THE IMPORTANCE OF VESICULAR-ARBUSCULAR MYCORRHIZAE (VAM) IN DECIDUOUS TROPICAL FOREST ECOSYSTEMS AT DOI SUTHEP-PUI NATIONAL PARK.

ABDUL MANAN

A THESIS SUBMITTED TO THE GRADUATE SCHOOL IN
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Thesis Title The Importance of Vesicular-Arbuscular Mycorrhizae (VAM) in Deciduous Tropical Forest Ecosystems at Doi Suthep-Pui National Park

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Abstract

A study was carried out to determine the prevalence of vesicular-arbuscular mycorrhizal infection amongst leguminous trees in Doi Suthep-Pui National Park and to investigate the effects of VAM on germination and growth rate of one of these species, *Albizia odoratissima*.

Ten tree species of the family Leguminoseae were selected for determination of VAM association. Three soil samples from around three adult trees of each species were collected for determination of VAM spore density using the wet sieving and decantation method and 6 seedlings of each species were collected for determination of infection rate using a staining technique. *Erythrina subumbrans* was selected at different altitudes to examine the relationship between altitude and VAM. Soil moisture, pH, field capacity and nutrients were analyzed to relate VAM abundance with soil properties. In addition, slope, canopy cover and micro-habitat characteristics were recorded to relate VAM association with environmental parameters. A pot experiment was undertaken with the following treatments: Ao (sterilized soil without *Glomus microcarpus* inoculum); A1 (sterilized soil

with 5g *G. microcarpus* inoculum/kg soil); A2 (sterilized soil with 10g *G. microcarpus* inoculum/kg soil) and A3 (sterilized soil with 15g *G. microcarpus* inoculum/kg soil). For each treatment, 100 seeds of *Albizia odoratissima* were germinated to evaluate the effect of *G. microcarpus* on germination rate and to evaluate VAM's effect on the growth of *Albizia odoratissima*. The experiment had a randomized complete block design with 4 replications.

All tree species were associated with VAM. The higher the elevation, the fewer VAM spores were observed which ranged from 104.11 - 169.67 per 50 g soil. The number of VAM spores was associated with environmental parameters and soil properties and the strongest correlated factor was soil pH (r=0.460). Both soil moisture and soil pH were positively correlated with infection rate of VAM in seedlings roots. *G. microcarpus* had no significant effect on germination rate of *Albizia odoratissima*. *G. microcarpus* inoculation significantly increased seedling growth 1, 2 and 3 months after inoculation ($p = 0.05$). In addition, VAM also significantly increased seedling dry weight ($p = 0.05$) and the highest increase was obtained with 15 g VAM inoculum/kg soil (A3). Thus, VAM can improve the growth of seedlings of *A. odoratissima*. Overall, it can be concluded that VAM could play an important role on the growth and survival of trees in tropical deciduous forest ecosystems.

ชื่อเรื่องวิทยานิพนธ์ ความสำคัญของเวสสิคูลา อาบัสคูลา ไมคอไรซา ในระบบนิเวศแบบป่าผลัดใบเขตร้อนบริเวณอุทยานแห่งชาติดอยสุเทพ-ปุย

ชื่อผู้เขียน นายอับดุล มานัน

วิทยาศาสตรมหาบัณฑิต สาขาวิชาการประเมินความเสี่ยงทางด้านสิ่งแวดล้อมในระบบนิเวศเขตร้อน

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บทคัดย่อ

การศึกษาเพื่อหาการเข้าสู่รากพืชของเวสสิคูลา อาบัสคูลา ไมคอไรซาในรากต้นไม้ตระกูล Leguminoseae บริเวณอุทยานแห่งชาติสุเทพ-ปุย และศึกษาผลของวีเอไมคอไรซาที่มีต่อการงอกและอัตรา การเติบโตของพืชชนิด Albizia odoratissima

ได้เลือกพืชในตระกูล Leguminoseae มา 10 ชนิด เพื่อศึกษาถึงความสัมพันธ์กับวีเอไมคอไรซา โดยเก็บตัวอย่างดินรอบๆรากต้นไม้แต่ละชนิดมาหาปริมาณความหนาแน่นของสปอร์ของวีเอไมคอไรซา โดยใช้วิธีการร่อนดินแบบเปียกและเก็บต้นกล้าของพืชแต่ละชนิดๆละ 6 ต้นมาย้อมสีรากเพื่อหาอัตราการเข้าสู่รากพืชของวีเอไมคอไรซา เลือกพืชชนิด Erythrina subumbrans เพื่อหาความสัมพันธ์ระหว่างความสูงของพื้นที่จากระดับน้ำทะเลกับวีเอไมคอไรซา วิเคราะห์ความชื้นของดิน ค่า pH ค่า field capacity และปริมาณธาตุอาหารในดินเพื่อศึกษาความสัมพันธ์ระหว่างวีเอไมคอไรซากับคุณสมบัติของดิน นอกจากนั้นยังได้บันทึกถึงค่า slope ค่าทรงพุ่มของต้นไม้และคุณสมบัติของ micro-habitat เพื่อหาความสัมพันธ์ระหว่างวีเอไมคอไรซากับปัจจัยในสิ่งแวดล้อม ทำการทดลองปลูกพืชชนิด Albizia odoratissima ในกระถางโดยแบ่งชุดการทดลองออกดังนี้ A_0 (ปลูกในดินที่ฆ่าเชื้อไม่ใส่สปอร์ของ Glomus microcarpus) ; A_1 (ปลูกในดินฆ่าเชื้อและใส่ดินที่มีสปอร์ของ G. microcarpus หนัก 5 กรัม ต่อดินที่ปลูกหนัก 1

กิโลกรัม); A_2 (ปลูกในดินฆ่าเชื้อและใส่ดินที่มีสปอร์ของ G. microcarpus หนัก 10 กรัมต่อดินที่ปลูกหนัก 1 กิโลกรัม) และ A_3 (ปลูกในดินฆ่าเชื้อและใส่ดินที่มีสปอร์ของ G. microcarpus หนัก 15 กรัม ต่อดินที่ปลูกหนัก 10 กรัม ในแต่ละชุดการทดลองใช้เมล็ดของ Albizia odoratissima จำนวน 100 เมล็ด ในการปลูกเพื่อประเมินผลของ G. microcarpus ต่ออัตราการงอกและต่อการเติบโตของพืชชนิดนี้ โดยวางแผนการทดลองเป็นแบบ ramdomized complete block design ทำ 4 ซ้ำ

ผลการศึกษาพบว่า ต้นไม้ทุกชนิดที่ศึกษามีวีเอไมคอไรซาอยู่ร่วมกับรากพืชด้วย ที่ระดับความสูงของพื้นที่จากระดับน้ำทะเลที่สูงขึ้นพบว่า จำนวนสปอร์ของวีเอไมคอไรซามีน้อยลง นับจำนวนสปอร์ได้อยู่ในช่วง 104.11-169.67 ต่อดิน 50 กรัม พบว่าจำนวนสปอร์ของวีเอไมคอ-ไรซามีความสัมพันธ์กับปัจจัยต่างๆในสิ่งแวดล้อม และคุณสมบัติของดินโดยเฉพาะ pH ของดิน ($r=0.460$) เป็นปัจจัยที่สำคัญและมีผลต่อจำนวนสปอร์มากที่สุดทั้งความชื้นในดินและ pH ของดินมีความสัมพันธ์ทางด้านบวกต่อการเข้าสู่รากพืชของต้นกล้า วีเอไมคอไรซาชนิด G. microcarpus มีผลต่ออัตราการงอกของ Albizia odoratissima อย่างไม่มีนัยสำคัญทางสถิติ การเพาะเชื้อวีเอไมคอไรซานี้มีผลช่วยเพิ่มการเติบโตของต้นกล้าที่อายุ 1, 2 และ 3 เดือน ภายหลังการเพาะเชื้ออย่างมีนัยสำคัญ ($p=0.05$) นอกจากนั้นวีเอไมคอไรซายังสามารถเพิ่มน้ำหนักแห้งของต้นกล้าอย่างมีนัยสำคัญ ($p=0.05$) การเพิ่มการเติบโตมีสูงสุดเมื่อเพาะด้วยเชื้อตั้งต้นหนัก 15 กรัม ต่อดิน 1 กิโลกรัม (A_3) จากการทดลองครั้งนี้สรุปได้ว่าวีเอไมคอไรซามีบทบาทสำคัญต่อการเติบโตและการอยู่รอดของต้นไม้ในระบบนิเวศแบบป่าผลัดใบเขตร้อน

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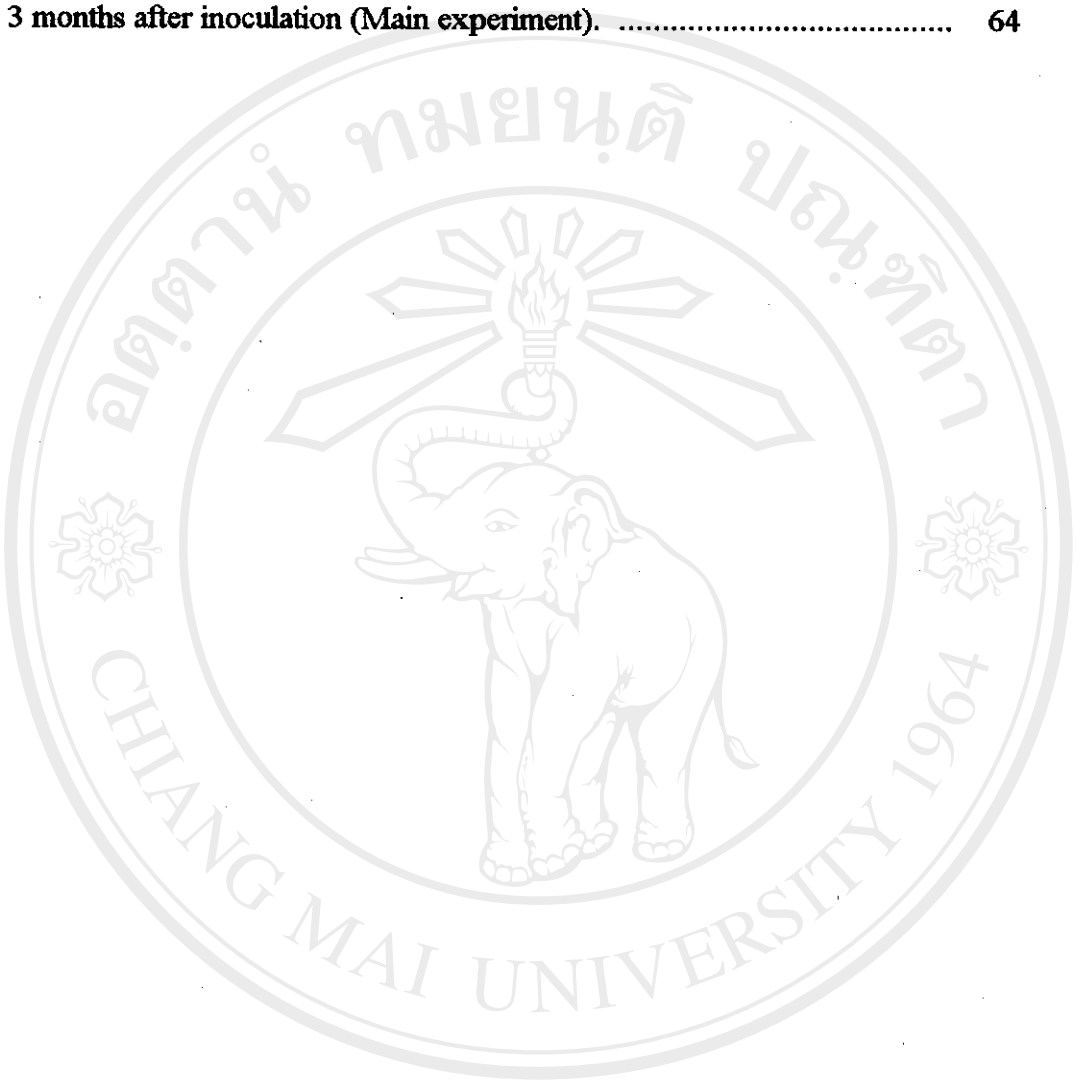

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1. Introduction

A mycorrhiza is a symbiotic association formed between the roots of a host plant and a fungus. By far the most common mycorrhizal association with plants in the tropics is the vesicular-arbuscular (VA) type, which produces fungal structures (vesicles and arbuscules) in the cortex region of roots (figure 1). Only few species form ectomycorrhizal associations (a fungal mantle of septate hyphae ensheathing the root and a hartig net composed of hyphae, which penetrates between and surrounds the cortical cells) (Powell and Bagyaraj, 1986).

It is now an established fact that in natural ecosystems most plants develop mycorrhizal associations. Some essential plant nutrients are transported through the mycorrhizal fungal network, rather than directly via root absorption. There is no doubt that the growth of many plants can be substantially improved if they possess a well-developed mycorrhizal system (Jeffries and Dodd, 1991).

Plant species with low root densities and poorly developed root hairs, such as leguminous trees, respond to mycorrhizal colonization over a greater range of soil fertility than do plants with dense root systems like grasses (Baylis, 1975). Some plant species are obligately mycorrhizal and cannot grow well without mycorrhizal fungi e.g *Araucaria* sp (Bevege, 1970).

Mycorrhizal associations mainly help plants enhance the rate of nutrient absorption by increasing the effective exploratory surface area of the root system, in addition to affecting plants' physiological activity and extending the nutrient depletion zone further

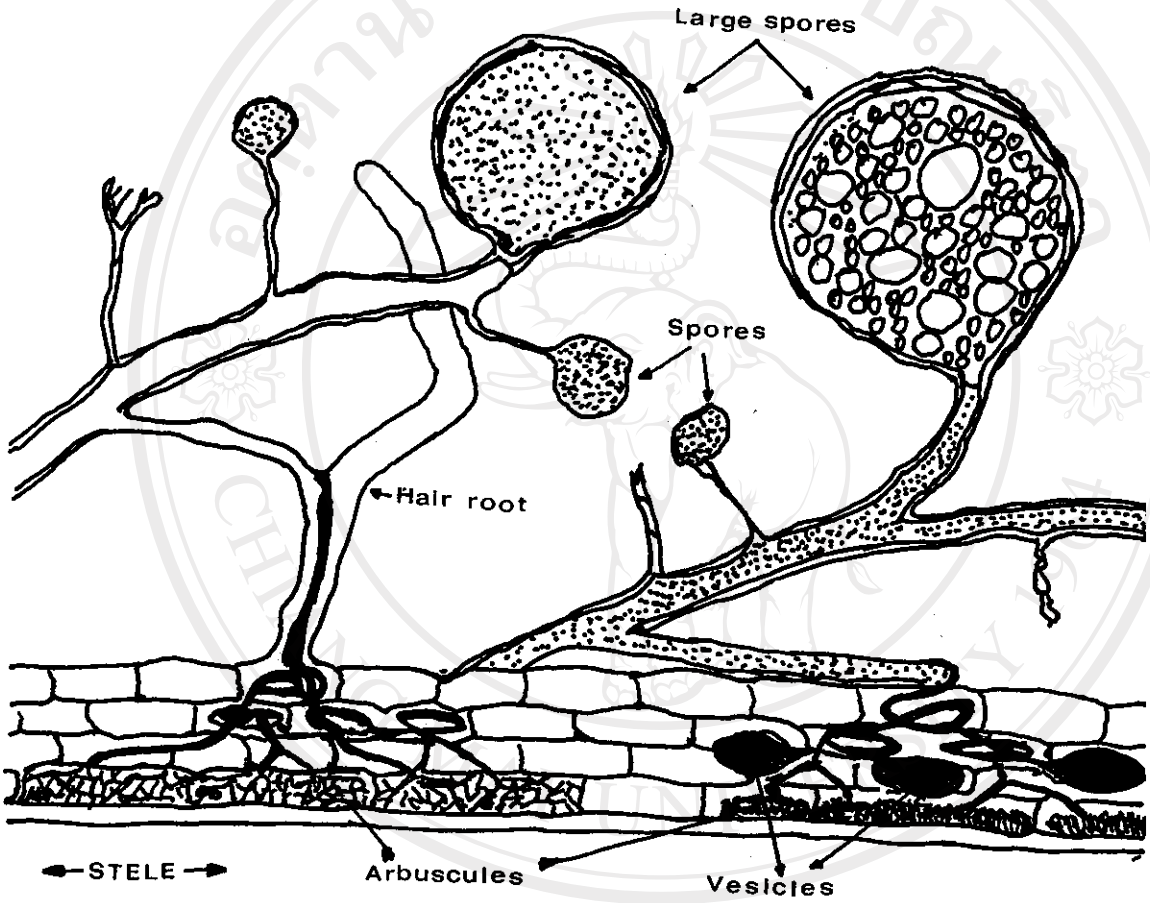


Figure 1. Diagram of vesicular-arbuscular mycorrhizae and its relationship with roots (Mosse, 1981).

from the root surface (Dighton, 1991, Fitter, 1991, and Hetrick, 1991). In addition, mycorrhizal fungi enhance the plant roots' ability to dissolve mineral nutrients from the soil solution before such nutrients can either be immobilized into the tissues of other organisms or are leached down the soil profile beyond the reach of root systems. Mycorrhizal plants have greater tolerance to drought, toxic metals, saline soil and root pathogens, than non-mycorrhizal plants (Caldwell and Virginia, 1989, Harley and Smith, 1983). Other benefits may also be associated with this symbiosis.

Interestingly, mycorrhizal fungi offer an environmentally sound, biological alternative to chemical fertilizers and pesticides for maintaining plant quality and productivity in agriculture, horticulture and forestry (Wood, 1992). The formation of mycorrhizae represent a special adaptation to surviving in unsuitable conditions (Allen, 1981). They may therefore become increasingly important in a changing global climate (Dixon, 1992).

It is well-known that myrorrhizae, especially the vesicular-arbuscular type, play an important role in tropical rain forest ecosystems and that they increase the fitness of tropical rain forest trees. Very few studies have been done on forest soils and forest plants with respect to vesicular-arbuscular mycorrhiza in deciduous tropical forest. Hence, this study attempted to determine whether mycorrhizae may be similarly important in tropical deciduous forest.

Thailand has a fairly large expanse of deciduous tropical forest, part of which is located in Doi Suthep-Pui National Park. There are two basic kinds of forest in the National Park; deciduous forest (from the lowlands up to about 950 m above sea level) and

evergreen forest (from about 950 m above sea level up to the summit of Doi Pui, 1,685 m above sea level). The vegetation of Doi Suthep-Pui National Park has been well described (Maxwell, 1988) and the most species-rich plant family found there is the Leguminoseae.

Although many studies have been carried out in this area, most of them relate to the diversity of plants (e.g. Elliott, *et al.*, 1989).

There is a growing concern in Thailand about the serious degradation of the forests and the significant reduction in the area of forest land. One remedy for this is the establishment of large-scale forest plantations (Khemnark,1980) and in this case mycorrhizae could play an important role by increasing the survival of trees. They could also help to produce seedlings of a wide variety of species for regeneration of natural forest ecosystems. Inoculation of pine roots with mycorrhizal fungi for forest plantations in Chiang Mai increases survival and growth rate of seedlings (Khemnark, 1980).

The aim of the study reported here was to determine the prevalence of vesicular-arbuscular mycorrhizal infection amongst leguminous trees in Doi Suthep-Pui National Park and to investigate the effects of vesicular-arbuscular mycorrhiza on the germination and growth rate of one of these species, *Albizia odoratissima*.

2. Review of Literature

Mycorrhiza literally means "fungus root" and by far the most common mycorrhizal association is the vesicular-arbuscular (VA) type (figure 1). It has been found in most plant families so far examined (Powell and Bagyaraj, 1986). VAM occur over a broad ecological range from aquatic to desert environments (Mosse, 1981) and have a widespread distribution geographically from the arctic and temperate regions to the tropics (Powell and Bagyaraj, 1986).

Interest in the mycorrhizal associations of tropical plants began almost one hundred years ago, when Treub (1885) recorded the VAM association with sugar cane in Java. The first extensive survey of the occurrence of VAM in tropical plants was carried out in 1896 by Jansen in Java. He found that 69 of 75 species examined, including bryophytes, vascular cryptogams, gymnosperm, monocotyledons and woody dicotyledons had VAM associations. Next, an extensive survey of VAM association in the tropics was carried out in 1949 by Johnston. He examined 93 species, including 13 species of forest tree and observed that 80, including all the forest trees, had VAM associations (Janos, 1981).

Recent studies have also been conducted in several tropical countries, such as India where VAM were found associated with the forest trees such as *Tectona* spp., *Terminalia* spp. and *Dalbergia* spp. and inoculating them with *Glomus* sp. increased growth and total biomass (Manoharachary and Rao, 1991). In addition, 53 tree species were screened, of which 42 were found associated with VAM and the infection of roots

ranged from 27 to 90 % (Nagarajan *et al.*, 1991). Bhattarai (1991) reported that the population density of VAM in a natural forest ecosystem of *Corchorus capsularis* ranged from 24-142 spores in 10 g soil with an infection rate ranging from 23-70 %. In addition, Porter (1979) reported that using the wet sieving technique, Ulva soil of Australia contained 95 spores per 50 g soil. Several studies have been conducted in Thailand, most of them related to agricultural activities. Only few studies have been related to forestry, such as the association of mycorrhiza with pine. Toyporn and Rangsichol (1991) reported that the number of VAM spores in upland rice in northern Thailand ranged from 0-174 spores in 100 g soil with an infection rate ranging from 0-83 %. In addition, Plikomol *et al.* (1991) identified the VAM in 43 soil samples from various areas in Chiang Mai Province and found nine *Glomus* spp. three *Gigaspora* spp. and two *Sclerocystis* spp. However, they didn't record the tree species with which the VAM were associated. The almost universal occurrence of the VAM association in the tropics has also been confirmed in the Philippines (Tupas and Sajise, 1976).

Almost all plants including the Graminae and Leguminoseae are capable of forming VAM with fungi from a single zygomycetous family, the Endogenaceae (Gerdemann, 1968). At present, over 107 species of fungi forming vesicular-arbuscular have been described in four genera (Gerdemann and Trappe, 1974). Many are cosmopolitan, but some may be strictly tropical, e.g. *Acalauspora foveata* and *A. tuberculata* (Janos, 1981). The vesicular-arbuscular endophytes are not host specific, although evidence is growing that certain endophytes may form preferential association with certain host plants (Mosse, 1981). John (1980) listed 64 VAM species, including many of economic importance from several habitats in Brazil.

It is by now largerly agreed that infection with VAM fungi increases growth in higher plants and that the main mechanism whereby this is achieved is an increase in the supply of phosphorus (Sanders *et al.*, 1975). This is because uptake of phosphate by plants is usually limited by the rate of movement of phosphates to the plant root, rather than by the rate of absorption at the root surface (Nye, 1977). In soils with high capacities to adsorb phosphate, phosphate concentration in the soil solution is extremely low and diffusion to the plant roots is very slow (Powell and Bagyaraj, 1986). These are the soils in which it is likely that the greatest benefits will be gained from VAM (Abbott and Robson, 1986).

There have been several claims that mycorrhizae have an ability to exploit nonlabile forms of soil phosphate such as tricalcium phosphate and rock phosphate (Smith, 1980).

It is likely that VAM fungi also increase the uptake of other nutrients that move to plant roots primarily by diffusion. Indeed, inoculation with VAM fungi alleviates zinc (La Rue *et al.*,1975) and copper deficiencies (Timmer and Leyden, 1978) in peach and citrus seedlings.

Cooper (1986) reported that investigations of VAM associations have generally centered on responses of the host to fungal infection. Positive host growth responses occur frequently in soils of low nutrient status and this effect is usually attributed to enhance nutrient uptake by mycorrhizal roots (Mosse, 1981). Interestingly, VAM can improve drought tolerance of plants (Aldon, 1975). However, the mechanisms whereby mycorrhizal infection might increase drought resistance or improve water flow through the plant are still unclear (Cooper, 1986). In addition, Powell and Bagyaraj (1986) reported that VAM fungi

improve the water relations of many plants, including forest trees. Cooper (1986) also reported that an interesting feature of all mycorrhizal associations is that, in contrast with pathogenic fungi, the mycorrhizal fungi symbionts fail to activate the host's defence mechanisms on infection and as the result of the host's metabolic response to infection, there are alterations in chitinase activity and phenol metabolism and increased production of phenolic compounds, oxidative enzymes, phytosterols, cytokinins and amino acids. Furthermore, VAM infection increases the host's resistance to invading fungal and nematode root pathogens.

Mycorrhizal fungi may be able to influence host growth by the production of hormonal compounds (Letham *et al.*, 1978). Various auxins, cytokinins, gibberellin, and vitamins are produced by ectomycorrhiza fungi in pure culture (Slankis, 1973). In contrast, the possible production of growth-promoting compounds by VAM fungi has been little investigated and studies are limited by inability to grow the fungi in culture (Slankis, 1973). Allen *et al.*(1980) reported that VAM infection can substantially increase cytokinin activity in leaves and roots of mycorrhizal plants. These increases have been cited as a probable cause of the altered growth habits of mycorrhizal plants in a grazing environment (Wallace, 1981).

Experiments to demonstrate the effects of mycorrhizae on growth are usually conducted using pots of sterilized soil, but field (Mosse and Hayman 1980) and nursery (Mikola, 1980) studies have shown similar effects. VAM inoculation can improve plant growth in the field and timber tree seedling survival (Mosse and Hayman, 1980). Thus, mycorrhizae may have the ability to influence the ecological fitness of plant species in natural vegetation (Janos, 1981).

A series of pot experiments with seedlings of 32 species of lowland tropical plants found that VAM improved the growth of 28 species, including all mature forest tree species tested but that the species differed in their degree of dependence on VAM. Some were able to grow without mycorrhizae, although mycorrhizae improved their growth, whilst others could neither grow nor survive without mycorrhizae (Janos, 1981). In addition, Janos (1981) reported that dependence of plant species on mycorrhizae can influence the composition of mature plant communities. Humid tropical soils under native vegetation (Redhead, 1977) and some tree plantations (Nadarajah, 1980) usually contain few spores, although Waidyanatha (1980) found very many spores in rubber plantations.

VAM increased seedling survival of three strongly mycorrhiza-dependent tropical tree species in mixed plots of nine competing species that included non-mycorrhizal and facultatively mycotropic ones (Janos, 1981). The experiment also suggested that VAM reduce differences in competitive ability among all species.

Bowen (1980) reported that VAM are an important component of forest ecosystems, central not only to the growth and vigor of trees, but also to the maintenance of soil fertility and nutrient cycles.

The fungi forming vesicular-arbuscular mycorrhizae have the largest known resting spores of any fungi. These spores can be isolated from soil by the wet sieving and decantation method (Gerdemann and Nelson, 1963; Daniels and Skipper 1983). A suspension of 50 g soil in 200 ml of water is passed through different sized sieves and finally examined under a binocular stereo microscope.

A root system colonized by VAM does not show any morphological variations from a normal root system. Infection can only be determined after staining the roots with trypan blue (Phillips and Hayman, 1970). The presence of vesicles and arbuscles is the diagnostic criterion for identifying a VAM fungus in a root (Kormanic and Mc Graw ,1983).

3. Description of The Study Area

This study was conducted in Doi Suthep-Pui National Park just a few km west of Chiang Mai city in Northern Thailand at approximately 18°50'N laltitude, 99°0'E longitude. Rising to 1,685 m above sea level, the mountain is part of a geologically ancient ridge forming the western boundary of the Ping river valley. The bedrock of the mountain is almost entirely granitic. Shale is found in a few places in the southern part of the mountain (Maxwell, 1988). Soils are generally deep and highly weathered, ranging from coarse grey sands on ridges to red-brown loams in gullies (Elliott *et al.*, 1989). Annual rainfall varies considerably with elevation, ranging from about 1,000 mm near the base of the mountain to just over 2,000 mm near the summit (Elliott *et al.*, 1989). Rainfall is usually none during December and January and peaks at monthly average of 45 mm in August (Maxwell, 1988). The temperature recorded at Chiang Mai ranges from a monthly mean of 20°C in December to nearly 31°C in April. It should be noted that the temperature from 1,000 m elevation to the summit of Doi Suthep-Pui is considerably less than in Chiang Mai city. Monthly rain and temperature of Doi Suthep and Chiang Mai are presented in figs. 2 and 3 respectively.

The area was declared a National Park in 1981 covering 261 km^2. In recent decades, the mountain has been settled in several areas by hilltribe folk who have, unfortunately, destroyed large portions of the original forest cover. Sadly, the west side of the park has been either virtually destroyed or severely disturbed in part because the preserve has been allowed to accommodate tourist resorts, government agencies, agricultural research stations, television relay towers and at least 500 hilltribes families, whilst undisturbed forest survives only on the eastern side of the mountain. The map of

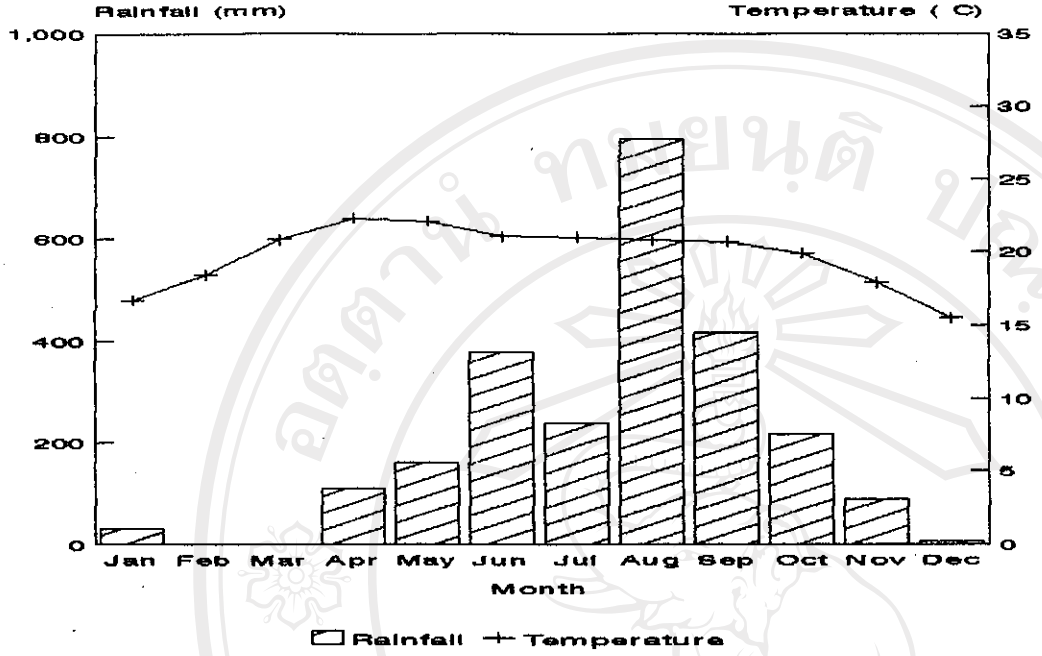


Fig.2. Mean monthly rainfall and temperature of Doi Suthep-Pui National Park.
(Recorded at Chiang Klan Station, 1400m ASL)

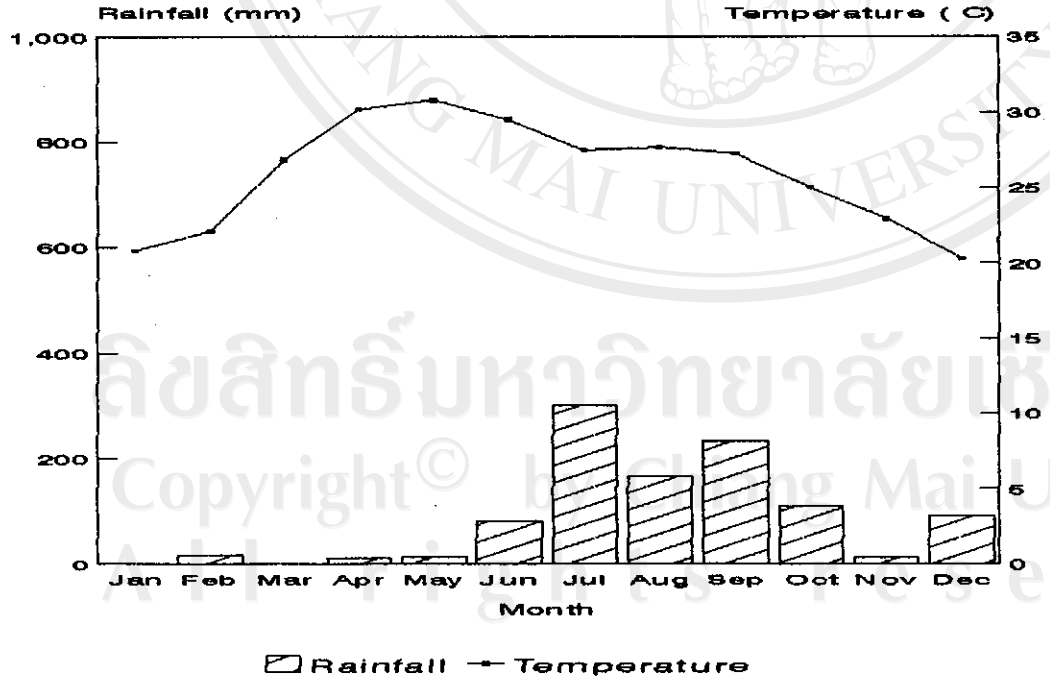


Figure 3. Mean monthly rainfall and temperature of Chiang Mai, 1992.
(Recorded at Airport, 350m ASL)

Doi Suthep-Pui National Park is presented in figure 4.

There are two basic kinds of forest in the park including deciduous forest (from the lowlands up to about 950 m above sea level) and evergreen forest (from about 950 m above sea level to the summit of Doi Pui, 1,685 m above sea level). In addition, there are deciduous forest associations, a deciduous dipterocarp oak association and a mix deciduous association.

Hosseus (1908) published the first account of the vegetation of Doi Suthep, followed by Kerr (1911) and Cockerell (1929). Later, Kuchler and Sawyer (1966) published a more thorough analysis of the vegetation of Doi Suthep, with a detailed vegetation map. They divided the forest into 10 different phytocenoses based on physical characteristics (e.g. life form, leaf shape etc.). Elliott *et al.* (1989) carried out a transect survey (0.828 ha) through monsoon forest and reported that deciduous forest on Doi Suthep contains more tree species than any other similar forest yet surveyed. There are 90 species per ha for trees of diameter at breast height of 10 cm or more and the most species-rich plant family found was the Leguminoseae. Maxwell (1988) provides the most recent and detailed description of the vegetation of Doi Suthep-Pui National Park.

Doi Suthep is not only an area exceptionally rich in species but it is also home to many endangered species and it is a study site for scientific research and education. At the moment, information about mycorrhiza in this area are still unavailable especially mycorrhizal associations with trees species in the two forest types of Doi Suthep-Pui National Park.

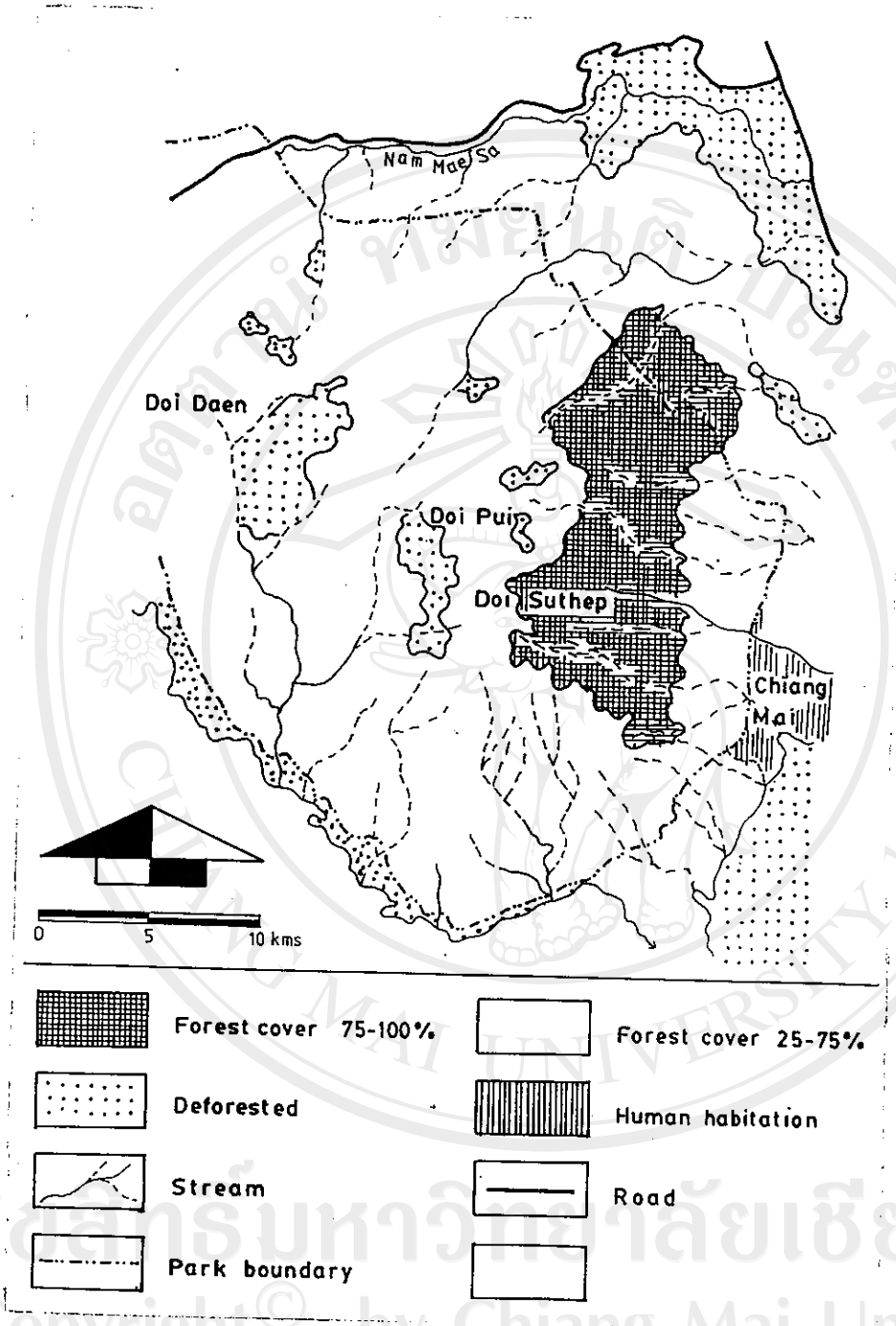


Figure 4. Map of Doi Suthep-Pui National Park, Chiang Mai Province (Round, 1984).

Reforestration efforts in the park have relied heavily on pine and *Eucalypthus* sp. (Round, 1984). Recent policy has changed and now there are some efforts to raise seedlings of a wide variety of tree species in nurseries, including some legumes, for forest restoration.

4. Materials and Equipments

1. Materials

a. 10 % KOH

b. 2 % HCl

c. 0.05 % Trypan blue lactophenol

d. Distilled water

e. Plastic bags

f. FAA (Formalin-Aceto-Alcohol)

g. Trays

h. Seeds of *Albizia odoratissima*

i. Spores of *Glomus microcarpus* from Department of Agriculture, Ministry of Agriculture and Cooperation, Thailand

2. Equipment

a. Binocular stereo microscope

b. Compound microscope

c. Balance

d. Pipette

e. Forceps

f. Sieve of 500 μm, 250 μm, 106 μm and 63 μm

g. Drill for soil samples

h. Tweezers

i. Stirrer

j. Pin (needle)

k. Erlenmeyer flask

l. Beaker glasses

m. Petri dish

n. Watch glasses

o. Flash bottles

p. Altimeter

q. Compass

r. Measuring tape

s. Calliper

5. Methods

A pilot study was conducted to assess the appropriate number of soil samples that should be collected from around each tree and the number of trees of each species that should be sampled. Eight soil samples were collected from each of 5 *Dalbergia fusca* trees at different places around the roots and examined. From this study it was concluded that three soil samples from each tree and three trees of each species constituted a sufficient sample because variability in spore counts between soil samples and between trees was very low (SD = 2.83 and 3.067 respectively) (see chapter 10.4. table 1 and 2).

Ten tree species of the family Leguminoseae were selected for determination of VAM association. The number of spores in soil collected from around the roots of adult trees was estimated using the modified wet sieving and decantation method (Gerdemann and Nicolson, 1963; Daniels and Skipper, 1983). A suspension of 50 g of soil was made and passed through sieves from a large mesh size (500 μm) to a smaller mesh size (63 μm). The liquid and very fine soil particles remaining after sieving were transferred to a petri dish and examined under a binocular stereo microscope to observe and count the spores. Identification of VAM was based on morphological characteristics including shape, color, size, structure and subtending hyphae. To count the spores, the liquid remaining after wet sieving was transferred to a watch glass from the petri dish with a drop pipet of 2 drops and diluted with distilled water. Then the spores were counted under a microscope. This technique was repeated until no more liquid remained. Both spores which floated and those which sank were counted. Most spores floated because their density was less than that of soil particles.

Of each species, 6 seedlings were collected for determination of VAM infection rate using a staining technique. Those seedlings were collected during rainy season (July-September) and the height of them ranged from 30-81 cm (CV= 27.72%). The fine roots of the seedlings were cleaned with tap water, placed in a glass beaker, covered with 10 % KOH solution for an hour at 90 °C in a well-ventilated exhaust hood and then placed in 2 % HCl for 2 minutes. After that the roots were washed with distilled water and boiled in 0.05 % trypan blue lactophenol solution for 3 minutes. The lactophenol solution was poured away and replaced with another 0.05 % trypan blue lactophenol solution. The specimens were soaked in this solution for 24 hours, after which the roots were examined under a binocular stereo microscope and compound microscope to observe the presence of vesicles and arbuscules.

To examine the relationship between habitat and VAM association, *Erythrina subumbrans* was selected as a species which grows at widely different altitudes from 360-1685 m above sea level and three trees were selected at each altitude.

Soil moisture, pH, field capacity and nutrients were analyzed to relate spore density and infection rate to soil properties. Soil was analysed using standard techniques at the central laboratory of the Faculty of Agriculture. In addition, slope, canopy cover and micro-habitat characteristics were recorded to relate spore density and infection rate with environmental parameters.

To determine the effects of VAM on the growth of leguminous trees, a pot experiment was conducted with *Albizia odoratissima*. Seeds were collected from the forest floor of Doi Suthep-Pui National Park and germinated in sterilized soil in the nursery

of the Biology Department and inoculated with *Glomus microcarpus*.

The arrangement of experiments was a complete random design with 4 treatments and 3 replications for a pilot experiment and 4 treatments and 4 replications for the main experiment. The treatments were as follows:

Ao = Sterilized soil without inoculum

A1 = Sterilized soil with 5 g VAM inoculum/kg soil

A2 = Sterilized soil with 10 g VAM inoculum/kg soil

A3 = Sterilized soil with 15 g VAM inoculum/kg soil

The parameters measured were :

a. The growth rate of seedlings, recorded monthly for 3 months.

b. The number of leaves, recorded monthly for 3 months.

c. The diameter of seedlings, recorded monthly for 3 months by callipers.

d. Infection rate was observed at the end of experiment.

f. Spore density in the soil was observed at the end of the experiment.

g. Dry weight of the seedlings was measured at the end of experiment.

To observe if VAM affected the germination rate, 100 seeds of *Albizia odoratissima* were used in each of the above treatments.

Analysis of the results were carried out as follows :

The number of spores was counted from the remaining sieved material from 50 g soil samples.

Infection rate of VAM was calculated by :

$$\frac{\text{Number of hair roots infected}}{\text{Total number of hair roots observed}} \times 100\%$$

6. Results

6.1. Number of Spores Around the Roots of Adult Trees

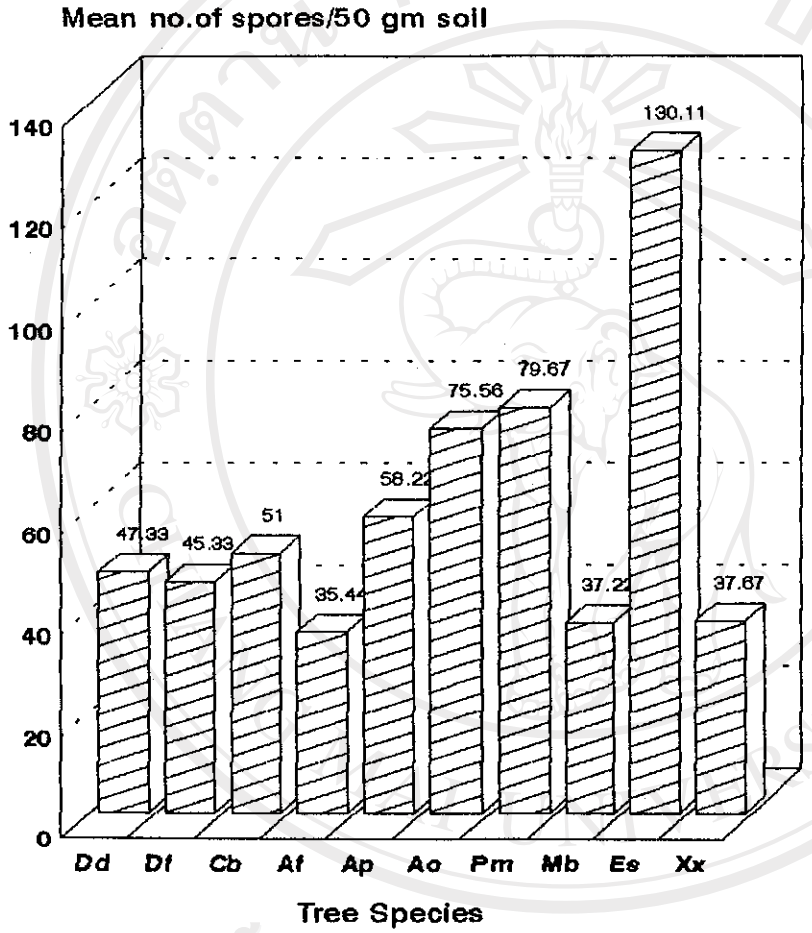
Figure 5, shows the VAM spore density in 50g soil collected from around the roots of 10 tree species of the family Leguminoseae. All selected tree species were associated with VAM and *Erythrina subumbrans* had the highest spore density (131.11 spores/50g) followed by *Pterocarpus macrocarpus* (79.67) and *Albizia odoratissima* (75.56), whilst the lowest was found in soil around roots of *Acrocarpus fraxinifolius*. Three common VAM were found including *Glomus* spp, *Gigaspora* spp and *Acalauspora* spp.

6.2. Infection Rate of Seedlings

The infection rates of seedlings of ten tree species are presented in figure 6. All species were infected. *Erythrina sumbumbrans* had the highest infection rate (59.03%) followed by *Albizia odoratissima* (53.29%) and *Pterocarpus macrocarpus* (51.06%) whilst the lowest infection was found in *Cassia bakeriana* (6.35%).

Least Significant Different (LSD) analysis revealed that infection rate in roots of seedlings showed significant differences amongst some of the species ($p < 0.05$) (table 1).

Fig.5. VAM spore density near adult trees means of 3 individuals/spp.



Abbreviations:

Dd=Dalbergia dongnaeinsis
Df= *Dalbergia fusca*
Cb=Cassia bakeriana
Af=Aorocarpus frexinifolius
Ap=Adenanthera pavonina
Ao=Albizia odoratissima
Pm=Pterocarpus macrocarpus
Mb=Millettia brandisiana
Es=Erythrina subumbrans
Xx=Xylia xylocarpa

Fig.6. Infection rate of VAM in seedling roots

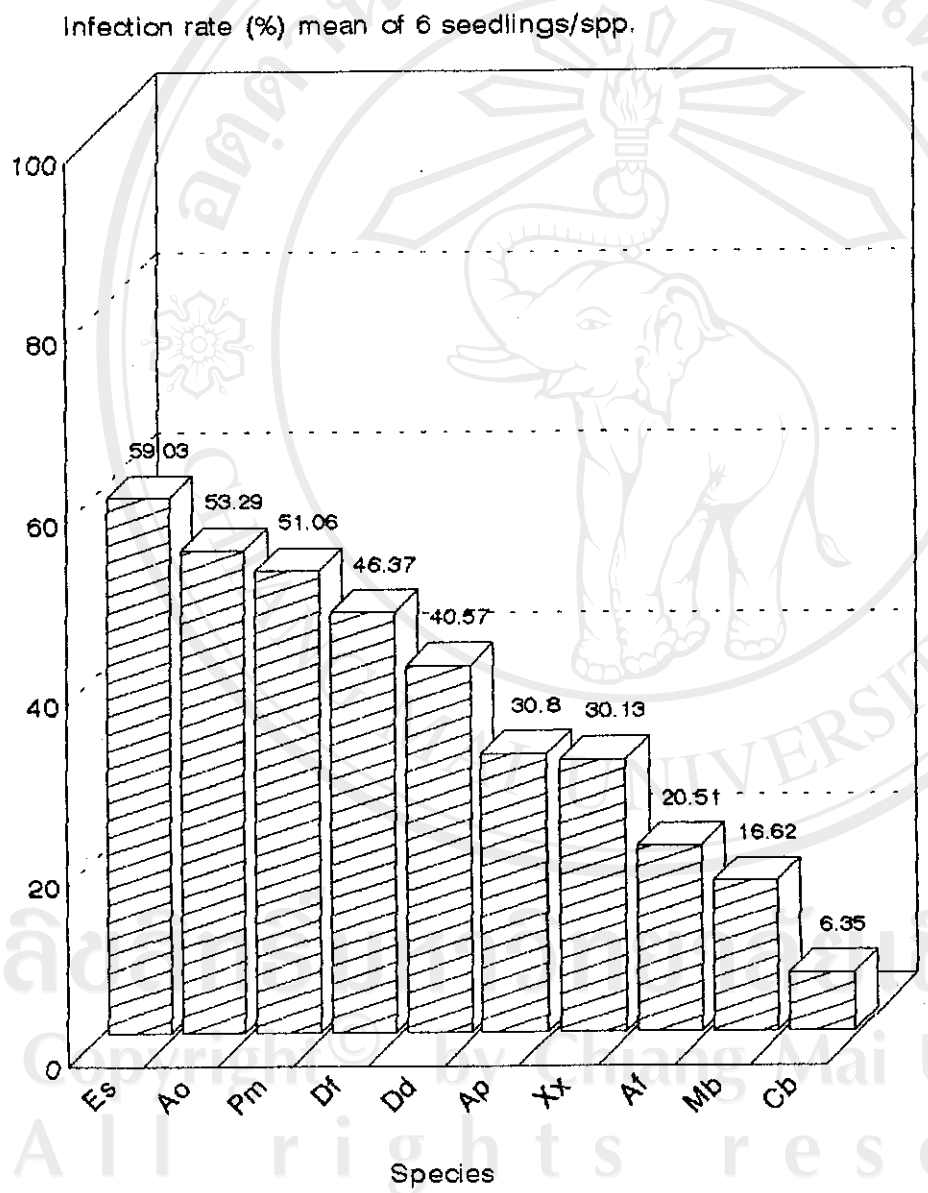


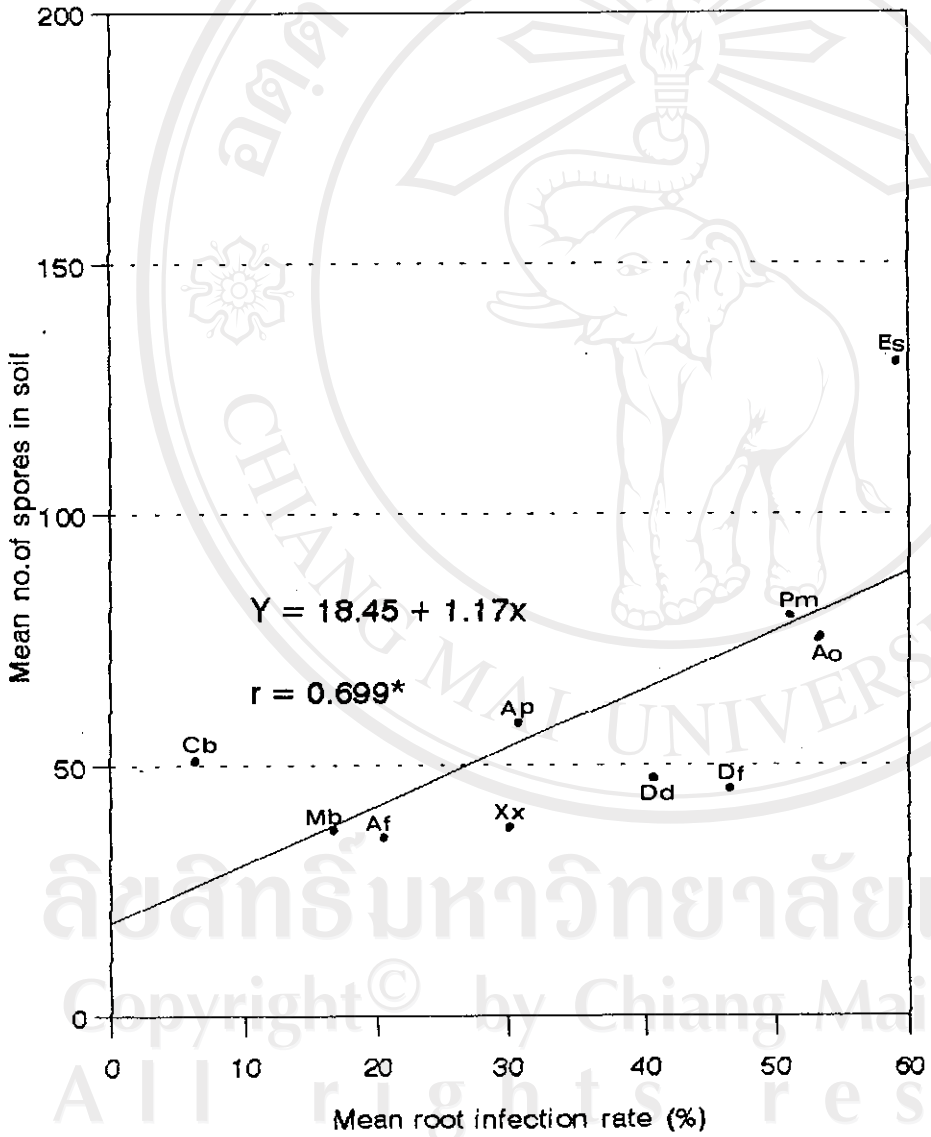
Table 1. Comparison of mean infection rate from 6 seedlings of 10 species using LSD.

Species	Mean of infection rate (%)	$LSD_{0.05}$
Erythrina subumbrans	59.03^{d}	
Albizia odoratissima	53.29^{cd}	
Pterocarpus macrocarpus	51.06^{cd}	
Dalbergia fusca	46.37^{cd}	
D. dongnaiensis	40.57^{cd}	23.19
Adenanthera pavonina	30.80^{bc}	
Xylia xylocarpa	30.13^{bc}	
Acrocarpus fraxinifolius	20.51^{ab}	
Millettia brandisiana	16.62^{ab}	
Cassia bakeriana	6.35^{a}	

Means followed by a common letter are not significantly different at the 5% level.

Infection rate of seedlings was positively correlated with spore density around adults (figure 7) with correlation coeficient (r) of 0.699 (p = 0.05).

Fig.7. Relationship between number of spore around adults and infection rate of seedlings



6.3. Relationships between Environmental Parameters and VAM Spore Density

VAM spore density tended to decrease with increasing slope. However, this relationship did not show linearity (figs. 8a-d).

Tree species which grew in deciduous forest tended to have increasing VAM spore density with increasing canopy cover (figs. 9a-c) and the influence of canopy cover on VAM spore density was greatest around *Pterocarpus macrocarpus* roots (figure 8b) than around the roots of other species. At higher altitudes, however, (evergreen forest) VAM spore density tended to decrease with increasing canopy cover (figure 9d). In addition, the spore density of VAM tended to increase in sites near dry stream beds in lowland deciduous forest. However, increases in spore density around the roots of both tree species were not high (figs.10a and 10b), whilst at higher altitudes (evergreen forest), spore density tended to decline with distance from streams as shown in figs.10c and 10d.

Moreover, at higher elevations lower VAM spore density was observed (figure 11). The number of spores at 350 m was statistically higher than that at 1000 m and 1350 m ($p = 0.05$), but it was not different compared with that at 550 m (table 2). The number of spores ranged from 104.11 - 169.67 per 50 g soil.

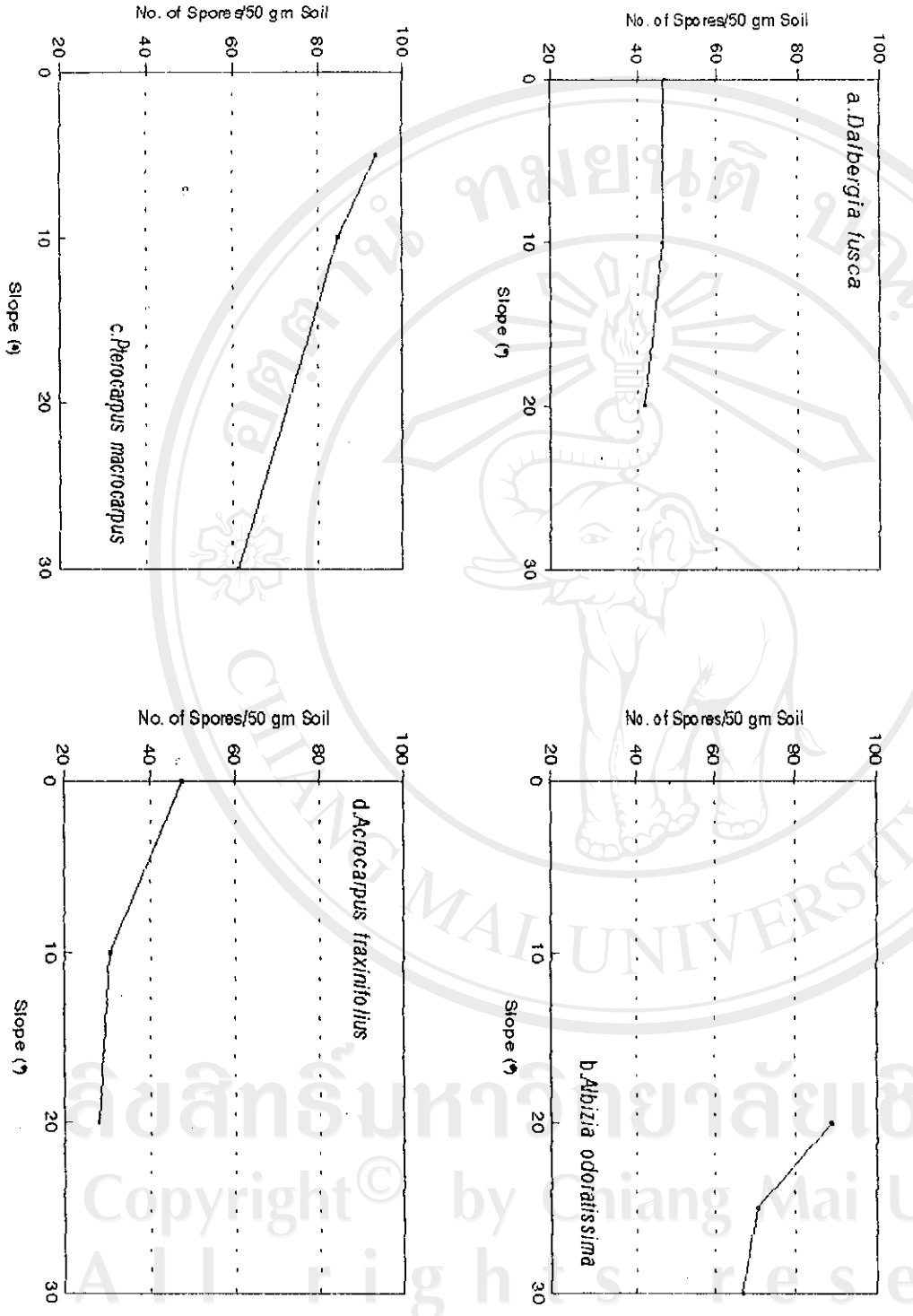


Fig.8. Relationship between slope and VAM spore density around adult tree roots.

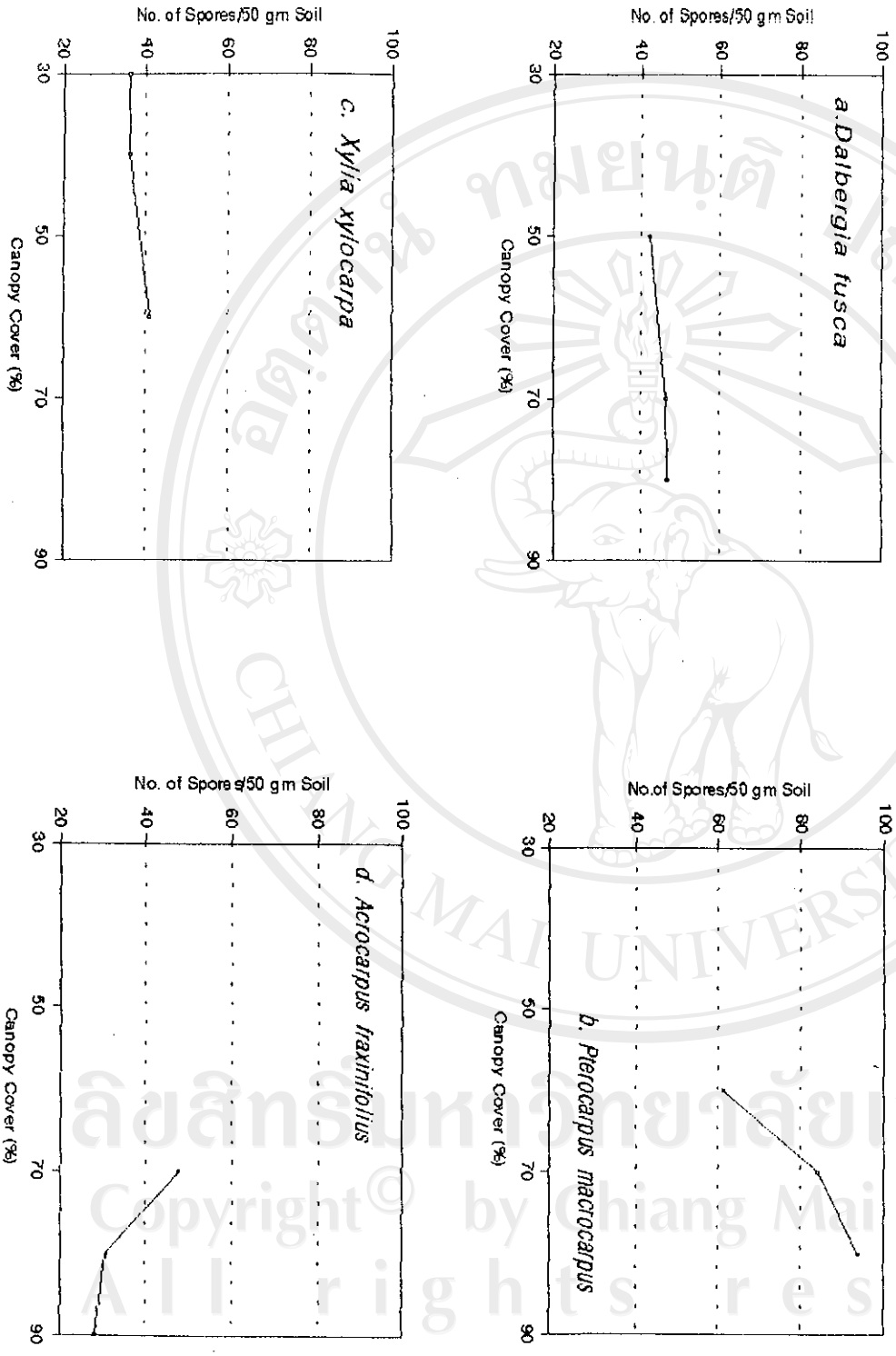


Fig.9. Influence of canopy cover on VAM spore density around adult tree roots.

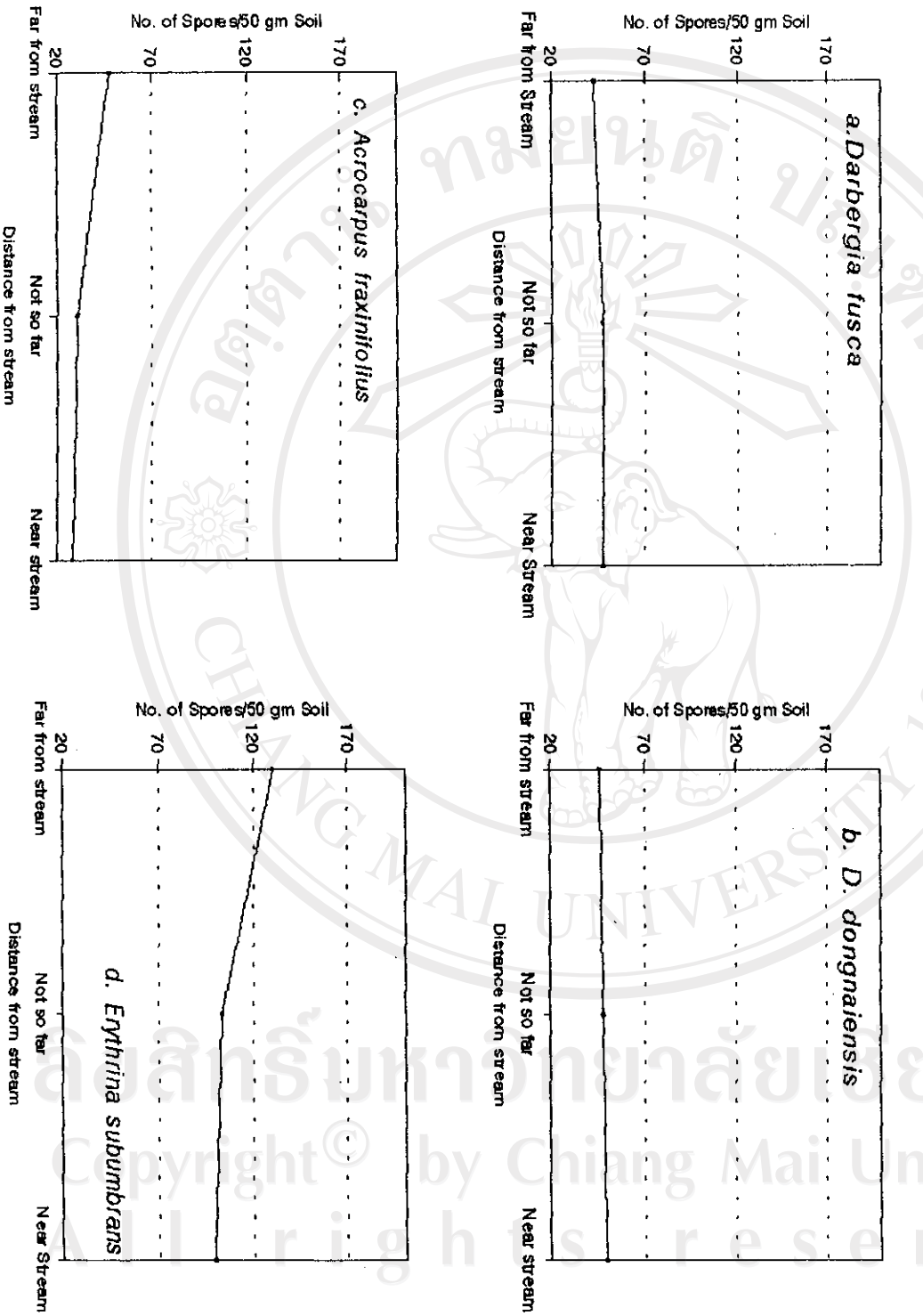


Fig.10. Relationship between distance from stream and VAM spore density around adult tree roots.

Figure 11. Relationship between altitude and VAM spore density around *Erythrina subumbrans* roots

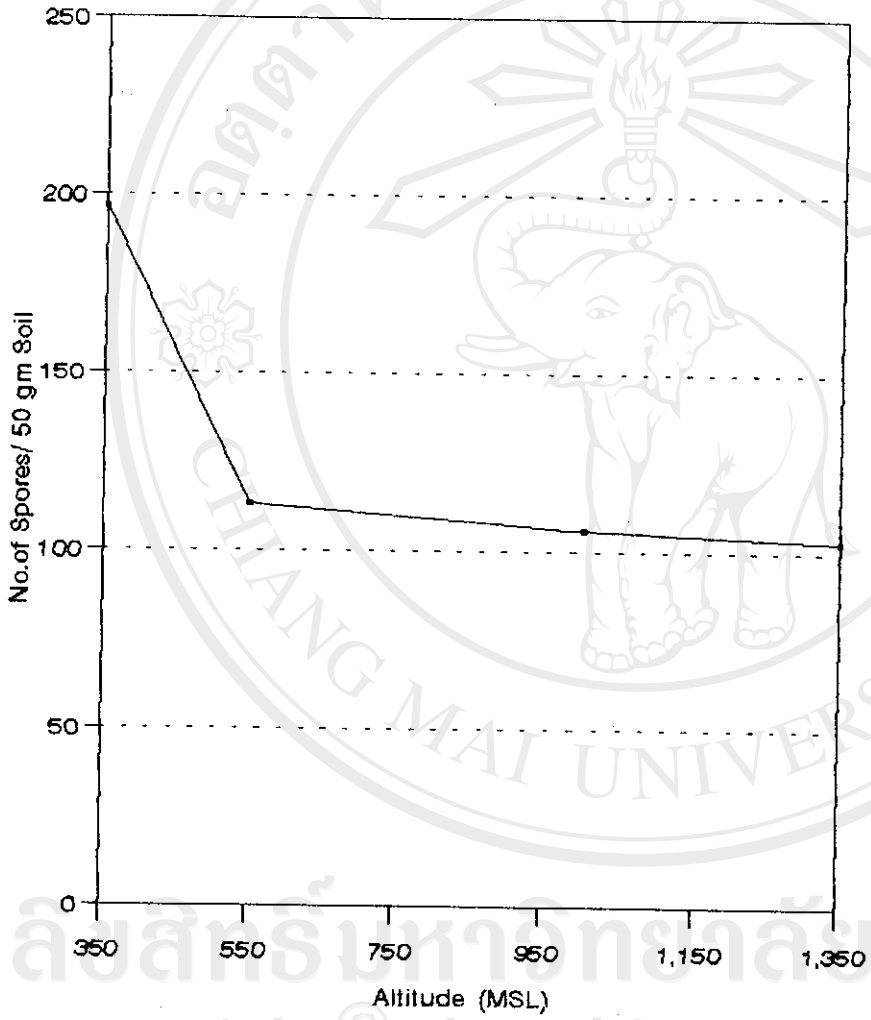


Table 2. Comparison of mean VAM spore density around *Erythrina sumbumbrans* roots at different altitudes using LSD.

Altitudes (MSL)	Mean of spore density* (spores)	$LSD_{0.05}$
350	169.67[b]	62.92
550	113.67[ab]	
1000	106.54[a]	
1350	104.11[a]	

Means followed by a common letter are not significantly different at the 5% level.

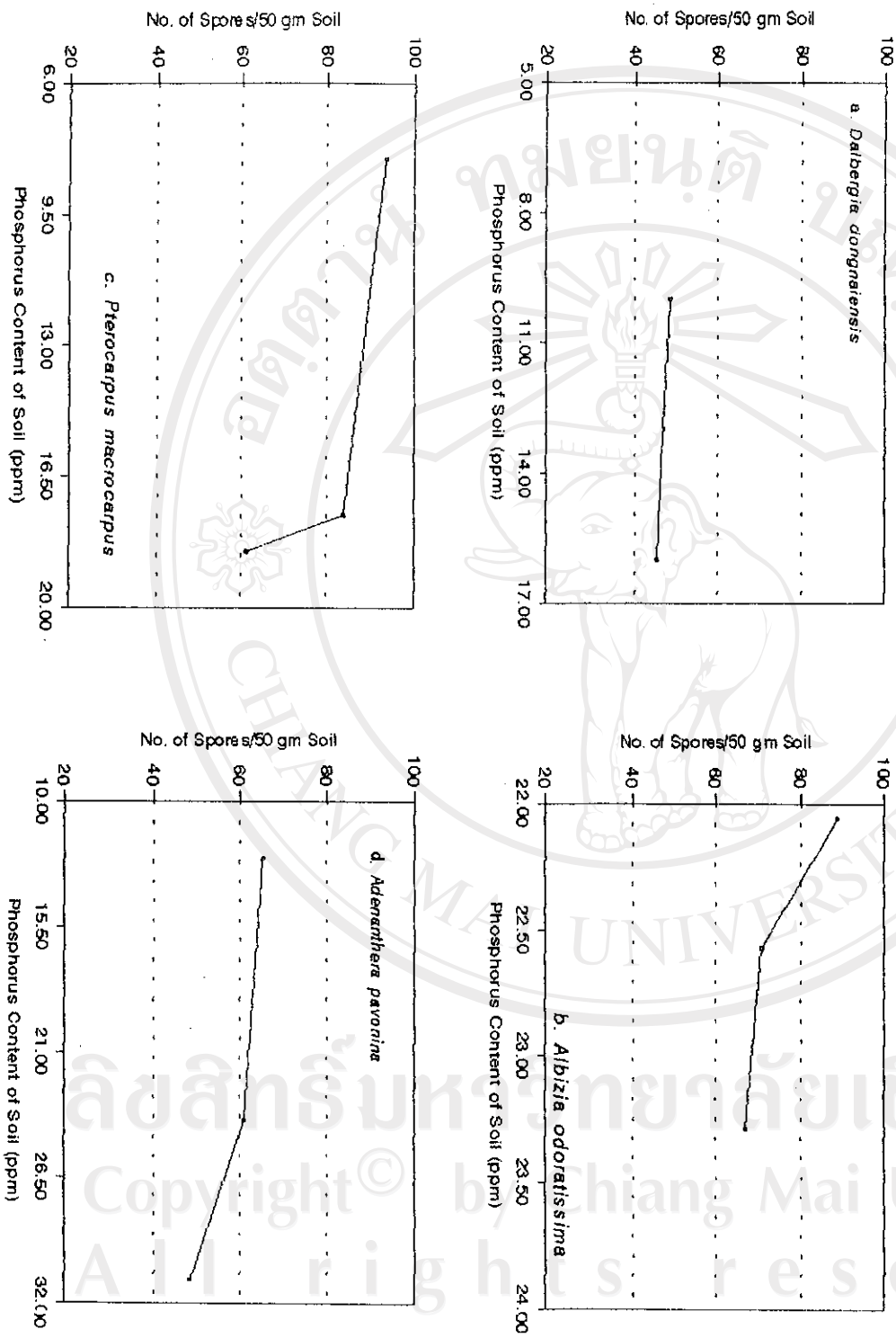
* = Average of 3 trees at each altitude.

6.4. Relationship between Soil Properties and VAM Association

Fertile soils tended to have low VAM spore density. Spore density was low in soils with high phosphorus (figs.12a-d). In contrast, spore density increased with increasing field capacity (figs. 13a-d). VAM spore density increased with increasing nitrogen content of soil (figs. 14a-d). Decreasing of VAM spore density around the roots of *Albizia odoratissima* and *Pterocarpus macrocarpus* with increasing nitrogen occurred more markedly than around the other observed species.

Soil moisture was positively correlated with infection rate (figs. 15a-c) with correlation coefficients (r) of 0.926, 0.954 and 0.915 respectively (p= 0.05). Furthermore,

Fig.12. Relationship between phosphorus and VAM spore density around adult tree roots.



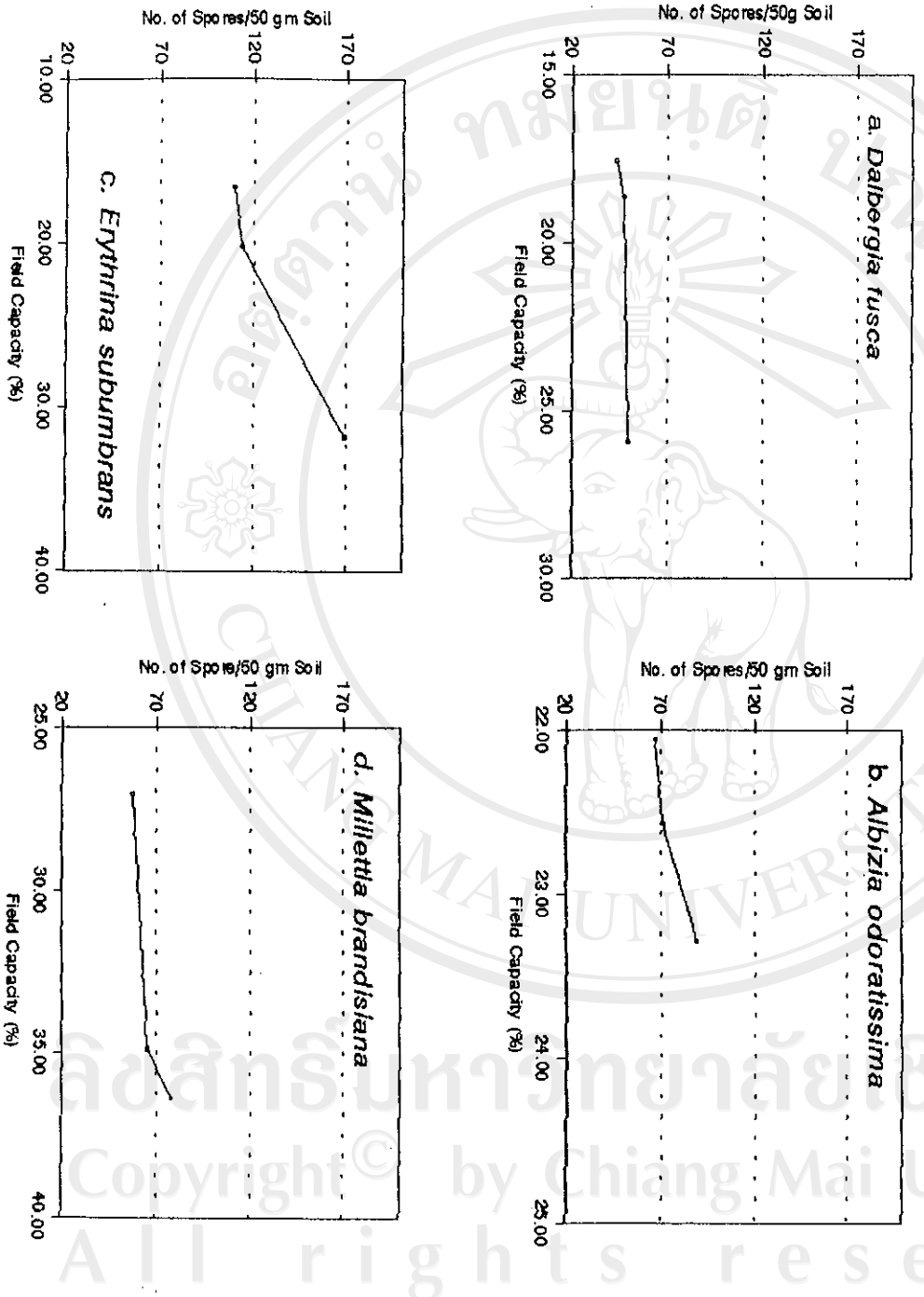


Fig.13. Relationship between field capacity and VAM spore density around adult tree roots.

Fig.14. Relationship between nitrogen and VAM spore density around adult tree roots.

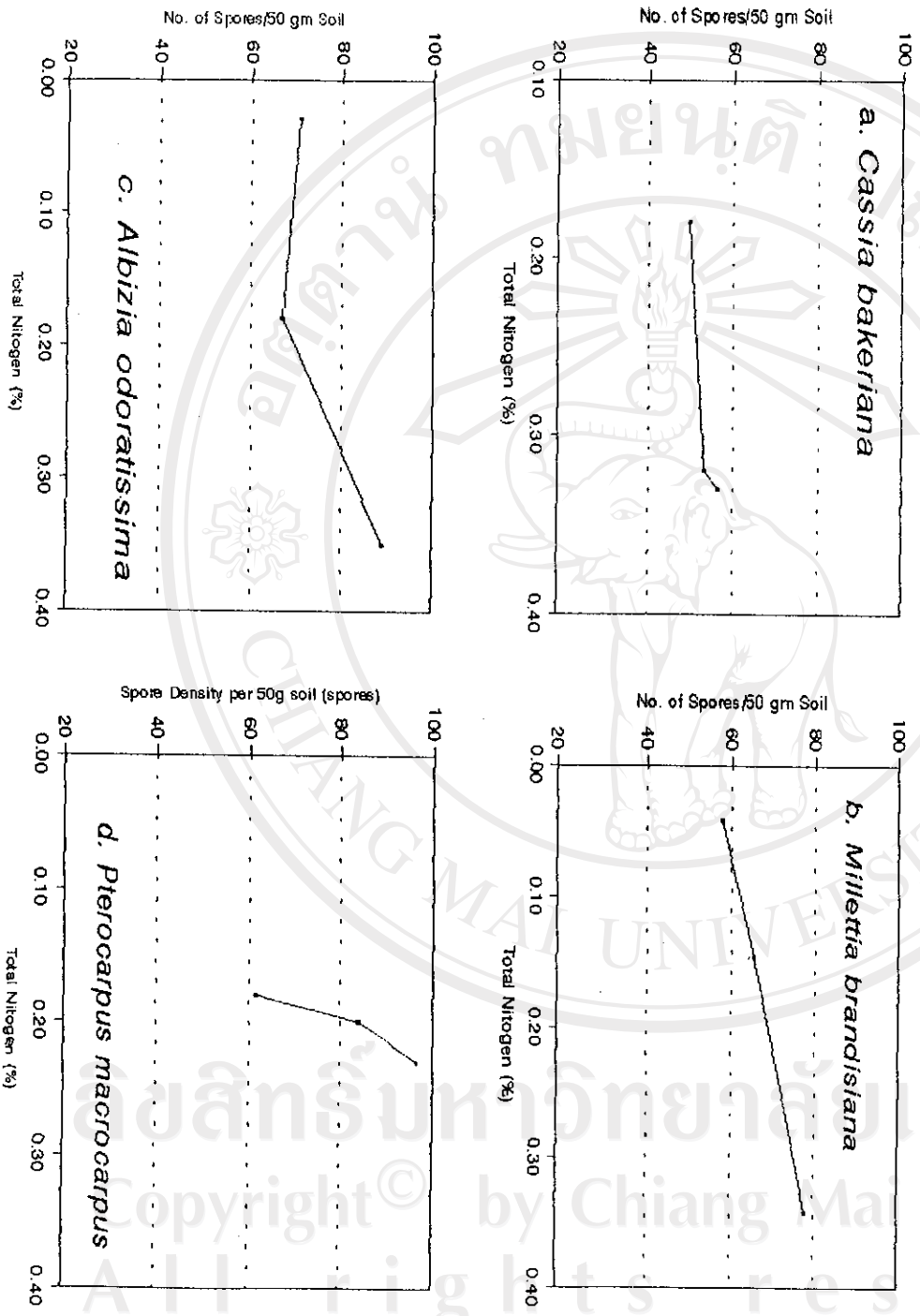
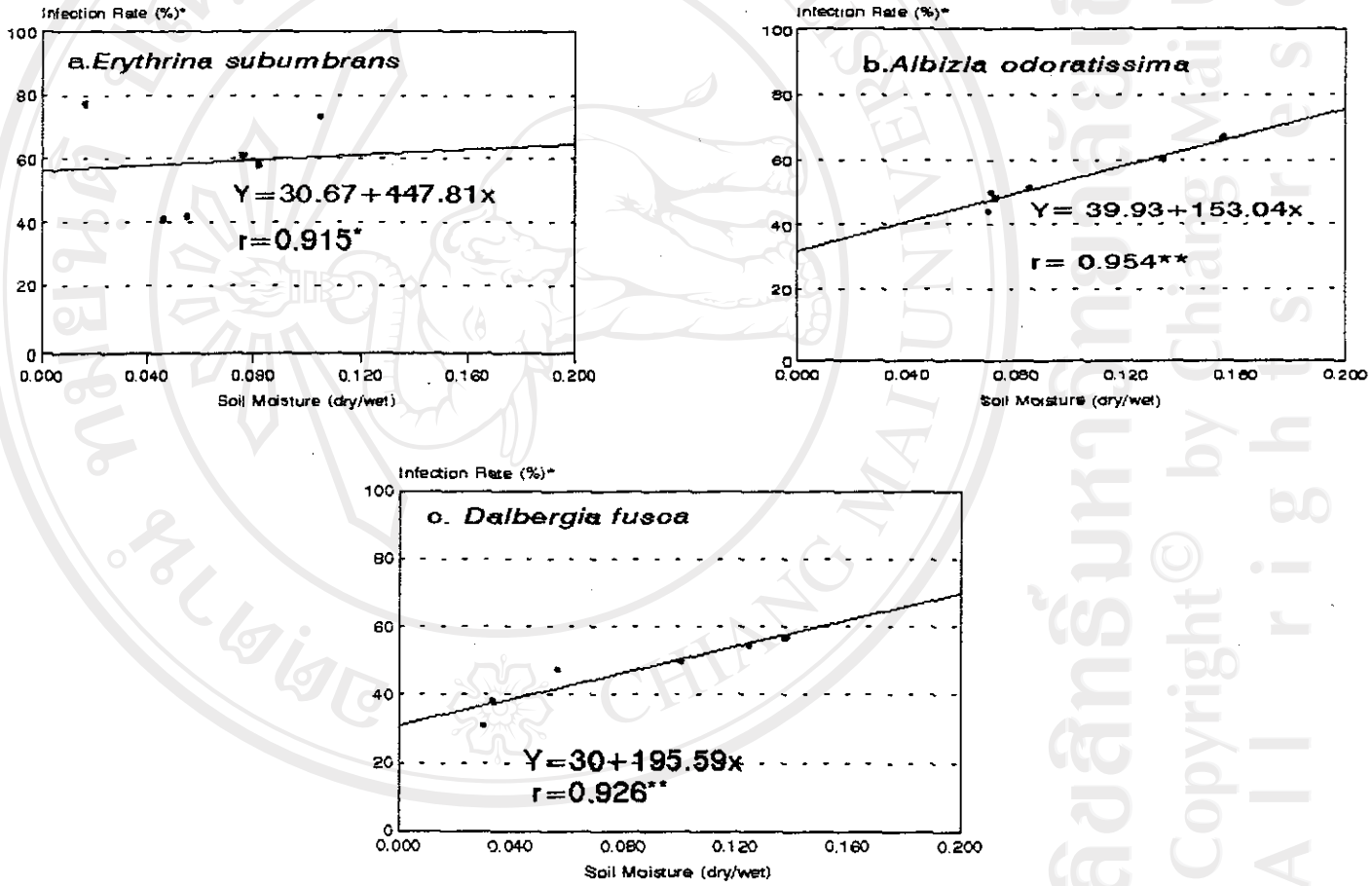


Fig.15. Relationship between soil moisture and infection rate of seedlings.



* mean of 6 seedlings/spp

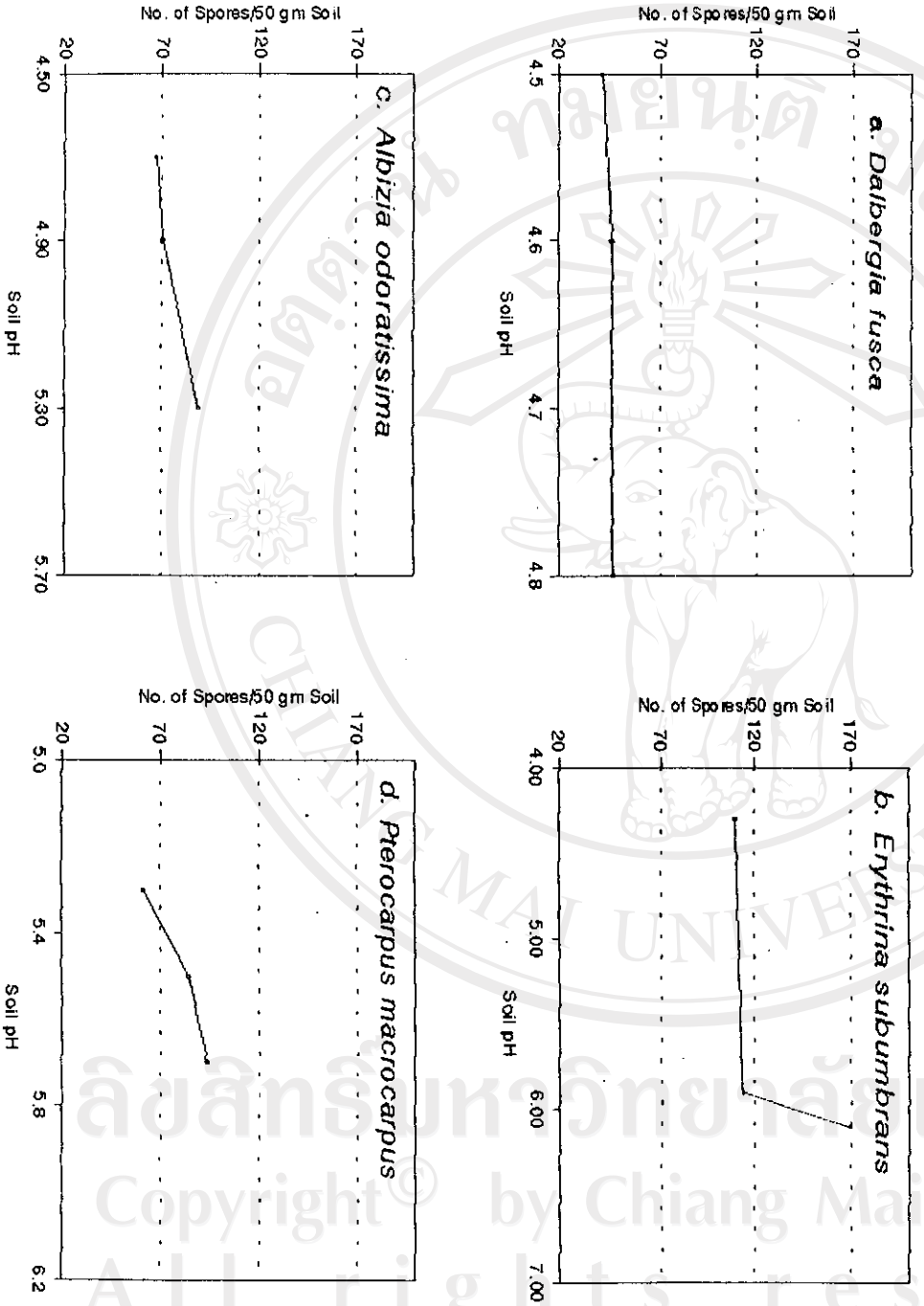


Fig.16. Relationship between pH and VAM spore density around adult tree roots.

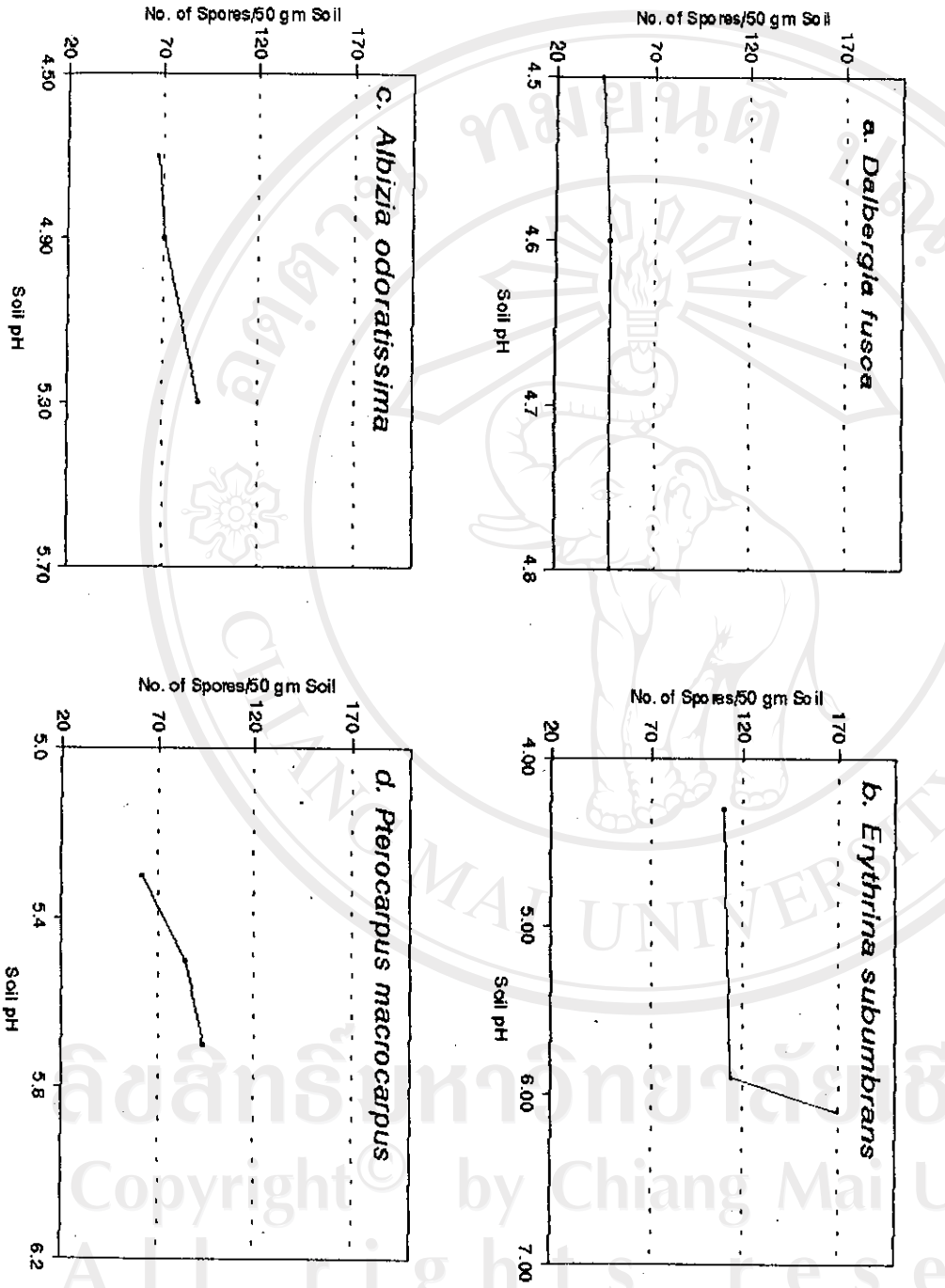


Fig.16. Relationship between pH and VAM spore density around adult tree roots.

soil pH was associated with increasing VAM spore density (figs. 16a-d). Regression analysis revealed that pH was positively correlated with infection rate (figs.17a-c) with correlation coefficients (r) of 0.928, 0.918 and 0.909 respectively ($p = 0.01$)

Overall, the correlation of all variables for both environmental parameters and soil properties with VAM spore density was low. Only 59.20 % of the variation can be explained using multiple regression analysis ($Y = -95.422 - 4.598x_1 + 0.525x_2 + 2.097x_3 - 0.876x_4 + 14.973x_5 - 0.026x_6 + 145.236x_7$) and the strongest correlated factor amongst them was pH which accounted for 46.0 % ($p<0.05$) of the variation ($Y = -48.776 + 22.028x$). The correlation coeficients of all factors with VAM spore density are presented in the appendix (Chapter 10.5. table 25).

6.5. Effect of VAM on Germination Rate of *Albizia odoratissima*

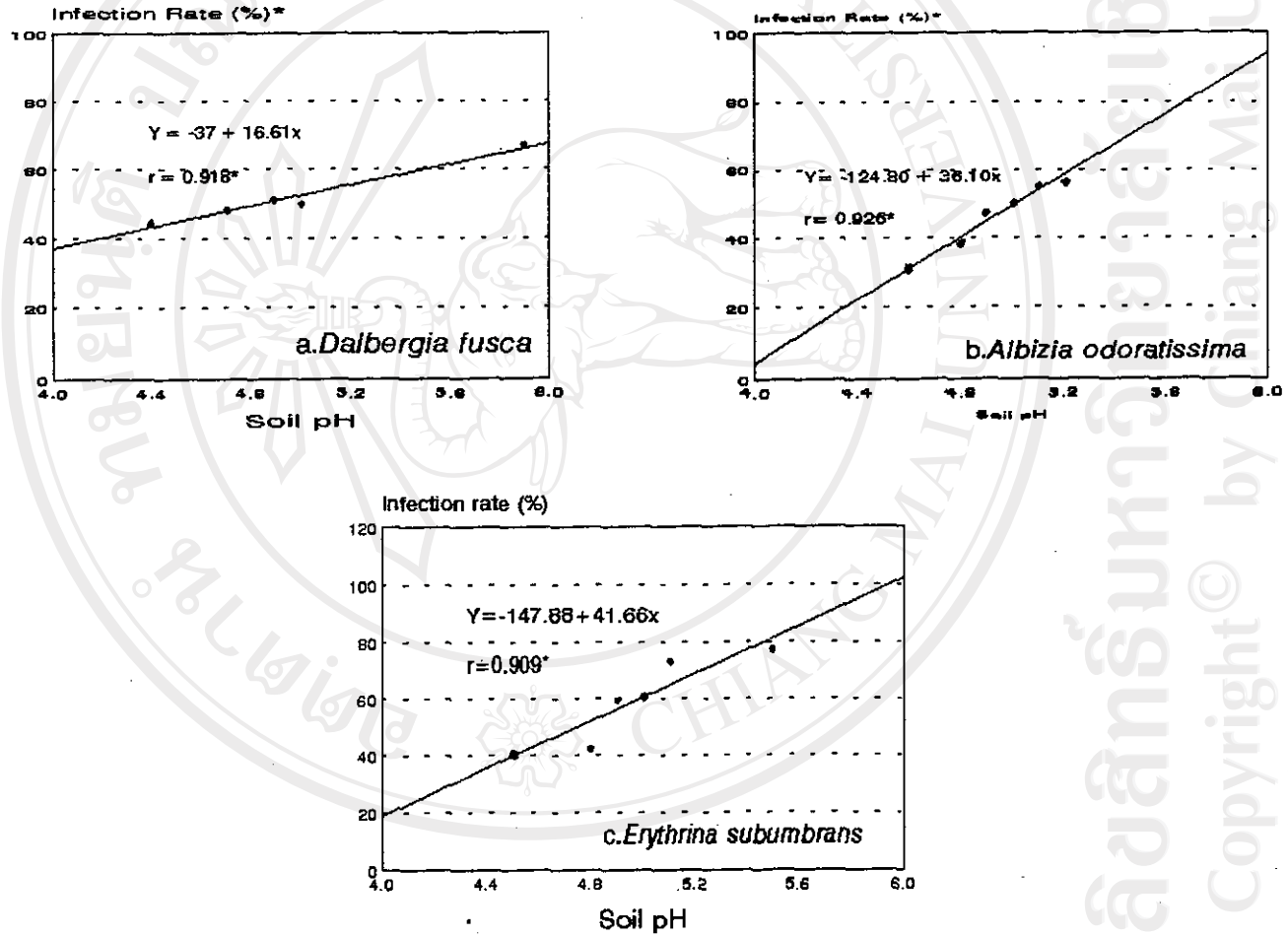
The effects of VAM inoculation on germination of *Albizia odoratissima* seeds is shown in figure 18. Increasing the dosage of the VAM inoculum increased germination rate. However, the effect was not statistically significant according to statistical tests devised by Robert (1963) (qouted by Bradbear, 1992).

6.6. Effect of VAM on the Growth of *Albizia odoratissima*

6.6.1. Pilot Experiment

The pilot experiment showed that VAM had a significant effect on growth rate, number of leaves of seedlings 1, 2 and 3 months after application (Chapter 10.5. tables 2

Fig. 17. Relationship between pH and infection rate of seedlings



* = mean of 6 seedlings/spp

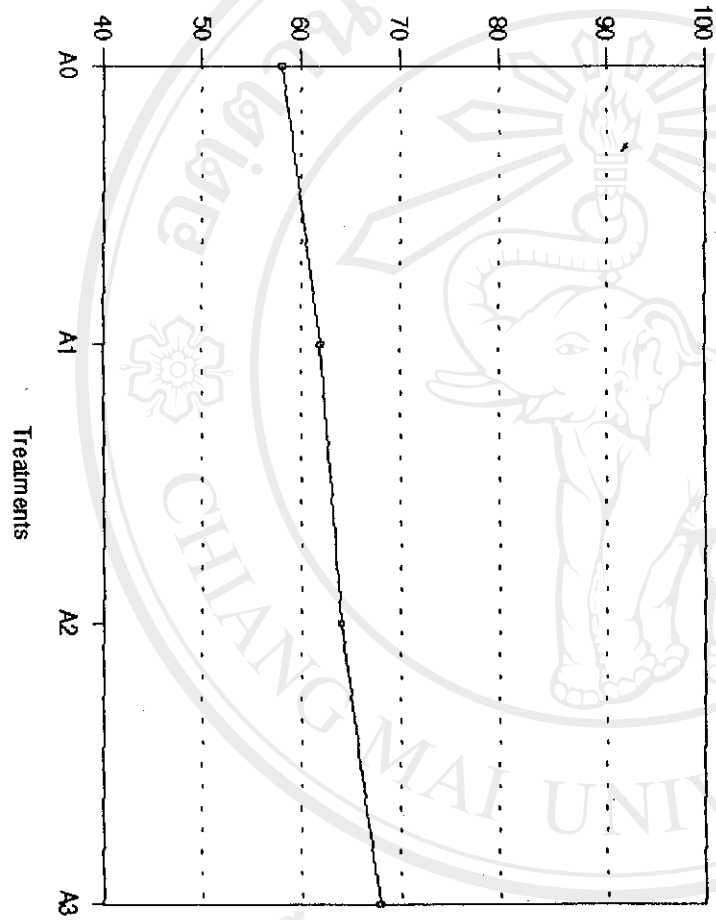


Fig.18. Effect of VAM on germination rate of *Albizia odoratissima* seeds after 30 days.

and 4), dry weight of seedlings (Chapter 10.5. table 8) and infection rate of the roots (Chapter 10.5. table 10). However, a significant effect of VAM on diameter occurred only 3 months after inoculation (Chapter 10.5. table 6).

The application up to 10g VAM inoculum/kg soil increased the growth rate, number of leaves, stem diameter and dry weight of seedlings, as well as infection rate of the roots (tables 3, 4, 5, 6 and 7) and significantly compared with control (Ao) and 5g VAM inoculum/kg soil (A1) (p = 0.05). However, these parameters were not significantly higher compared with 15g VAM inoculum/kg soil (A3).

Spore density in the soil at end of experiment was higher with 15g VAM inoculum/kg soil (A3), significantly higher, compared with the control (Ao) and inoculation with 5g VAM inoculum/kg soil (A1), but not significantly higher compared with A2 (p = 0.05) (table 8).

Table 3. Effect of VAM on the growth rate of *Albizia odoratissima*, mean of 9 seedlings (Pilot study).

Treatments	Growth rate (cm)		
	1 month	2 months	3 months
A0	1.71^{a}	1.95^{a}	2.33^{a}
A1	1.89^{a}	2.35^{a}	3.11^{b}
A2	3.34^{b}	4.68^{b}	6.24^{c}
A3	2.08^{a}	4.04^{b}	6.02^{c}
$LSD_{0.05}$	0.54	0.61	0.63

Means followed by a common letter in columns are not significantly different at the 5 % level.

Table 4. Effect of VAM on number of leaves of *Albizia odoratissima*, mean of 9 seedlings (Pilot study).

Treatments	Number of leaves		
	1 month	2 months	3 months
A0	5.17[a]	6.92[a]	7.36[a]
A1	5.42[a]	7.50[a]	8.13[b]
A2	7.17[b]	13.00[b]	13.81[c]
A3	6.25[a]	10.50[b]	11.61[c]
$LSD_{0.05}$	0.93	1.22	1.38

Means followed by a common letter in columns are not significantly different at the 5 % level.

Table 5. Effect of VAM on stem diameter of *Albizia odoratissima* after 3 months, mean of 9 seedlings (Pilot study).

Treatments	Mean stem diameter (mm)	$LSD_{0.05}$
A0	1.73[a]	0.62
A1	2.31[a]	
A2	3.37[b]	
A3	3.31[b]	

Means followed by a common letter are not significantly different at the 5 % level.

Table 6. Effect of VAM on dry weight of *Albizia odoratissima* seedlings after 3 months (Pilot study).

Treatments	Mean dry weight (gm)	$LSD_{0.05}$
A0	1.805[a]	1.71
A1	3.309[a]	
A2	7.325[b]	
A3	6.101[b]	

Means followed by a common letter are not significantly different at the 5 % level.

Table 7. Effect of VAM inoculum on the infection rate of *Albizia odoratissima* seedling roots after 3 months (Pilot study).

Treatments	Mean of infection rate (%)	$LSD_{0.05}$
A0	1.63[a]	3.87
A1	13.68[a]	
A2	58.35[b]	
A3	57.87[b]	

Means followed by a common letter are not significantly different at the 5 % level.

Table 8. Effect of initial VAM inoculum on the number of VAM spores in the soil 3 months after application (Pilot study).

Treatments	Mean spore density (spores)	$LSD_{0.05}$
A0	2.45^a	
A1	63.00^b	
A2	175.11^c	12.01
A3	180.44^c	

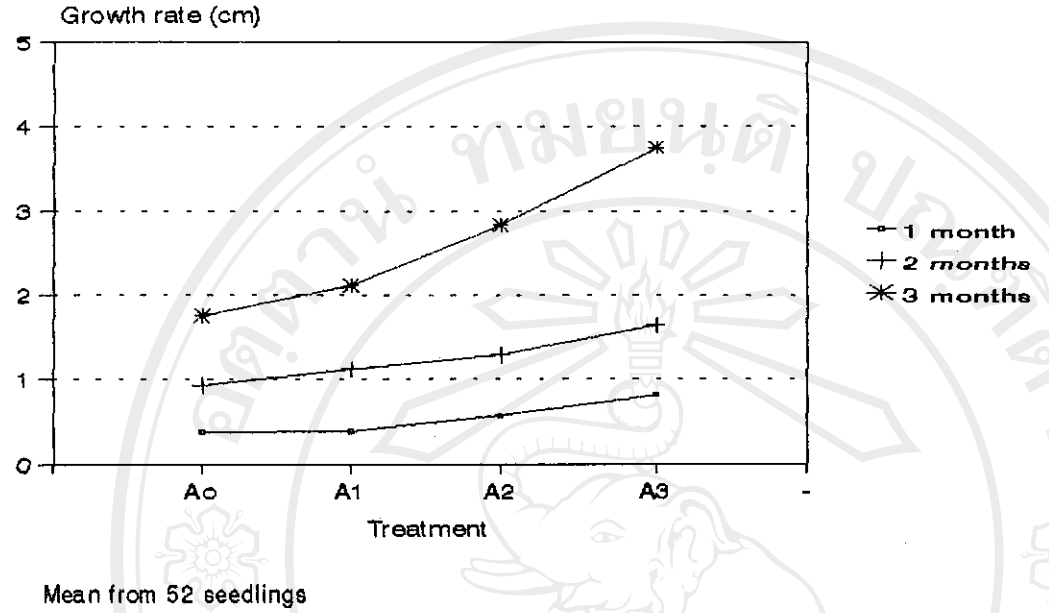
Means followed by a common letter are not significantly different at the 5 % level.

6.6.2. Main Experiment

6.6.2.1. Effect of VAM on Seedling Growth Rate

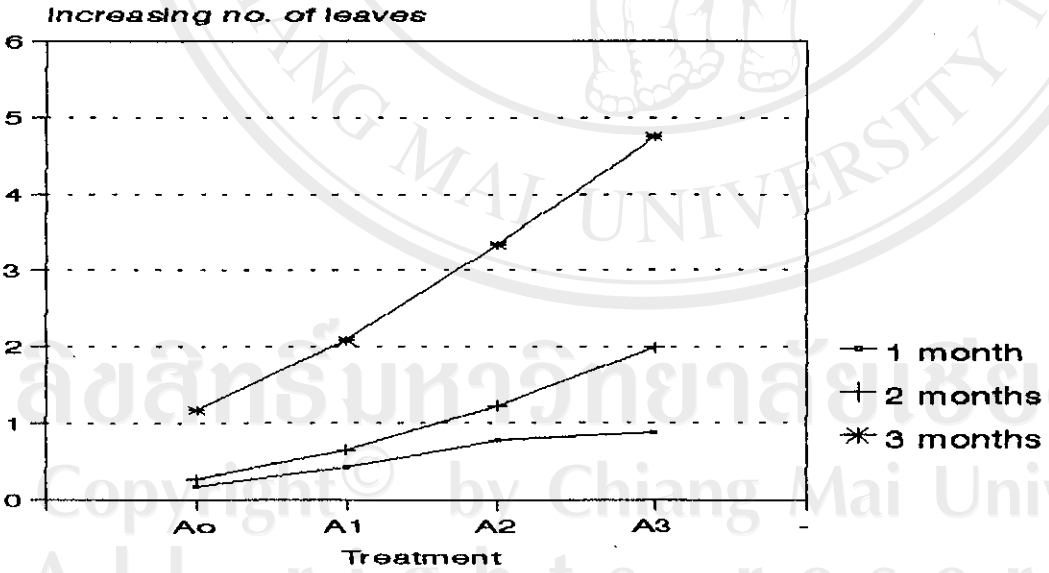
Growth rates 1, 2 and 3 months after inoculation are presented in figure 19. Growth rate increased with increasing application of VAM inoculum.

Statistical analysis (ANOVA) revealed that VAM had a significant effect on the growth rate of seedlings 1, 2 and 3 months after inoculation (Chapter 10.5. table 14). Application of 15 g VAM inoculum/kg soil (A3) significantly increased growth rate compared with control (Ao) and 5 g VAM inoculum/kg soil (A1), but was not significantly different compared with 10 g VAM inoculum/kg soil (A2) (table 9) (p = 0.05).



Mean from 52 seedlings

Fig.19. Effect of VAM on growth rate of *Albizia odoratissima* 1,2 and 3 months after inoculation.



Mean from 52 seedlings

Fig.20. Effect of VAM on number of leaves of *A. odoratissima* 1,2 and 3 months after inoculation.

Table 9. Effect of VAM on growth rate of *Albizia odoratissima* seedlings, mean of 52 seedlings (Main experiment).

Treatments	Growth rate (cm)		
	1 month	2 months	3 months
A0	0.38[a]	0.93[a]	1.76[a]
A1	0.39[a]	1.12[a]	2.11[a]
A2	0.57[a]	1.29[ab]	2.83[b]
A3	0.82[b]	1.65[b]	3.76[c]
$LSD_{0.05}$	0.22	0.46	0.51

Means followed by a common letter in columns are not significantly different at the 5 % level.

6.6.2.2. Effect of VAM on Number of Leaves

Figure 20 shows that the number of leaves increased with increasing application of VAM inoculum.

Statistical analysis (ANOVA) revealed that VAM significantly increased the number of seedling leaves 1, 2 and 3 months after inoculation (Chapter 10.5 table 16). Application of 15 g VAM inoculum/kg soil (A3) had greatest effect on increasing the number of leaves; significantly higher (p = 0.05) compared with control (Ao) and 5 g VAM inoculum/kg soil (A1) and 10 g VAM inoculum/kg soil (A2), after 2 and 3 months application (table 10).

Table 10. Effect of VAM on number of leaves of *Albizia odoratissima*, mean of 52 seedlings (Main experiment).

Treatments	Number of leaves		
	1 month	2 months	3 months
A0	0.18[a]	0.28[a]	1.18[a]
A1	0.43[b]	0.66[b]	2.08[b]
A2	0.78[c]	1.23[c]	3.33[c]
A3	0.88[c]	2.00[d]	4.75[d]
$LSD_{0.05}$	0.19	0.32	0.69

Means followed by a common letter in columns are not significantly different at the 5 % level.

6.6.2.3. Effect of VAM on Stem Diameter

Figure 21 shows that steam diameter increased with increasing VAM inoculum. However, according to statistical analysis (ANOVA) this effect was not significant ($p = 0.05$) (Chapter 10.5 table 18).

6.2.4. Effect of VAM on Dry Weight of Seedlings

Figure 22 shows that dry weight increased with increasing application of VAM inoculum.

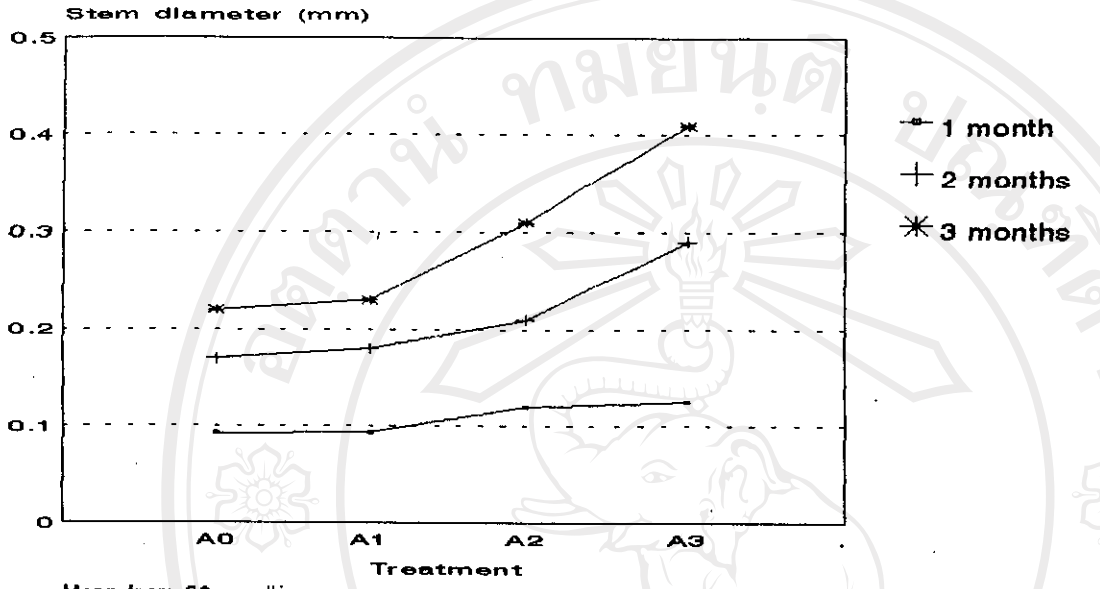


Fig.21. Effect of VAM on stem diameter of *A. odoratissima* seedlings 1,2 and 3 months after inoculation.

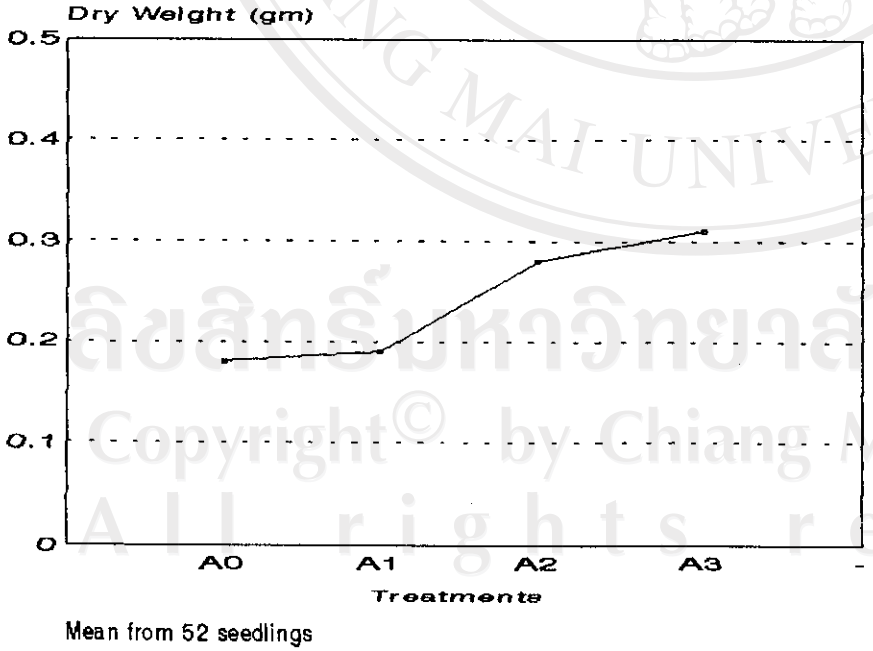


Fig.22. Effect of VAM on dry weight of *A. odoratissima* seedlings 3 months after inoculation.

Statistical analysis (ANOVA) revealed that VAM had a significant effect on increasing seedling dry weight (Chapter 10.5. table 20). Application of 15 g VAM inoculum/kg soil (A3) had the greatest effect; significantly higher compared with the control (Ao) and 5 g VAM inoculum/kg soil (A1), but not significantly higher (p = 0.05) compared with 10 g VAM inoculum/kg soil (A2) (table 11).

Table 11. Effect of VAM on *Albizia odoratissima* seedling dry weight after 3 months, mean of 52 seedlings (Main experiment).

Treatments	Mean dry weight (gm)	$LSD_{0.05}$
A0	0.1811[a]	
A1	0.1905[a]	
A2	0.2813[b]	0.039
A3	0.3100[b]	

Means followed by a common letter are not significantly different at the 5 % level.

6.6.2.5. Effect of Initial VAM Inoculum on the Number of VAM Spores.

Spore densities at the end of experiment are presented in appendix (Chapter 10.5. table 22).

Analysis of variance revealed that initial VAM inoculum significantly increased the number of VAM spores (p= 0.01) (Chapter 10.5. table 24). Application with 15 g VAM

inoculum/kg soil (A3) had greatest effect; significantly higher compared with the control (Ao) and with 5 g VAM inoculum/kg soil (A1), but not significantly higher compared (p = 0.05) with 10 g VAM inoculum/kg soil (A2) (table 12).

Table 12. Effect of initial VAM inoculum on VAM spore density 3 months after application, mean of 52 samples (Main experiment).

Treatments	Spore density (spores)	$LSD_{0.05}$
A0	1.25^{a}	
A1	26.08^{b}	
A2	71.67^{c}	13.99
A3	76.09^{c}	

Means followed by a common letter are not significantly different at the 5 % level.

6.6.2.6. Effect of VAM inoculation on the infection rate of *Albizia odoratissima* roots.

The Infection rates of seedlings are presented in an appendix (Chapter 10.5. table 23).

Statistical analysis (ANOVA) revealed that inoculation had a significant effect on the infection rate of seedling roots (p= 0.01) (Chapter 10.5. table 24). Application of 15 g VAM inoculum/kg soil (A3) had greatest effect, with an infection rate significantly higher than that of control (Ao) and with 5 g VAM inoculum/kg soil (A1). However it was not

significantly higher (p= 0.05) compared with 10 g VAM inoculum/kg soil (A2) (table 13).

Table 13. Comparison of mean of infection rate of seedling roots given different treatments using LSD (Main experiment).

Treatments	Mean of infection rate (%)	$LSD_{0.05}$
A0	7.58[a]	15.75
A1	40.26[b]	
A2	57.66[c]	
A3	59.88[c]	

Means followed by a common letter are not significantly different at the 5 % level.

7. Discussion

7.1. VAM Association with Tree Species

Harley and Smith (1983) and Malajczuk *et al.* (1992) reported that tree species of the family Leguminoseae (e.g. *Albizia* sp., *Dalbergia* spp., *Pterocarpus* spp., *Erythrina* sp. and some *Cassia* spp.) were associated with VAM, which agrees with the results reported here.

In general, the rates of infection reported in this study were lower than those reported for tropical rain forest studies which ranged up to 100 %, mostly for plants belonging to the family Leguminoseae e.g. *Sesbania* sp. (Janos, 1981). It is well-known that in tropical rain forests VAM can cross-infect from root to root (Janos, 1981) so VAM colonization is higher. Though, may be in deciduous tropical forest cross infection can also occur via roots, because in this study plants that grew close to each other had more or less the same VAM spore density and infection rate.

VAM fungi are remarkably nonhost-specific. Although, certain VAM species may be more efficient at stimulating the growth of certain plant species than others, each VAM fungus is generally able to colonize every VAM host species. This might explain the differences in spore density amongst the tree species observed in this study. Mosse (1981) also stated that, although all susceptible plant species seem able to form VAM with any of the VAM fungi species, there is some degree of preferential association. VAM may multiply better when associated with certain plant species. In addition, Schenck and Kinloch (1980) explained that VAM sporulation and colonization are affected by the host,

in addition to the soil and the fungus itself. Only in a suitable host can VAM spores germinate very well. Bevege (1970) explained that VAM were preferentially associated with particular plant species. For example, *Gigaspora gigantia* was found more commonly with maize and tobacco than with soybean. In addition, in the Sierra del Rossaria forest Cuba, VAM colonization occurs at different levels in different tree species. The highest infection rate was found with *Oxandra lanceolata,* ranging up to 97.8 % ; much higher than observed in Doi Suthep-Pui National Park (table 3). Similarly, Elumalia and Kauran (1991) observed that VAM infection in *Casuarina equisetifolia* in a coastal region in India ranged from 20 % to 80 % (table 14), also higher compared with the study reported here. In contrast, the infection rates reported here were higher compared with those found in one study in Nepal (table 15) which ranged from 8 to 40 % (Bhattarai, 1991), but spore density was lower.

Soil in the Nepalese study contained 2.4 to 14.2 spores per g soil; much higher than in the present study (table 14). However, the spores densities reported in this study were higher compared with those in USA, Thailand (upland rice) and Ulva soil in Australia (table 14). The differences in spore density maybe related to the different forest types and particularly different tree species where soil was collected.

Table 14. Comparison of VAM occurances between previous studies from several countries with the present study.

Country	Forest type	Range of spore density/g soil	Range of infection rate (%)	Author	Year
Australia	Ulva soil	1.9	-	Porter	1979
India	Tropical costal forest.	-	27-90	Elumalia& Kauran	1991
Cuba	Mix primary forest.	-	5.9-97.8	Herrera, *et al.*	1991
Nepal	Nat. forest.	2.4-14.2	8-78*	Bhattarai	1991
Thailand	Upland rice field.	0-1.74	0-83	Toyporn & Rangsichal	1991
U.S.A (Kansas)	Tallgrass praire	0.0002-0.8	-	Gibson & Hetrick	1988
My study	Deciduous forest	0.71-2.62	6.35-59.03		1994

* mean of 5 study sites, presented in more detail in table 15.

Table 15. VAM colonization of Jute (*Corchorus capsularis*) in the 5 study sites, Nepal.

Study sites	Infection rate (%)
Khaireni	23-56
Bharatpur	30-78
Mangalpur	10-51
Dibyanagar	8-40
Jagathur	not available data

VAM are particularly important for legumes because: (1) nodulation and symbiotic nitrogen fixation by rhizobia require adequate P and VAM improve the supply of this element and (2) root systems of legumes are relatively restricted, so VAM increase the ability of roots to absorb nutrients. Mosse (1981) also stated that symbiotic N fixation by rhizobia in legume nodules has a high P requirement. The percentage of P in nodules is 2 to 3 times greater than that in roots on which they are borne and compounds rich in P are energy sources for N fixation.

The methods used to determine VAM spore density in the present study are perhaps associated with some error in the counting of spores. It is therefore, important to discuss this and suggest improvements for further similar studies especially in tropical forest ecosystems. The wet sieving technique and counting method employed in this study are still efficient because spore density of VAM found here were low, ranging from 0.71 - 2.60 spores/g soil, therefore the error in the counting of spores would also be low, but if VAM spore density exceeds 20 spores/g soil, this counting technique is not efficient and the error will be high. Therefore, I suggest using "an eelworm counting slide " in the future which would be more accurate. In addition, a new method to determine accurately the number of spores in soil has been developed e.g. the "Most Probable Number (MPN) Method ". The recovery rate of spores recorded by the MPN method is higher compared with the wet sieving technique, as has been documented in Ulva soil in Australia, where 95 spores/50 gm soil were recorded with wet sieving technique and 752 spores/50 gm soil with the MPN method (Porter, 1979).

In fact, several studies have been conducted to determine the association of trees with VAM, using spore density as an indicator of infection, determined by the wet sieving

technique. Some references state that VAM spores are distributed over a wide range of environments from aquatic ecosystems to deserts, but VAM spores are only dominant if present around the roots of the plant host (Gerdemann, 1968). Extramatrical hyphae of VAM spores are associated with hair roots of host plants as shown in figure 1. So spores in soil around roots most probably come from those roots. Similarly, Nemec (1974) (quoted by Mosse, 1981) reported that soil adhering to roots usually contains the most spores.

Two alternative techniques could be employed for further studies to minimize the error of extracting and counting spores from soil; (1) the wet sieving technique to extract spores and "an ellworm counting slide" to count spores and (2) The Most Probable Number method.

To isolate spores from soil for an inoculum source it is often necessary to distinguish between living and dead spores. Not many references regarding the determination of living and dead spores are available. Mosse (1981) explains that most spores which float on water are alive. However, a recent study by Pachart (unpublished) which cultured spores, using agar media, found that spores which sunk grow after 4 weeks, whilst those which floated didn't. Similarly, I observed that most spores of *Glomus macrocarpum* used in my experiment sunk but caused a significant effect on the growth of *Albizia odoratissima*.

Determination of living and dead VAM spores should be investigated in more detail because maybe difefrent species behave differently. Therefore, further studies should be carried out on the spores of different species both those which sink and those which float.

7.2. Association of VAM with Soil Properties

Spore density tended to decrease with increasing phosphorus content, in contrast to nitrogen, field capacity, soil organic matter and pH which had a positive relationship with VAM fungi.

It is well-known that a change in the moisture content of a soil alters its chemical and physical properties and its development, as well as the activity of the fungi present in it. Khan (1975) reported on the dependence of the spread of VAM spores in soil on soil moisture. VAM spores germinate better and there is a higher rate of colonization, if soil moisture approaches the field capacity (Powell and Bagyaraj, 1986). VAM have a favorable effect on plant growth where the soil moisture is low, but better sporulation occurs when soil moisture approaches field capacity. Below or above this value, sporulation is low (Powell and Bagyaraj, 1986).

pH is associated with VAM fungal spores and colonization, but germination and infection seem to occur within a range which is still acceptable for plant growth. Schench (1984) reported that the optimum pH for VAM fungal spore germination probably differs with each VAM species and the environment to which each is indigenous. For example, *Glomus mosseae*, is common in alkaline flatland soils with pH ranging from 6 to 9. In comparison, *Gigaspora coralloidea* germinates best in more acidic soils (pH 4 to 6). Walker (1983) observed that pH had little effect on VAM in the range 4.2 - 7.0 in the perennial grass *Festuca ovina*. Similarly, Sparling and Tinker (1978) reported that pH had little effect on VAM in the range 4.9 - 6.2 at three grassland sites. Interestingly, Mosse (1981) observed that in a very acidic soil (pH below 4.5) no spores were found, whereas

almost all species of VAM found were present at pHs above 5.5. Walker (1983) explained that very acidic soil contains high concentrations of Al which might be responsible for inhibition of VAM. However, most references state that it is difficult to interpret how pH influences spore germination and infection rate.

Increased VAM spore density with increasing soil field capacity is perhaps related to the amount of organic matter in the soil because field capacity is influenced by organic matter which absorbs water. Organic matter also has an influence on increasing spore density since it is a major source of soil nitrogen. From these inter-relationships organic matter maybe the most important factor influencing VAM spore density, since it also determines field capacity and nitrogen content of soil. Similarly, Anderson *et al.* (1984) reported that VAM fungi are positively correlated with percentage soil organic matter. Moreover, Bevege (1970) found that root colonization (infection) and sporulation increase as nitrogen content increases if phosphorus levels are moderate. At higher levels of phosphorus, however, nitrogen application is inhibitory. In addition, Gibson and Hetrick (1988) gave a similar explanation that total spore number was positively correlated with nitrogen because of the organic matter content of soil. However, how organic matter and nitrogen affect the sporulation is still unclear.

According to Cooper (1986) the number of VAM spores decreases with increasing soil phosphorus, because soil phosphorus may cause root phosphorus to increase. Thus membrane phospholipids of the roots also increase, causing decreased root membrane permeability. Therefore, root leakage of reducing carbohydrate decreases and eventually, the formation of VAM decreases. Many publications agree that increased phosphorus content of the soil reduces sporulation, but they fail to determine whether it is phosphorus

in the soil or in the host plants which affects fungal development. Some authors suggest that sporulation and infection of mycorrhiza is regulated by soil phosphorus and that there are a threshold level of soil phosphorus, below which mycorrhizal development, both sporulation and infection, can be maintained at a high level. Others suggest that sporulation and infection are more likely to be regulated by the P content of the plant tissue, rather than by that of the soil, but the mechanism whereby the internal P content of the host regulates sporulation and infection is not clear. Powell and Bagyaraj (1986) suggested that plant P status can affect the soluble carbohydrate content of the root and root exudations, thereby regulating the carbon available to VAM fungi.

It is widely accepted that maximum root colonization (infection) and sporulation occur in soils of low fertility. Phosphorus may significantly reduce root colonization if present at high levels (Hayman, 1970). Simanungkalit (1991) observed that the efficiency of VAM fungi with host symbiosis decreases with increased P levels in the soil. Furthermore, Powell and Bagyaraj (1986) observed that a high level of phosphorus is likely to decrease VA mycorrhizal infection and sporulation.

Moreover, spores of VAM fungi appear to be controlled by soil temperature, moisture, pH and nutrients which also induce plant seed germination. Thus, spores of VAM fungi germinate when newly formed growing roots are likely to be present (Powell and Bagyaraj, 1986).

7.3. Associations between VAM and Environmental Parameters

The explanation for increasing spore density with decreasing slope could be that on

very steep slopes, there is high probability of run-off, which sweep away VAM spores. VAM spores are thus washed away down hill. According to Gibson and Hetrick (1988), spore density of VAM decreases from the top to the bottom of slopes and the abundance of VAM fungi is different on different slopes e.g. *Glomus mosseae* is most abundant at the top of slopes, whereas *Schelorocystics sinuasa* is most abundant at the bottom of slopes. He also reported that the distribution of VAM is clearly related to topography. Usually VAM are most abundant at the top of slopes or on gentle slopes.

VAM spore density increased with increasing tree canopy cover especially in deciduous forest, because in this habitat plants get more light to produce photosyntate which will be translocated to the roots as carbohydrate, providing a food source for VAM fungi. Normally, the greater the concentration of photosyntate in the roots, the higher the infection rate of VAM. In addition, light can also increase the soil temperature which increases VAM spore germination, as explained by Koske (1987). In contrast, in evergreen forest fewer VAM spores were observed with increasing canopy cover, because the trees grew close to each other; canopy cover is denser and light cannot penetrate to the soil, so soil temperature is very low and this inhibits sporulation and colonization of VAM. Both temperature and light have a significant influence on colonization and sporulation of VAM fungi under greenhouse conditions. Higher soil temperature generally results in greater root colonization and increased sporulation (Furlan and Fertin, 1973). Increased light intensity increases photosynthesis which increases colonization (Ferguson, 1981 and Khan, 1975). Moreover, low light intensity can significantly reduce root colonization and sporulation (Ferguson, 1981). In general, infection and development of normal VAM can take place even in poor light, but the effect is often less than in full light (Moawad, 1981).

May be soil temperature could also be responsible for differences in spore density at different altitudes, because at lower altitudes temperature is higher than at higher altitudes, where low temperature has severe effect upon both host plant and fungus growth (Powell and Bagyaraj, 1986). It appears that in most cases VAM colonization and spore production increase with increasing temperature until growth of the host plant is severely inhibited (Ferguson, 1981). For example, *Glomus fasciculatum* has higher spore density on sudangrass as the temperature increases up to 30°C, but when temperature is 15°C (cold season) VAM colonization and sporulation are inhibited. In contrast, Mohankumar and Mahadevan (1988) reported that the spore density of VAM was higher in high altitude grassland at 900 m than mixed deciduous forest at 740 m and in teak forest at 150 m. This is because in the grassland more light penetrate, into the soil, increasing soil temperature increase up to a suitable level for sporulation.

Most trees around streams in deciduous forest tended to have a higher association with VAM, because in this habitat soil moisture was higher, field capacity was higher, nitrogen content was higher, phosphorus content was low and soil pH was higher, compared to those trees far from streams. The relationship between soil moisture, pH, nitrogen and phosphorus content of soil has been explained above.

Overall, this study could provide information, particularly to the Royal Forestry Department, to help establish nurseries with the 10 tree species of the family Leguminoseae observed using VAM inoculum. VAM could play an important role in increasing the growth and survival of trees in tropical deciduous forest ecosystems. So, VAM can be used to restore degraded tropical deciduous forest ecosystems, particularly with mycorrhizal

plants e.g. Legumes.

I suggest further studies should test the VAM inoculation treatment with the nine other tree species of Leguminoseae, either in a greenhouse or field, under shaded conditions to examine the effects of VAM on these tree species. After this step, I believe that the results will be more valuable and applicable.

7.4. Effects of VAM on Germination and Growth of *Albizia odoratissima*

The seeds of *Albizia odoratissima* may contain enough minerals and food reserves so that germination of them was not significantly affected by VAM. In addition, absorption of P through roots is not necessary for germination because seeds do not have roots. Therefore, germination cannot be affected by VAM. Another factor maybe due to the suitable soil conditions, because of watering so may be soil moisture is sufficient for seeds to germinate. Further experiments should be carried out in the field under shade and controlled period and amount of watering.

In contrast, Osonubi (1991) carried out a similar experiment, inoculating VAM on *Gmelina* sp. seeds. He found that seed inoculation increased the germination percentage (32 and 95 % for uninoculated and inoculated seeds, respectively). However, this study was conducted for 5 weeks longer than I did.

The results showed that VAM could greatly increase the growth of *Albizia odoratissima*, because VAM enhances the ability of seedling roots to absorb nutrients.

There are three possible explanations for the greater uptake of mineral nutrients by mycorrhizal plants compared to non-mycorrhizal plants. First, mycorrhizae may increase nutrient uptake, by reducing the distance that nutrients must diffuse to plant roots (Powell and Bagayaraj 1986). Secondly, mycorrhizal roots may differ from non-mycorrhizal roots in the relationship between rate of nutrient absorption and nutrient concentration at the absorbing surface (Islam and Ayanaba, 1981). Finally, mycorrhizal hyphae may chemically modify the availability of nutrients for uptake by plants (Powell and Bagayaraj, 1986). According to Abbott and Robson (1986) VAM increase nutrient uptake from soil primarily by shortening the distance that nutrients must diffuse through soil to roots. It is likely , therefore, that the effects of VAM in increasing nutrient uptake is most marked for nutrients which move to roots principally by diffusion and for plant species with coarse roots and sparse, short root hairs (Baylis, 1975).

Phosphate is the main nutrient enhanced by VAM and leguminous plants (e.g. *Albizia odoratissima*) have a high requirement for phosphorus. With a sufficient supply of phosphorus, the roots system is improved and nodulation well developed. In addition, absorption of other nutrients is increased. I observed that the more nodules on the roots, the higher the growth rate, number of leaves, diameter and dry weight of seedlings (table 16). In addition, the number of nodules increased with increasing the VAM inoculum (table 16). May be, therefore, growth of seedlings was enhanced because nodulation in legumes plays an important role, in which there is a bacteria called *Rhizobium* which fixes nitrogen from the atmosphere and converts it into NH_3, thus improving nitrogen nutrition of the host.

NH_3 is a component of protein formation and protoplasm which increases the

vegetative growth of seedlings. According to Asimi *et al.* (1980) (quoted by Pearson and Diem (1987)) nodulation and nitrogen fixing increases in the presence of VAM. The tripartite association of flowering plants, mycorrhizal fungi and nitrogen-fixing bacteria has been a subject of interest since 1896 when Jense (quoted by Powell and Bagyaraj (1986)) described such a symbiotic association in legumes. He found that several legumes grew poorly and failed to nodulate in autoclaved soil unless they were mycorrhizal. Those plants with mycorrhizae contained higher concentrations of nitrogen than plants without. This is because VAM increases the ability of *Rhizobium* to fix nitrogen (Schenck, 1984). In addition, Crush (1974) reported that VAM strongly stimulate growth and nodulation of legumes (e.g. in *Centrosema pubescens*, *Stylosanthes* and *Trifolium*) and most plants that have more nodules display better growth, compared to those without nodules. Abbott and Robson (1986) observed that mycorrhizal legumes exhibit increased nodulation and nitrogen fixation compared to non-mycorrhizal plant, when phosphorus supply limits the growth of non-mycorrhizal plants.

Table 16. Relationship between number of nodules and seedling growth 3 months after inoculation (Main experiment)

Treatment	No.of nodules	Growth rate (cm)	No.of leaves	Diameter (mm)	Dry weight (gm)
A0	2.0	1.76	1.18	0.22	0.1811
A1	6.33	2.11	2.08	0.23	0.1905
A2	29.67	2.83	3.33	0.31	0.2813
A3	36.67	3.76	4.75	0.41	0.3100

However, the effects of mycorrhizae on nodulation and nitrogen fixing can be completely overcome by increasing phosphorus supply in the non-mycorrhizal treatments, but it is not efficient economically.

VAM increased the growth of seedlings by enhancing phosphate absorption, so a depletion zone of phosphate ions builds up rapidly around an actively absorbing root hair. Hyphal strands of the fungi go beyond the depletion zone and explore a greater volume of soil for mineral elements. Once a phosphate ion has been absorbed by the hyphae, it is transported back to the roots to be utilized for further root and shoot growth. In addition, VAM can improve the uptake of other elements such as zinc, copper and sulfur and interestingly, VAM have an outstanding effects on soil aggregations which are very important for plant growth.

The improvement of seedlings growth could also be related to the level of infection of the roots, because I observed that seedlings which inoculated with 15g VAM inoculum/kg soil (A3) had a greater infection rate (table 13), display better growth (tables 9 and 10) and have a higher dry weight of seedlings (table 11) .

8. Conclusion

Based on the results of this investigation it can be concluded that:

1. All selected tree species of Leguminoseae were associated with vesicular-arbuscular mycorrhiza (VAM), if these species are used for reforestration programs, use of VAM inoculum might be beneficial e.g. to raise seedlings in nurseries.

2. Environmental parameters and soil properties were associated with the occurences of vesicular-arbuscular mycorrhiza (VAM) and the strongest correlated factor was soil pH.

3. Vesicular-arbuscular mycorrhiza (VAM) had no effect on germination rate of *Albizia odoratissima* seeds after 1 month's treatment.

4. Vesicular-arbuscular mycorrhiza (VAM) can greatly increased the growth and yield *A. odoratissima*.

5. So, vesicular-arbuscular mycorrhiza (VAM) could play an important role in the survival and growth of trees in tropical deciduous forest ecosystems.

10. Appendix

10.1. Layout of experiment (RCB Design)

I	II	III	IV
A0	A1	A0	A2
A2	A3	A1	A3
A1	A2	A3	A1
A3	A0	A2	A0

N

10.2. Result of Soil Analysis

Lab. No.	Field No.	% OM	% N Tot.	P ppm	P.C (%)	K ppm	Texture			
							sand (%)	silt (%)	clay (%)	Des.
49	Af1	7.72	0.331	25	19.65	58.65	60.8	13.7	25.5	SCL
50	Af2	7.97	.336	32	19.96	53.55	60.6	16.5	22.9	SCL
51	Af3	7.34	.324	32	19.41	53.55	61.0	13.9	25.1	SCL
52	Dd1	5.43	.281	16	18.14	109.7	68.0	13.7	18.3	SL
53	Dd2	5.17	.246	10	17.43	76.50	68.2	13.7	18.1	SL
54	Dd3	4.60	.240	13	22.06	107.1	54.4	20.1	25.5	SCL
55	Df1	2.71	.202	10	17.56	86.7	58.6	16.7	24.7	SCL
56	Df2	2.75	.202	9.5	18.64	89.25	59.0	18.5	22.5	SCL
57	Df3	4.73	.231	9	22.94	58.65	56.4	16.1	27.5	SCL
58	Ap1	3.51	.188	12.5	18.46	89.25	51.0	5.3	43.7	SL
59	Ap2	2.24	.139	31	14.47	84.15	60.6	15.3	24.1	SCL
60	Ap3	2.49	.143	24	16.04	112.2	55.6	19.3	25.1	SCL
61	Pm1	4.31	.176	17.5	21.50	63.95	54.6	22.9	22.5	SCL
62	Pm2	3.77	.203	14.5	30.62	56.10	52.6	26.3	21.1	SCL
63	Pm3	3.13	.226	8	31.55	68.85	46.6	27.9	25.5	L
64	Mb1	2.56	.146	12.5	27.62	94.35	38.4	31.1	30.5	CL
65	Mb2	5.39	.042	10	34.88	89.25	44.4	31.1	24.5	L
66	Mb3	8.1	.342	15	36.39	99.45	38.6	32.7	28.7	CL
67	Bs1	3.77	.262	10	16.57	35.70	66.0	14.4	19.7	SL
68	Bs2	6.63	.309	7.5	31.81	91.8	58.2	14.4	27.5	SCL
69	Bs3	5.43	.177	16	20.25	73.95	61.4	13.9	24.7	SCL
70	Ao1	6.07	.181	95	22.06	147.9	53.6	19.4	27.1	SCL
71	Ao2	7.46	.295	33.5	27.41	221.9	49.8	21.4	28.9	SCL
72	Ao3	7.54	.353	20	23.59	61.2	53.4	18.9	27.7	SCL
73	Cb1	3.42	.177	43.5	23.29	130.1	53.2	15.2	31.7	SCL
74	Cb2	6.06	.319	34.5	22.57	135.2	61.8	14.6	23.7	SCL

Continued

75	Cb3	6.70	.326	21	24.44	227	56.8	12.9	25.3	SCL
76	Xx1	7.46	.339	16	30.30	158.1	50.8	20.1	29.1	SCL
77	Xx2	5.68	.312	7.5	28.99	188.7	45.8	21.4	32.9	SCL
78	Xx3	7.79	.342	10	28.65	232.1	47.9	19.6	32.5	SCL

Des. = Description
L = Loam
SL = Sandy Loam
SC = Sandy Clay
CL = Clay Loam
SCL = Sandy Clay Loam
FC = Field Capacity
Af = *Acrocarpus fraxinifolius*
Dd = *Dalbergia dongnaeinsis*
Df = *D. fusca*
Ap = *Adenanthera pavonina*
Pm = *Pterocarpus macrocarpus*
Mb = *Millettia brandisiana*
Es = *Erythrina subumbrans*
Ao = *Albizia odoratissima*
Cb = *Cassia bakeriana*
Xx = *Xylia xylocarpa*

3. Data of observation for the ten tree species.

Species	Slope	C.Cover(%)	S.Moist	pH	OM(%)	N (%)	P(ppm)	%FC	No.of spore around adults. Mean of three samples	Infection rate(%) Mean of six seedlings
Df1	20	50	0.018	4.5	2.71	0.202	10	17.56	42.33	
Df2	10	80	0.02	4.6	2.75	0.202	9.5	18.64	46.67	46.37
Df3	0	50	0.023	4.8	4.73	0.231	9	22.94	47	
Dd1	20	80	0.031	4.3	5.43	0.281	16	18.14	45.67	
Dd2	25	80	0.031	4.3	5.17	0.246	10	17.43	49	40.57
Dd3	25	80	0.103	4.4	4.6	0.24	13	22.06	47.33	
Cb1	10	80	0.064	4.4	3.42	0.177	43.5	23.29	49.67	
Cb2	25	90	0.043	5.7	6.06	0.319	34.5	22.57	53.33	6.35
Cb3	30	80	0.058	6.4	6.7	0.326	21	24.44	56.33	
Xx1	20	40	0.103	4.4	7.46	0.339	16	30.3	36	
Xx2	25	30	0.099	4.4	5.68	0.312	7.5	28.99	36	30.13
Xx3	25	60	0.091	4.5	7.79	0.342	10	28.65	41	
Ao1	30	80	0.056	4.7	6.07	0.295	95	22.06	67	
Ao2	25	80	0.069	4.9	7.46	0.353	33.5	22.57	70.67	53.29
Ao3	20	80	0.075	5.3	3.54	0.181	20	23.29	89	
Af1	10	90	0.235	4.8	7.72	0.331	25	19.65	30.67	
Af2	20	70	0.23	4.5	7.97	0.336	32	19.96	28	20.51
Af3	5	80	0.225	5.2	7.34	0.324	32	19.41	47.67	
Ap1	0	80	0.134	6.9	3.51	0.188	12.5	18.46	65.33	
Ap2	10	70	0.105	4.9	2.24	0.139	31	14.47	48.33	30.8
Ap3	10	70	0.091	5.8	2.49	0.143	24	16.06	61	
Mb1	10	70	0.079	4.6	2.56	0.146	12.5	27.62	58	
Mb2	30	80	0.085	5.2	0.39	0.042	10	34.88	65.67	16.62
Mb3	30	70	0.076	5.3	8.1	0.342	15	36.39	78	
Pm1	30	60	0.039	5.5	4.31	0.226	17.5	21.5	84	
Pm2	10	70	0.086	5.3	3.77	0.203	14.5	30.62	61.33	51.06
Pm3	10	80	0.065	5.7	3.13	0.176	8	31.55	93.66	
Es1	5	80	0.228	5.9	3.77	0.177	10	20.25	113.67	
Es2	5	80	0.088	6.1	6.63	0.309	7.5	31.81	169.67	59.03
Es3	5	80	0.264	4.3	5.43	0.262	16	16.57	110	

Df = *Dalbergia fusca*
Dd = *D. dongnaeinsis*
Cb = *Cassia bakeriana*
Xx = *Xylia xylocarpa*
Ao = *Albizia odoratissima*
Af = *Acrocarpus fraxinifolius*
Mb = *Millettia brandisiana*
Pm = *Pterocarpus macrocarpus*
Ap = *Adenanthera pavonina*
Es = *Erythrina subumbrans*

10.5. Statistical analysis.

A. Pilot Experiment.

Table 1. Original data for analysis of variance (ANOVA) of growth rate of *Albizia odoratissima* 1, 2 and 3 months after inoculation.

a. After 1 month

Treatments	Mean of growth rate (cm)			Total	Mean
	Rep. I	Rep. II	Rep. III		
Ao	1.875	1.425	1.825	5.125	1.708
A1	2.125	1.555	2.000	5.675	1.892
A2	2.500	3.650	3.875	10.025	3.342
A3	2.250	1.675	2.300	6.225	2.075
Rep. total	8.750	8.300	10.000		
Grand total				27.050	
Grand Mean					2.254

b. After 2 months

Treatments	Mean of growth rate (cm)			Total	Mean
	Rep. I	Rep. II	Rep. III		
Ao	1.950	1.825	2.075	5.850	1.950
A1	2.400	2.300	2.350	7.050	2.350
A2	4.000	4.750	5.275	14.025	4.680
A3	4.300	4.100	3.725	12.135	4.040
Rep. total	12.650	12.975	13.425		
Grand total				39.060	
Grand Mean					3.255

Continued of table 1

c. After 3 months

Treatments	Mean of growth rate (cm)			Total	Mean
	Rep. I	Rep. II	Rep. III		
Ao	2.450	2.030	2.520	7.000	2.330
A1	2.900	3.220	3.210	9.330	3.110
A2	5.650	7.040	6.040	18.730	6.240
A3	4.990	6.320	6.750	18.060	6.020
Rep. total	15.990	18.610	18.520		
Grand total				53.120	
Grand Mean					4.430

Table 2. Analysis of variance (ANOVA) on the effect of VAM inoculation on growth rate of *Albizia odoratissima*

a. After 1 month

SV	df	SS	MS	Computed F	Tabular F. 5%	Tabular F. 1%
Replication	2	0.39	0.19	0.87ns	5.14	10.92
Treatment	3	4.93	1.64	7.41*	4.76	9.78
Error	6	1.34	0.22	-		
Total	11	6.66				

CV = 9.85 %

Continued of table 2

b. After 2 months

SV	df	SS	MS	Computed F	Tabular F. 5%	Tabular F. 1%
Replication	2	0.04	0.02	0.01ns	5.14	10.92
Treatment	3	17.53	5.84	21.17**	4.76	9.78
Error	6	1.66	0.28	-		
Total	11	18.23				

CV = 16.14 %

c. After 3 months

SV	df	SS	MS	Computed F	Tabular F. 5%	Tabular F. 1%
Replication	2	1.11	0.56	1.87ns	5.14	10.92
Treatment	3	35.87	11.96	39.87**	4.76	9.78
Error	6	1.81	0.30	-		
Total	11	38.79				

CV = 12.36 %

ns = not significant
* = significant at 5 % level.
** = significant at 1 % level.

Table 3. Original data for analysis of variance (ANOVA) of number of leaves of *Albizia odoratissima* 1, 2 and 3 months after inoculation.

a. After 1 month

Treatments	Mean number of leaves			Total	Mean
	Rep. I	Rep. II	Rep. III		
Ao	4.75	4.25	6.00	15.50	5.17
A1	5.75	5.75	4.75	16.25	5.42
A2	6.00	9.75	5.75	21.50	7.17
A3	6.25	7.25	5.25	18.75	6.25
Rep. total	22.750	27.000	21.750		
Grand total				71.500	
Grand Mean					5.96

b. After 2 months

Treatments	Mean number of leaves			Total	Mean
	Rep. I	Rep. II	Rep. III		
Ao	7.50	6.75	6.50	20.75	6.92
A1	6.50	7.00	9.00	22.50	7.50
A2	11.70	14.50	12.75	39.00	13.00
A3	10.00	11.00	10.50	31.50	10.05
Rep. total	35.75	39.25	38.75		
Grand total				113.75	
Grand Mean					9.48

continued of table 3

c. After 3 months

Treatments	Mean number of leaves			Total	Mean
	Rep. I	Rep. II	Rep. III		
Ao	8.33	6.83	6.92	22.08	7.36
A1	7.80	7.17	9.42	24.39	8.13
A2	12.58	15.42	13.42	41.42	13.81
A3	10.83	11.76	12.25	34.84	11.61
Rep. total	39.54	41.18	42.01		
Grand total				122.73	
Grand Mean					10.23

Table 4. Analysis of Variance (ANOVA) on the effect of VAM inoculation on number of leaves of *Albizia odoratissima*

a. After 1 month

SV	df	SS	MS	Computed F	Tabular F. 5%	Tabular F. 1%
Replication	2	3.89	1.95	2.17ns	5.14	10.92
Treatment	3	13.36	4.45	4.94*	4.76	9.78
Error	6	5.37	0.90	-		
Total	11	22.89				

CV = 15.92 %

Continued of table 4

b. After 2 months

SV	df	SS	MS	Computed F	Tabular F. 5%	Tabular F. 1%
Replication	2	1.79	0.90	0.81ns	5.14	10.92
Treatment	3	71.76	23.92	21.65**	4.76	9.78
Error	6	6.63	1.12	-		
Total	11	80.18				

CV = 11.09 %

c. After 3 months

SV	df	SS	MS	Computed F	Tabular F 5%	Tabular F 1%
Replication	2	0.79	0.40	0.28ns	5.14	10.92
Treatment	3	82.06	27.35	18.99**	4.76	9.78
Error	6	8.62	1.44	-		
Total	11	91.47				

CV = 11.73 %

ns = not significant
* = significant at 5 % level.
** = significant at 1 % level.

Table 5. Original data for analysis variance (ANOVA) of stem diameter of *Albizia odoratissima* 1, 2 and 3 months after Inoculation.

a. After 1 month

Treatments	Mean of diameter (mm)			Total	Mean
	Rep. I	Rep. II	Rep. III		
Ao	1.513	1.100	1.700	4.313	1.438
A1	1.263	1.938	1.875	5.076	1.692
A2	1.438	2.000	1.875	5.313	1.771
A3	1.188	1.788	1.750	4.726	1.575
Rep. total	5.402	6.826	7.200		
Grand total				19.428	
Grand Mean					1.619

b. After 2 months

Treatments	Mean of diameter (mm)			Total	Mean
	Rep. I	Rep. II	Rep. III		
Ao	1.525	1.105	1.763	4.393	1.464
A1	1.338	2.000	2.156	5.494	1.831
A2	1.719	2.943	2.375	7.036	2.345
A3	1.188	1.950	1.815	6.140	2.047
Rep. total	6.956	7.998	7.998		
Grand total				20.060	
Grand Mean					1.922

Continued of table 5

c. After 3 months

Treatments	Mean of diameter (mm)			Total	Mean
	Rep. I	Rep. II	Rep. III		
Ao	1.95	1.15	2.10	5.20	1.73
A1	2.11	2.25	2.56	6.92	2.31
A2	2.44	3.96	3.71	10.11	3.37
A3	3.52	3.29	3.13	9.94	3.31
Rep. total	10.02	10.65	11.50		
Grand total				32.17	
Grand Mean					2.68

Table 6. Analysis of variance (ANOVA) on the effect of VAM inoculation on stem diameter of *Albizia odoratissima*

a. After 1 month

SV	df	SS	MS	Computed F	Tabular F. 5%	Tabular F. 1%
Replication	2	0.46	0.23	3.37ns	5.14	10.92
Treatment	3	0.20	0.07	0.97ns	4.76	9.78
Error	6	0.41	0.07			
Total	11	1.06				

CV = 16.12 %

b. After 2 months

SV	df	SS	MS	Computed F	Tabular F. 5%	Tabular F. 1%
Replication	2	0.20	0.10	0.46ns	5.14	10.92
Treatment	3	1.24	0.41	1.88ns	4.76	9.78
Error	6	1.32	0.22	-		
Total	11	2.76				

CV = 11.43 %

c. After 3 months

SV	df	SS	MS	Computed F	Tabular F. 5%	Tabular F. 1%
Replication	2	0.28	0.14	0.48ns	5.14	10.92
Treatment	3	5.74	1.91	6.59*	4.76	9.78
Error	6	1.75	0.29	-		
Total	11	7.77				

CV = 13.46 %

Table 7. Original data for analysis of variance (ANOVA) of dry weight of *Albizia odoratissima* at the end of experiment.

Treatments	Mean of dry weight (g)			Total	Mean
	Rep. I	Rep. II	Rep. III		
Ao	1.915	1.937	1.564	5.415	1.805
A1	2.163	2.915	4.851	9.928	3.309
A2	5.783	9.154	7.038	21.974	7.325
A3	6.046	7.274	4.983	18.302	6.101
Rep. total	15.907	21.280	18.436		
Grand total				55.619	
Grand Mean					4.635

Table 8. Analysis of variance (ANOVA) on the effect of VAM inoculation on increasing dry weight of *Albizia odoratissima*

SV	df	SS	MS	Computed F	Tabular F. 5%	Tabular F. 1%
Replication	2	3.62	1.81	1.24ns	5.14	10.92
Treatment	3	57.45	19.15	13.12**	4.76	9.78
Error	6	8.76	1.46	-		
Total	11	69.81				

CV = 16.06 %

Table 9. Original data for analysis of variance (ANOVA) of infection rate in roots of *Albizia odoratissima* at the end of experiment.

Treatments	Mean of infection rate (%)			Total	Mean
	Rep. I	Rep. II	Rep. III		
Ao	0	4.88	0	4.88	1.63
A1	20.00	13.89	7.14	41.03	13.68
A2	58.33	56.47	60.26	175.06	58.35
A3	60.27	48.84	64.49	173.60	57.87
Rep. total	138.60	124.08	131.89		
Grand total				394.57	
Grand Mean					32.88

Table 10. Analysis of variance (ANOVA) on the effect of VAM inoculation on infection rate in roots of *Albizia odoratissima*

SV	df	SS	MS	Computed F	Tabular F. 5%	Tabular F. 1%
Replication	2	26.40	13.20	0.38ns	5.17	10.92
Treatment	3	7856.29	2618.76	74.63**	4.76	9.78
Error	6	210.54	35.09	-		
Total	11	8093.23				

CV = 14.71 %

Table 11. Original data for analysis of variance (ANOVA) of number of spore in pot treatment at the end of experiment.

Treatments	Mean number of spore			Total	Mean
	Rep.I	Rep.II	Rep. III		
Ao	5	1.67	0.67	7.34	2.45
A1	58.33	72.33	58.33	188.99	63.00
A2	179.33	170.33	175.67	525.33	175.11
A3	182.33	169.67	189.33	541.33	180.44
Rep. total	424.99	414.00	424.00		
Grand total				1262.99	
Grand Mean					89.47

Table 12. Analysis of Variance (ANOVA) on the effect of VAM inoculation on number of spore in pot treatment at the end of experiment.

SV	df	SS	MS	Computed F	Tabular F. 5%	Tabular F. 1%
Replication	2	18.48	9.24	0.15ns	5.17	10.92
Treatment	3	68664.97	22888.32	360.90**	4.76	9.78
Error	6	380.52	63.42	-		
Total	11	69045.49				

CV = 8.90 %

B. Main experiment

Table 13. Original data for analysis of variance (ANOVA) of growth rate of *Albizia odoratissima* 1,2 and 3 months after inoculation.

a. After 1 month

Treatments	Mean of growth rate (cm)				Total	Mean
	Rep.I	Rep.II	Rep.III	Rep.IV		
Ao	0.52	0.41	0.36	0.22	1.51	0.378
A1	0.39	0.27	0.31	0.60	1.57	0.393
A2	0.75	0.44	0.59	0.48	2.26	0.565
A3	0.89	0.97	0.68	0.73	3.27	0.818
Rep. total	2.55	2.09	1.94	2.03		
Grand total					8.61	
Grand Mean						0.538

Continued of table 13

b. After 2 months

Treatments	Mean of growth rate (cm)				Total	Mean
	Rep.I	Rep.II	Rep.III	Rep.IV		
Ao	1.28	1.28	0.48	0.66	3.70	0.925
A1	0.79	1.32	0.78	1.54	4.43	1.108
A2	1.29	1.32	1.29	1.24	5.14	1.285
A3	1.75	1.93	1.57	1.35	6.60	1.650
Rep. total	5.11	5.85	4.12	4.79		
Grand total					19.87	
Grand Mean						1.242

c. After 3 months

Treatments	Mean of growth rate (cm)				Total	Mean
	Rep.I	Rep.II	Rep.III	Rep.IV		
Ao	1.95	2.13	1.45	1.51	7.04	1.76
A1	1.76	2.77	1.75	2.14	8.42	2.12
A2	2.54	2.90	3.02	2.85	11.31	2.83
A3	3.97	3.72	3.43	3.90	15.02	3.76
Rep. total	10.22	11.52	9.65	10.40		
Grand total					41.79	
Grand Mean						2.61

Table 14. Analysis of variance (ANOVA) on the effect of VAM inoculation on growth rate of *Albizia odoratissima*.

a. After 1 month

SV	df	SS	MS	Computed F	Tabular F. 5%	Tabular F. 1%
Replication	3	0.06	0.02	1.00ns		
Treatment	3	0.50	0.17	8.89**	3.89	6.99
Error	9	0.17	0.02	-		
Total	15	0.73				

CV = 26.19%

b. After 2 months

SV	df	SS	MS	Computed F	Tabular F. 5%	Tabular F. 1%
Replication	3	0.39	0.13	1.52ns		
Treatment	3	1.48	0.49	5.79*	3.89	6.99
Error	9	0.43	0.05	-		
Total	15	2.30				

CV = 17.67 %

c. After 3 months

SV	df	SS	MS	Computed F	Tabular F. 5%	Tabular F. 1%
Replication	3	0.46	0.15	1.50ns		
Treatment	3	9.34	3.11	31.10**	3.89	6.99
Error	9	0.86	0.10	-		
Total	15	10.66				

CV = 12.12 %

Table 15. Original data for analysis of variance (ANOVA) of number of leaves of *Albizia odoratissima* 1, 2 and 3 months after inoculation.

a. After 1 month

Treatments	Mean number of leaves				Total	Mean
	Rep.I	Rep.II	Rep.III	Rep.IV		
Ao	0.20	0.10	0.20	0.20	0.70	0.175
A1	0.30	0.50	0.60	0.30	1.70	0.425
A2	0.70	0.90	0.80	0.70	3.10	0.775
A3	0.80	1.10	1.00	0.60	3.50	0.875
Rep. total	2.00	2.60	2.60	9.00		
Grand total					9.00	
Grand Mean						0.563

b. After 2 months

Treatments	Mean number of leaves				Total	Mean
	Rep.I	Rep.II	Rep.III	Rep.IV		
Ao	0.30	0.30	0.20	0.30	1.10	0.275
A1	0.50	0.50	0.85	0.80	2.65	0.663
A2	1.00	1.40	1.30	1.20	4.90	1.225
A3	2.10	2.20	2.10	1.60	8.00	2.000
Rep. total	3.90	4.40	4.45	3.90		
Grand total					16.65	
Grand Mean						1.04

Continued of table 15

c. After 3 months

Treatments	Mean number of leaves				Total	Mean
	Rep.I	Rep.II	Rep.III	Rep.IV		
Ao	1.10	1.40	0.90	1.30	4.70	1.18
A1	1.80	1.90	2.10	2.50	8.30	2.08
A2	4.10	3.20	2.50	3.50	13.30	3.33
A3	5.10	4.10	4.90	4.90	19.00	4.75
Rep. total	12.10	10.60	10.40	12.20		
Grand total					45.30	
Grand Mean						2.83

Table 16. Analysis of Variance (ANOVA) on the effect of VAM inoculation on number of leaves of *Albizia odoratissima*.

a. After 1 month

SV	df	SS	MS	Computed F	Tabular F. 5%	Tabular F. 1%
Replication	3	0.13	0.043	3.13ns		
Treatment	3	1.25	0.416	30.57**	3.89	6.99
Error	9	0.12	0.014	-		
Total	15	1.50				

CV = 20.71 %

Continued of table 16

b. After 2 months

SV	df	SS	MS	Computed F	Tabular F. 5%	1%
Replication	3	0.07	0.023	0.59ns		
Treatment	3	6.74	2.245	57.56**	3.89	6.99
Error	9	0.35	0.039	-		
Total	15	7.16				

CV = 18.99 %

C. After 3 months

SV	df	SS	MS	Computed F	Tabular F. 5%	1%
Replication	3	0.68	0.228	1.32ns		
Treatment	3	28.96	9.650	52.16**	3.89	6.99
Error	9	1.67	0.185	-		
Total	15	31.31				

CV = 15.20 %

Table 17. Original data for analysis of variance (ANOVA) of stem diameter of *Albizia odoratissima* 1, 2 and 3 months after inoculation.

a. After 1 month

Treatments	Mean of diameter (mm)				Total	Mean
	Rep.I	Rep.II	Rep.III	Rep.IV		
Ao	0.150	0.120	0.050	0.050	0.370	0.093
A1	0.100	0.175	0.050	0.050	0.375	0.094
A2	0.150	0.061	0.120	0.150	0.481	0.120
A3	0.225	0.150	0.025	0.100	0.500	0.125
Rep. total	0.625	0.506	0.245	0.350		
Grand total					1.726	
Grand Mean						0.108

b. After 2 months

Treatments	Mean of diameter (mm)				Total	Mean
	Rep.I	Rep.II	Rep.III	Rep.IV		
Ao	0.200	0.295	0.050	0.125	0.670	0.168
A1	0.125	0.175	0.225	0.175	0.700	0.175
A2	0.225	0.100	0.350	0.150	0.825	0.206
A3	0.340	0.400	0.225	0.175	1.140	0.285
Rep. total	0.890	0.970	0.850	0.625		
Grand total					3.335	
Grand Mean						0.208

Continued of table 17

c. After 3 months

Treatments	Mean of diameter (mm)				Total	Mean
	Rep.I	Rep.II	Rep.III	Rep.IV		
Ao	0.330	0.300	0.075	0.170	0.875	0.220
A1	0.200	0.200	0.330	0.175	0.905	0.230
A2	0.305	0.220	0.500	0.225	1.250	0.310
A3	0.200	0.500	0.320	0.630	1.650	0.410
Rep. total	1.040	1.220	1.220	1.200		
Grand total					4.680	
Grand Mean						0.290

Table 18. Analysis of variance (ANOVA) on the effect of VAM inoculation on stem diameter of *Albizia odoratissima*

a. After 1 month

SV	df	SS	MS	Computed F	Tabular F. 5%	Tabular F. 1%
Replication	3	0.46	0.23	3.37ns		
Treatment	3	0.20	0.07	0.97ns	3.89	6.99
Error	9	0.41	0.07	-		
Total	15	1.06				

CV = 16.12 %

Continued of table 18

b. After 2 months

SV	df	SS	MS	Computed F	Tabular F. 5%	1%
Replication	3	0.017	0.0057	0.58ns		
Treatment	3	0.035	0.0120	1.22ns	3.89	6.99
Error	9	0.088	0.0098			
Total	15	0.140				

CV = 4.76 %

c. After 3 months

SV	df	SS	MS	Computed F	Tabular F. 5%	1%
Replication	3	0.005	0.0017	0.074ns		
Treatment	3	0.097	0.0320	1.390ns	3.89	6.99
Error	9	0.208	0.023	-		
Total	15	0.310				

V = 15.17 %

Table 19. Original data for analysis of variance (ANOVA) of dry weight of *Albizia odoratissima* 3 months after application.

Treatments	Mean of dry weight (g)				Total	Mean
	Rep.I	Rep.II	Rep.III	Rep.IV		
Ao	0.190	0.143	0.234	0.157	0.724	0.181
A1	0.169	0.243	0.165	0.185	0.762	0.191
A2	0.289	0.219	0.332	0.284	1.125	0.281
A3	0.342	0.355	0.211	0.332	1.241	0.310
Rep. total	0.990	0.960	0.942	0.958		
Grand total					3.852	
Grand Mean						0.241

Table 20. Analysis of variance (ANOVA) on the effect of VAM inoculation on increasing dry weight of *Albizia odoratissima*

SV	df	SS	MS	Computed F	Tabular F. 5%	Tabular F. 1%
Replication	3	0.007	0.0002	0.067ns		
Treatment	3	0.051	0.0170	5.670*	3.89	6.99
Error	9	0.027	0.0030	-		
Total	15	0.079				

CV = 22.73 %

Table 21. Original data for analysis of variance (ANOVA) of number of spore in pot treatment at the end of experiment.

Treatments	Mean number of spore				Total	Mean
	Rep.I	Rep.II	Rep.III	Rep.IV		
Ao	1	2.00	1.67	0.33	5.00	1.25
A1	26.00	27.67	28.33	22.33	104.33	26.08
A2	87.00	61.00	77.00	61.67	286.67	71.67
A3	97.00	60.33	72.00	74.67	304.00	76.09
Rep. total	211.00	151.00	179.00	159.00		
Grand total					700.00	
Grand Mean						43.75

Table 22. Analysis of variance (ANOVA) on the effect of initial VAM inoculum on number of spore at the end of experiment.

SV	df	SS	MS	Computed F	Tabular F. 5%	Tabular F. 1%
Replication	3	536.00	178.67	2.33ns		
Treatment	3	15751.36	5250.45	68.60**	3.89	6.99
Error	6	688.89	76.54	-		
Total	15	16956.25				

CV = 19.99 %

Table 23. Original data for analysis of variance (ANOVA) of infection rate of seedling roots at the end of experiment.

Treatments	Mean infection rate				Total	Mean
	Rep.I	Rep.II	Rep.III	Rep.IV		
Ao	15.63	10.34	0	4.35	30.32	7.58
A1	33.33	59.26	31.25	37.21	161.05	40.26
A2	51.61	50.66	60.26	68.09	230.62	57.66
A3	61.76	52.78	64.52	60.47	239.53	59.88
Rep. total	162.33	173.04	156.03	170.12		
Grand total					661.52	
Grand Mean						41.35

Table 24. Analysis of Variance (ANOVA) on the effect of VAM inoculation on infection rate of seedlings at the end of experiment.

SV	df	SS	MS	Computed F	Tabular F. 5%	Tabular F. 1%
Replication	3	44.47	14.82	0.15		
Treatment	3	7003.61	2334.54	24.09**	3.89	6.99
Error	6	872.32	96.92	-		
Total	15	7920.40				

CV = 23.81 %

Table 25. Correlation coeficients of all independent variables with VAM spore density.

Variables	Correlation coeficient with VAM spore density
Canopy cover	0.2354 (P= 0.210)
Field capacity	0.2974 (P= 0.110)
Nitrogen	0.0569 (P= 0.579)
Organic matter	0.0284 (P= 0.882)
Phosphorus	-0.1177 (P= 0.535)
Slope	-0.1954 (P= 0.301)
pH	0.4595 (P= 0.011)

10.4. The alternative percentage values (y) which are significant different from a value (x) when using 100 seeds in each treatment. The table includes Yates' correction. (Robert, 1963)

x	y	y	x	y	y	x	y	y	x	y	y
0	6	-	-	-	-	-	-	-	-	-	-
1	8	-	26	41	13	51	66	36	76	88	62
2	10	-	27	42	14	52	67	37	77	89	63
3	12	-	28	43	15	53	68	38	78	90	64
4	13	-	29	44	16	54	69	39	79	91	65
5	15	-	30	45	17	55	70	40	80	91	66
6	16	0	31	46	18	56	71	41	81	92	67
7	18	0	32	47	18	57	72	42	82	93	68
8	19	1	33	48	19	58	73	43	83	93	70
9	20	1	34	49	20	59	74	44	84	94	71
10	21	2	35	50	21	60	75	45	85	95	72
11	23	2	36	51	22	61	75	46	86	96	73
12	24	3	37	52	23	62	76	47	87	96	75
13	25	4	38	53	24	63	77	48	88	97	76
14	27	4	39	54	25	64	78	49	89	98	77
15	28	5	40	55	25	65	79	50	90	98	78
16	29	6	41	56	26	66	80	51	91	99	80
17	30	7	42	57	27	67	81	52	92	99	81
18	32	7	43	58	28	68	82	53	93	100	82
19	33	8	44	59	29	69	82	54	94	100	84
20	34	9	45	60	30	70	83	55	95	-	85
21	35	9	46	61	31	71	84	56	96	-	87
22	36	10	47	62	32	72	85	57	97	-	88
23	37	11	48	63	33	73	86	58	98	-	90
24	38	12	49	64	34	74	87	59	99	-	92
25	40	13	50	65	35	75	87	60	100	-	94

10.7. List of the 10 tree species.

1. *Acrocarpus fraxinifolius* Wight ex Arn.
2. *Adenanthera pavonina* L. var. microsperma (Teijsm. & Binn.) Niels.
3. *Albizia odoratissima* (L.f.) Bth.
4. *Cassia bakeriana* Craib
5. *Dalbergia dongnaiensis* Pierre
6. *Dalbergia fusca* Pierre
7. *Erythrina subumbrans* (Hassk.) Merr.
8. *Millettia brandisiana* Kurz
9. *Pterocarpus macrocarpus* Kurz
10. *Xylia xylocarpa* Roxb. var. kerri (Craib & Hutch.) Niels.

10. 8. Photographs.

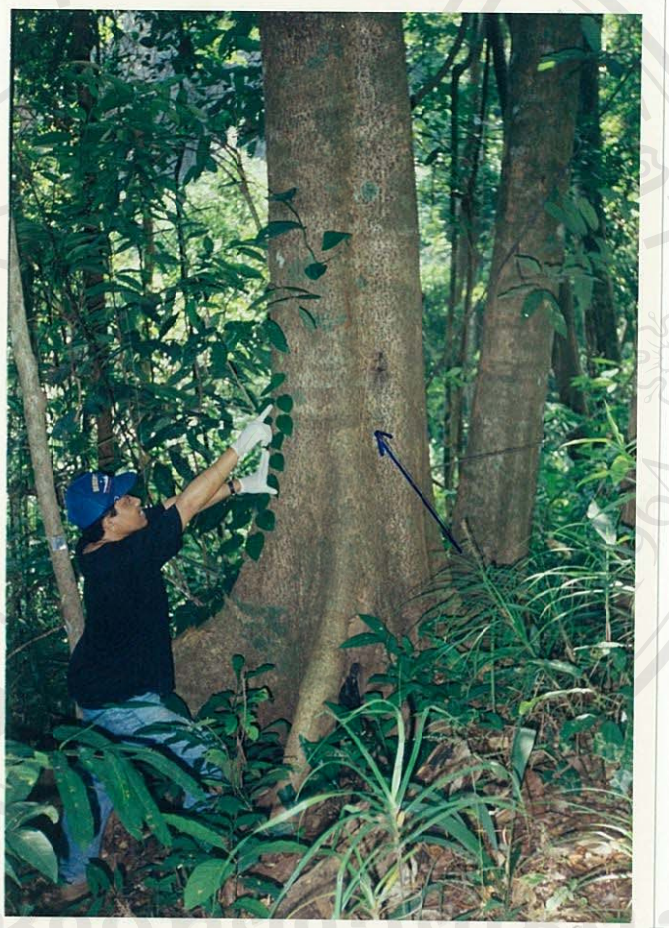


Fig.1. Photo of *Acrocarpus fraxinifolius* Wight ex Arn. (adult tree)

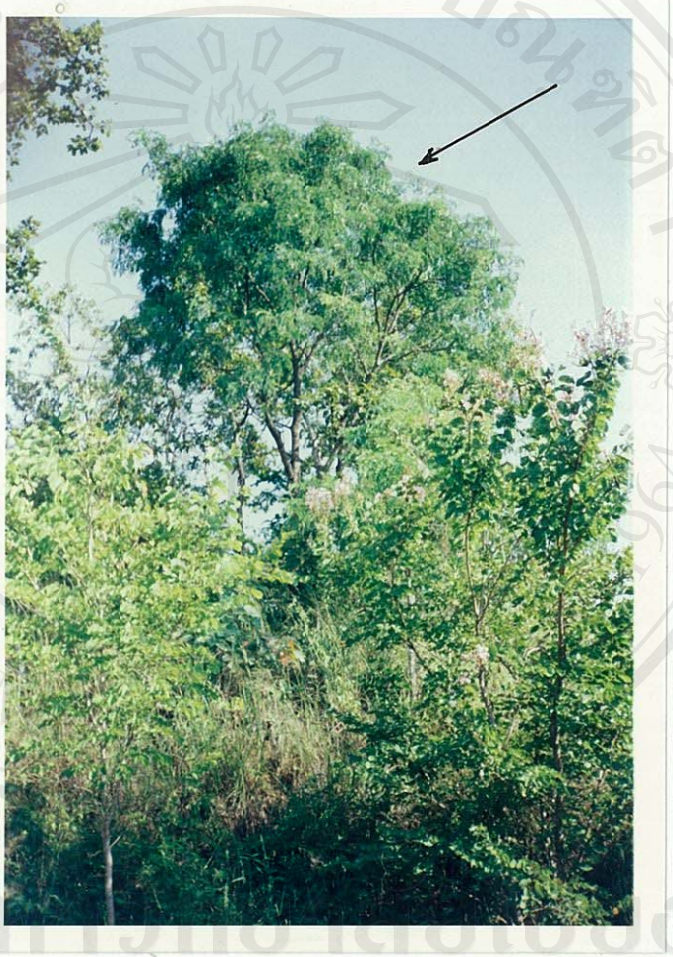


Fig. 2. Photo of *Albizia odoratissima* (L.f) Bth. (adult tree)

Fig. 3. Photo of *Dalbergia fusca* Pierre (adult tree)

Fig. 4. Photo of *Erythrina subumbrans* (Hassk.) Merr.
(adult tree and seedlings)

Fig. 5. Photo of experiment on the effect of *Glomus microcarpus* on germination of *Albizia odoratissima* seeds.

Fig. 6. Photo of *A. odoratissima* seedlings given different treatment of inoculation.

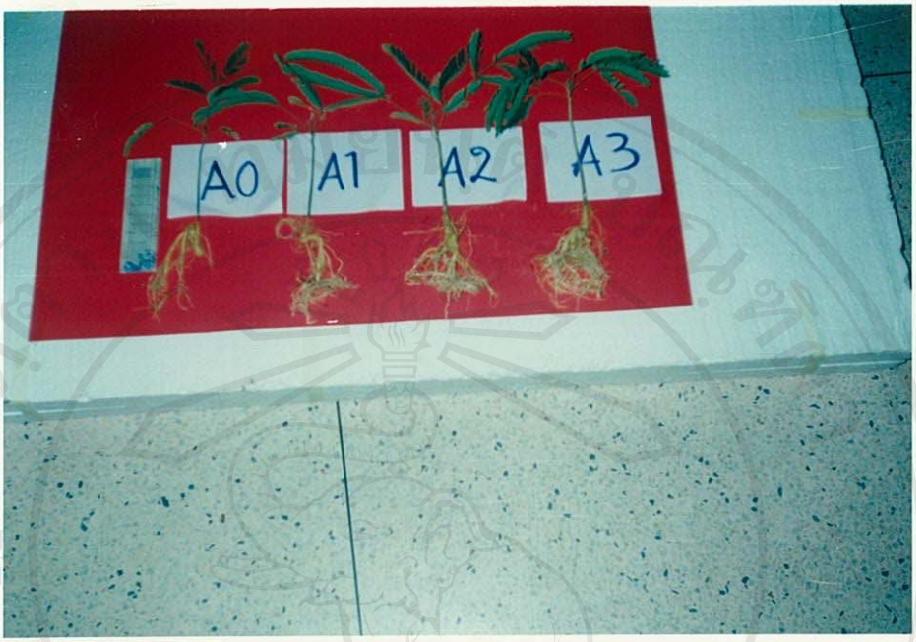


Fig. 7. Photo showing the root system of *A. odoratissima* seedlings given different treatment of inoculation.

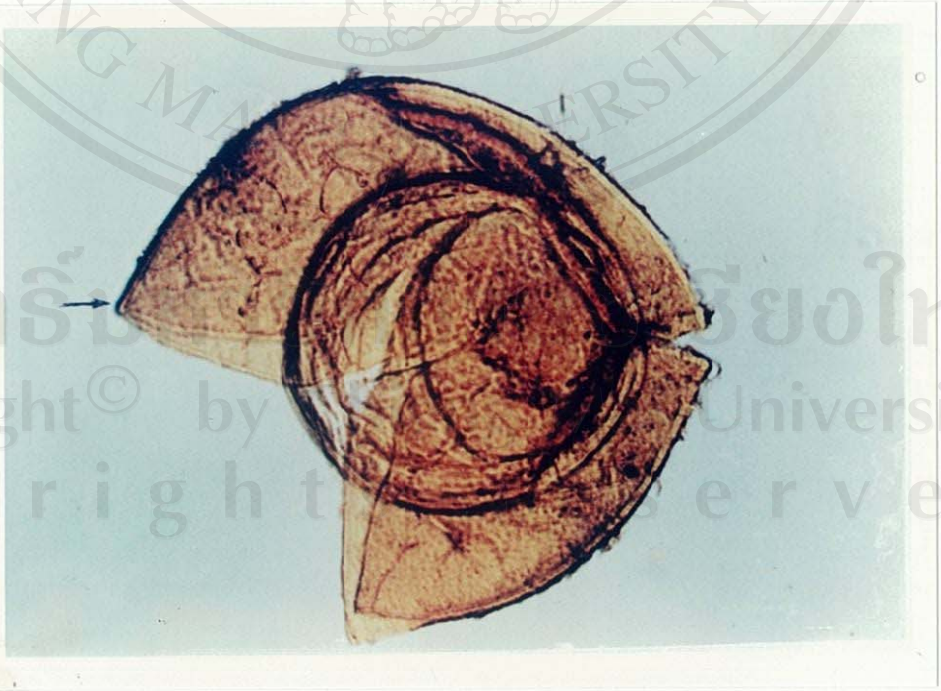


Fig. 8. Photo of *Glomus microcarpus* spore.

Curriculum Vitae

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