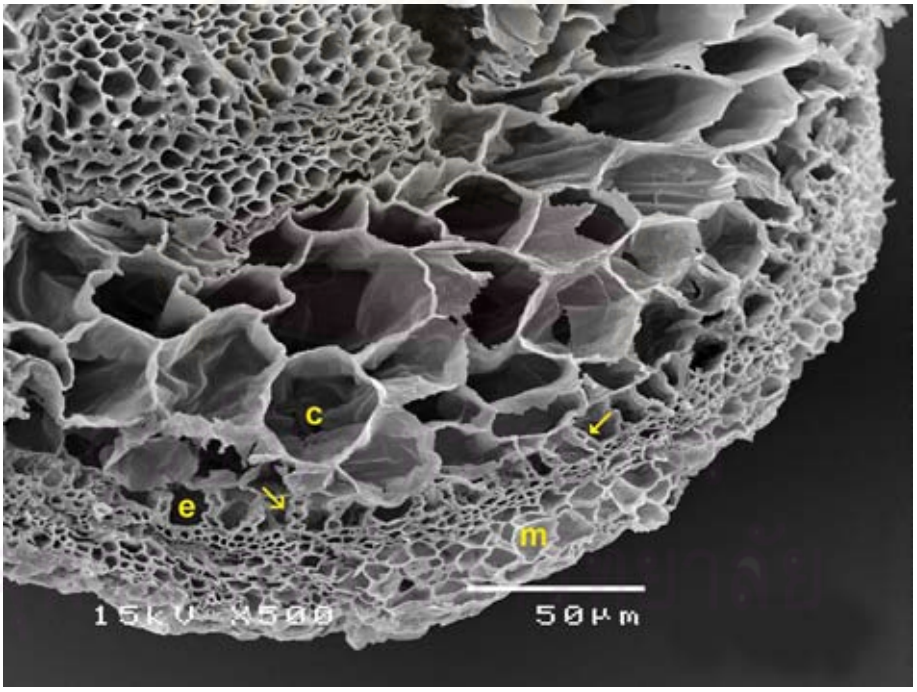


Fig. 4.25 Scanning electron micrograph showed mantle sheath (m) and Hartig net (arrow) of *Dipterocarpus intricatus* Dyer associated with *Astraeus asiaticus* Phosri et al.. Hartig net were observed around epidermal cells (e) and not detected in cortex (c).

There were more than one morphotype appearing on 12 month old inoculated seedlings. Not only *A. asiaticus*, *A. odoratus* and *P. abditus* ECM morphotypes occurred on roots of inoculated dipterocarp seedling but also golden-brown ECM morphotype did in roots of inoculated dipterocarp seedling. Golden-brown ECM morphotype was also observed in the root of control seedlings. Natural contaminant golden-brown morphotype were thelephoroid using morphological identification (Fig. 4.26). The contaminant was identified as *Tomentella* sp. by comparing ITS sequence to a previously published database from DDBJ/EMBL/GenBank and UNITE using algorithm blasn.

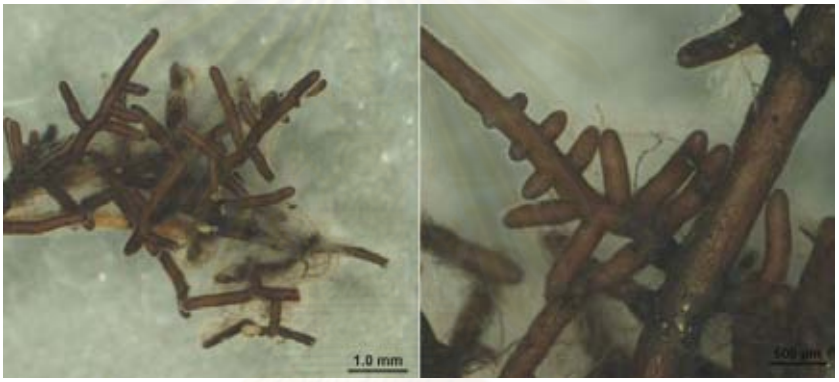


Fig. 4.26 *Tomentella* sp. ectomycorrhizas were characterized by golden-brown mantle colour to dark-brown root tips.

Eighteen months old of all seedlings inoculated with ECM fungi at the beginning of the experiment formed ectomycorrhizas at the end of the experiment. ECM morphotypes were found take the same as the ones at 12 months. However, the ECM fungi that colonized the control seedlings were not only *Tomentella* sp. but also pale brown ECM roots, as *L. vinaceoavellanea* by morphological features and molecular identification which were occurred. Moreover, sporocarps of *L. vinaceoavellanea* were found in control seedling pot of *D. alatus* (Fig.4.27). ECM fungal contaminants occurred on the inoculated or control seedlings were similar to those in the other previous studies. For instance, *Thelephora* were found on Douglas-fir seedlings (Jones et al., 1997) and often considered early stage, commonly colonizing jack pine seedlings in nursery stock

before planting (Visser, 1995). Moreover, the other contaminant such as *Laccaria* sp. was also observed in the roots of control seedlings of *S. pinanga* (Turjaman et al., 2005). It was possible that wind, rain and/or insects carried the contaminant ECM fungal inoculums and possible incomplete surface seed sterilization.

Fig. 4.27 *Laccaria vinaceoavellanea* associated with *Dipterocarpus alatus* Roxb.. Basidiomes of *Laccaria vinaceoavellanea* Hongo appeared in control seedling pot, Scale bar = 1 cm (A). Ectomycorrhizas of *Laccaria vinaceoavellanea* (B,C).

All of ECM fungi varied considerably in their ability to establish ECM roots. The intensity of infection at the end of experiments by 3 ECM fungi tested was different on different hosts. The percentage of ECM colonization on dipterocarps ranged from 13.27 to 74.13% (Fig. 4.27). Using the morphotyping approach, inoculated ECM fungi were more abundant than ECM fungi contaminant. In addition, dark-brown *Tomentella* sp. ECM morphotype was dominant in control seedlings compared to the pale brown *L. vinaceoavellanea* morphotype.

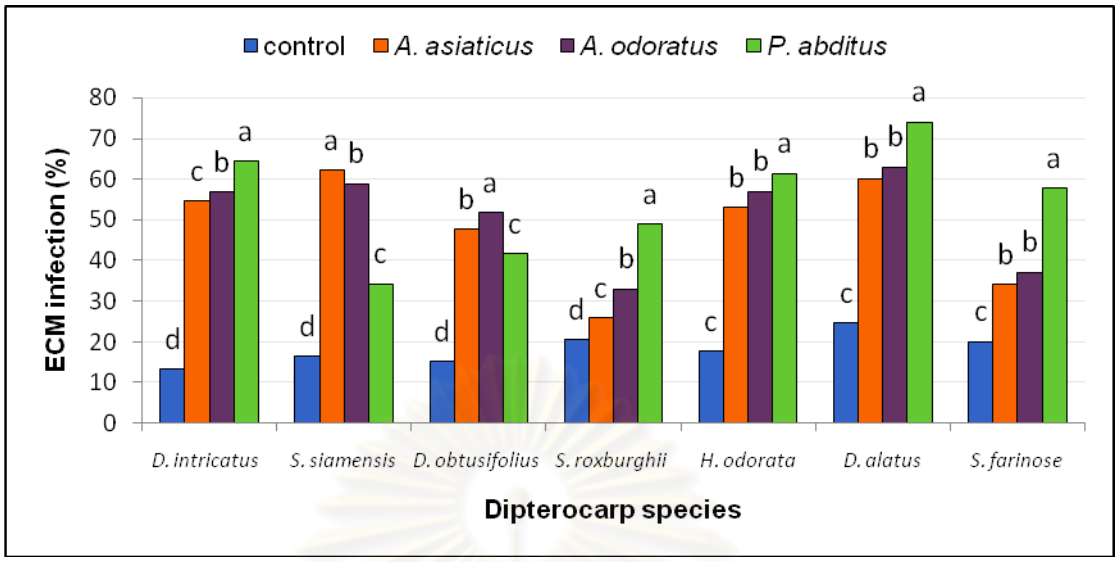


Fig. 4.27 Percentage of ECM infection of 18 months-old of 7 dipterocarp species seedlings previously non-inoculated (control) or inoculated with *Astraeus asiaticus* Phosri et al., *Astraeus odoratus* Phosri et al.and *Pisolithus abditus* Karn. et al.. Different letters within a column indicate significant effect of inocula on the growth of each dipterocarp species (Duncan test, $P<0.05$).

The percentage of ECM infection of inoculated seedlings were significantly higher than control seedlings in all treatments (Fig.4.27). The highest percentage of ECM infection was 74.13% in *D. alatus* inoculated with *P. abditus* (Appendix H).

4.2.4.3 Measurements and harvest of Dipterocapaceae seedlings

Effects of inoculation with 3 ECM fungi on growth stimulation of shoot height, stem diameter, shoot dry weight and ECM colonization of 7 dipterocarp seedlings 18 months old were analyzed using one-way ANOVA, Duncan tests. Mean values were compared in each dipterocarp species and ECM fungus inoculation.

Shoot height, stem diameter, shoot dry weight of inoculated dipterocarp seedlings were significantly higher than control seedlings. Inoculation with *A. asiaticus* and *A. odoratus* enhanced the growth of *S. siamensis*, *D. obtusifolius* and *D. intricatus* more than *S. roxburghii*, *D. alatus*, *H. odorata*, *S. farinose*. Whereas, *P. abditus* increased growth of *S. roxburghii*, *D. alatus*, *H. odorata* and *S. farinosa* more

than *D. obtusifolius*, *S. siamensis* and *D. intricatus*. The height of seedlings inoculated with ECM fungi increased up to 6.49-51.94 %. The stem diameter enlarged from 4.61% to 50.11 % and shoot dry weight increased the range from 2.63% to 64.38%. Summarizes result was done by comparing the success of different host plants and ECM inoculants. The results were examined by dividing fungi into ecological groups based on where the fungi originated; viz. either occur in evergreen or dry forests. The *P. abditus* found in the evergreen *D. alatus* plantation was more effective than the fungi which were found in the dry dipterocarp forests associated with evergreen dipterocarp species such as *D. alatus*, *H. odorata*, *S. roxburghii* and *S. farinose*. On the other hand, the *P. abditus* was less effective when associated with dry dipterocarp species such as *S. siamensis*, *D. obtusifolius* and *D. intricatus* which were themselves stimulated growth by *A. asiaticus* and *A. odoratus* (Fig. 4.28 and 4.29).

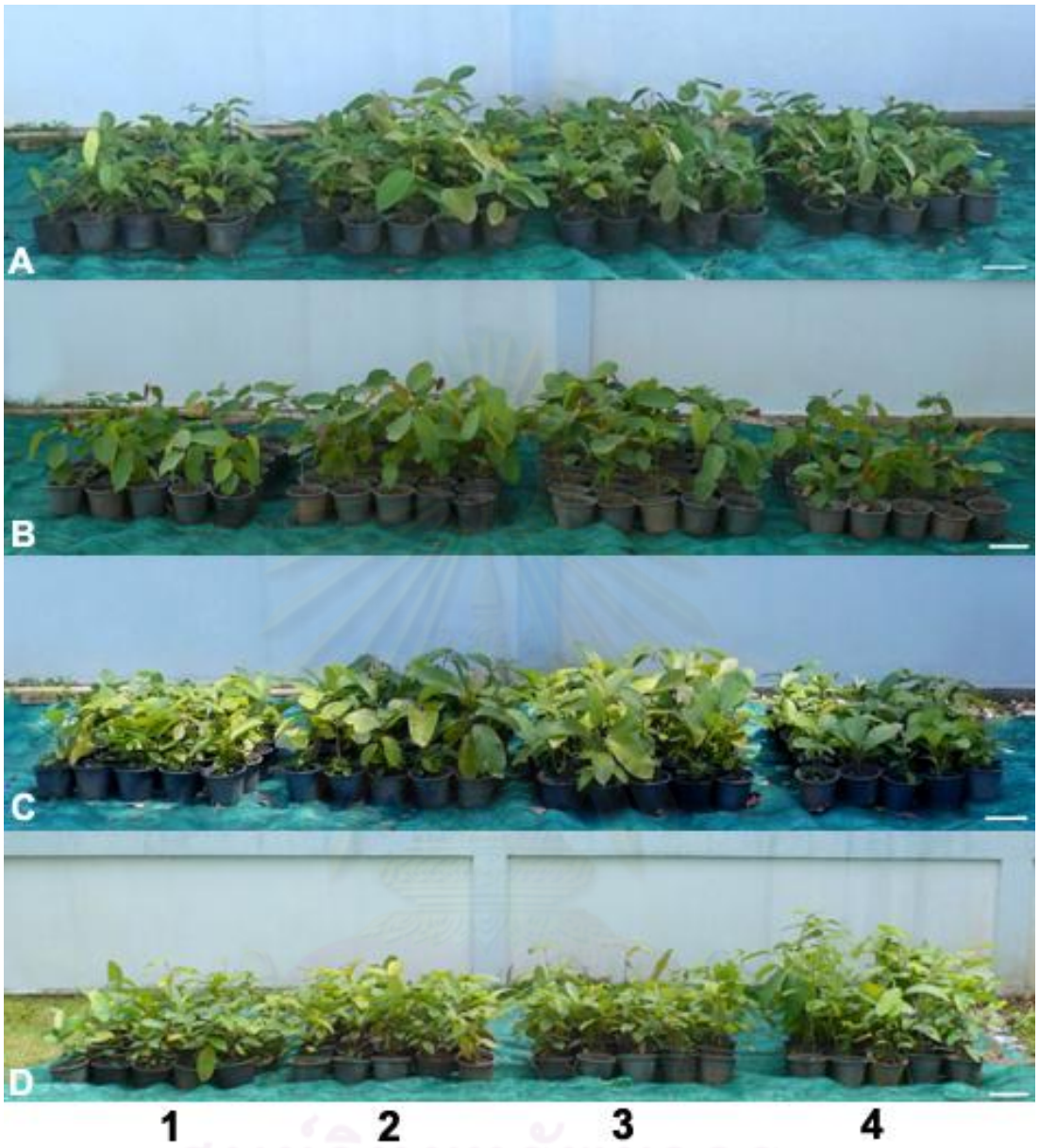


Fig. 4.28 Growth response of dipterocarp seedlings when inoculated with 1;control, 2; *Astraeus asiaticus* Phosri et al., 3; *Astraeus odoratus* Phosri et al., 4;*Pisolithus abditus* Karn. et al. after 18 months. (A; *Dipterocarpus obtusifolius* Teijsm ex Miq., B; *Shorea siamensis* Miq, C; *Dipterocarpus intricatus* Dyer, D; *Shorea roxburghii* G.Don, E; *Hopea odorata* Roxb., F; *Dipterocarpus alatus* Roxb. ex G.Don and G; *S. farinose* C.E.C. Fischer). Scale bars = 10 cm.

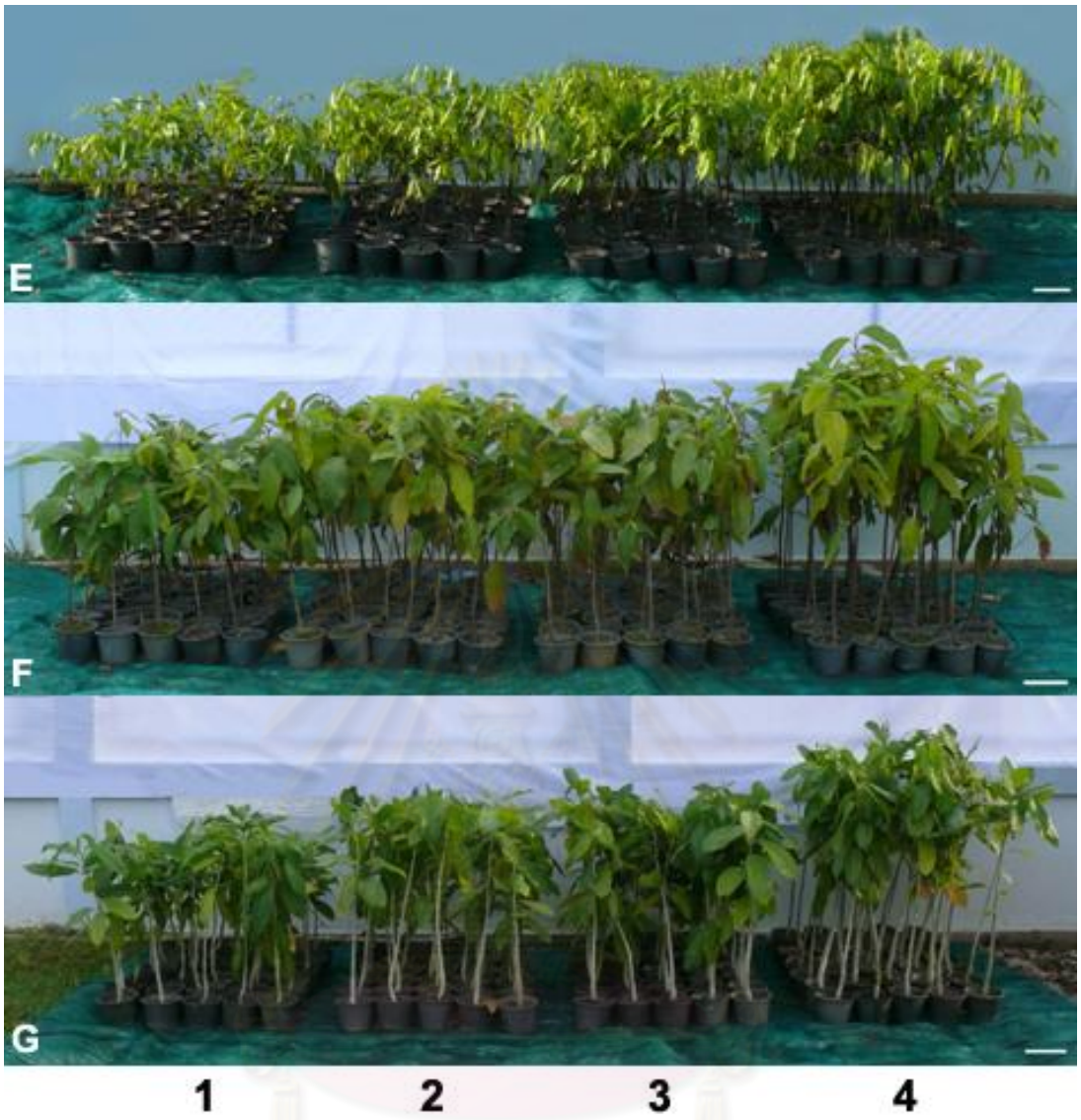


Fig. 4.28 (continued) Growth response of dipterocarp seedlings when inoculated with 1;control, 2; *Astraeus asiaticus* Phosri et al., 3; *Astraeus odoratus* Phosri et al., 4;*Pisolithus abditus* Karn. et al. after 18 months. (A; *Dipterocarpus obtusifolius* Teijsm ex Miq., B; *Shorea siamensis* Miq, C; *Dipterocarpus intricatus* Dyer, D; *Shorea roxburghii* G.Don, E; *Hopea odorata* Roxb., F; *Dipterocarpus alatus* Roxb. ex G.Don and G; *S. farinose* C.E.C. Fischer). Scale bars = 10 cm.

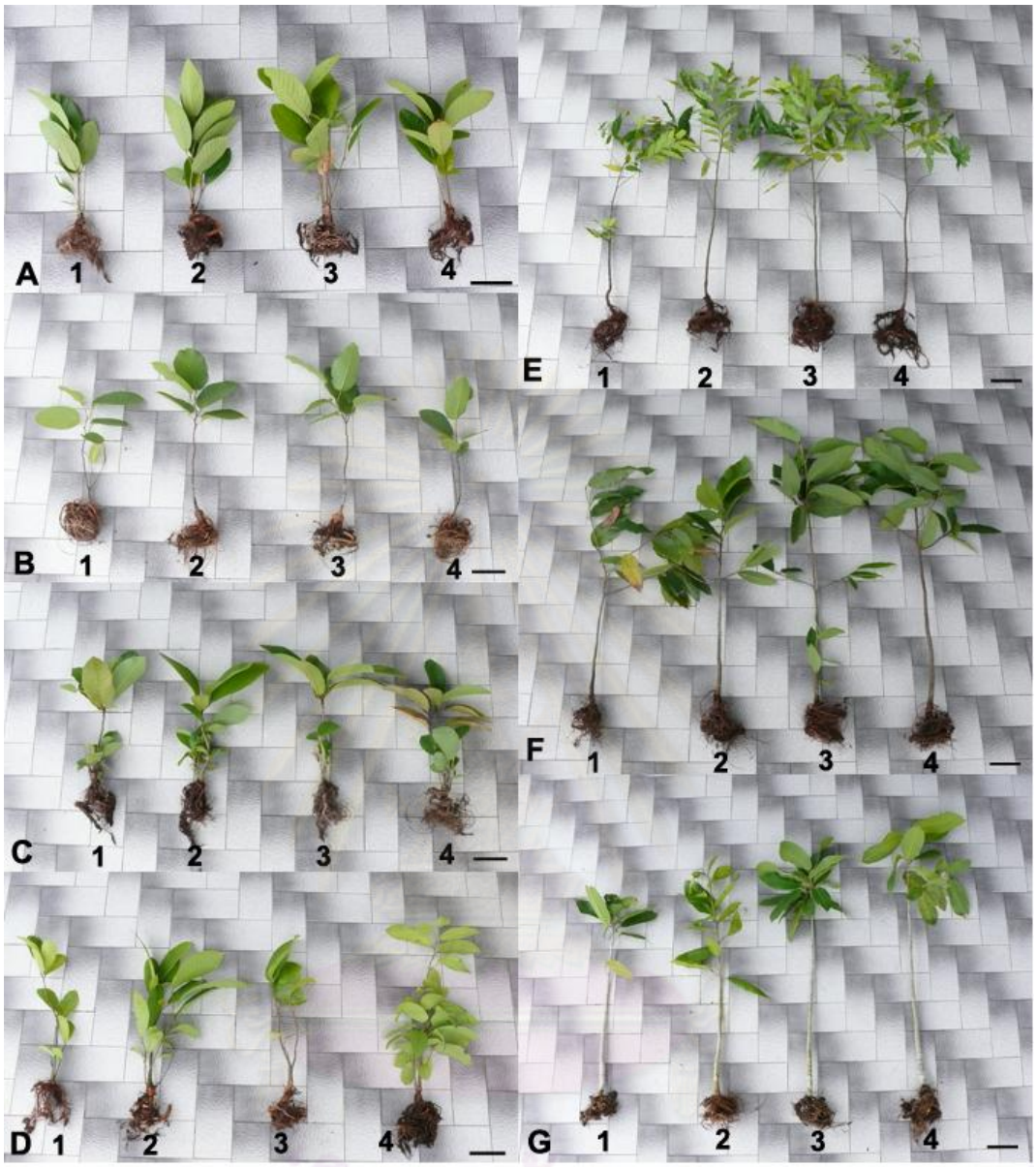


Fig. 4.29 Growth response of dipterocarp seedlings when inoculated with 1;control, 2; *Astraeus asiaticus* Phosri et al., 3; *Astraeus odoratus* Phosri et al., 4;*Pisolithus abditus* Karn. et al. after 18 months. (A; *Dipterocarpus obtusifolius* Teijsm ex Miq., B; *Shorea siamensis* Miq, C; *Dipterocarpus intricatus* Dyer, D; *Shorea roxburghii* G.Don, E; *Hopea odorata* Roxb., F; *Dipterocarpus alatus* Roxb. ex G.Don and G; *S. farinose* C.E.C. Fischer). Scale bars = 10 cm.

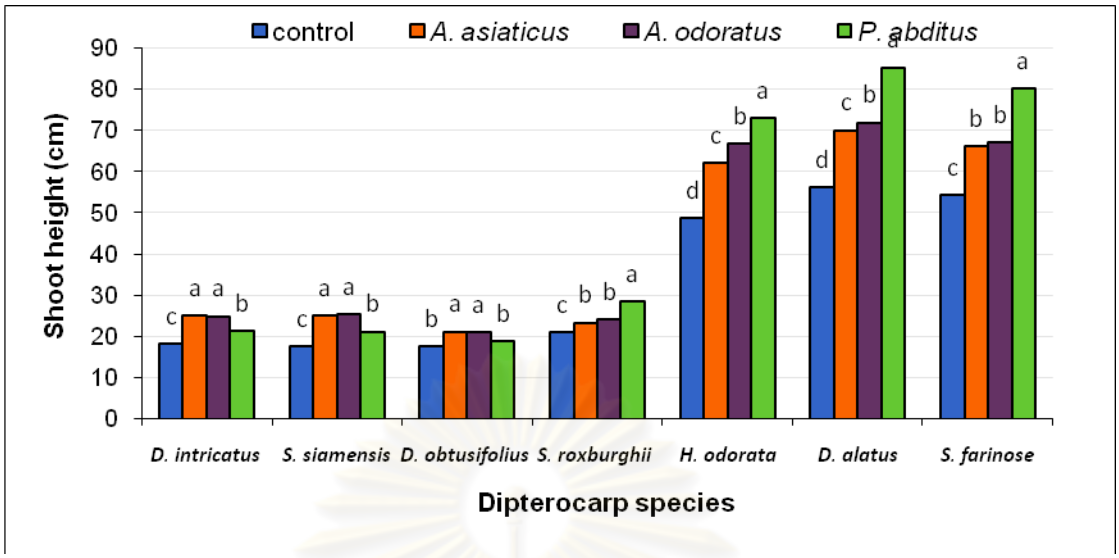


Fig. 4.30 Shoot height after 18 months old of 7 dipterocarp species seedlings uninfected and infected with *Astraeus asiaticus* Phisri et al., *Astraeus odoratus* Phisri et al.and *Pisolithus abditus* Karn. et al.. Different letters within a column indicate significant different among each inocula on the growth of each dipterocarp species (Duncan test, $P<0.05$).

Shoot height of 18 months old inoculated seedlings with mycorrhizal fungi was significantly higher than non-inoculated seedlings in all treatments, except *D. obtusifolius* seedlings were inoculated with *P. abditus* (Fig.4.30). The highest increase of shoot height was 51.94% in *D. alatus* when inoculated with *P. abditus* (Appendix H).

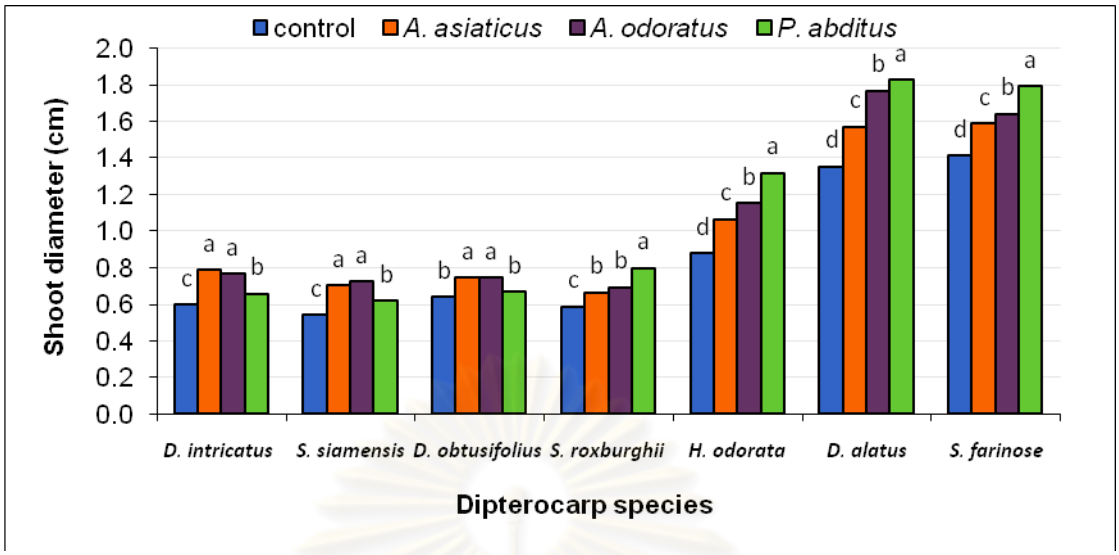


Fig. 4.31 Shoot diameter after 18 months old of 7 dipterocarp species seedlings uninfected and infected with *Astraeus asiaticus* Phisri et al., *Astraeus odoratus* Phisri et al.and *Pisolithus abditus* Karn. et al.. Different letters within a column indicate significant different among each inocula on the growth of each dipterocarp species (Duncan test, $P<0.05$).

The shoot diameter of inoculated seedlings was significantly higher than control seedlings in all treatments, except *D. obtusifolius* seedlings were inoculated with *P. abditus* (Fig.4.31). The highest increase of shoot diameter was 50.11% in *H. odorata* when inoculated with *P. abditus* (Appendix H).

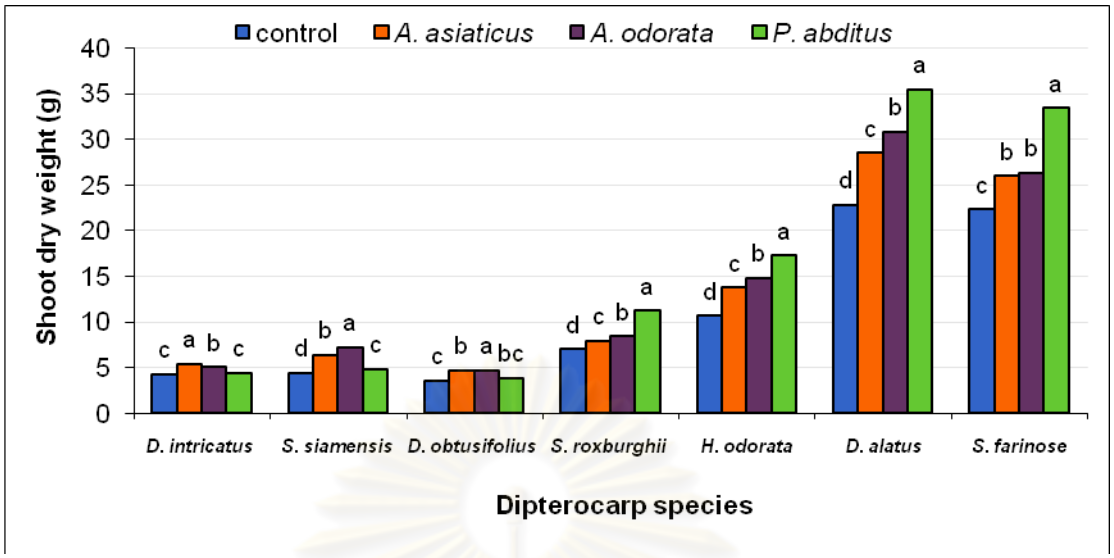


Fig. 4.32 Shoot dry weight after 18 months old of 7 dipterocarp species seedlings uninfected and infected with *Astraeus asiaticus* Phisri et al., *Astraeus odoratus* Phisri et al.and *Pisolithus abditus* Karn. et al.. Different letters within a column indicate significant different among each inocula on the growth of each dipterocarp species (Duncan test, $P<0.05$).

The shoot dry weight of inoculated seedlings was significantly higher than control seedlings in all treatments (Fig.4.32). The effect of *P. abditus* in *D. intricatus* was exception. The highest increase of shoot dry weight was 64.38% in *S. siamensis* when inoculated with *A. odoratus* (Appendix H).

This study described for the first time a significant positive effect of mycorrhizal association with *A. asiaticus*, *A. odoratus* and *P.abditus* on the growth of 7 dipterocarp species including *D. intricatus*, *S. siamensis*, *D. obtusifolius*, *S. roxburghii*, *Hopea odorata*, *D. alatus* and *S. farinosa* seedlings in unsterilized soils. These results supported previously reported beneficial effects of ECM fungi on the growth of dipterocarps seedlings. For instant, Yazid et al. (1994) reported that a selected strain of *P. tinctorius* prepared on cardboard was able to stimulate growth of *H. odorata* and *Hopea helferi* under control conditions. An average of 80% of the root tips in the *P. tinctorius* inoculated *H. odorata* and *H. helferi* seedlings were infected at the end of 9 months. The shoot height of *H. odorata* and *H. helferi* were increased 82% and 75%

more than control seedlings. Shoot dry weight was increased by 7.3 and 3.6 times, respectively. Turjaman et al. (2005) reported that ECM colonization with *P. arhizus* and *Scleroderma* sp. improved the shoot growth of *S. pinanga* 7 months after spore inoculation. With inoculation with *P. arhizus* showed the height of *S. pinanga* incleased by 86% and with inoculation of *Scleroderma* sp. by 71%. In contrast, Hadi et al. (1991) found that *P. tinctorius* had no significant effect on the growth of *H. odorata* and *S. pinanga*.

Although , ectomycorrhizal colonization and percentage of increased growth stimulation found in this study was less than those previous reported by Yazid et al. (1994) and Turjaman et al. (2005). Experimental design of this study and those previous studies had different method. Yazid et al. (1994) planted 1 month-old seedling of each species into clear perpex rhizotrons (30 cm x 15 cm x 1 cm) filled with steam sterilized forest soil. There were six seedlings of each species for each treatment. One side of the rhizotron was inoculated with a prepared on cardboard. This method was usually practiced in the laboratory and was not appropriate for mass production of seedling in nursery.

Turjaman et al. (2005) inoculated *S. pinanga* with spores of *P. arhizus* and *Scleroderma* sp. which collected from basidiocarps. One of major disadvantages of spore inoculum obtained from ECM fungi is lack of standard laboratory tests to determine spore viability and it is not possible to inoculate with a specific fungal isolate due to inherent variability among spores. Sufficient sporocarps of many ECM fungi may not be available all time. Sporocarps should be collected and stored large numbers of fruiting bodies when they are abundance only in fruiting season. In addition, formation of ectomycorrhiza by basidiospores usually take time longer than vegetative inoculum of the same fungi. Moreover, spore inoculum type could be contaminated with undesirable or pathogenic fungi

This suggested inoculum size and inoculum types of the ECM fungi may affected the ECM development of the tested dipterocarp species. The above results need to be viewed more critically. Then, inoculum size and inoculum types of *A. asiaticus*, *A. odoratus* and *P. abditus* inoculum on mycorrhizal infection and growth of

dipterocarp seedlings in nursery will be investigated. However, the results of signficantly improved growth stimulation in dipterocarp seedlings with selected mycorrhizal fungi that could be used to produced good quality inoculated seedlings in commercial nursery system. These inoculated dipterocarps seedlings could to be improve the establishment of seedlings in reforestation program of clear-cut forests or afforestation of disturbed sites, especially in degraded area such as abandoned farmland, abandoned surface mine sites or mine-waste spoil where low or no indigenous mycorrhizal inoculum.

CHAPTER V

CONCLUSIONS

The aboveground and underground ECM communities were characterized using morphological and molecular techniques. Thirty aboveground sporocarps and 12 underground ECM species were found in a natural dry dipterocarp forest. Three ECM found underground produced T-RFLP patterns that matched T-RFLP from sporocarps. The patterns in ECM abundance observed aboveground do not correlated with patterns observed underground.

Nine aboveground sporocarps and 4 underground ectomycorrhizal species were found in a *Dipterocarpus alatus* plantatipn. All of four underground ECM taxa had T-RFLP patterns identical with those of aboveground sporocarps. The patterns in ECM abundance observed aboveground correlated with patterns observed underground.

The ECM fungal community structure of each morphotype changed depending on the season. Ordination analyses showed that underground ECM community structure was significantly correlated with soil properties, in particular soil moisture content. In the natural dry dipterocarp forest and the *D. alatus* plantation, decreased soil moisture content gave lower diversity, colonization and shifted the composition of ECM fungal communities.

The percentage of ECM colonization on dipterocarps ranged from 26.27 to 74.13%. Shoot growth of inoculated dipterocarps seedlings were significantly higher than control seedlings. Colonization of *Shorea siamensis, S. roxburghii*, *S. farinosa, Dipterocarpus intricatus* , *D. obtusifolius* , *D. alatus* and *Hopea odorata* roots by ECM fungi, *Astraeus ordoratus*, *A. asiaticus* and *Pisolithus abditus* enhanced shoot height, stem diameter and shoot dry weight up to 51.94 %, 50.11% and 64.38%, respectively.

The results of signficantly improved growth stimulation in dipterocarp seedlings with selected mycorrhizal fungi indicated that they could be used to produce good quality inoculated seedlings in commercial nursery system. These inoculated

dipterocarps seedlings could to improve the establishment of seedlings in reforestation programmes of clear-cut forests or afforestation of disturbed sites, especially in degraded areas such as abandoned farmland, abandoned surface mine sites or mine-waste where low or no indigenous mycorrhizal inoculums existed.

Considerations for future studies:

Some issues remain to be addressed in future studies. First, while the results suggest that seasonal drought could directly impact on the ECM, the sampling cannot distinguish seasonal from annual variations. Studies examining ECM communities across contiguous seasons are therefore necessary to assess the real impact of the summer drought period. Second, while we have documented the turnover of species on ECM active roots. It is possible that their hyphae remain active and are receiving carbon from another part of the site that is still colonized by an active root. In addition, ECM fungi can survive in dry soil during the summer in the form of resting hyphae, rhizomorphs or sclerotia. The ability to consider multiple forms of the fungi will therefore greatly enhance our understanding of temporal changes in ECM communities. Third, while soil sampling was only set up to test ECM community structure within plots. It is possible that their ectomycorrhizas remain in other parts of the study site. These compartment was not representative of overall ECM diversity in the study site. Studies examining ECM communities across the study site including additional soil core samples in random directions across the site and under ECM sporocarps are therefore necessary to assess the real impact of the ECM community.

Inoculum size and inoculum types of the ECM fungi affect on ECM development of the tested dipterocarp species. Thus, inoculum size and inoculum types of *A. asiaticus*, *A. odoratus* and *P. abditus* on mycorrhizal infection and growth of dipterocarp seedlings in nursery will be investigated.

CHAPTER VI

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APPENDICES

APPENDIX A

Media and chemical reagents

1. Modified Melin-Norkrans (MMN) (Marx, 1969)

Malt extract	3.00	g
Glucose	10.00	g
KH_2PO_4	0.50	g
$(NH_4)_2HPO_4$	0.25	g
$MgSO_4.7H_2O$	0.15	g
$CaCl_2$	0.05	g
$FeCl_3$ (1% solution)	1.20	g
NaCl	0.025	g
Thiamine HCl	100	µg
Agar	15.00	g

Dissolve with distilled water 900 ml thoroughly and adjust pH with HCl to pH 5.8. After that the distilled water was added to reach 1000 ml. Sterilization in a autoclave at 121°C and pressure at 15 pounds/square inch for 15 minutes.

2. Malt Extract Agar (MEA)

Malt Extract	20.00	g
Glucose	20.00	g
Peptone	1.00	g
Agar	15.00	g

Dissolve with distilled water 900 ml thoroughly and adjust pH with HCl to pH 5.8. After that the distilled water was added to reach 1000 ml. Sterilization in a autoclave at 121°C and pressure at 15 pounds/square inch for 15 minutes.

3. Malt Extract Broth (MEB)

Malt Extract	20.00	g
Glucose	20.00	g
Peptone	1.00	g

Dissolve with distilled water 900 ml thoroughly and adjust pH with HCl to pH 5.8. After that the distilled water was added to reach 1000 ml. Sterilization in a autoclave at 121°C and pressure at 15 pounds/square inch for 15 minutes.

4. Fertilizer low phosphorus

NH_4NO_3	30	g
Na_2HPO_4	46	g
KCl	18	g
$CaCl_2$	28	g
$MgSO_4$	60	9
H_3BO_3	0.64	g
$ZnSO_4.7H_2O$	0.44	g
$MnCl_2.4H_2O$	2.5	g
FeEDTA	36.2	g
$Na_2MoO_4.2H_2O$	2.50	mg
$CuSO_4.5H_2O$	1.50	mg

Dissolve with distilled water 1,000 ml thoroughly for stock solution (NB. recommend separate dissolve). Mixed 1 ml of each stock solution and added water to reach 1000 ml.

5. Washing buffer

PVP (Polyvinylpyrrolidone)	2	g
Ascorbic acid	1.76	g
1 N Tris-HCl (pH 8.0)	20	ml
2-Mercaptoethanol	4	ml

Mix PVP, Ascorbic acid, Tris-HCl and 2-Mercaptoethanol. After that the distilled water was added to reach 200 ml and mix thoroughly. Keep at 4°C .

6. 2X CTAB lysis buffer

CTAB	4	g
1 N Tris-HCl (pH 8.0)	20	ml
0.5 N EDTA (pH 8.0)	8	ml
Sodium chloride (NaCl)	16.36	g
2-Mercaptoethanol	1	ml

Mix CTAB, 0.5 N EDTA, NaCl and 2-Mercaptoethanol. After that the distilled water was added to reach 200 ml and mix thoroughly. Keep at room temperature.

7. Choloroform/Isoamyl alcohol (24:1 v/v)

Choloroform	192	ml
Isoamyl alcohol	8	ml

Tris-HCl pH 8.0

Tris base	121	g
Distilled water	800	ml

Dissolve Tris base thoroughly and adjust pH with HCl to pH 8. After that the distilled water was added to reach 1000 ml. Autoclave at 121°C and pressure at 15 pounds/square inch for 15 minutes. Keep at room temperature.

8. Tris-EDTA buffer (TE buffer)

1 N Tris-HCl ; pH 7.4, 7.5 or 8.0	10	ml
0.5 N EDTA ; pH 8.0	2	ml

Mix Tris-HCl ; pH 7.4, 7.5 or 8.0 and EDTA ; pH 8.0. After that the distilled water was added to reach 1000 ml. Autoclave at 121°C and pressure at 15 pounds/square inch for 15 minutes. Keep at room temperature.

9. 0.5 N EDTA (Ethylenediamine Tetraacetic acid)

EDTA (Ethylenediamine Tetraacetic acid)	86.10	g
Distilled water	800	ml

Dissolve EDTA thoroughly and adjust pH with NaOH to pH 8. After that the distilled water was added to reach 1000 ml. Autoclave at 121°C and pressure at 15 pounds/square inch for 15 minutes. Keep at 4°C.

APPENDIX B

Clearing and Staining Method

(Kormanik and McGraw, 1982 modified from Phillips and Hayman, 1970)

1. Wash root sample and transfer to beaker
2. Clear in 10% potassium hydroxide solution in a water bath or hot plate or autoclave at 90°C. Length of time required varies greatly with materials. Dark coloured roots may be bleached with hydrogen peroxide.
3. Drain and wash thoroughly.
4. Acidify cleared root by immersion in 1% hydrochloric acid. Length of time in acid may be important and although momentary immersion may suffice, it may be found necessary to acidify for much longer, even overnight.
5. Stain for 10-30 minutes in 0.01% acid fuchsin or 0.05% trypan blue in lactoglycerol at 90°C.
6. Drain and wash thoroughly.
7. Destain roots in 14:1: 1 lactic acid : glycerol : water overnight or longer.
8. Mount roots in destaining solution on a microscope slide

APPENDIX C

Protocol for Scanning Electron Microscope (SEM) preparation

1. Fresh specimens were prepared for SEM by fixed in 2.5% glutaraldehyde 0.1 M phosphate buffer at pH 7.2 for 2 hr at 5 $^{\circ}$C.
2. Specimens were then rinsed twice in phosphate buffer for 10 min. of each and once in distilled water for 10 min.
3. Dehydration process was achieved by passing the specimens through the series of 30, 50, 70 and 95% ethanol for 10 min of each and finally three changes of absolute ethanol for 10 min each.
4. Specimens were dried in critical point drying machine (Balzers model CPD 020).
5. The dried specimens were fixed to brass stubs with double sticker tape and then coated with gold in sputter coating machine (Balzer model SCD 040).
6. Specimens were observed under SEM (JEOL model JSM 5410LV) with an accelerating voltage of 15 KV
7. Photographs were recorded with computer.

From Scientific and Technological Research Equipment Centre, Chulalongkorn University

APPENDIX D

Analysis of soil properties

1. Analysis of soil organic matter

Soil organic matter (OM) is made up of the decaying remains of plants and animals. It is a relatively small component of the soil's mass, but has a disproportionately important role in controlling the physical and chemical character of the soil, including its fertility and aggregate stability. This method is known as 'loss on ignition'. Organic matter is composed of carbon compounds which when heated to high temperatures are converted to CO_2 and water. In the ignition process a dry solid sample is heated to a high temperature. The organic matter in the soil and some volatile, non-organic chemicals from the sample are given off as gases. This results in a change in weight that allows for calculation of the organic content of the soil sample. Weighing the sample before and after this treatment, taking account of the weight of the crucible holding the soil, gives an estimate of the proportion of the sample's weight that was organic matter.

$$\% \text{ organic matter} = \frac{[((B - A) - (C + A)) - A]}{(B - A)} \times 100$$

Where:

Crucible weight = A

Dry soil + crucible = B

Fired soil + crucible = C

2. Analysis of soil moisture content

2.1 Clean and dry the container and weigh (A). Place a sample of about 10 g of soil in the container (B).

2.2 Place the container in the oven and dry 110 °C for 4 h to a constant weight.

2.3 After drying, remove the container from the oven, allow to cool and weigh the container with contents to 0.1 g (C).

Calculate the moisture content of the soil as a percentage of the dry soil weight.

$$MC\% = \frac{B - C}{C - A} \times 100$$

Where:

A = Weight of tin (g)

B = Weight of moist soil + tin (g)

C = Weight of dried soil + tin (g)

3. Analysis of soil pH

The most common method for measuring soil pH employs a glass electrode pH meter in a soil-water mixture. Five grams of air-dried soil are mixed with 10 ml of water for 5 sec and allowed to stand for 10 min.

APPENDIX E

Soil properties

Table E1 Soil properties of dipterocarp forest and *D. alatus* plantation

	2005		2006	
	Dry season	Rainy season	Dry season	Rainy season
Dipterocarp forest				
Organic matter (%)	2.08±0.42(a)	2.45±0.30(a)	1.58±0.18(b)	2.30±0.25(a)
Moisture content (%)	5.39±0.61(b)	8.71±0.48(a)	2.64±0.20(b)	8.53±0.33(a)
pH	5.62±0.52(c)	6.60±0.70(a)	6.14±0.38(b)	6.85±0.12(a)
***D. alatus* plantation**				
Organic matter (%)	2.38±0.32(c)	2.90±0.35(a)	2.46±o.25(c)	2.82±0.22(b)
Moisture content (%)	6.60±0.34(c)	10.67±0.64(a)	6.48±o.39(c)	10.02±0.54(b)
pH	6.22±0.50(b)	6.84±0.24(a)	6.23±0.38(b)	6.89±0.20(a)

Different letters within a row indicate significant different among each inocula (Duncan test, $P<0.05$)

E1. Statistic analysis data by Oneway – ANOVA of soil properties in dipterocarp forest

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Organic matter	Between Groups	1.765	3	.588	9.090	.002
	Within Groups	.777	12	.065		
	Total	2.541	15			
Moisture content	Between Groups	99.963	3	33.321	188.395	.000
	Within Groups	2.122	12	.177		
	Total	102.085	15			
pH	Between Groups	3.349	3	1.116	24.880	.000
	Within Groups	.538	12	.045		
	Total	3.888	15			

Post Hoc Tests

Homogeneous Subsets

Organic matter

Duncan[a]

		Subset for alpha = .05	
TIME	N	1	2
3.00	4	1.5750	
1.00	4		2.0825
4.00	4		2.3025
2.00	4		2.4525
Sig.		1.000	.073

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Moisture content

Duncan[a]

		Subset for alpha = .05		
TIME	N	1	2	3
3.00	4	2.6400		
1.00	4		5.3850	
4.00	4			8.5250
2.00	4			8.7100
Sig.		1.000	1.000	.546

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

pH

Duncan[a]

TIME	N	Subset for alpha = .05		
		1	2	3
1.00	4	5.6500		
3.00	4		6.1450	
2.00	4			6.6025
4.00	4			6.8475
Sig.		1.000	1.000	.128

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

E2. Statistic analysis data by Oneway – ANOVA of soil properties in *Dipterocarpus alatus* plantation

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Organic matter	Between Groups	.807	3	.269	86.602	.000
	Within Groups	.037	12	.003		
	Total	.844	15			
Moisture content	Between Groups	58.912	3	19.637	1132.376	.000
	Within Groups	.208	12	.017		
	Total	59.120	15			
pH	Between Groups	1.632	3	.544	191.733	.000
	Within Groups	.034	12	.003		
	Total	1.666	15			

Post Hoc Tests
Homogeneous Subsets

Organic matter

Duncan[a]

TIME	N	Subset for alpha = .05		
		1	2	3
1	4	2.3800		
3	4	2.4575		
4	4		2.8150	
2	4			2.9050
Sig.		.073	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Moisture content

Duncan[a]

TIME	N	Subset for alpha = .05		
		1	2	3
3	4	6.4650		
1	4	6.6050		
4	4		10.0100	
2	4			10.6750
Sig.		.159	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

pH

Duncan[a]

TIME	N	Subset for alpha = .05	
		1	2
1	4	6.2200	
3	4	6.2350	
2	4		6.8375
4	4		6.8925
Sig.		.697	.170

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

APPENDIX F

Table F1 Relative frequency of ectomycorrhizal community in dipterocarp forest

ECM taxa	Dry season 05	Rainy season 05	Dry season 06	Rainy season 06
Sebacina sp.	9.30	5.71	0	6.09
Astraeus asiaticus	19.77	10.47	19.40	7.83
Russulaceae	12.78	17.15	0	17.39
Thelephoraceae 1	10.47	3.81	11.94	2.61
Lacctarius sp.1	8.14	11.43	0	16.52
Tomentella sp.1	5.82	10.47	17.91	6.09
Tomentella sp.2	3.49	6.67	0	7.83
Cenococcum geophilum	20.93	12.38	29.85	12.17
Thelephoraceae 2	0	5.71	0	6.09
Lactarius sp.2	0	1.91	0	2.61
Lactarius volemus	9.30	5.72	11.94	4.35
Mycoamaranthus cambodgensis	0	8.57	8.96	10.42

Table F2 Relative abundance of ectomycorrhizal community in dipterocarp forest

ECM taxa	Dry season 05	Rainy season 05	Dry season 06	Rainy season 06
Sebacina sp.	6.62	5.71	0	5.15
Astraeus asiaticus	14.35	13.18	20.38	10.04
Russulaceae	7.14	14.30	0	16.85
Thelephoraceae 1	13.77	6.12	12.85	8.07
Lacctarius sp.1	7.64	9.59	0	10.26
Tomentella sp.1	11.29	10.46	15.83	10.43
Tomentella sp.2	10.53	9.17	0	8.19
Cenococcum geophilum	22.05	13.20	23.82	11.41
Thelephoraceae 2	0	5.07	0	5.62
Lactarius sp.2	0	2.04	0	3.21
Lactarius volemus	6.61	4.36	12.54	4.40
Mycoamaranthus cambodgensis	0	6.80	14.58	6.37

Table F3 Relative frequency of ectomycorrhizal community in *D. alathus* plantation

ECM taxa	Dry season 05	Rainy season 05	Dry season 06	Rainy season 06
Russula siamensis	19.02	33.89	24.36	32.12
Pisolithus abditus	21.20	18.16	20.05	18.40
Lactarius piperatus	20.67	25.52	23.21	26.62
Scleroderma columnare	39.11	22.43	32.38	22.85

Table F4 Relative abundance of ectomycorrhizal community in *D. alathus* plantation

ECM taxa	Dry season 05	Rainy season 05	Dry season 06	Rainy season 06
Russula siamensis	23.70	39.18	19.95	35.27
Pisolithus abditus	18.06	21.92	22.41	21.58
Lactarius piperatus	18.59	23.30	17.57	20.82
Scleroderma columnare	39.65	15.60	40.07	22.33

APPENDIX G

G1. Data analysis of Nonmetric Multidimensional Scaling (NMS) ordination of combined 2005 and 2006 ECM fungi colonizing roots in dipterocarp forest

Coefficients of determination for the correlations between ordination distances and distances in the original n-dimensional space:

	R Squared	
Axis	Increment	Cumulative
1	.219	.219
2	.206	.425
3	.226	.651

Increment and cumulative R-squared were adjusted for any lack of orthogonality of axes.

Axis pair	r	Orthogonality,% = 100(1-r^2)
1 vs 2	-0.090	99.2
1 vs 3	0.033	99.9
2 vs 3	-0.019	100.0

Number of entities = 128
Number of entity pairs used in correlation = 8128
Distance measure for ORIGINAL distance: Sorensen (Bray-Curtis)

G2 Data analysis of Canonical correspondence analysis (CCA) in dipterocarp forest

AXIS SUMMARY STATISTICS
Number of canonical axes: 3
Total variance ("inertia") in the species data: 2.4829

	Axis 1	Axis 2	Axis 3
Eigenvalue	0.136	0.046	0.012
Variance in species data			
% of variance explained	5.5	1.8	0.5
Cumulative % explained	5.5	7.3	7.8
Pearson Correlation, Spp-Envt*	0.673	0.399	0.239
Kendall (Rank) Corr., Spp-Envt	0.444	0.241	0.169

* Correlation between sample scores for an axis derived from the species data and the sample scores that are linear combinations of the environmental variables. Set to 0.000 if axis is not canonical.

MULTIPLE REGRESSION RESULTS:
Regression of plots in species space on environm

	Canonical Coefficients					
	Standardized			Original Units		
Variable	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3
S.Dev						
1 pH	-0.082	-0.514	-0.182	-0.172	-1.083	-0.382
0.475E+00						
2 OM	0.034	0.386	-0.627	0.110	1.236	-2.010
0.312E+00						
3 MC	-0.585	0.020	0.663	-0.295	0.010	0.335
0.198E+01						

FINAL SCORES and raw data totals (weights) for 12 species

	Raw Data			
	Axis 1	Axis 2	Axis 3	Totals
1 Seba	-0.474821	0.779006	0.284344	451.0000
2 Astr	0.182137	0.114019	0.130515	1018.0000
3 Russ	-0.801973	-0.084933	-0.085322	1116.0000
4 Thep1	0.762894	-0.077186	0.555159	719.0000
5 Lact1	-0.554121	-0.024336	-0.176825	674.0000
6 Tome1	0.058842	-0.124304	0.257718	1109.0000
7 Tome2	-0.323723	0.842206	0.018196	629.0000
8 Ceno	0.465819	0.317967	-0.464902	1407.0000
9 Thep2	-1.145483	-0.882568	0.392203	356.0000
10 Lact2	-0.875423	-1.014103	-0.131904	132.0000
11 Lact3	1.236673	-0.406963	0.244696	453.0000
12 Myco	0.344743	-0.898380	-0.570017	565.0000

CORRELATIONS AND BIPLOT SCORES for 3 environm

	Correlations*			Biplot Scores		
Variable	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3
1 pH	-0.680	-0.664	-0.313	-0.413	-0.307	-0.104
2 OM	-0.854	0.309	-0.418	-0.519	0.143	-0.139
3 MC	-0.994	0.111	0.019	-0.603	0.051	0.006

* Correlations are "intraset correlations" of ter Braak (1986)

INTER-SET CORRELATIONS for 3 environm

	Correlations		
Variable	Axis 1	Axis 2	Axis 3
1 pH	-0.457	-0.265	-0.075
2 OM	-0.575	0.123	-0.100
3 MC	-0.669	0.044	0.005

Note: Obtain joint plots or biplots by selecting GRAPH, then
requesting "Joint plots" from the GRAPH menu.

MONTE CARLO TEST RESULTS - EIGENVALUES

		Randomized data			
	Real data	Monte Carlo test, 998 runs			
Axis	Eigenvalue	Mean	Minimum	Maximum	p
1	0.136	0.035	0.011	0.084	0.0010
2	0.046	0.019	0.004	0.045	
3	0.012	0.009	0.001	0.028	

p = proportion of randomized runs with eigenvalue greater than or equal to the observed eigenvalue; i.e.,
p = (1 + no. permutations >= observed)/(1 + no. permutations)
p is not reported for axes 2 and 3 because using a simple randomization test for these axes may bias the p values.

MONTE CARLO TEST RESULTS - SPECIES-ENVIRONMENT CORRELATIONS

		Randomized data			
	Real data	Monte Carlo test, 998 runs			
Axis	Spp-Envt Corr.	Mean	Minimum	Maximum	p
1	0.673	0.366	0.214	0.546	0.0010
2	0.399	0.279	0.119	0.444	
3	0.239	0.199	0.086	0.344	

p = proportion of randomized runs with species-environment correlation greater than or equal to the observed species-environment correlation; i.e.,
p = (1 + no. permutations >= observed)/(1 + no. permutations)
p is not reported for axes 2 and 3 because using a simple randomization test for these axes may bias the p values.

CORRELATIONS AND BIPLOT SCORES for 1 environm

	Correlations*			Biplot Scores		
Variable	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3
1 MC	-1.000	0.000	0.000	-1.000	0.000	0.000

* Correlations are "intraset correlations" of ter Braak (1986)
Set to 0.000 if axis is not canonical.

INTER-SET CORRELATIONS for 1 environm

	Correlations		
Variable	Axis 1	Axis 2	Axis 3
1 MC	-0.666	0.000	0.000

Set to 0.000 if axis is not canonical.

Note: Obtain joint plots or biplots by selecting GRAPH, then requesting "Joint plots" from the GRAPH menu.

MONTE CARLO TEST RESULTS -- EIGENVALUES

		Randomized data			
	Real data	Monte Carlo test, 999 runs			
Axis	Eigenvalue	Mean	Minimum	Maximum	p
1	0.135	0.020	0.002	0.055	0.0010
2	0.345	0.380	0.354	0.383	
3	0.324	0.322	0.312	0.324	

p = proportion of randomized runs with eigenvalue greater than or equal to the observed eigenvalue; i.e.,
p = (1 + no. permutations >= observed)/(1 + no. permutations)
p is not reported for axes 2 and 3 because using a simple randomization test for these axes may bias the p values.

MONTE CARLO TEST RESULTS -- SPECIES-ENVIRONMENT CORRELATIONS

		Randomized data			
	Real data	Monte Carlo test, 999 runs			
Axis	Spp-Envt Corr.	Mean	Minimum	Maximum	p
1	0.666	0.284	0.096	0.471	0.0010
2	0.000	0.000	0.000	0.000	
3	0.000	0.000	0.000	0.000	

p = proportion of randomized runs with species-environment correlation greater than or equal to the observed species-environment correlation; i.e.,
p = (1 + no. permutations >= observed)/(1 + no. permutations)
p is not reported for axes 2 and 3 because using a simple randomization test for these axes may bias the p values.

CORRELATIONS AND BIPLOT SCORES for 1 environm

	Correlations*			Biplot Scores		
Variable	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3
1 OM	-1.000	0.000	0.000	-1.000	0.000	0.000

* Correlations are "intraset correlations" of ter Braak (1986)
Set to 0.000 if axis is not canonical.

INTER-SET CORRELATIONS for 1 environm

	Correlations		
Variable	Axis 1	Axis 2	Axis 3
1 OM	-0.585	0.000	0.000

Set to 0.000 if axis is not canonical.
Note: Obtain joint plots or biplots by selecting GRAPH, then requesting "Joint plots" from the GRAPH menu.

MONTE CARLO TEST RESULTS -- EIGENVALUES

		Randomized data			
	Real data	Monte Carlo test, 999 runs			
Axis	Eigenvalue	Mean	Minimum	Maximum	p
1	0.106	0.020	0.003	0.055	0.0010
2	0.343	0.380	0.353	0.383	
3	0.324	0.322	0.312	0.324	

p = proportion of randomized runs with eigenvalue greater
than or equal to the observed eigenvalue; i.e.,
p = (1 + no. permutations >= observed)/(1 + no. permutations)
p is not reported for axes 2 and 3 because using a simple
randomization test for these axes may bias the p values.

MONTE CARLO TEST RESULTS -- SPECIES-ENVIRONMENT CORRELATIONS

		Randomized data			
	Real data	Monte Carlo test, 999 runs			
Axis	Spp-Envt Corr.	Mean	Minimum	Maximum	p
1	0.585	0.287	0.104	0.462	0.0010
2	0.000	0.000	0.000	0.000	
3	0.000	0.000	0.000	0.000	

p = proportion of randomized runs with species-environment
correlation greater than or equal to the observed
species-environment correlation; i.e.,
p = (1 + no. permutations >= observed)/(1 + no. permutations)
p is not reported for axes 2 and 3 because using a simple
randomization test for these axes may bias the p values.

CORRELATIONS AND BIPLOT SCORES for 1 environm

	Correlations*			Biplot Scores		
Variable	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3
1 pH	-1.000	0.000	0.000	-1.000	0.000	0.000

* Correlations are "intraset correlations" of ter Braak (1986)
Set to 0.000 if axis is not canonical.

INTER-SET CORRELATIONS for 1 environm

	Correlations		
Variable	Axis 1	Axis 2	Axis 3
1 pH	-0.553	0.000	0.000

Set to 0.000 if axis is not canonical.

Note: Obtain joint plots or biplots by selecting GRAPH, then
requesting "Joint plots" from the GRAPH menu.

MONTE CARLO TEST RESULTS -- EIGENVALUES

		Randomized data			
	Real data	Monte Carlo test, 999 runs			
Axis	Eigenvalue	Mean	Minimum	Maximum	p
1	0.084	0.020	0.004	0.062	0.0010
2	0.377	0.380	0.354	0.383	
3	0.324	0.322	0.311	0.324	

p = proportion of randomized runs with eigenvalue greater
than or equal to the observed eigenvalue; i.e.,
p = (1 + no. permutations >= observed)/(1 + no. permutations)
p is not reported for axes 2 and 3 because using a simple
randomization test for these axes may bias the p values.

MONTE CARLO TEST RESULTS -- SPECIES-ENVIRONMENT CORRELATIONS

		Randomized data			
	Real data	Monte Carlo test, 999 runs			
Axis	Spp-Envt Corr.	Mean	Minimum	Maximum	p
1	0.553	0.287	0.128	0.505	0.0010
2	0.000	0.000	0.000	0.000	
3	0.000	0.000	0.000	0.000	

p = proportion of randomized runs with species-environment
correlation greater than or equal to the observed
species-environment correlation; i.e.,
p = (1 + no. permutations >= observed)/(1 + no. permutations)
p is not reported for axes 2 and 3 because using a simple
randomization test for these axes may bias the p values.

G3. Data analysis of Nonmetric Multidimensional Scaling (NMS) ordination of combined 2005 and 2006 ECM fungi colonizing roots in *D. alatus* plantation

Coefficients of determination for the correlations between ordination
distances and distances in the original n-dimensional space:

	R Squared	
Axis	Increment	Cumulative
1	.135	.135
2	.107	.241
3	.199	.440

Increment and cumulative R-squared were adjusted for any lack of orthogonality of axes.

Axis pair	r	Orthogonality,% = 100(1-r^2)
1 vs 2	0.031	99.9
1 vs 3	-0.172	97.0
2 vs 3	-0.092	99.2

Number of entities = 128
Number of entity pairs used in correlation = 8128
Distance measure for ORIGINAL distance: Sorensen (Bray-Curtis)

G4 Data analysis of Canonical Correspondence Analysis (CCA) ordination of combined 2005 and 2006 ECM fungi colonizing roots in *D. alatus* plantation

AXIS SUMMARY STATISTICS
Number of canonical axes: 3
Total variance ("inertia") in the species data: 1.3801

	Axis 1	Axis 2	Axis 3
Eigenvalue	0.022	0.014	0.000
Variance in species data			
% of variance explained	1.6	1.0	0.0
Cumulative % explained	1.6	2.7	2.7
Pearson Correlation, Spp-Envt*	0.211	0.180	0.019
Kendall (Rank) Corr., Spp-Envt	0.114	0.020	0.014

* Correlation between sample scores for an axis derived from the species data and the sample scores that are linear combinations of the environmental variables. Set to 0.000 if axis is not canonical.

MULTIPLE REGRESSION RESULTS:
Regression of soils in species space on environm

	Canonical Coefficients						
	Standardized			Original Units			
Variable	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3	S.Dev
1 pH	-0.399	-0.017	-0.254	-1.275	-0.054	-0.812	0.313E+00
2 OM	0.034	0.591	-0.004	0.134	2.356	-0.017	0.251E+00
3 MC	0.690	-0.483	0.189	0.386	-0.270	0.106	0.179E+01

FINAL SCORES and raw data totals (weights) for 4 species

	Raw Data			
	Axis 1	Axis 2	Axis 3	Totals
1 Scle	-0.537133	-0.196200	-0.068169	1783.0000
2 Russ	0.460563	-0.153784	-0.073598	2119.0000
3 Lact	0.022050	-0.150717	0.216992	1311.0000
4 Piso	-0.038319	0.709987	-0.005674	1230.0000

CORRELATIONS AND BIPLOT SCORES for 3 environm

	Correlations*			Biplot Scores		
Variable	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3
1 pH	0.665	-0.050	-0.745	0.258	-0.017	-0.084
2 OM	0.762	0.538	-0.359	0.295	0.186	-0.040
3 MC	0.909	-0.055	-0.413	0.352	-0.019	-0.047

* Correlations are "intraset correlations" of ter Braak (1986)

INTER-SET CORRELATIONS for 3 environm

	Correlations		
Variable	Axis 1	Axis 2	Axis 3
1 pH	0.140	-0.009	-0.014
2 OM	0.161	0.097	-0.007
3 MC	0.192	-0.010	-0.008

Note: Obtain joint plots or biplots by selecting GRAPH, then requesting "Joint plots" from the GRAPH menu.

MONTE CARLO TEST RESULTS - EIGENVALUES

		Randomized data			
		Real data	Monte Carlo test, 998 runs		
Axis	Eigenvalue	Mean	Minimum	Maximum	p
1	0.022	0.028	0.004	0.092	0.5866
2	0.014	0.008	0.000	0.055	
3	0.000	0.001	0.000	0.012	

p = proportion of randomized runs with eigenvalue greater than or equal to the observed eigenvalue; i.e.,
p = (1 + no. permutations >= observed)/(1 + no. permutations)
p is not reported for axes 2 and 3 because using a simple randomization test for these axes may bias the p values.

MONTE CARLO TEST RESULTS - SPECIES-ENVIRONMENT CORRELATIONS

		Randomized data			
		Real data	Monte Carlo test, 998 runs		
Axis	Spp-Envt Corr.	Mean	Minimum	Maximum	p
1	0.211	0.239	0.092	0.478	0.6607
2	0.180	0.132	0.018	0.325	
3	0.019	0.041	-0.022	0.176	

p = proportion of randomized runs with species-environment correlation greater than or equal to the observed species-environment correlation; i.e.,
p = (1 + no. permutations >= observed)/(1 + no. permutations)
p is not reported for axes 2 and 3 because using a simple randomization test for these axes may bias the p values.

CORRELATIONS AND BIPLOT SCORES for 1 env

	Correlations*			Biplot Scores		
Variable	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3
1 MC	1.000	0.000	0.000	0.370	0.000	0.000

* Correlations are "intraset correlations" of ter Braak (1986)
Set to 0.000 if axis is not canonical.

INTER-SET CORRELATIONS for 1 env

	Correlations

Variable	Axis 1	Axis 2	Axis 3
1 MC	0.191	0.000	0.000

Set to 0.000 if axis is not canonical.Note: Obtain joint plots or biplots by selecting GRAPH, then requesting "Joint plots" from the GRAPH menu.

MONTE CARLO TEST RESULTS -- EIGENVALUES

		Randomized data			
	Real data	Monte Carlo test, 998 runs			
Axis	Eigenvalue	Mean	Minimum	Maximum	p
1	0.019	0.012	0.000	0.064	0.1932
2	0.555	0.559	0.514	0.564	
3	0.452	0.455	0.418	0.459	

p = proportion of randomized runs with eigenvalue greater than or equal to the observed eigenvalue; i.e.,
p = (1 + no. permutations >= observed)/(1 + no. permutations)
p is not reported for axes 2 and 3 because using a simple randomization test for these axes may bias the p values.

MONTE CARLO TEST RESULTS -- SPECIES-ENVIRONMENT CORRELATIONS

		Randomized data			
	Real data	Monte Carlo test, 998 runs			
Axis	Spp-Envt Corr.	Mean	Minimum	Maximum	p
1	0.191	0.157	0.010	0.398	0.2853
2	0.000	0.000	0.000	0.000	
3	0.000	0.000	0.000	0.000	

p = proportion of randomized runs with species-environment correlation greater than or equal to the observed species-environment correlation; i.e.,
p = (1 + no. permutations >= observed)/(1 + no. permutations)
p is not reported for axes 2 and 3 because using a simple randomization test for these axes may bias the p values.

CORRELATIONS AND BIPLOT SCORES for 1 Env

	Correlations*			Biplot Scores		
Variable	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3
1 OM	1.000	0.000	0.000	0.362	0.000	0.000

* Correlations are "intraset correlations" of ter Braak (1986)
Set to 0.000 if axis is not canonical.

INTER-SET CORRELATIONS for 1 Env

	Correlations		
Variable	Axis 1	Axis 2	Axis 3
1 OM	0.185	0.000	0.000

Set to 0.000 if axis is not canonical.
Note: Obtain joint plots or biplots by selecting GRAPH, then requesting "Joint plots" from the GRAPH menu.

MONTE CARLO TEST RESULTS -- EIGENVALUES

		Randomized data			
		Real data	Monte Carlo test, 998 runs		
Axis	Eigenvalue	Mean	Minimum	Maximum	p
1	0.017	0.013	0.000	0.069	0.2573
2	0.552	0.559	0.518	0.564	
3	0.459	0.455	0.415	0.459	

p = proportion of randomized runs with eigenvalue greater than or equal to the observed eigenvalue; i.e.,
p = (1 + no. permutations >= observed)/(1 + no. permutations)
p is not reported for axes 2 and 3 because using a simple
randomization test for these axes may bias the p values.

MONTE CARLO TEST RESULTS - SPECIES-ENVIRONMENT CORRELATIONS

		Randomized data			
		Real data	Monte Carlo test, 998 runs		
Axis	Spp-Envt Corr.	Mean	Minimum	Maximum	p
1	0.185	0.159	0.016	0.369	0.3403
2	0.000	0.000	0.000	0.000	
3	0.000	0.000	0.000	0.000	

p = proportion of randomized runs with species-environment correlation greater than or equal to the observed species-environment correlation; i.e.,
p = (1 + no. permutations >= observed)/(1 + no. permutations)
p is not reported for axes 2 and 3 because using a simple randomization test for these axes may bias the p values.

CORRELATIONS AND BIPLOT SCORES for 1 environm

	Correlations*			Biplot Scores		
Variable	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3
1 pH	1.000	0.000	0.000	0.317	0.000	0.000

* Correlations are "intraset correlations" of ter Braak (1986)
Set to 0.000 if axis is not canonical.

INTER-SET CORRELATIONS for 1 environm

	Correlations		
Variable	Axis 1	Axis 2	Axis 3
1 pH	0.140	0.000	0.000

Set to 0.000 if axis is not canonical.
Note: Obtain joint plots or biplots by selecting GRAPH, then requesting "Joint plots" from the GRAPH menu.

MONTE CARLO TEST RESULTS -- EIGENVALUES

		Real data	Randomized data Monte Carlo test, 998 runs		
Axis	Eigenvalue	Mean	Minimum	Maximum	p
1	0.010	0.011	0.000	0.092	0.4444
2	0.558	0.559	0.468	0.564	
3	0.455	0.455	0.420	0.459	

p = proportion of randomized runs with eigenvalue greater than or equal to the observed eigenvalue; i.e.,
p = (1 + no. permutations >= observed)/(1 + no. permutations)
p is not reported for axes 2 and 3 because using a simple randomization test for these axes may bias the p values.

MONTE CARLO TEST RESULTS -- SPECIES-ENVIRONMENT CORRELATIONS

		Real data	Randomized data Monte Carlo test, 998 runs		
Axis	Spp-Envt Corr.	Mean	Minimum	Maximum	p
1	0.140	0.151	0.011	0.419	0.5435
2	0.000	0.000	0.000	0.000	
3	0.000	0.000	0.000	0.000	

p = proportion of randomized runs with species-environment correlation greater than or equal to the observed species-environment correlation; i.e.,
p = (1 + no. permutations >= observed)/(1 + no. permutations)
p is not reported for axes 2 and 3 because using a simple randomization test for these axes may bias the p values.

APPENDIX H

Table H1 Shoot growth and ectomycorrhizal colonization of dipterocarp seedlings with the ectomycorrhizal fungi.

Dipterocarp species	Treatments	Shoot height (cm)	Collar diameter (mm)	Shoot dry weight (g)	ECM colonization (%)
D. obtusifolius	Control	17.73±0.18(d)	0.64±0.05(b)	3.58±0.20(b)	15.27±0.92(d)
	A. asiaticus	21.99±1.44(a)	0.74±0.03(a)	4.62±0.15(a)	47.73±0.20(c)
	A. odoratus	21.25±0.45(b)	0.74±0.03(a)	4.67±0.83(a)	51.79±3.27(b)
	P. abditus	18.87±0.71(c)	0.67±0.04(b)	3.84±0.14(b)	41.64±2.72(a)
S. siamensis	Control	17.81±0.44(d)	0.54±0.03(c)	4.37±0.62(c)	16.33±1.13(d)
	A. asiaticus	25.30±0.40(a)	0.70±0.03(a)	6.32±0.56(a)	62.27±0.42(a)
	A. odoratus	25.56±1.58(b)	0.72±0.06(a)	7.18±0.51(a)	58.84±0.92(c)
	P. abditus	21.33±0.80(c)	0.61±0.04(b)	4.77±0.45(b)	34.25±2.50(a)
D. intricatus	Control	18.42±0.74(d)	0.59±0.03(c)	4.25±0.24(c)	13.27±1.10(b)
	A. asiaticus	25.11±1.50(b)	0.78±0.02(a)	5.35±0.20(a)	54.56±2.08(c)
	A. odoratus	25.06±1.10(a)	0.76±0.02(a)	4.99±0.64(a)	56.82±2.21(b)
	P. abditus	21.50±0.30(c)	0.65±0.04(b)	4.37±0.65(b)	64.43±0.90(a)
S. roxburghii	Control	21.16±1.67(d)	0.58±0.03(c)	4.07±0.26(c)	20.61±0.94(d)
	A. asiaticus	23.50±1.17(c)	0.66±0.03(b)	5.43±0.21(a)	26.27±0.32(c)
	A. odoratus	24.31±1.09(b)	0.69±0.02(b)	5.62±0.07(a)	33.02±2.57(b)
	P. abditus	28.62±1.04(a)	0.79±0.05(a)	4.32±0.14(b)	48.93±1.33(a)
H. odorata	Control	48.97±0.42(d)	0.88±0.03(d)	10.62±0.22(d)	17.75±1.74(d)
	A. asiaticus	62.26±1.67(c)	1.06±0.02(c)	13.71±0.92(c)	53.03±1.97(c)
	A. odoratus	66.88±0.91(b)	1.15±0.04(b)	14.73±0.49(b)	57.09±2.72(b)
	P. abditus	73.09±1.30(a)	1.32±0.03(a)	17.24±0.58(a)	61.47±1.47(a)

Dipterocarp species	Treatments	Shoot height (cm)	Collar diameter (mm)	Shoot dry weight (g)	ECM colonization (%)
D. alatus	Control	56.25±0.76(d)	1.35±0.02(d)	22.72±0.58(d)	24.69±1.97(d)
	A. asiaticus	70.17±2.37(c)	1.57±0.03(c)	28.47±0.96(c)	60.25±1.83(c)
	A. odoratus	72.09±1.50(b)	1.76±0.03(b)	30.73±1.59(b)	63.07±1.17(b)
	P. abditus	85.47±1.22(a)	1.82±0.04(a)	35.42±0.94(a)	74.13±2.08(a)
S. farinose	Control	54.52±0.77(d)	1.41±0.04(c)	22.30±0.70(d)	20.09±1.36(d)
	A. asiaticus	66.41±0.90(c)	1.58±0.05(b)	25.90±1.20(c)	34.20±0.66(c)
	A. odoratus	67.12±2.39(b)	1.64±0.03(b)	26.28±0.49(b)	37.13±0.80(b)
	P. abditus	80.23±1.17(a)	1.79±0.08(a)	33.38±1.00(a)	57.80±1.73(a)

Different letter within a column indicate significant different among each inocula (Duncan test, $P<0.05$)

H1. Statistic analysis data by Oneway – ANOVA of percentage of colonization in dipterocarp seedlings

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
D. obtusifolius	Between Groups	4784.694	3	1594.898	1840.071	.000
	Within Groups	6.934	8	.867		
	Total	4791.629	11			
S. siamensis	Between Groups	4229.153	3	1409.718	543.847	.000
	Within Groups	20.737	8	2.592		
	Total	4249.890	11			
D. intricatus	Between Groups	2430.307	3	810.102	382.725	.000
	Within Groups	16.933	8	2.117		
	Total	2447.240	11			
S. roxbuighii	Between Groups	1360.880	3	453.627	95.635	.000
	Within Groups	37.947	8	4.743		
	Total	1398.827	11			
H. odorata	Between Groups	3605.868	3	1201.956	513.345	.000
	Within Groups	18.731	8	2.341		
	Total	3624.600	11			
D. alatus	Between Groups	4144.067	3	1381.356	335.552	.000
	Within Groups	32.933	8	4.117		
	Total	4177.000	11			
S. farinose	Between Groups	2180.627	3	726.876	224.575	.000
	Within Groups	25.893	8	3.237		
	Total	2206.520	11			

Post Hoc Tests

Homogeneous Subsets

D. obtusifolius

Duncan[a]

		Subset for alpha = .05			
Treatment	N	1	2	3	4
1.00	3	13.2667			
2.00	3		54.5569		
3.00	3			56.8196	
4.00	3				64.4275
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

S. siamensis

Duncan[a]

Treatment	N	Subset for alpha = .05			
		1	2	3	4
1.00	3	16.3333			
4.00	3		34.2510		
3.00	3			58.8392	
2.00	3				62.2667
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

D. intricatus

Duncan[a]

Treatment	N	Subset for alpha = .05			
		1	2	3	4
1.00	3	15.2667			
4.00	3		41.6000		
2.00	3			47.7333	
3.00	3				51.8000
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

S. roxbuighii

Duncan[a]

Treatment	N	Subset for alpha = .05			
		1	2	3	4
1.00	3	20.6000			
2.00	3		26.0000		
3.00	3			33.0000	
4.00	3				48.9333
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

H. odorata

Duncan[a]

Treatment	N	Subset for alpha = .05			
		1	2	3	4
1.00	3	17.7529			
2.00	3		53.0314		
3.00	3			57.0667	
4.00	3				61.4667
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

D. alatus

Duncan[a]

Treatment	N	Subset for alpha = .05		
		1	2	3
1.00	3	24.6000		
2.00	3		60.2000	
3.00	3		63.0667	
4.00	3			74.1333
Sig.		1.000	.122	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

S. farinose

Duncan[a]

Treatment	N	Subset for alpha = .05		
		1	2	3
1.00	3	20.0667		
2.00	3		34.2000	
3.00	3		37.1333	
4.00	3			57.8000
Sig.		1.000	.081	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

H2. Statistic analysis data by Oneway – ANOVA of shoot height in dipterocarp seedlings

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
D. obtusifolius	Between Groups	35.801	3	11.934	16.994	.001
	Within Groups	5.618	8	.702		
	Total	41.419	11			
S. siamensis	Between Groups	121.734	3	40.578	46.404	.000
	Within Groups	6.996	8	.874		
	Total	128.730	11			
D. intricatus	Between Groups	83.673	3	27.891	35.121	.000
	Within Groups	6.353	8	.794		
	Total	90.026	11			
S. roxbuighii	Between Groups	87.306	3	29.102	55.980	.000
	Within Groups	4.159	8	.520		
	Total	91.465	11			
H. odorata	Between Groups	942.632	3	314.211	832.917	.000
	Within Groups	3.018	8	.377		
	Total	945.650	11			
D. alatus	Between Groups	1286.439	3	428.813	741.795	.000
	Within Groups	4.625	8	.578		
	Total	1291.064	11			
S. farinose	Between Groups	998.256	3	332.752	157.170	.000
	Within Groups	16.937	8	2.117		
	Total	1015.193	11			

Post Hoc Tests

Homogeneous Subsets

D. obtusifolius

Duncan[a]

Treatment	N	Subset for alpha = .05	
		1	2
1.00	3	17.7300	
4.00	3	18.8733	
3.00	3		21.2533
2.00	3		21.9867
Sig.		.133	.315

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

S. siamensis

Duncan[a]

TM	N	Subset for alpha = .05		
		1	2	3
1.00	3	17.8100		
4.00	3		21.3267	
2.00	3			25.2967
3.00	3			25.5633
Sig.		1.000	1.000	.736

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

D. intricatus

Duncan[a]

Treatment	N	Subset for alpha = .05		
		1	2	3
1.00	3	18.4233		
4.00	3		21.4967	
2.00	3			24.4433
3.00	3			25.0633
Sig.		1.000	1.000	.419

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

S. roxbuighii

Duncan[a]

Treatment	N	Subset for alpha = .05		
		1	2	3
1.00	3	21.1633		
2.00	3		23.5000	
3.00	3		24.3133	
4.00	3			28.6200
Sig.		1.000	.204	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

H. odorata

Duncan[a]

Treatment	N	Subset for alpha = .05			
		1	2	3	4
1.00	3	48.9678			
2.00	3		62.2630		
3.00	3			66.8783	
4.00	3				73.0936
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

D. alatus

Duncan[a]

Treatment	N	Subset for alpha = .05			
		1	2	3	4
1.00	3	56.2533			
2.00	3		70.1733		
3.00	3			72.0900	
4.00	3				85.4733
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

S. farinose

Duncan[a]

Treatment	N	Subset for alpha = .05		
		1	2	3
1.00	3	54.5200		
3.00	3		65.6200	
2.00	3		66.4133	
4.00	3			80.2333
Sig.		1.000	.523	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

H3. Statistic analysis data by Oneway – ANOVA of shoot diameter in dipterocarp seedlings

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
D. obtusifolius	Between Groups	.038	3	.013	25.778	.000
	Within Groups	.004	8	.000		
	Total	.042	11			
S. siamensis	Between Groups	.062	3	.021	63.694	.000
	Within Groups	.003	8	.000		
	Total	.065	11			
D. intricatus	Between Groups	.073	3	.024	55.783	.000
	Within Groups	.003	8	.000		
	Total	.076	11			
S. roxbuighii	Between Groups	.068	3	.023	21.399	.000
	Within Groups	.008	8	.001		
	Total	.077	11			
H. odorata	Between Groups	.301	3	.100	162.161	.000
	Within Groups	.005	8	.001		
	Total	.306	11			
D. alatus	Between Groups	.411	3	.137	454.084	.000
	Within Groups	.002	8	.000		
	Total	.413	11			
S. farinose	Between Groups	.230	3	.077	104.584	.000
	Within Groups	.006	8	.001		
	Total	.236	11			

Post Hoc Tests

Homogeneous Subsets

D. obtusifolius

Duncan[a]

		Subset for alpha = .05	
Treatment	N	1	2
1.00	3	.6360	
4.00	3	.6653	
2.00	3		.7407
3.00	3		.7757
Sig.		.143	.088

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

S. siamensis

Duncan[a]

Treatment	N	Subset for alpha = .05		
		1	2	3
1.00	3	.5407		
4.00	3		.6147	
2.00	3			.7017
3.00	3			.7203
Sig.		1.000	1.000	.240

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

D. intricatus

Duncan[a]

Treatment	N	Subset for alpha = .05		
		1	2	3
1.00	3	.5943		
4.00	3		.6533	
3.00	3			.7630
2.00	3			.7830
Sig.		1.000	1.000	.273

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

S. roxbuighii

Duncan[a]

Treatment	N	Subset for alpha = .05		
		1	2	3
1.00	3	.5840		
2.00	3		.6607	
3.00	3		.6897	
4.00	3			.7943
Sig.		1.000	.308	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

H. odorata

Duncan[a]

Treatment	N	Subset for alpha = .05			
		1	2	3	4
1.00	3	.8760			
2.00	3		1.0620		
3.00	3			1.1497	
4.00	3				1.3150
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

D. alatus

Duncan[a]

TM	N	Subset for alpha = .05			
		1	2	3	4
1.00	3	1.3493			
2.00	3		1.5660		
3.00	3			1.7613	
4.00	3				1.8220
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

S. farinose

Duncan[a]

Treatment	N	Subset for alpha = .05			
		1	2	3	4
1.00	3	1.4087			
3.00	3		1.5167		
2.00	3			1.5840	
4.00	3				1.7883
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

H4. Statistic analysis data by Oneway – ANOVA of shoot dry weight in dipterocarp seedlings

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
D. obtusifolius	Between Groups	2.428	3	.809	28.845	.000
	Within Groups	.224	8	.028		
	Total	2.653	11			
S. siamensis	Between Groups	15.666	3	5.222	304.719	.000
	Within Groups	.137	8	.017		
	Total	15.803	11			
D. intricatus	Between Groups	3.023	3	1.008	45.999	.000
	Within Groups	.175	8	.022		
	Total	3.199	11			
S. roxbuighii	Between Groups	29.732	3	9.911	456.363	.000
	Within Groups	.174	8	.022		
	Total	29.906	11			
H. odorata	Between Groups	67.599	3	22.533	119.670	.000
	Within Groups	1.506	8	.188		
	Total	69.105	11			
D. alatus	Between Groups	250.100	3	83.367	71.133	.000
	Within Groups	9.376	8	1.172		
	Total	259.476	11			
S. farinose	Between Groups	193.579	3	64.526	80.849	.000
	Within Groups	6.385	8	.798		
	Total	199.963	11			

Post Hoc Tests

Homogeneous Subsets

D. obtusifolius

Duncan[a]

		Subset for alpha = .05		
Treatment	N	1	2	3
1.00	3	4.2533		
4.00	3	4.3653		
3.00	3		4.9933	
2.00	3			5.3467
Sig.		.437	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

S. siamensis

Duncan[a]

Treatment	N	Subset for alpha = .05			
		1	2	3	4
1.00	3	4.3680			
4.00	3		4.7653		
2.00	3			6.3247	
3.00	3				7.1800
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

D. intricatus

Duncan[a]

Treatment	N	Subset for alpha = .05		
		1	2	3
1.00	3	3.5780		
4.00	3	3.8373	3.8373	
2.00	3		3.9573	
3.00	3			4.9060
Sig.		.064	.350	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

S. roxbuighii

Duncan[a]

TM	N	Subset for alpha = .05			
		1	2	3	4
1.00	3	6.9633			
2.00	3		7.8700		
3.00	3			8.3933	
4.00	3				11.1800
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

H. odorata

Duncan[a]

Treatment	N	Subset for alpha = .05			
		1	2	3	4
1.00	3	10.6180			
2.00	3		13.7100		
3.00	3			14.7296	
4.00	3				17.2405
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

D. alatus

Duncan[a]

Treatment	N	Subset for alpha = .05			
		1	2	3	4
1.00	3	22.7247			
2.00	3		28.4733		
3.00	3			30.7340	
4.00	3				35.4153
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

S. farinose

Duncan[a]

Treatment	N	Subset for alpha = .05		
		1	2	3
1.00	3	22.2953		
2.00	3		25.8973	
3.00	3		26.2787	
4.00	3			33.3767
Sig.		1.000	.615	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

BIOGRAPHY

Miss Sunadda Yomyart was born on November 3, 1977 in Chanthaburi province, Thailand. She graduated with Bachelor Degree of Science in Microbiology (1999) and Master Degree of Science in Industrial Microbiology (2003), Department of Microbiology, Faculty of Science, Chulalongkorn University, Thailand in 2003. After graduation M. Sc., she continued her Ph.D. in Biotechnology, Program in Biotechnology Faculty of Science, Chulalongkorn University (2003). Throughout her Ph.D. study, she had received the financial support from the Royal Golden Jubilee for Ph.D. Scholarship and partial financial support from the Graduate School, Chulalongkorn University.

Academic Presentation

1. Yomyart, S., Piapukiew, J., Watling, R., Wu, B., Hogetsu, T., and Sihanonth, P. 2007. Community structure of ectomycorrhizal fungi in a dipterocarp forest in Thailand. International Symposium on Microbial Ecology, ISME Asia 2007. 15-17 September. Matsuyama, Japan. (Poster)
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Publication

1. Yomyart, S., Piapukiew, J., Watling, R., Whalley, A. J. S., and Sihanonth, P. 2006. *Russula siamensis*: a new species of anulate *Russula* from Thailand. Mycotaxon 95:247-254.