ECOPHYSIOLOGICAL STUDIES ON PRODUCTIVITY OF ECTOMYCORRHIZAE IN **PINUS KESIYA** (Royle Ex. Gordon)

ABSTRACT

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THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENT
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To

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ABSTRACT

Seasonal study on ectomycorrhizae and mycorrhizal fungi of khasi pine (*Pinus kesiya* Royle ex. Gordon) in 2, 4, 11 and 17 years old pine plantations was carried out in West Khasi Hills of Meghalaya.

Thirteen mycorrhizal fungi forming ectomycorrhiza with khasi pine were observed. Diversity of index of mycorrhizal fungi was directly proportional to the age of the pine stand. Maximum number of fungal species was observed in oldest stand. Evenness of the sheathing mycorrhizal fungi was also increased with the increase in age of pine.

The sporocarps of *Boletus luteus, Scleroderma aurantium, Tricholoma saponaceum* and *Hygrophorus limacinus* were observed as an early colonizing fungi with P. kesiya. However, in older platations *Russula lepida* and *Amanita phalloides* were observed as late stage fungi. *B.luteus* and *S. aurantium* were dominant species in all the pine stands. Sporocarps of mycorrhizal fungi were maximum during rainy season and minimum during winter months.

A positive correlation was observed between the colonization of myocrrhizae and population of mycorrhizae with soil moisture, soil *p*H, total nitrogen, available phoshphorus, exchangeable potassium and organic matter of soil.

Colonization and population of mycorrhizae showed a uniform pattern of seasonality in all the pine plantations. Maximum colonization and population of mycorrhizae was observed during rainy (Aug-Oct) season and minimum during winter (Feb) season. Positive correlation was observed between colonization of mycorrhizae and population of ectomycorrhizae in all the pine plantations.

Mycorrhizospheric region had more of N,P,K and organic matter than non-mycorrhizospheric ones.

Microbial enzyme activities (dehydrogenase, urease and acid phosphatase) were higher during rainy season than in winter. All the enzyme activities were positively correlated with population of bacteria and fungi. Microbial enzyme activity and microbial population of bacteria and fungi was higher in mycorhizosphere (MR) than in non-mycorrhizospheric region.Population of bacterial, and fungi, and CO_2 evolution were maximum during rainy season and minimum during winter months. A positive correlation was observed between CO_2 evolution and microbial population, dehydrogenase activity and CO_2 evolution, phosphatase activity and microbial population.

A significant variation was observed in number of microbes and enzyme activities within different age plantations, sampling periods and mycorrhizospheric and non-mycorrhizospheric regions.

Different mycorrhizal fungi varied in their colonizing ability of roots of pine and production of ectomycorrhizae Ectomycorrhizal productivity was increased with inoculation of various mycobionts Marginal increase in productivity of mycorrhizae was observed after 45 days in all treatments. Maximum productivity was observed in *Laccaria laccata* inoculatged seedlings followed by *Boletus luteus* inoculated ones. Minimum productivity was observed in the seedlings with *P.tinctorius* inoculation.

Root surface phosphatase activity was always higher than in soil. Root activity was maximum in *L.laccata* inoculated seedlings, which was 11 times more than in the soil. Minimum activity was observed with *P.tinctorius* inoculation. A significant positive correlation was observed between the root surface phophatase activity and mycorrhizal productivity and mycorrhizal infection.

Insignificant variation in seedling and biomass and mycorrhizal infection was observed within the various mycobiont inoculated pine seedlings. After 180 days, colonization of mycorrhiza was maximum in *S.aurantium* (85%) inoculated seedlings in forest soil and in *B.luteus* inoculated ones in degraded soil. Productivity of mycorrhizae was maximum with *L.laccata* and minimum with *P.tinctorius* inoculated seedlings grown on forest soil.

Whereas, in degraded soil maximum and minimum productivity was observed in *B.luteus* and *C.graniforme* inoculated seedlings respectively. All the mycorrhizal symbionts exhibited a promotory effect on the growth of pine seedlings in forest and degraded soils. Significantly highest stimulation in growth of seedlings was observed by *L.laccata* .It followed a definite trend of growth stimution by *S.aurantium* > *C.radiata* > *B.luteus* > *P.tinctorius* > *C.graniforme* in forest soil. Whereas in degraded soil the order was *B.luteus* > *L.laccata* > *S.aurantium* > *C.radiata* > *P.tinctorius* > *C.graniforme*. Seedling growth was 3 times more in forest soil than in degraded soil in all the mycobiont treatments. Maximum productivity of mycorrhizae was observed in degraded soil seedlings inoculated with *B.luteus* than in forest soil propagated by *L.laccata* . Similarly mycorrhizal infection was highest in degraded soil (85%)in seedling inoculated with *B.luteus*. Shoot height, root length, seedling volume and biomass and nutrients contents in shoot and roots of seedlings grown in forest soil with various mycobionts showed comparatively better improvement than those grown in degraded soil.

Colonization of mycorrhizae of pine seedlings was maximum by *B.luteus* fungus on grass litter amended soil (85%) and minimum by *P.tinctorius* on pine fresh amended soil (61%).It showed a significant positive correlation with

seedling volume, biomass, root phosphorus, P transolocation, phosphatase activity and mycorrhizal productivity with the various litter amendments. Productivity of mycorrhizae was maximum in *B.luteus* inoculated with grass-litter amended soil and minimum in those inoculated seedlings with *P.tinctorius*, grown on unamended soil .Productivity of mycorrhiza showed a significant positive correlation with seedling volume, root phosphorus, P-translocation, biomass and phosphatase activity in all the treatments except with Biomass in pine fresh amended soil and phosphatase activity in unamended soil inoculated with *B.luteus*.

Maximum phosphatase activity of root was observed in grass litter amended soil having *B.luteus* inoculated seedlings.

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I certify that the thesis entitled "Ecophysiological studies on productivity of ectomycorrhizae in *Pinus Kesiya* (Royle Ex.Gordon)", submitted by Mr. C.Satyajit Rao, for the Degree of Doctor of Philosophy of the North-Eastern Hill University, Shillong, embodies the record of original investigation carried out by him under my supervision. He has been duly registered and the thesis presented is worthy of being considered for the award of the Ph.D. degree. This work has not been submitted for any Degree of any other University.

G. D. Sharma

(G.D. Sharma)
Supervisor

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Satyajit Rao

June,1994 *(C.Satyajit Rao)*

TO

MY LATE GRANDMOTHER

GENERAL INTRODUCTION

The mycorrhizal association between various fungi and plant roots is mutualistic and is of an ecologically better adapted, which play an exceedingly influential part in natural and in man-made ecosystems. Both partners derive nutritional benefits in the association. Based on morphological characters mycorrhizal association may be ecto, endo or ectendo types. Ectomycorrhizal association is mainly formed by higher basidiomycetes. Some zygosporic species of phycomycetes have also been reported to form such association (Trappe,1977). Ectomycorrhizae are more common in timber yielding tree species like oak, birch, larch and pines.

The Khasi pine (*Pinus kesiya* Royle ex.Gordon), an indigenous timber yielding tree is an early successional dominant species which grows on denuded hills of North-Eastern India. The Khasi pine harbours the ecto or ect-endomycorrhizal association (Sharma,1981; Kumar,1990; Jha,1990).

Survival and establishment of plant species depends upon a number of ecological factors (Grime,1979), but ectomycorrhizal association is especially adapted to nutrient stress condition. It is an additional eco-physiological aid to plant species growing in a temperate conditons (Mexal,1980).

Ectomycorrhizae help in the establishment, survival and better growth of forest tree species (Kropp and

Langlois,1990). Ecotmycorrhizae are particularly important in the uptake of nutrients that are found in weak concentrations in the soil solution or that are relatively immobile, particularly phosphorus, which is either not accessible or is available in less amount to non-mycorrhizal plants (Theodorou,1971; Ho and Zak,1979). Production of growth hormones (Ulrich,1966;Gogala,1967; Miller,1971; Slankis,1973) and growth regulators (Shemakhanova,1962; Turner,1962) has been reported which are responsible for the changed morphology in ectomycorrhizal association. Mycorrhizae are able to resist the root pathogens, that otherwise would attack the plant roots (Perrin,1985; Duchesne *et al*,1989). They also decrease soil toxicity and increase resistance to extreme soil temperature (Lapeyrie *et al*,1984; Heinrich *et al*,1989). Resistance of mycorrhizae to certain root pathogens, better accessbility to soil nutrients and tolerance to drought renders better survival of seedlings than without mycorrhizal association (Harley and Smith,1983). In absence of mycorrhizae, seedlings of pine become yellow, show stunted growth and ultimately may die (Sharma,1981b).

The rhizosphere of mycorrhizae, or mycorrhizosphere (Rambelli,1973) is rich in bacteria, diatoms, actinomycetes and other fungi. The bacteria are intimately associated with the hyphal cells of the mantle and form a nutritional relationship with fungus (Foster and Marks,1967). The

mycorrhizospheric environment differs from soil factors such as O_2 and CO_2 concentration as well as being a zone of intense microbial activity. Numerous authors have documented some portion of the rhizosphere microbiota associated with ectomycorrhizal roots of trees (Harley and Waid,1955; Kozlowski,1971; Lupi-Mosca and Marchisio,1985; Summerbell,1989). Mycorrhizae themselves normally carry a high population of micro-organisms in the "mycorrhizosphere" Davey,(1971). Katznelson *et al*,(1962) recorded upto ten times the number of bacteria in mycorrhizosphere as in rhizosphere. Bacteria play an active role in the mycorrhizal complex of higher plants as they do on their leaves (Wichner and Libbert,1968). Rhizosphere micro-organisms benefit plants by various means (Trappe,1977). Bacteria, fungi and actinomycetes in the mycorrhizosphere produce substances which influence mycorrhizal fungi, subsequently affecting plant growth (Rozycki and Skrzelczyk,1986; Ames,1987; Strzelczyk *et al*,1986)

Soil microbial enzymes are important in mineralization and play an important role in initial decomposition of organic matter (Kiss *et al*,1978). The activity of any particular enzyme in soil is a composite of activities associated with various biotic, and abiotic components (Burns,1982: Tiwari *et al*,1987a). The enzyme activity of a soil depends not only on the so-called abiontic factors (Skujins,1976), which include

extracellular enzymes, active enzymes within dead cells and those associated with the dead cell fragments, but also the activity is associated with the living microbial cells.

Dehydrogenase activity in soil provides correlative information on the biological activity and microbial population. Microbial biomass and their dehydrogenase activity (Ladd,1978) have been observed to be influenced by the rhizosphere of the host plant (. Ross, 1971; Speir *et al*,1980) and their microbial parameters have been used to study the turnover of naturally occuring organic matter (Spalding,1980). Urease activity generally correlates with organic matter content due to its existence as a complex with organic constituents (Skujins,1973,1976). Besides temperature, soil factors such as moisture content, *p*H, organic matter and number of micro-organisms also affect the urease activity in soil (Skujins,1967, Jha *et al*,1992)

Phosphatase is thought to be directly related to the level of organic phosphorus in the soil. Acid phosphatase, which is present in plant roots (Rogers *et al*,1942) and predominant in acid soils is believed to be responsible for hydrolysis of organic phosphorus in soils. Therefore, the activity of this enzyme is significant in *P* cycling and in plant nutrition. Phosphatases are also produced by ectomycorrhizal fungi (Ho and Zak,1979; Dighton,1983).

Phosphatase activity and its role in *P*-uptake is well documented (Koychler et al 1988 == Jha *et al*,1991).

Increased uptake and mobilization of phosphorus by ectomycorrhizal plants has been attributed to increased fungal phosphatase activity (Alexander and Hardy,1981; Antibus *et al*,1981). Acid phosphatase activity in fungal mantle and the mycelium hydrolyses the complex organic phosphorus, which improves the nutrient uptake (Ho and Zak,1979; Ho,1989).

The formation of mycorrhiza and its efficiency may vary under different soil types (Lee,1982). Ectomycorrhiza formation is affected by substrate *p*H, soil microbiological activity and substrate fertility (Bouvin,1986; Marx and ,198). Drainage and accumulation of organic carbon are also important factors for the colonization of mycorrhizal fungi (McAfee and Fortin,1989). Edaphic conditions, biological factors, soil fertility and management practices affect mycorrhiza development and function (Gianinazzi et al ,1984; Kropp and Langlois,1990).

The concept of ectomycorrhizal synthesis on tree seedlings in nurseries with specific fungi and their ecological adaptation to the planting site was originally developed by Moser (1958). This subject has been reviewed comprehensively (Bowen,1965; Mikola,1973; Trappe,1977; Marx,1980; Molina and Trappe,1982; Browning and Whitney,1993).

Partial or unsuccessful ectomycorrhizal infection is often the limiting factor in experiments intended to explore applications for mycorrhizal fungi. It has been shown that infection depends on many factors, such as the type of soil (Bjorkman,1942; Chilvers and Pryor ,1965) availability of plant mineral nutrients (Mulette,1976) and soil micro-organisms (Garbaye,1983). Infection is also presumably dependant on the inherent receptivity of the root and inherent infectivity of the fungus (Lapeyrie and Bruchet,1985). There are few field studies on the effectiveness of ectomycorrhizal inoculation and lack of knowledge of the behaviour of ectomycorrhizal seedlings in different types of soils (Kropp and Langlois,1990). Thus, it is essential to carefully choose ectomycorrhizal association that is ecologically adapted to reforestation sites. (Perry *et al*,1987).

North-Eastern region of India is very rich in forest resources. However, shifting cultivation coupled with industrialization, urbanization and increased demand of wood has led to the excessive deforestation in the hills, resulting in the formation of denuded wastelands. High precipitation and hilly topography with less vegetation has further fastened the process of depletion of rich soils in these areas. Inspite of favourable climatic conditions, the soil is unable to suppport the growth of trees under natural conditions. The population

of symbionts and other beneficial microbes and microbial activity in such soils have reduced and resulted in poor availability of nutrients (Sharma,1981a,1983).

To successfully apply ectomycorrhiza research to forestry, we must consider and understand the ecophysiological functions of the symbiosis as well as the relationship among the fungi, their host, and the sites onto which they are planted (Kropp and Langlois,1990)). Harley (1969) has suggested taht abundant development of mycorrhizal short roots are essential for tree growth on infertile sites.

Therefore, it was planned, to study the population of mycobionts in the pine plantations of different successional stages growing in the highest precipitation receiving area of the world, undernatural conditions and study the predominant ectomycorrhizal fungi as bioinoculants to assess their efficiency in the establishment and survival of pine seedlings under different edaphic conditiions. The present investigation was therefore aimed to study the following aspects.

- Seasonal variation in ectomycorrhizae, mycorrhizal fungi and soil characters in different age groups of pine.
- Seasonal variation in microbes (Bacteria and Fungi) and microbial activity (Phosphatase, dehydrogenase and urease) in root region of pine (*Pinus kesiya* Royle ex.Gordon).

- Production of ectomycorrhizae with different mycorrhizal fungi and their influence on phosphatase activity of roots.

- Efficiency of ectomycorrhizae formed by different mycobionts in degraded and forest soils.

- Effect of organic amendments on the development of ectomycorrhizae and their efficiency in P-uptake and growth of seedlings under glass house condition.

REVIEW OF LITERATURE

In 1842, Vittadini proposed that tree rootlets are nourished by certain fungal mycelia which mantle them, as observed by him more than a decade earlier. This hypothesis was elaborated to a theory of mutualistic symbiosis by Frank (1885), who named the fungus root organ as "mycorrhiza". The association of fungal hyphae with the roots of orchids and of *Monotropa* was noticed by Reissek (1847) and Kamienski (1881) respectively. Frank (1885) recognised two types of mycorrhiza: ectotrophic and endotrophic. Later on, based on the morphology, Harley and Smith (1983) have classified mycorrhiza into ectomycorrhiza, ectendomycorhiza, endomycorrhiza, arbutoid, monotropoid, ericoid and orchidaceous type.

Marks and Foster (1973) concluded after cytological studies of different fungi that mycorhizal association is truely a symbiotic one like lichens.

Fontana (1962) surveyed mycorrhizal association in 14 tree species from plains, hills and mountains of Italy which possessed ectotrophic mycorrhiza. Richard and Wilson (1963) noticed the variation in the development of mycorrhiza by adding carbohydrates.

Miller (1967) isolated and identified three different kinds of cytokinins from *Rhizopogan roseolus*, a mycorrhizal

fungus, which may be responsbile for the change in the morphology of ectomycorrhizal roots.

Peglar and Fiard (1979) reported that ectomycorrhiza are most prevalent in areas having variable seasons.

Harley and Smith (1983) have the opinion that ectomycorrhizal association may exist in areas where vegetative activity is restricted during some period of the year for edaphic, climatic and other environmental reasons.

Trappe (1962,1977) has estimated over 2000 fungal species as potential ectomycorrhizal symbionts in natural conditions. Most of these species have been identified by their fruit bodies.

Lamb (1979) has reported 40 ectomycorrhizal species in *Pinus elliotii* stand. Sharma (1981) has reported 33 fungal species in east khasi hills of Meghalaya with *Pinus kesiya*. Thomas and Jackson(1983) have reported 24 different fungi with *Pinus sitchnsis*.

Trappe and Strand (1969) have reported that the fungi like *Thelephora terrestris, Laccaria spp., Hebeloma spp. and Inocybe sp.* were associated with young trees. Usher and Parr (1977) also reported that diversity of fungal species increased with the increase in the tree age and decline in thick canopy.

Chu Chou (1979) made a detail observation of mycorrhizal succession with pine. They found several fruiting bodies of *Hebeloma crustiliniforme, Laccaria laccata* and two species of Rhizopogan in tree nursery. Their production was continued upto 3-5 years after transplanting of pine seedlings but *H.crustiliniforme* production ceased and was superceded by *Inocybe spp. and Suillus spp.* After 10 years of transplantation *Amanita muscar.* and *Scleroderma spp.* were observed.

Last *et al*(1981) observed that increase in number of fruit bodies of *Amanita muscaria* was directly correlated with the increased age of *Pinus patula* tree.

Sharma (1981b) have reported that sporocarp production and species occurrence were significantly influenced by the age of the host.

Mason *et al* (1983) have suggested an order of sequential arrangement of mycorrhizal fungi. They recorded that during the second to fourth year after planting birch species, *Hebeloma, Inocybe, Laccaria and Thelephera* colonize quickly after which other fruiting bodies like *Cortinarius, Leccinum and Russula* appeared. Last *et al*(1983) have suggested that sporocarps of sheathing mycorrhizal fungi do not occur at random. Their distribution is dependant on host, time and space. They discovered that some fruiting bodies emerged in the early

stage of mycorrhizal succession and other at later stage.

Last and Flemming (1985) suggested that sporocarps of ectomycorrhizal fungi were mostly arranged in rings around the trees but some of them were arranged linearly, seemingly along with secondary thickened roots.

Last *et al* (1987) observed succession of sporocarps which seems to reflect, in large measure, the different abilities of early and late stage fungi to form mycorrhizas on roots growing in soils with accumulation of recalcitrant leaf litter.

Termorshuizen (1993) observed the nitrogen fertilizer to affect the ectomycorrhizae and their fungal sporocarps in young stands of *Pinus sylvestris.*

Marx (1975) found that seedlings with ectomycorrhizae could survive and grow better than non-mycorrhizal seedlings. Marx *et al*(1976) concluded that *Pisolithus tinctorius* enhanced the growth of *Pinus taeda, P.viriginiata* and *P.strobus.*

Trappe (1977) emphasized about the selection of ectomycorrhizal fungi in forest nurseries practice. He suggested the screening of mycobiont in laboratory followed by experimental inoculation of seedlings and planting them at the different sites.

Lee (1982) reported that *P.tinctorius* stimulated the growth of pine seedlings in nursery soil, but *T.terrestris* failed to do so.

Marx *et al* (1982) emphasized the case of *P.tinctorius* symbiont for inoculation of pine seedlings in extreme soil conditions like, higher temperature, drought and low *p*H of soil.

Chu Chou and Grace (1979) found that *Rhizopogan spp.* were more efficient in increasing the growth of radiata pine than *Laccaria laccata* and *H.Crustiliniforme*.

Gibson and Deacon (1990) observed mycorrhiza formation depends on fungal growth, host genotype and also on external factors including availability of nutrients such as carbohydrates, nitrogen and phosphorus.

Bevege (1970) observed that mycorrhiza of hop, pine is beneficial in phosphorus and nitrogen uptake only when nutrient level in soil is high.

Marx *et al* (1977) reported that high level of N and P in soil decreases the sucrose content of short roots of lobolly pine and the susceptibility of pine to *P.tinctorius*.

Bledsoe and Zasoski (1983) reported that Douglas fir seedlings with mycorrhizae grew taller, increased root and

shoot dry weight and accumulated more N and P with *H.crustiliniforme* than control.

Ford *et al* (1985) reported that after 10 months of pine seedlings transplantation in green house, mycorrhizal fungi enhanced the seedlings growth significantly than control. The performance of *S.aurantium* was superior over the other mycorrhizal fungi in increasing the P-uptake.

Holopainen (1993) reported that depending on the soil characteristics and concentration of other nutrients, there exists an optimum level of N for maximal mycorrhizal development.

Worley and Hacskaylo (1959) observed a reduction in mycorrhizal formation with decrease in soil water availability. Theodorou (1977) suggested that mycorrhizal infection was greatest when the soil water content was from 13 to 20 % and was significantly depressed at 27 % moisture content.

Marx *et al* (1970) established a linear relationship between dry weight of the mycobiont and the temperature. Cline *et al* (1987) observed maximum growth of *Cenococcum geophilum* and *Suillus granulatus* at 27°C and *Pisolithus tinctorius* at 32°C. Harley (1969) reported that acidic *p*H was better for the mycelial growth of mycorrhizal fungi.

Harley and Smith (1983) expressed that the change in *p*H may not be due to absorption of nitrogen compounds, but a result of organic acids which may be inhibitory.

Katznelson *et al* (1962) recorded upto ten times the number of bacteria in mycorrhizosphere as in rhizosphere.

Foster and Marks (1967) and Oswald and Ferchan,1968) concluded that the immediate environment of fungal hyphae and mycorrhizae is rather different from surrounding soil and adjacent uninfected roots thus leading to increased microbial activity in the mycorrhizosphere.

Davey (1971) observed that mycorrhizae themselves normally carry a high population of micro-organisms in the mycorrhizopshere. Seasonal variation in the rhizospheric microbial population of *Pinus radiata* was investigated by Rambelli *et al* (1972) who observed an extremely irregular distribution of the microflora.

Lenhard (1956) for the first time observed the dehydrogenase activity in soil by using Triphenyl tetrazolium chloride salt.

Skujins (1976) studied the seasonal variation in enzyme activity. He recorded that once the enzyme is stabilized in the soil, it develops resistance to humidity, temperature and other environmental changes.

Burns (1982) suggested that total activity in soil is comprised of the activity associated with diffrent soil constitu ents like, microorganisms, cell debris, clay and humic colloids.

Dormar *et al*(1984) found that maximum activity was in fescue grassland site regardless of grazing intensity. Tiwari *et al* (1987_a) mentioned that moisture plays a significant role in the variation of dehydrogenase activity during summer season.

Dalal (1975) has shown that *u*rease activity was significantly related with organic carbon, total nitrogen and cation exchange capacity of soil.

Skujins (1976) reported that urease produced by microbes was correlated with organic matter. Speir (1977) correlated the Urease activity positively with soil *p*H. Rao and Ghai (1985) found that Urease activity was positively correlated with organic carbon and N and negative correlation was observed with soil *p*H and $CaCO_3$ content of soil.

Tiwari *et al* (1987_b) noted higher *u*rease activity during summer season on the surface soil which was attributed to high organic carbon, bacterial population temperature and favourable moisture content of soil.

Ridge and Rovira (1971) observed an increased

phosphatase activity on the surface of mycorrhiza and correlated to the enhanced P mineralization from complex organic p compounds into more readily absorbed forms of phosphorus.

Ho and Zak (1979) suggested that mantle and mycelium of ectomycorrhizae catalyze and hydrolyse the complex organic phosphorus more efficently than non-mycorrhizal roots leading to increased available form of phoshporus in mycorrhizospheric region.

Alexander and Hardy (1981) have noticed that phoshpatase activity of mycorrhizal roots was inversely proportional in P deficient soil. Haussling and Marscher (1989) observed acid phosphatase activity was 2.5 times more in the rhizosphere of Norway spruce than in the bulk soil.

Farrell and Leaf (1974) and Keyes and Grier (1981) suggested that fine root biomass may be higher in sites low in nutrients than those showing no nutrient deficiencies. Turner and Long (1975) observed that ectomycorrhizal root biomass appeared to vary directly with changes in above ground biomass with increasing stand age.

Vogt *et al* (1983) observed that stand developmental stage may also have a strong influence on the amount of mycorrhizal infection of roots and suggested that mycorrhizal

biomass productivity be related to the age of stand and canopy closure of trees.

Lindeberg (1944) and Melin (1946) observed that leaf litter and humus contain substances which may increase the growth of mycorrhizal fungi in culture.

Mikola (1948) showed that extracts of fresh leaves or litter of pine had inhibitory affect on the growth of *Cenoccocum graniforme* and *Lactarius tormines* in high concentrations. Bowen and Theodorou (1967) reported that 80-93% of phosphorus in decomposing *P.radiata* litter was in various forms and much of this may have been bound up in microbial cells and is not available to other micro-organisms until death of the cell.

Mikola (1973) recommended inoculation with forest humus to guarantee rapid mycorrhizal infection.

Prescott *et al* (1993) observed the pattern of nutreint immobilization and found the release could be very different in litter types with a different structure or chemistry.

STUDY SITE, CLIMATE AND VEGETATION

Study Site

Four sites of pine stands (*Pinus kesiya* Royle ex.Gordon) of different age groups representing different stages of their growth, growing in Nongstoin area, West Khasi Hills, Meghalaya were selected for the present study. The pine stands were of 2,4,11 and 17 years old. The sites were situated at an altitude of 1500 m msl. The area lies between $25^{o}35'$N latitude and $91^{o}31'$ longitude (Fig.1). Due to shifting cultivation and heavy precipitation, the hill tops are denuded. The forests are mostly confined to grooves and valleys.

Soil

The Shillong plateau embracing the Garo, Khasi and Jaintia Hills of Meghalaya is made up of largely pre-cambrian rocks acutely folded and steeply dipping with an overturned fringe of mesozoic and tertiary sediments. The rock distribution in the plateau is an ancient mass of genesis much intruded by a coarse granite (Pascoe,1950). Sandstones, limestones and conglomerates with subordinate clay superimposed over these rocks also occur in the Shillong plateau (Zimba,1977). In general the soil is acidic and is red lateritic in nature. The soil of different pine

plantations varied in texture. The physical and chemical properties of soil are shown in the subsequent chapters of the thesis.

SEASONS

Based on the meterological conditions of the region, the year may be broadly classified into rainy, winter and spring seasons.

(i) Rainy season

Rainy season starts from June and ends in October. The maximum precipitation occurs between June to September. On an average the area receives a rainfall of 2520 mm per annum.

(ii) Winter Season

It extends from November to February. The period is characterised with very low temperature and less rainfall (Figure 2). The average minimum and maximum temperature was recorded as 8.6^{o}C to 27.5^{o}C respectively.

(iii) Spring season

It starts from March and ends by May. The period is characterized with high velocity of wind, less humidity and moderate temperature with occasional rains.

VEGETATION

The vegetation of all the pine plantations was almost similar. All the studied sites were found to be pure pine stands. The grasslands represent most important vegetation type of the region. They consists of dominant grass species like *Paspalam dilatatum, Imperata cylindrica, Arundinella benghalensis, Bothriochloa fimbriostris, Panicum auritum and Capillipidium assimile* and the herbs belonging to *Osbeckia sp.*, *Rubus sp.*, *Anaphalis contorta and Desmodium sp.* etc.,

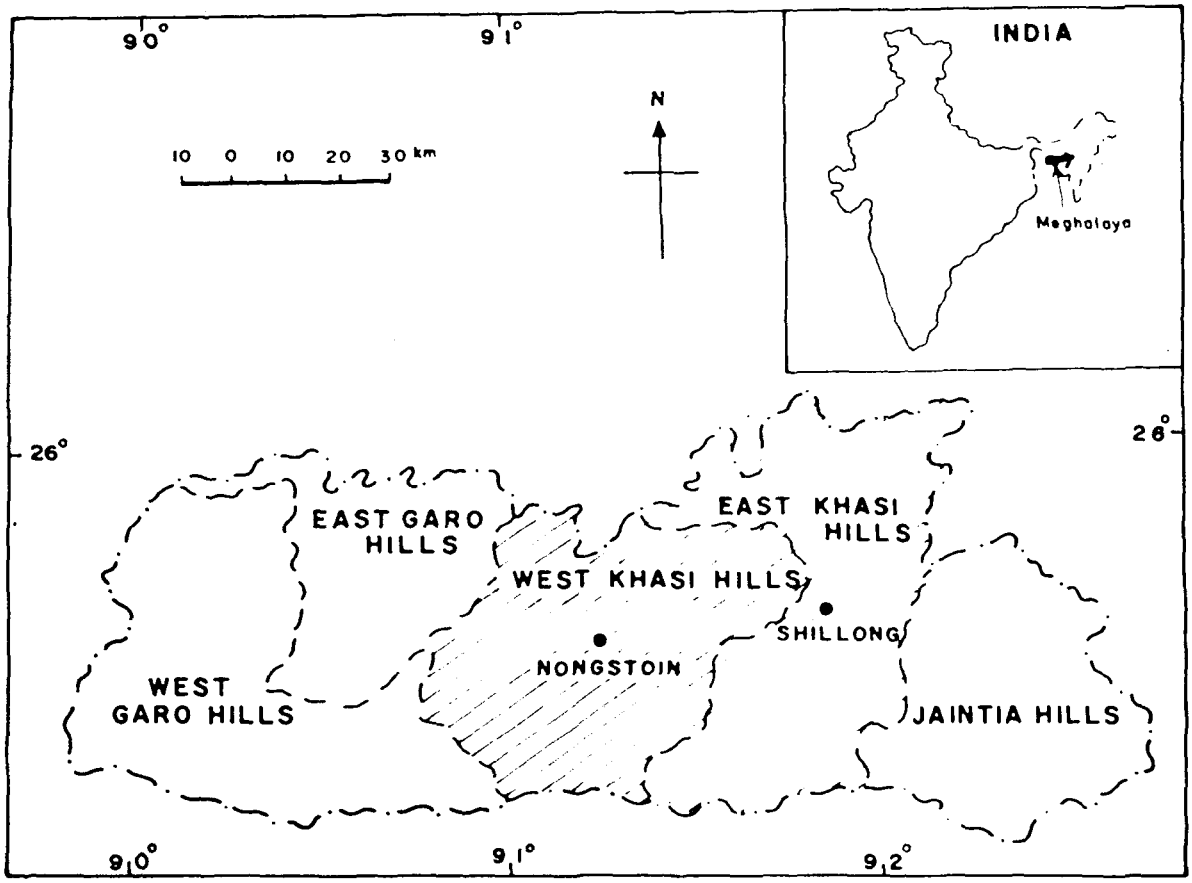


Fig. 1. Study area (shown hatched) in the State of Meghalaya (India).

Figure 2 : Meteorological data for maximum (-o-) and minimum (-●-) temperature (^{o}C) and rainfall (cm) for the period of investigation (April,1986 to February 1988)

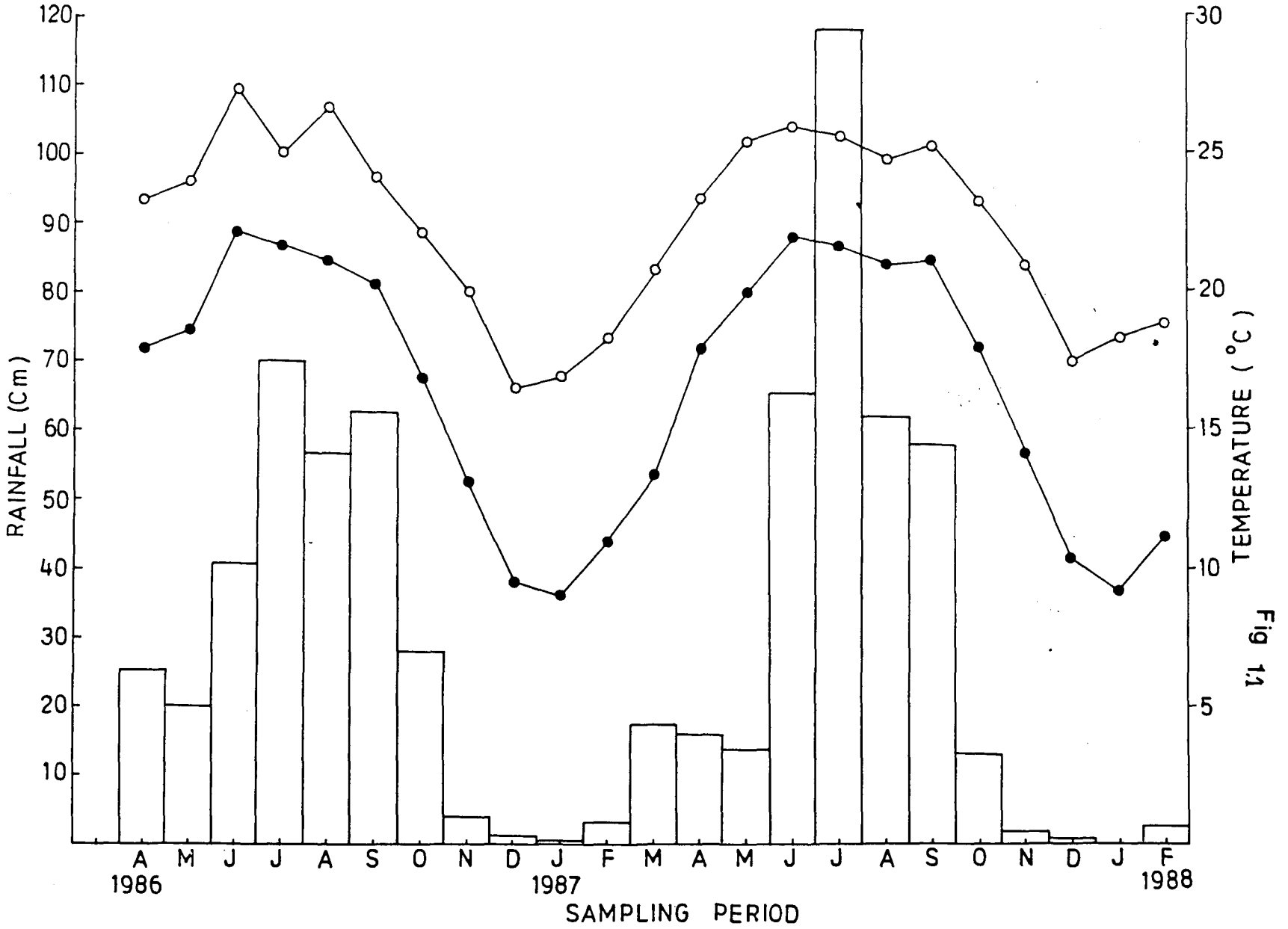

RAINFALL (Cm)
TEMPERATURE (°C)
120
110
100
90
80
70
60
50
40
30
20
10
30
25
20
15
10
5
A M J J A S O N D J F M A M J J A S O N D J F
1986
1987
1988
SAMPLING PERIOD

Fig 1.1

CHAPTER I

SEASONAL VARIATION IN ECTOMYCORRHIZAE, MYCORRHIZAL FUNGI AND SOIL CHARACTERS IN DIFFERENT AGE GROUPS OF PINE

INTRODUCTION

It is estimated that more than 2000 species of fungi have the potential to form sheathing mycorrhiza (Trappe,1977). The sporocarps of sheathing mycorrhizal fungi produce spores and disseminate over a large distance to colonize the roots of trees under favourable conditions. Some investigations on the spatial and temporal distribution of ectomycorrhizal fungi have been carried out in temperate conditions (Deacon *et al*, 1983; Mason *et al* , 1983;Fleming *et al*.,1984; Last *et al* , 1987). The sporocarps are usually absent during winter and dry periods of the year (Wilkins and Harris,1946) and exhibit a temporal variation (Hora, 1959). They may also form the fairy rings (Ford *et al* 1980).

The production of sporocarps is adversely affected by defoliation indicating a close relationship with host plant (Last *et al*, 1979). They either may belong to early stage or late

stage fungi by their occurrence in young and old plantations respectively (Last *et al*,1987). Their production may be enhanced by litter accumulation and decomposition (Mason and Last,1986) and extracellular microbial enzymes (Vogt *et al*,1983). Colonization of mycorrhiza is affected by nutrients in soil and in host plant (Menge *et al*,1978;Graham *et al* 1981, Plenchette,1982) Usually the mineral fertilizers decrease the mycorrhizal colonization and production of sporocarps (Marx and Barnett,1974; Alexander and Fairley,1983; Gagnon *et al*,1988), whereas, Bjorkman(1942) and Bevege (1970) were of the opinion that optimum nutrients in soil favour the colonization of mycorrhiza. Moisture and aeration of soil may also influence the colonization (Harley,1969; Mikola,1969; Medve,1978). Soil temperature may have some effect on the production of new root tips (Barney,1951) and the mycorrhizal growth (Lyr and Hoffman,1967), however, variation in temperature may be effective to change the mycorrhizal colonization (Theodorou and Bowen,1971). The intensity of mycorrhiza, therefore, is largely confined to the upper most surface of soil (Lyr,1963).

Relationship between the age of the plants and the succession of ectomycorrhizal fungi has been investigated in recent years (Sharma,1981; Mason *et al*.1983; Last *et al*,1987). but no attempt has been made to study their distribution in the regenerated pines of different age groups in the degraded lands.

The large scale deforestation on eastern hills of Himalayan ranges in India is causing an ecological imbalance. Large areas are denuded and degraded with a poor growth of vegetation annually in these areas. The physical and chemical properties of soil vary from place to place, which may affect the establishment of pine plantations and colonization of mycorrhizae. Therefore, to understand the ecology of ectomycorrhizal fungi, their colonization with pine roots in different soil types having varying physico-chemical characters with different age groups of pine stands was undertaken.

MATERIALS AND METHODS

Selection of site :

Four sites of pine stands (*Pinus kesiya* Royle Ex.Gordon) of different age groups representing different stages of their growth, growing in Nongstoin area, in West Khasi Hills, Meghalaya were selected for the present study. The pine stands were of 2,4,11 and 17 years old, situated at an altitude of 1500 m msl. The area lies between 25° 35'N latitute and 91° 31'E longitude.

Soil sampling :

Soil sampling in the various stands was done at a bimonthly interval from April,1986 to February,1988. From each

plantation, ten soil monoliths of 15 cm x 15 cm x 15 cm along with the roots of pine were collected from 5 randomly places . From each monolith soil samples, mycorrhizal and non-mycorrhizal regions were taken separately as mycorrhizospheric (the rhizosphere region of mycorrhizal roots) soil sample and non-mycorrhizospheric soil samples in sterilized polythene bags and were brought to the laboratory for further study.

ECOLOGY OF ECTOMYCORRHIZAL FUNGI:

(1) Relative abundance:

Occurrence and abundance of ectomycorrhizal fungi were determined by collecting their sporocarps from each pine stand from April,1986 to February,1988 at bimonthly intervals. Sporocarps were collected from 10 m^{-2} area from each pine stand in five replicates. To ensure the mutual relationship between the sporocarps and the roots of pine tree hyphal connections were traced out from the base of the sporocarps to the mantle of the mycorrhizal roots. The sporocarps of different species of ectotrophs were brought to the laboratory in sterilized container for identification. Sporophores of higher basidiomycetes were identified as suggested by Singer (1962,1975),Bakshi (1974) and Chinery (1983).

Relative abundance of the ectomycorrhizal fungi was determined with the formula.

$$\text{Relative Abundance (\%)} = \frac{\text{Total number of individual sp.}}{\text{Total no. of individuals of all fungal species}} \times 100$$

The ectomycorrhizal fungi were grouped in different frequency classes, as dominant (81-100%), common (61-80%), frequent (41-60%), occasional (21-40%) and rare (1-20%) as suggested by Vittol (1976).

II) Diversity and Evenness:

Shannon diversity index for ectomycorrhizal fungi was calculated as suggested by Margalef (1968).

$$\bar{H} = -\sum_{i=1}^{s} (ni/N) \log_e (ni/N)$$

where, $\bar{H}$ = Diversity index

$\bar{N}$ = Total number of individuals of all the species

ni = Total number of individuals of the individual species

With the help of the values of Diversity Index, Evenness of ectomycorrhizal fungi was also calculated as suggested by Pielou (1966).

$$e = \bar{H}/\log_e S$$

where, e = Evenness

$\bar{H}$ = Diversity index

S= Number of species

(iii) Succession:

Ectomycorrhizal fungi occurrence was categorized on the basis of their early and late appearance as "early successional" and "late successional" fungi. Ectomycorrhizal fungi which appeared just after the first shower of monsoon were grouped in 'early successional category, whereas those fungi which required more precipitation and appeared late in the seasons were grouped in the 'late successional' category.

Early and late-stage fungi were categorized on the basis of their appearance in different ages of pine stands. 'Early-stage' fungi were those fungi which were associated with the young pine trees, whereas, those occurred in old pine stands were termed as 'late-stage' fungi.

iv) Assessment of ectomycorrhizae

Pine roots were sorted out manually from the soil collected in monoliths (15cm x 15cm x 15 cm), at bimonthly interval from each pine stand. The roots were washed gently several times by tap water in a tray. Thereafter, with the help of binocular microscope mycorrhizal infection percentage and the population of ectomycorrhizae/cm was calculated by the following formula (Sharma,1981b)

$$\text{Mycorrhizae (\%)} = \frac{\text{Total number of mycorrhizal rootlets}}{\text{Total number of rootlets}} \times 100$$

Total population of mycorrhizae was recorded on per centimeter of the mean root length.

$$\text{Number of ectomycorrhizae/cm} = \frac{\text{Total no. of ectomycorrhiza}}{\text{Total length of main root (cm)}}$$

Determination of Physico-chemical characters of the soil:

i) Soil temperature

Soil temperature was measured with help of soil thermometer in degree celsius. It was bored into the soil at the depth of 15 cm for five minutes and the temperature was noted down.

ii) pH and soil moisture content.

For measuring *p*H, 10 g of soil from mycorrhizal and non-mycorrhizal regions was suspended in 50 ml double glass distilled water. The soil suspension was stirred on magnetic stirrer for 10 minutes and pH was read out by electronic digital *p*H meter. Moisture content of soil was determined by drying 10g freshly collected soil in a hot air oven at 105^{o} C for 24 hours.

The dried soil was cooled at room temperature in a dessicator and finally weighed on an electronic balance. Percentage of soil moisture was calculated by the formula given below.

$$\text{Soil moisture content (\%)} = \frac{\text{Loss in weight by drying (g)}}{\text{Initial Sample weight (g)}} \times 100$$

(iii) Soil Organic matter:

The organic matter of mycorrhizal and non-mycorrhizal soil was determined by the method of Walkely and Black (1934). One gram dried sieved soil (0.2 mm mesh) was taken in a 500 ml conical flask and 10 ml of $K_2Cr_2O_7$ (1N) solution was added. It was mixed gently and left for 30 minutes. To this mixture, 200 ml of distilled water was added along with 10 ml of 85% orthophosphoric acid and 0.2 g of NaF. Six drops of Diphenylamine were mixed as an indicator in this solution. The solution was titrated with 0.5N ferrous sulphate solution. Organic matter in the soil was calculated by the following formula.

$$\text{Organic matter (\%)} = \frac{V_1 - V_2}{W} \times .003 \times 100 \times 1.724$$

where V_1 = Volume of $K_2Cr_2O_7$ (ml.)

V_2 = Volume of $FeSO_4$ (ml.)

W = Weight of soil (g)

1.724 is a constant factor (Jackson,1973).

(iv) Total Nitrogen content in soil:

Kjeldahl digestion method was followed for the determination of total nitrogen (Jackson,1973) of mycorrhizal and non-mycorrhizospheric soils. To 0.5 g sieved soil(0.2 mm mesh) in a 100 ml Kjeldahl flask, 2g potassium sulphate-

mercuric oxide mixture (20:1) and 3 ml of H_2SO_4 were added. The digestion was carried out on a digestion unit. At the end of digestion, when the colour of the solution turned transparent, the heating was stopped and the flasks were allowed to cool. The content was diluted with 50 ml distilled water. The solution was then ready for the determination of total nitrogen by spectrophotometer.

To 2 ml digested solution in a 50 ml volumetric flask, 8 ml alkaline Rochille reagent (60 g of sodium-potassium tartrate in 600 ml distilled water) was added and mixed. After that 1 ml sodium nitroprusside solution (0.16 %, w/v) was added and mixed. Now 2 ml solution of sodium phenol reagent (50 g phenol was dissolved in 250 ml 40 % NaOH and diluted to 400 ml with distilled water) was added and mixed. Finally 1 ml sodium hypochlorite reagent (1N) was added and the volume of the solution in the volumetric flask was made to 50 ml by adding distilled water and mixed well. The flasks were then kept on a water bath at 40°C for 20 minutes to develop blue colour of the solution. Later on the flask was allowed to cool and the optical density of the solution was measured at 625 nm against blank. A standard curve was prepared using NH_4^+ nitrogen and with the help of the standard curve, percentage of total nitrogen was calculated as follows.

$$\text{Nitrogen}(\%) = \frac{C(\text{mg}) \times \text{solution volume (ml)}}{10 \times \text{aliquot (ml)} \times \text{sample weight (g)}}$$

where, C= mg NH_4^+ nitrogen obtained from the standard curve.

(v) Soil Phosphorus:

The available phosphorus was extracted by ammonium fluoride extraction solution method (Allen,1974). The extraction solution was prepared by mixing 1.11 g NH_4F in 4.61 ml of 6N HCl and total volume was made to 1 litre with distilled water. 2.850 g sieved soil was taken into 100 ml conical flask in which 20 ml of extraction solution was added and stirred for 15 minutes on mechanical shaker. The extract was filtered through Whatman No.44 filter paper. Then, 2 ml aliquot was taken into 50 ml volumetric flask and 5 ml of water was also added. Thereafter, 2 ml of ammonium molybdate and stannous chloride was mixed and total volume was prepared to 50 ml by adding distilled water and left for 30 minutes. The optical density of the solution was measured on spectrophotometer at 700 nm wave length and converted into known units through the standard calibration curve and calculated into percentage phosphorus by the following formula:

$$\text{Available P (\%)} = \frac{C_{(mg)} \times \text{solution volume}_{(ml)}}{10 \times \text{aliquot}_{(ml)} \times \text{sample weight}_{(mg)}}$$

Where, C = concentration reading (mg) in the aliquot.

(vi) Exchangeable potassium in soil:

The potassium was extracted in ammonium acetate solution (*p*H-7) (Allen,1974). The latter was prepared by mixing 575 ml

glacial acetic acid with 600 ml of ammonium solution and diluted to 10 litres with distilled water. The *p*H was adjusted to 7±0.5 with the help of acetic acid or ammonia solution. Ten grams dried, sieved soil was taken into 500 ml conical flask and to it was added 250 ml extraction solution and stirred for an hour, thereafter, filtered through Whatman no. 44 filter paper. The exchangeable potassium of filtrate was read out by flame-photometer and converted into known unit through standard curve and percent potassium was calculated by the following formula:

$$\text{Exchangeable K (\%)} = \frac{C_{(ppm)} \text{ from graph X solution volume}_{(ml)}}{10 \text{ X sample weight}_{(g)}}$$

RESULTS

Sporocarps of twelve sheathing mycorrhizal fungi in the first year and thirteen in the second year of investigation were observed (Table 1.1). Few of them were observed for a short duration, while other occurred for a longer period. The frequency of occurrence of mycorrhizal fungi was maximum during rainy season and minimum or absent during winter season. *Boletus luteus, Suillus granulatus, Scleroderma aurantium* occurred abundantly throughout the study period (Plate 1).

In two year old stand few sheathing mycorrhizal fungi sporocarps namely *Boletus luteus, Suillus granulatus. Scleroderma aurantium and B.edulis* were observed. Sporocarps

of *Tricholoma saponaceum, Hygrophorus limacinus, Lactarius sp.* were observed only during winter period in both the years.

In four year old stand, sporocarps of *Lactarius deliciosus, Boletus luteus* occurred throughout the year whereas *Scleroderma aurantium.* occurred only during winter months. Abundance of sporocarps reduced during winter months.

In 11 year old plantation, *Tricholoma saponaceum and Boletus sp.* occurred throughout the year whereas *Amanita muscaria, A. Panthenina, Lactarius sp., Russula sp. Suillus granulatus and Scleroderma sp.* were absent during winter months. *A. patherina* and *Lactarius sp.* occurred during rainy season.

In the 17 years old plantation maximum number of sporocarps of sheathing mycorrhizal fungi was observed during rainy season and minimum during winter season. *A.muscaria, B.edulis and S.granulatus* occurred only during rainy season. The population of *Scleroderma sp* . was highest in 2 years old stand. *Boletus sp.* occurred in more numbers throughout the year, showing a broad ecological amplitude.

Sclerotia of *Cenococcum sp.* were noticed in all the plantations and throughout the study period. Diversity of sheathing mycorrhizal fungi was directly proportional to the age of the pine stand *i.e.,* more fungal species were observed in old stand (Table 1.1a)

Evenness of sheathing mycorrhizal fungi also increased with the increasing age of the pine stand except during winter in 11 years old pine plantation (Table 1.2b).

Colonization and population of ectomycorrhizal fungi

Population of ectomycorrhizae showed a uniform pattern of variation in all the plantations. Maximum population was observed during the month of October and minimum in December and February months. (Figure 1.1a).

Colonization of mycorrhizal fungi (%) also showed a uniform pattern of variation in all the plantations. Maximum colonization of mycorrhizae was observed in August,1986 and ctober,1987 and minimum during winter months. Positive correlation was observed between colonization of mycorrhizae and population of ectomycorrhizae in all the plantations (Fig. 1.1b).

Physico-chemical characters of the soil

Temperature of the soil was low in winter months in all the four plantations and increased slowly towards the April months and reached to maximum in June. Soil temperature showed a significant variation within the different age plantations ($p < 0.05$), sampling periods ($p < 0.01$), mycorrhizospheric and non-mycorrhizospheric region ($p < 0.01$) and showed correlation between age of plantation with sampling periods (Table 1.4).

Soil moisture content

In 2, 11 and 17 years old pine stands, lowest amount of soil moisture was observed during December but in 4 year old plantation, it was during October. Maximum soil moisture was during August-October months in all the four stands. It was comparatively less in the non-mycorrhizospheric region than in mycorrhizospheric ones. Similar trend in soil moisture contents was observed in all the plantations (Fig.1.2). Soil moisture showed a significant variation within the different age plantations ($p < 0.02$), sampling periods ($p < 0.01$) and mycorrhizal and non-mycorrhizal region ($p < 0.01$) (Table 1.4). A significant positive correlation was observed between soil moisture and population of mycorrhizae and mycorrhizal infection in all the plantations (Table 1.3).

Soil *p*H

The *p*H of soils of mycorrhizospheric (MR) and non-mycorrhizospheric (NMR) regions showed a similar pattern of variation in their respective plantations. Low *p*H of the soil was observed during June in 2,4,11 and 17 years old plantations. High *p*H values in 2 year old pine stand were found in February in MR region and in October in NMR region. In 4 year old plantation, high *p*H values were observed in December in MR region and in August in NMR region. In 11 and 17 years old plantation high *p*H values were observed in February in MR region and in August in NMR region (Fig.1.3). Soil *p*H showed a significant variation within the different

age plantations ($p < 0.01$), sampling periods ($p < 0.01$) and mycorrhizal and non-mycorrhizal region ($p < 0.01$) Table 1.4. A positive correlation was observed between soil *p*H and population of mycorrhizae in 2,11 and 17 year old pine plantations and a negative correlation in 4 year old plantation in the mycorrhizosphere.

Total Nitrogen

Total nitrogen in the soils of MR region and NMR region showed a uniform pattern of variation in all the plantations. Soil nitrogen was maximum during August month in 2 and 11 year old plantations in MR and NMR regions and lowest nitrogen content in 2 year old plantation in April and February in MR and NMR regions respectively. In 4 year and 11 year old plantation, lowest nitrogen content was observed in June and April respectively in both the regions whereas in 17 year old plantation highest level of nitrogen was observed in October and August months in MR and NMR regions respectively. Lowest nitrogen content was observed in April month in MR and NMR regions (Fig. 1.5). Total nitrogen of the soil showed a significant variation within the different age plantations ($p < 0.01$), sampling periods ($p < 0.01$) and mycorrhizal and non-mycorrhizal region (Table 1.4). A positive correlation was observed between total nitrogen of soil and population of mycorrhizae in 11 and 17 years old plantation while positive correlation between total nitrogen and mycorrhizal infection

was obtained in 17 years old plantation only. (Table 1.3).

Available Phosphorus

Phosphorus content of the soil showed a variation during the study period. Phosphorus contents in NMR region were comparatively less than in the MR region. In the 2 year old pine stand maximum phosphorus content was observed in December and February in MR and NMR regions respectively and lowest amount in October and August in MR and NMR regions respectively. In 4 year old pine plantation, maximum amount was observed during august in MR and NMR regions and minimum in October in MR and NMR regions. In 11 year old plantation, maximum amount of P was observed during August and April in MR and NMR regions respectively, whereas, minimum amount was observed during October and February in MR and NMR regions respectively. In 17 year old plantation maximum amount of P was observed during June in MR and NMR regions and minimum during December months (Fig. 1.6). Phosphorus content of soil showed a significant variation within the different age plantations ($p < 0.05$), Sampling periods ($p < 0.05$) and mycorrhizal and non-mycorrhizal region ($p < 0.01$) (Table 1.4). A positive correlation was observed between soil phosphorus and population of mycorrhizae in 2 and 17 years old plantation and with mycorrhizal infection in 2,4 and 17 years old pine plantations (Table 1.3).

Exchangeable potassium

Exchangeable potassium was significantly higher in the MR region than in the NMR region. In 2 year old plantation, maximum K amount was present during August in MR and NMR regions and minimum during February. In 4 years old stand maximum amount of potassium was found in August and December for MR and NMR regions respectively. In 11 years old stand maximum amount was observed in June and August for MR and NMR regions respectively whereas minimum was observed in February and December in MR and NMR regions respectively. In the oldest pine stand, maximum amount of exchangeable potassium was observed in August in both MR and NMR regions and minimum in February and December in MR and NMR regions respectively (Fig. 1.7). Exchangeable potassium of soil showed a significant variation within the different age plantation ($p < 0.01$), Sampling periods ($p < 0.01$) and mycorrhizal and non-mycorrhizal region (Table 1.4). A positive correlation was observed between exchangeable potassium and mycorrhizal infection in 17 years old plantation (Table 1.3).

Organic matter

The organic matter of soil varied greatly in the different pine stands. It was less in NMR regions than in MR ones which increased with the increase in the age of the pine plantation. In 2 year old plantation, maximum organic matter

was observed during October in both the regions and minimum during December. In 4 year old plantation maximum organic matter was observed in October and August in MR and NMR regions respectively and minimum during June and December in MR and NMR regions. In 11 year old plantation maximum and minimum amounts of organic carbon were observed in October and August in MR and NMR regions respectively and minimum during June and December in MR and NMR regions. In 11 years old plantation maximum and minimum amount were observed in August and December for MR and NMR regions respectively. In 17 year old plantation maximum amount was observed during February in both the regions and minimum in December and June in MR and NMR regions respectively (Fig. 1.4). Soil organic matter showed a significant variation within the different age plantations ($P < 0.01$), Sampling periods ($P < 0.01$) and mycorrhizal and non-mycorrhizal region ($p < 0.01$) (Table 1.4). A significant positive correlation was observed between soil organic matter and mycorrhizal population and mycorrhizal infection in all the pine plantations (Table 1.3).

DISCUSSION

In natural conditions various fungal species are reported of forming mycorrhizal association with trees (Last *et al* ,1987). Some of them are short lived while others are alive for a longer period (Marx and Bryan,1969).

In the present investigation thirteen species of ectomycorrhizal fungi from the different age groups of pine stands were identified. The low number of sporocarps during winter months was attributed to the low temperature and moisture in soil (Sharma,1981b). Steep fall in temperature, moisture and needle fall influenced the supply of organic nutrients to sheathing mycorrhizal fungi and also their growth (Hacskaylo,1965). Defoliation has been correlated to the fall in the population of symbiotic fungi (Last *et al*,1979). Some species of pseudosheathing mycorrhizal fungi may also be saprotrophs which are not influenced by the host whereas other true symbionts depend for their growth only on the host (Linde berg,1970).

A positive correlation between the population of ectomycorrhizal fungi and soil moisture, temperature, rainfall, pH, available phosphorus, total nitrogen and organic matter was observed. Very low soil moisture, temperature, rainfall, pH, amount of phosphorus and organic matter adversely affected the production of sporocarps, either individually or combined (Wilkins and Harris, 1946. and in Lahio,1970). Increased acidity (pH) in winter due to litter fall along with low soil moisture and temperature, seemed to have adverse effect on sporocarps production. They also caused the death of mycorrhizal root and fungal hyphae which may cut off the link of symbiont from the host plant and ultimately affect the development of mycobiont. Low temperature may inhibit the germination of mycorrhizal

spores which is related to sporocarps production (Bowen and Theodorou,1973).

High relative abundance of sporocarps of sheathing mycorrhizal fungi in older stands during rainy season than young stands may be attributed to better soil moisture, optimum temperature and organic carbon. Soil N,P and K were suitable for the production of sporocarps. Thick layer of pine litter in old plantation might also help in conserving the moisture content and water evaporation from soil surface and thus responsible for maintaining suitable soil temperature and organic matter which can be important source of energy for the production of sporocarps (Mason and Last,1986). Availability of suitable amount of nutrients in old stand, due to decomposition of litter by microbial activity, also was inducive for sporocarps production (Hora,1959;Shubin *et al*,1977). Probably, acidic nature of soil also might have helped the sporocarps emergence (Wasterlund,1982). *Suillus sp* . is adapted to low *p*H and compete better with other microbes in the rhizosphere (Ohenoja,1988). Therefore, an optimum *p*H and nutrient equilibrium in soil may be an important regulatory factor in the production of fruiting bodies of the ectotrophs.

The sporocarps of *Boletus,Scleroderma,Tricholoma and Hygrophorus spp.* were observed as an early colonizing fungi in the young pine stands. However, in older plantations of pine

Boletus, Amanita, Russula and Cenococcum sp.. were observed frequently as later stage fungi (Last and Flemming,1985). Sporocarps of *Russula and Amanita* were observed in young plantation. It might be due to variation in the nutrient secretion by the host and environmental conditions. However, occurrence of late stage fungi *i.e., Amanita, Scleroderma and Cenococcum sp.* has broad ecological amplitude (Mason *et al*,1983,1986). The sclerotia of *Cenococcum sp.* were observed during late rainy season when the moisture content of soil was moderate and *p*H was slightly less. However, *Cenococcum sp.* was reported to be prevalent at comparatively dried sites (Trappe,1962) indicating that the fungus was well adapted to the broad edaphic conditions of pine in West Khasi Hills of Meghalaya.

Early and late stage fungi may be equally efficient to develop mycorrhiza with roots (Mason,1980). However, they vary in their nutrient demand (Dighton and Mason,1985). The late stage fungi unlike early stage ones are unable to form mycorrhiza due to their low competitive ability than other micro-organisms while growing in first rotation in unsterile forest soil (Mason and Last,1986). *B.luteus and S.granulatus* produced sporocarps constantly in all the pine plantations. However, *B.luteus* was dominant mycorrhizal species in the younger stands. Results have shown that *B.luteus* and *S.granulatus* had the characteristic of both early and late

stage fungi. The early stage mycorrhizal fungi are better adapted for colonization in mineral soil (Alvarez *et al*,1979), whereas, late stage ones prefer more organic substrate (Harvey *et al*,1979) and easily colonize the roots which have been amended with pine litter (Harvey *et al*,1976; Alvarez *et al*,1979). Late stage mycorrhizal fungi were encountered higher in number in older pine stands showing a direct correlation with organic matter (Tyler,1984). Mason and Last (1986) observed that early stage fungi need very less amount of carbohydrates for their rapid growth than late stage ones but the decomposition of litter produced some exogenous enzymes increasing the availability of energy to the late stage fungi. Absence of late stage fungi from younger pine stand could be related to the low amount of pine litter and less carbohydrate than older stands which reduced the availability of extracellular enzymes to them (Vogt *et al*,1983). However, soil type and genotypic differences could modify the population of early stage sheathing mycorrhizal fungi on younger trees (Last *et al*,1984).

The sporocarps of sheathing mycorrhizal fungi *i.e., Suillus sp.,Russula sp. and Lactarius sp.* have been reported to form the fairy rings around the birch trees (Becker,1956;Mason *et al*,1983), but no ring formation of sporocarps was observed with Khasi pine. Most of the sporocarps were noticed as scattered or in small patches.

Better colonization and infection of mycorrhizal fungi with pine roots in all the pine stands was significantly correlated to soil moisture, *p*H, temperature, organic matter, available phosphorus which could be due to the pine litter decomposition accelerating the availability of carbohydrate which stimulated the colonization of fungi (Herman *et al*,1977).

Increased nitrogen in mycorrhizospheric and non-mycorrhizal region during the rainy season could be attributed to the favourable temperature and moisture which enhanced microbial mineralization of nitrogen. Death of mycorrhizal roots and hyphae strands of mycorrhizae and other microbes due to low temperature and moisture in the winter months decreased the availability of N in both the regions (Gagnon *et al*,1988).

Phosphorus content in MR and NMR regions was significantly higher during rainy season (August) which is positively correlated with mycorrhizal colonization, indicating their increased solubilizing capability, diffusion surface area and volume of root in soil. Significant increase in phosphorus in MR than NMR region in all the plantations was due to mycorrhizal symbiosis.(Heap and Newman, 1980; Maghembe and Readhead,1984; Marx *et al*,1985; Heinrich and Patrick, 1986). Better utilizing efficiency of the fixed phosphate in

adverse environmental conditions by the mycorrhiza has been assigned to their high amount of phosphatase activity (Flick, 1984; Korchler et al, 1988; Ho, 1989).

Amount of exchangeable potassium in both MR and NMR regions followed the similar trend like of phosphorus. The increased K uptake by mycorrhiza is mainly associated with improved growth of the plants (Hatch, 1937 and Maghembe and Redhead, 1984).

Significantly more amount of nutrients in mycorrhizosphere than non-mycorrhizospheric region reflects the importance of mycorrhiza in mineralization. Such affinity can therefore be exploited by selecting the early and late colonizing mycorrhizal fungi depending upon the age of the afforested trees.

Table 1.1 Abundance (%) of sporocarps of ectomycorrhizal fungi in different age groups of pine(*Pinus kesiya*) stands

Ectomycorrhizal fungi	year of sampling	2 years			4 years			11 years			17 years		
		spring	Rainy	Winter	Spring	Rainy	Winter	Spring	Rainy	Winter	Spring	Rainy	Winter
Amanita muscaria	1986							17	4			5	
	1987-88							16	9			8	
A. phalloides	1986										6	5	
	1987-88										11	6	
A. pantherina	1986					4			4				
	1987-88					6			5				
Tricholoma saponaceum	1986			11		14	6	5	14	13		4	7
	1987-88			13		6	5	4	14	18		6	12
Hygrophorus limacinus	1986			11									4
	1987-88			13		6						3	8
Lactarius deliciosus	1986			11	20	18	12		7		10	9	
	1987-88			13	22	20	25		11		15	11	
Russula lepida	1986									4	10	4	
	1987-88								5	4	13	6	
R. delica	1986							11			10	10	
	1987-88	6						23	5		11	8	
Boletus edulis	1986											4	
	1987-88												
B. luteus	1986	25	33		25	23	12	27	17	8	23	15	
	1987-88	20	22		26	27	10	26	13	14	17	14	8
Suillus granulatus	1986		8		18	14		11	10			10	
	1987-88		24		22	13		13	13			10	
Scleroderma aurantium	1986	38	34	11			6		7		3	4	
	1987-88	46	22	13			10				4	2	
Cenococcum graniforme (Sclerotia)	1986	37	25	56	37	27	64	29	34	79	38	30	89
	1987-88	28	35	48	30	22	50	18	25	64	29	26	72

Table 1.2

(a) Diversity index of ectomycorrhizal fungi in different age groups of pine stands

Age of pine stand (yrs)	Sampling period (season)					
	Spring		Rainy		Winter	
	1986	1987	1986	1987	1987	1988
2	0.870	1.21	0.592	1.07	1.550	1.430
4	1.320	1.33	0.972	1.53	0.670	1.301
11	1.638	1.37	1.85	1.98	0.651	0.980
17	1.650	1.87	2.124	1.30	0.408	0.890

(b) Evenness of ectomycorrhizal fungi in different age groups of pine stands

Age of pine stand (yrs)	Sampling period (season)					
	Spring		Rainy		Winter	
	1986	1987	1986	1987	1987	1988
2	0.261	0.387	0.238	0.313	0.646	0.528
4	0.456	0.415	0.314	0.449	0.241	0.434
11	0.566	0.46	0.498	0.492	0.204	0.296
17	0.952	0.487	0.537	0.377	0.122	0.278

Table 1.3 : Correlation coefficient (r) value between population of ectomycorrhizae, mycorrhizal infection and the various physico-chemical characters of the soil in different pi plantation.

Physico-chemical characters	Mycorrhizae/cm.				Mycorrhizal infection			
	A	B	C	D	A	B	C	D
1. Soil moisture	0.61*	0.64*	0.70**	0.69**	0.59**	0.68**	0.58*	0.74**
2. Organic carbon	0.57*	0.62*	0.67*	0.59*	0 65*	0.57*	0.79**	0.60*
3. Soil pH	0.62*	-0.47	0.80***	0.75**	0.52	0.32	0.11	0.42
4. Total Nitrogen	0.39	0.57	0.67*	0.75**	0.25	0.48	0.54	0.68*
5. Available Phosphorus	0.70**	0.20	0.242	0.60*	0.62*	0.57*	0.37	0.64*
6. Exchangable Potassium	0.10	0.28	0.13	0.36	0.37	0.49	0.52	0.59*

A = 2 Years old plantation
B = 4 Years old plantation
C = 11 Years old plantation
D = 17 Years old plantation

* = Significant at P<0.05
** = Significant at P<0.01
*** = Significant at P<0.001

Table 1.4 . Analysis of variance (ANOVA) values (f) within the different age plantations, sampling periods and mycorrhizal and non-mycorrhizal region of various parameters in the pine plantations.

Source of Variation	D.F.	Parameters						
		Total Nitrogen	Available Phosphorus	Exchangeable Potassium	Soil *pH*	Soil moisture	Organic matter	Soil temperature
1. Row-effects	3	76.54**	3.46*	34.89**	37.77**	28.57**	4.35**	4.12**
2. column-effects	5	16.84**	3.04*	10.61**	22.69**	67.38**	18.21**	124.24**
3. Layer-effects	1	93.51**	97.79**	114.37**	11.11**	22.61**	167.12**	3.02**
4. Row-column	15	0.29	1.21	2.67**	3.4**	2.54**	4.32**	2.35**
5. Row-Layer	3	5.90**	1.17	15.68**	0.36	0.75	4.75**	0.06
6. Column-layer	5	0.26	0.95	3.25*	1.46	0.47	0.59	0.11
7. Row-Column-Layer	15	0.18	0.71	1.74*	1.66	0.35	1.25	0.07

Row = Pine plantations
Column = Sampling periods
Layer = Mycorrhizal and non-mycorrhizal regions.
D.F. = Degrees of freedom

* = Significant at P < 0.05 level
** = Significant at P < 0.01 level.

Figure 1.1: Bimonthly changes of population of ectomycorrhiza/cm(a) and colonization of mycorrhiza(%)(b) in different pine stands

Fig 14

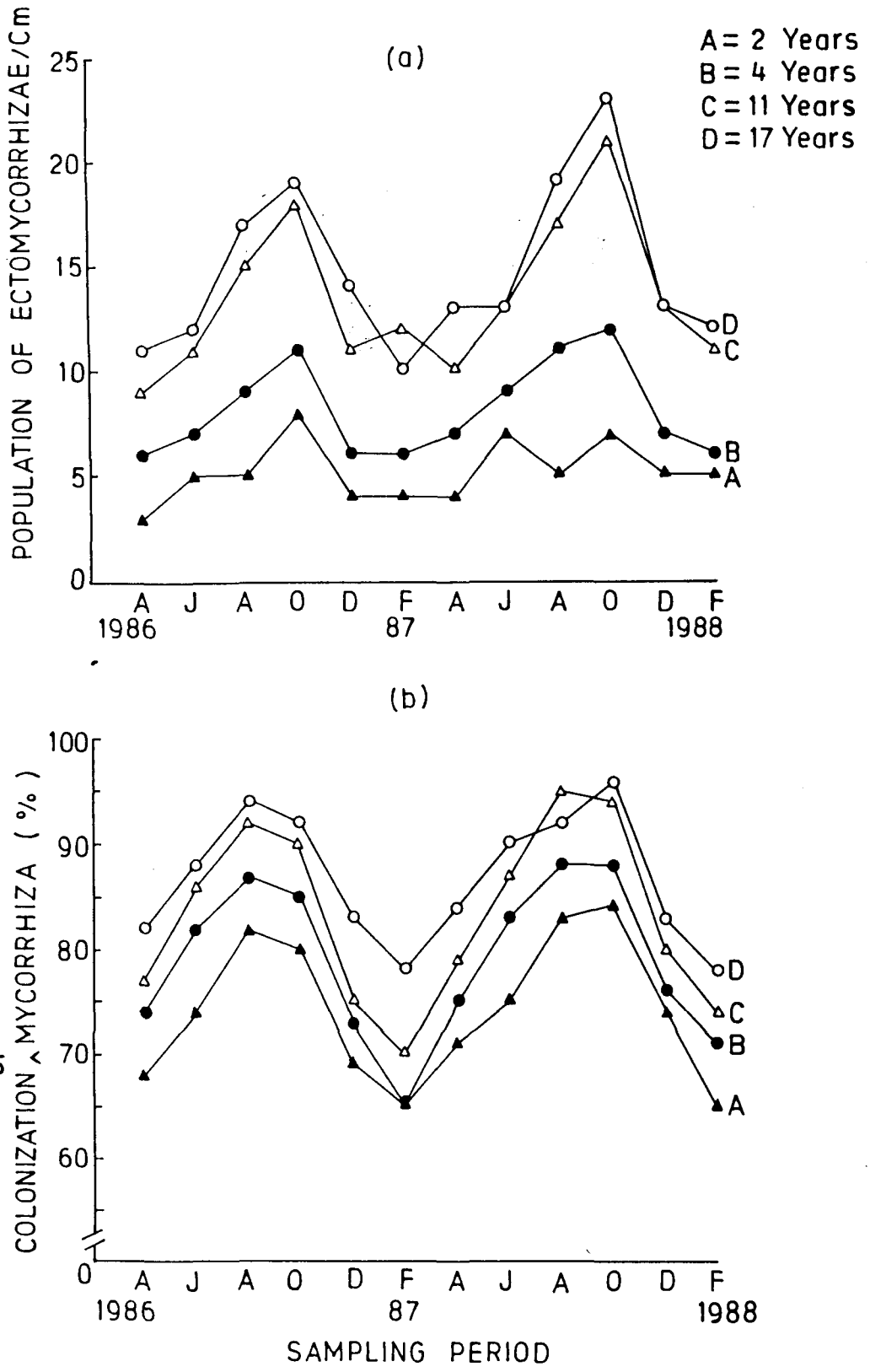

(a)
A = 2 Years
B = 4 Years
C = 11 Years
D = 17 Years
POPULATION OF ECTOMYCORRHIZAE/Cm
25
20
15
10
5
0
D
C
B
A
A J A O D F A J A O D F
1986
87
1988
(b)
COLONIZATION OF MYCORRHIZA (%)
100
90
80
70
60
0
D
C
B
A
A J A O D F A J A O D F
1986
87
1988
SAMPLING PERIOD

Figure 1.2: Bimonthly changes of soil moisture content(%) in mycorrhizospheric(MR)(-o-) and non-mycorrhizospheric (NMR)(-●-) regions in different pine stands.

Fig 1.2

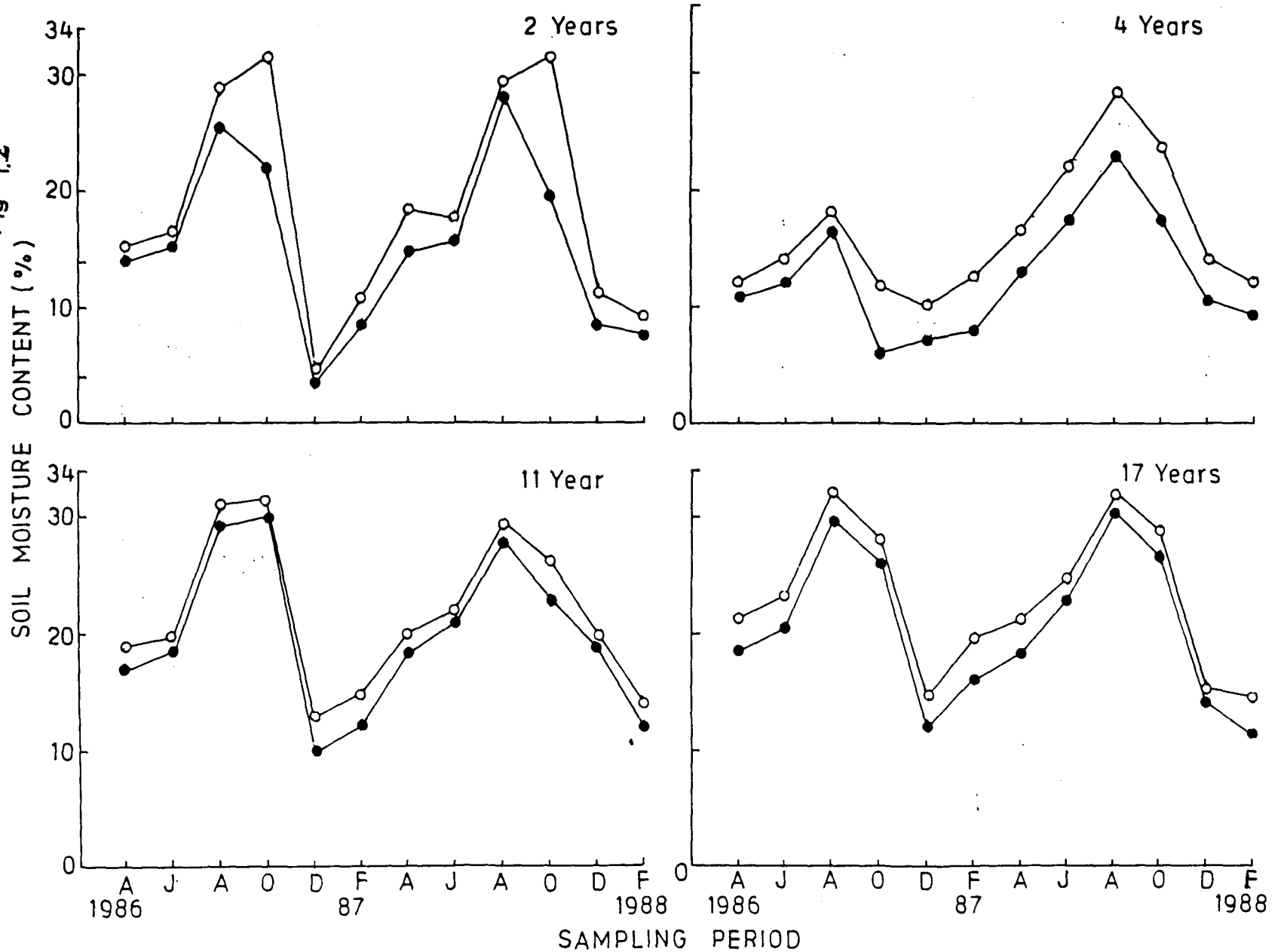

2 Years
4 Years
11 Year
17 Years
SOIL MOISTURE CONTENT (%)
34
30
20
10
0
A J A O D F A J A O D F
1986
87
1988
SAMPLING PERIOD

Figure 1.3: Bimonthly changes of soil *p*H in mycorrhizospheric(MR)(-o-) and non-mycorrhizospheric(-●-) (NMR) regions in different pine stands.

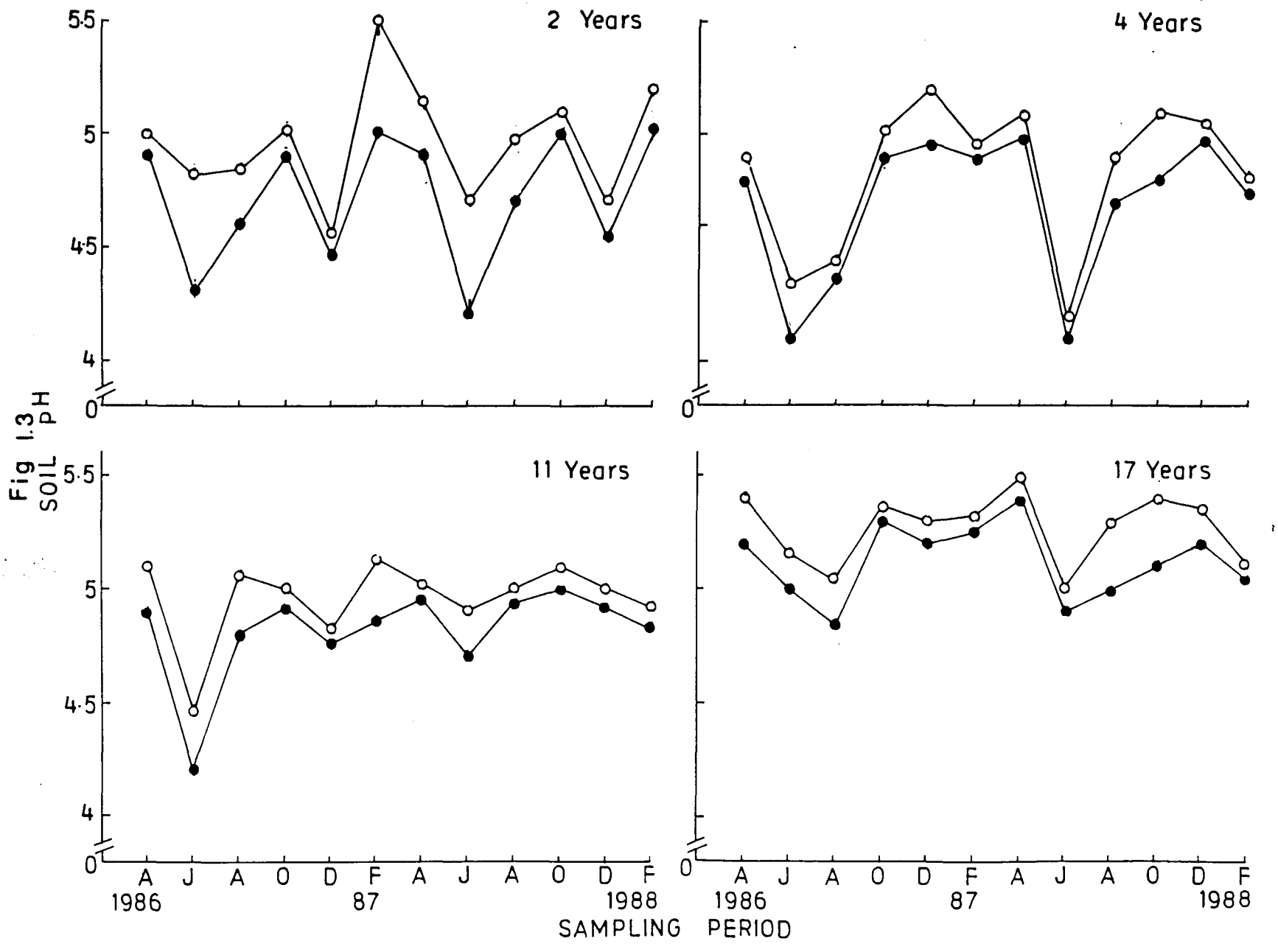


Fig 1.3 SOIL PH

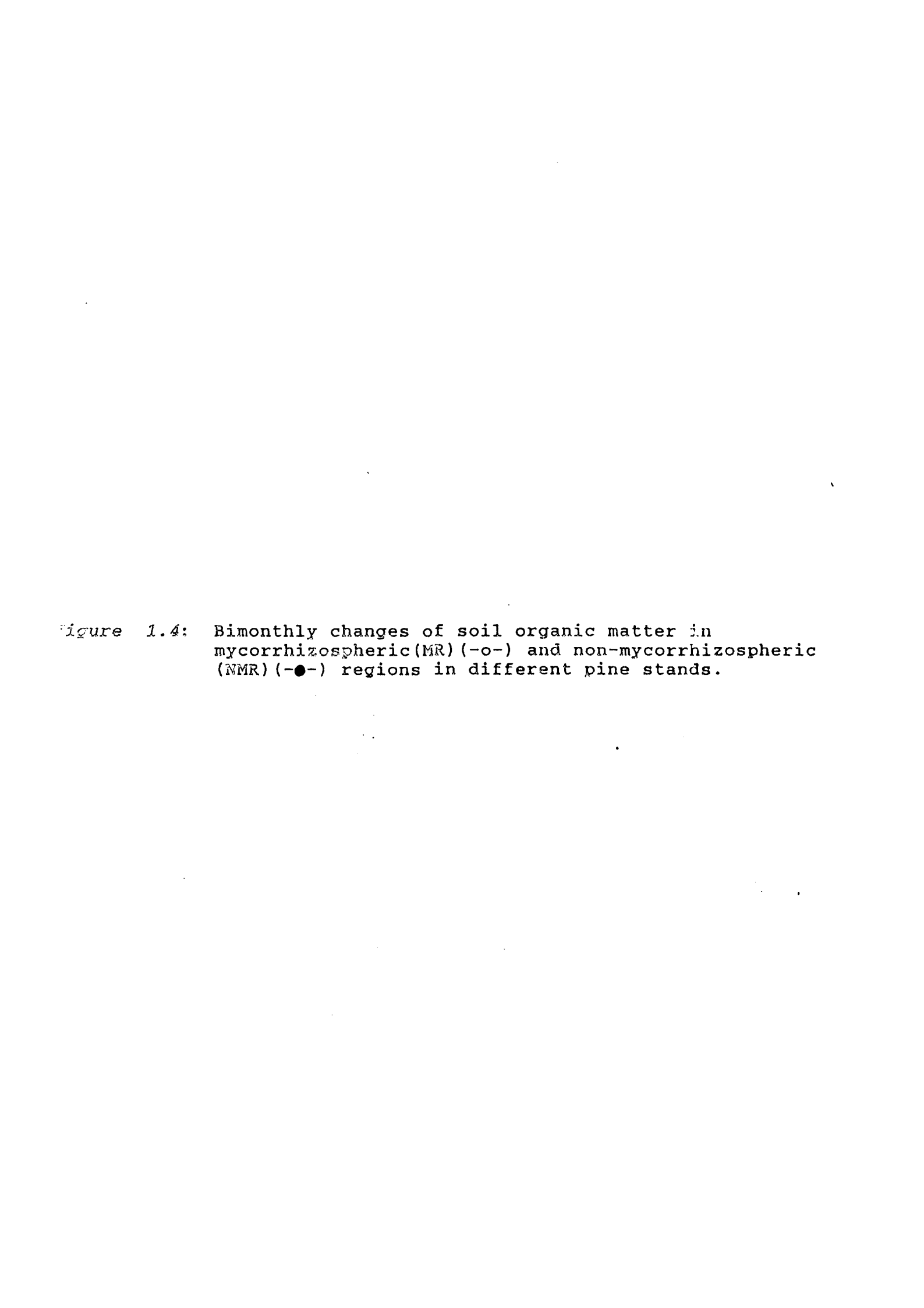


Figure 1.4: Bimonthly changes of soil organic matter in mycorrhizospheric(MR)(-o-) and non-mycorrhizospheric (NMR)(-●-) regions in different pine stands.

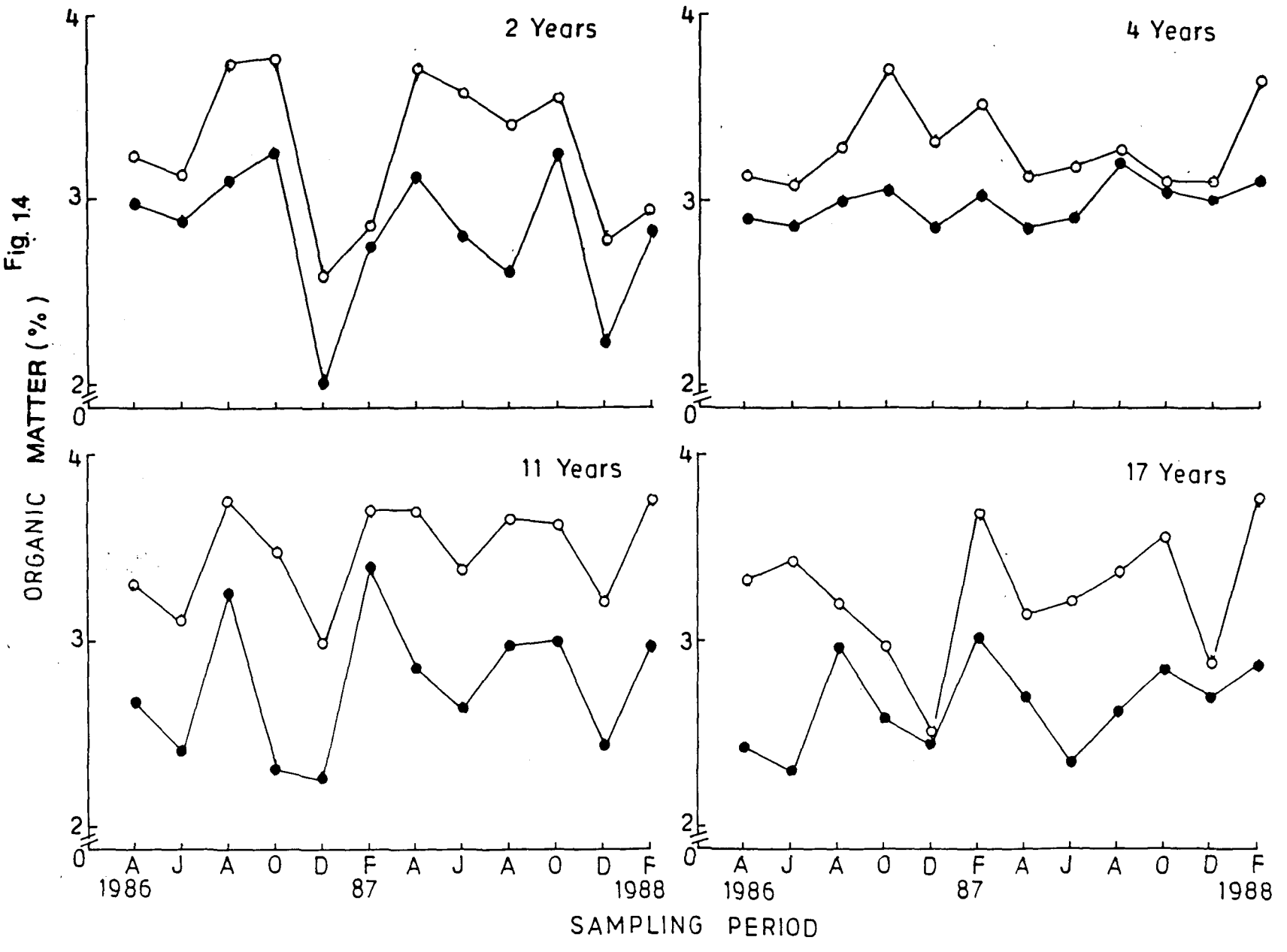

2 Years
4 Years
11 Years
17 Years
ORGANIC MATTER (%)
4
3
2
0
A J A O D F A J A O D F
1986
87
1988
SAMPLING PERIOD

Fig. 14

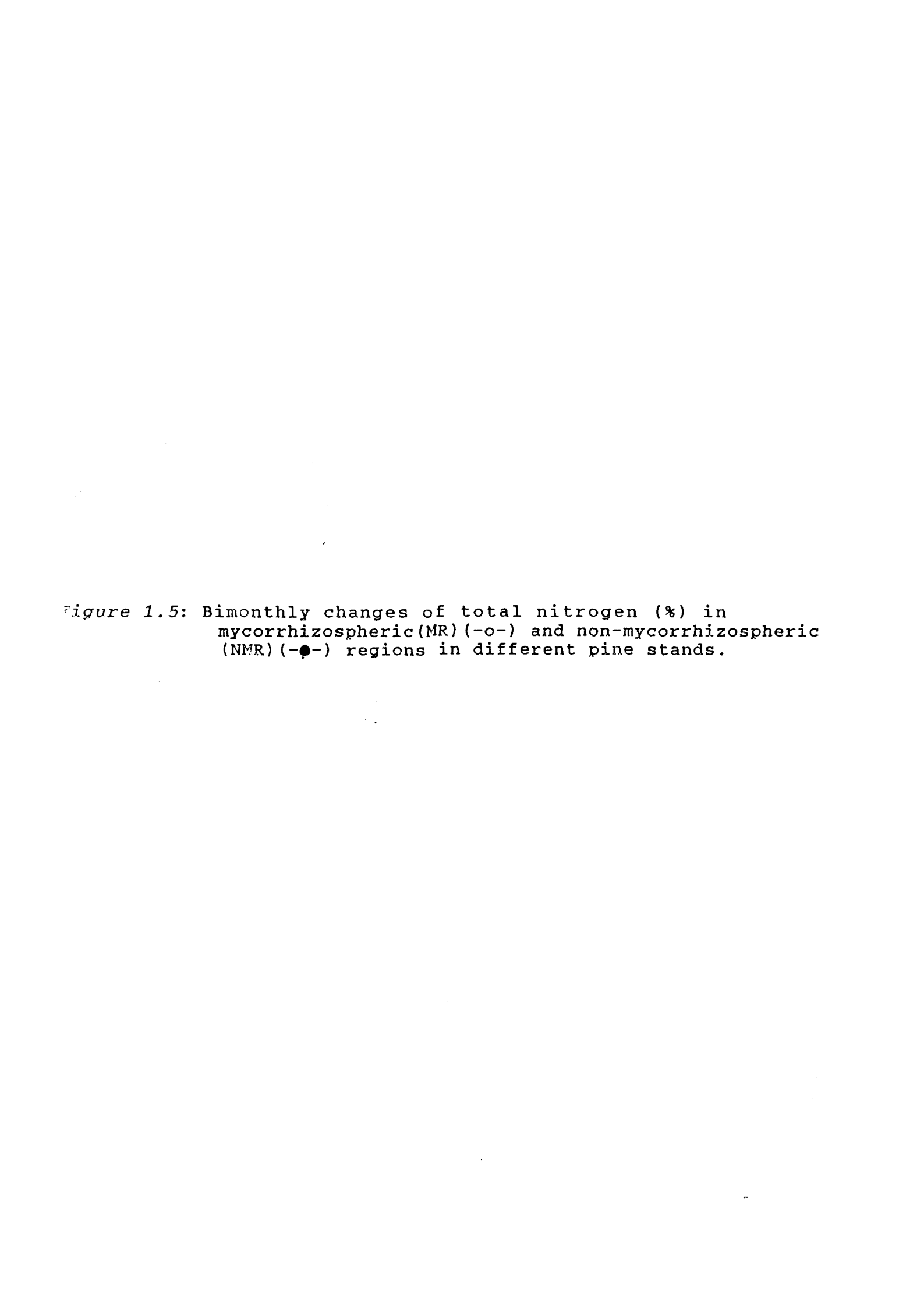


Figure 1.5: Bimonthly changes of total nitrogen (%) in mycorrhizospheric(MR)(-o-) and non-mycorrhizospheric (NMR)(-●-) regions in different pine stands.

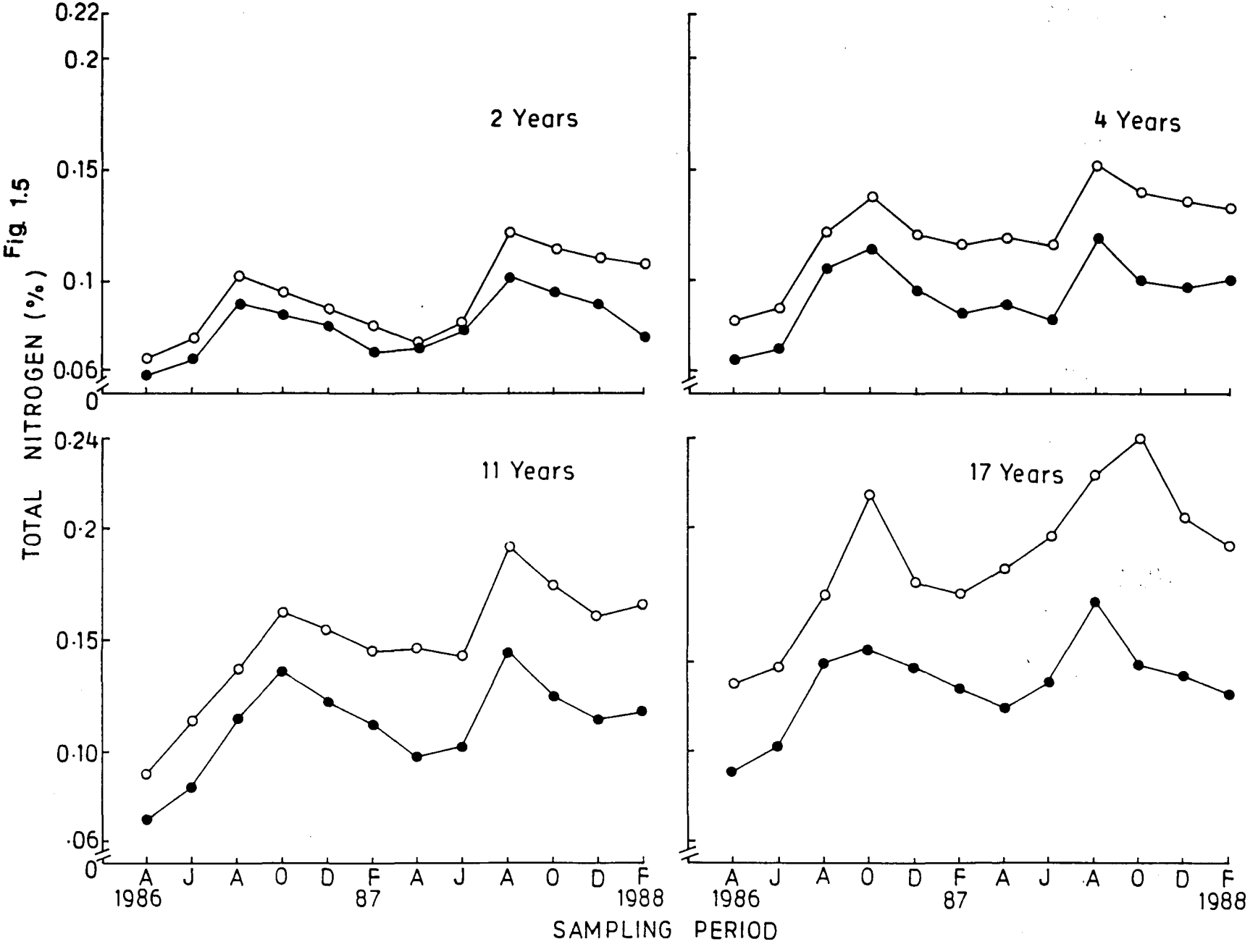


Fig. 1.5
TOTAL NITROGEN (%)
0·22
0·2
0·15
0·1
0·06
0
2 Years
4 Years
0·24
0·2
0·15
0·10
·06
0
11 Years
17 Years
A J A O D F A J A O D F
1986
87
1988
SAMPLING PERIOD

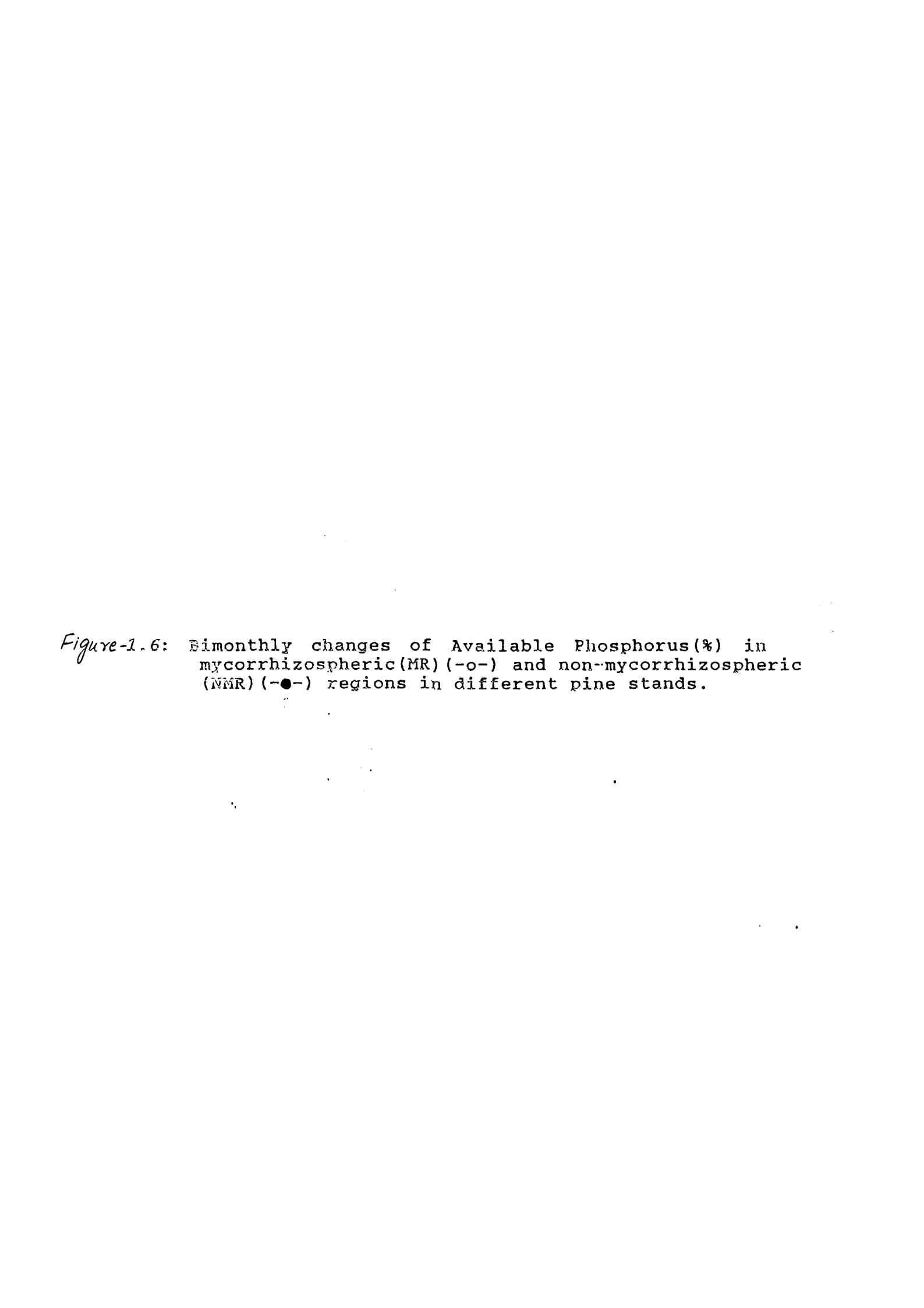


Figure-1.6: Bimonthly changes of Available Phosphorus(%) in mycorrhizospheric(MR)(-o-) and non-mycorrhizospheric (NMR)(-●-) regions in different pine stands.

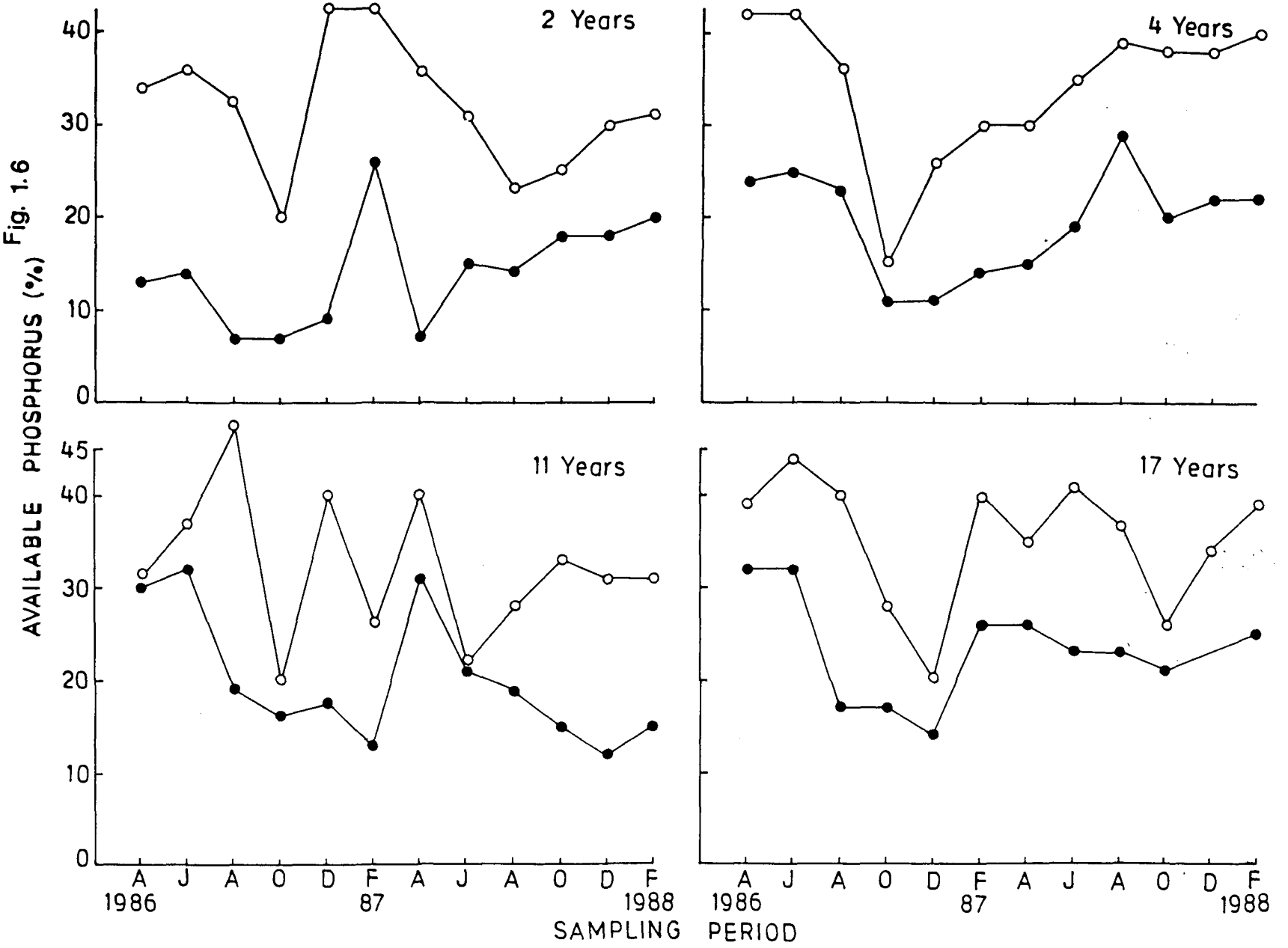


Fig. 1.6
AVAILABLE PHOSPHORUS (%)
2 Years
4 Years
11 Years
17 Years
0
10
20
30
40
45
A J A O D F A J A O D F
1986
87
1988
SAMPLING PERIOD

Figure 1.7: Bimonthly changes of Exchangeable potassium (%) in mycorrhizospheric(MR)(-o-) and non-mycorrhizospheric (NMR)(-●-) regions in different pine stands.

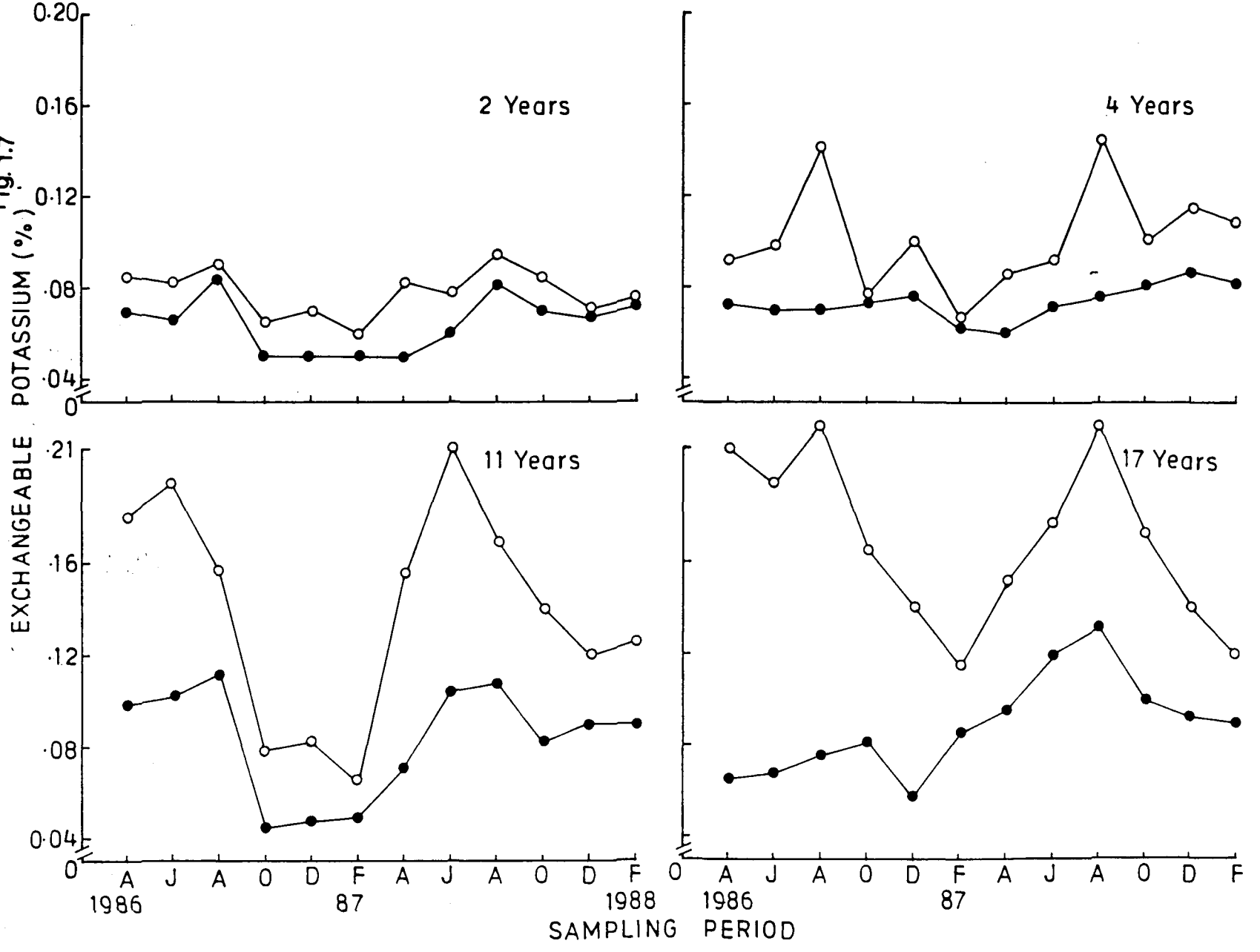
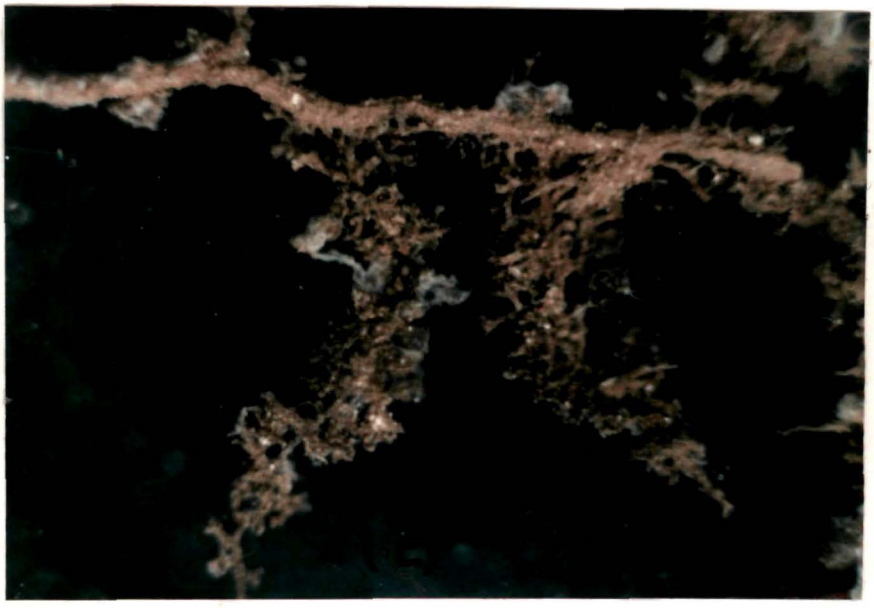


Fig. 1.7
EXCHANGEABLE POTASSIUM (%)
0·20
0·16
0·12
·08
·04
0
2 Years
4 Years
·21
·16
·12
·08
0·04
0
11 Years
17 Years
A J A O D F A J A O D F
1986
87
1988
SAMPLING PERIOD

Plate 1: (a). Sporocarps of *Boletus edulis*.
(b). Sporocarps of *Russula lepida*.
(c). Mycorrhiza of pine with *B.luetus* as mycobiont.

PLATE 1



CHAPTER II

SEASONAL VARIATION IN MICROBES (BACTERIA & FUNGI) AND MICROBIAL ACTIVITY (PHOSPHATASE, DEHYDROGENASE AND UREASE) IN ROOT REGION OF PINE (*PINUS KESIYA* Royle Ex.Gordon)

INTRODUCTION:

The biological activity in soil provides better insight in understanding the transformation of organic matter (Bishop and God-frey 1983). Soil enzyme activity estimates are often used as indices of microbial activity and soil fertility (Skujins,1978). In addtion to the fundamental properties of enzymes in soil, the data from enzymes assays have been interpreted periodically as a guide to soil productivity (Kiss *et al.*, 1978), an indirect measure of microbial biomass e.g. dehydrogenase assays (Ladd, 1978) a consequence of the rhizosphere effect (Spier *et al*, 1980), an indication of the soil's potential for degrading naturally occuring organic matter (Spalding,1980), an index of total biological activity of soil which could be used for practical purposes in agriculture and for describing the biological status of the environment (Nannipieri,1985) and in reclamation management .

In case of ectomycorrhizal zone, the fungal colonization brings changes in the morphology and physiology of root. The qualitative and quantitative changes of microbial population in the rhizosphere of mycorrhizal and non-mycorrhizal roots have been reported (Katzelson et al 1962;Sharma,1981a). Some studies on the microbial metabolic activities in soil are available (Dormaar *et al* 1984 and Dodd *et al*, 1987).

Phosphatase activity is directly related to the level of organic phosphorus in the soil. Roots can hydrolyse organic and insoluble phosphate in soil by the production of phosphatases which are also produced by a large number of soil microbes (Beever and Burns, 1980) and ectomycorrhizal fungi (Dighton, 1983; Dodd *et al*, 1987). Release of phosphorus due to microbial activity plays an important role in phosphorus cycle in semi-natural ecosystems (Harrison,1978; Miller *et al*, 1970). The intensity of phosphatase activity in soils is therefore, important in reflecting the rate of phosphorus cycling through soils (Zantua and Bremner, 1977).

Urease is produced by active soil microorganisms. Urease activity generally correlates with organic matter due to its existence as a complex with organic constitutents (Skujins, 1976). It may be affected by temperature, soil factors, such as moisture content, *p*H, organic matter and number of microorganisms (Skujins,1967; Dalal,1975 and Toole *et al*,1985).

Seasonal changes in carbon content, dehydrogenase, phosphatase and urease activities has been studied by (Dormari *et al*,1984). Dehydrogenase, urease and bacteria population has been studied by Tiwari *et al* (1987*a*). Procedure to measure rates of nutrient transformations in complex systems such as soil require an understanding of microbial population activity and enzyme production .

However, a seasonal change in the population of microbes in mycorrhizal zone influenced by the age of trees is not studied.

The rhizosphere of the mycorrhiza, or "mycorrhizosphere" (Rambelli,1973) is rich in bacteria, actinomycetes and other fungi. The bacteria are intimately associated with the hyphal cells of the mantle and form a nutritional relationship with fungus (Foster and Marks,1967). Thus, the surface of the mantle provides a complex ecological niche for soil inhabiting organisms. Bacteria play an active role in the mycorrhizal complex of higher plants as they do on leaves (Wichner and Libbert,1968). The mycorrhizosphere environment differs from soil in factors such as *p*H, chemical composition, oxygen and carbon-dioxide concentrations as well as being a zone of intesnse microbial activity. Mycorrhizae may harbour a high population of microorganisms in the mycorrhizosphere (Davey,1971; Sharma,1981*a*)

The present study was, therefore, conducted to investigate the seasonal relationship of microbial enzymes

(dehydrogenase urease,and phophotase) with microbial population (bacteria and fungi) in the mycorrhizospheric and non-mycorrhizospheric regions of four pine plantations at their different stages of regenaration.

Materials and methods :

Selection of site:

The four sites of pine stands (*Pinus kesiya* Royle ex.Gordon) of different age groups were selected in West Khasi Hills,Meghalaya as described in Chapter (I).

Soil sampling procedure

Soil sampling to study enzyme activites and microbial populations was done at bimonthly interval from April,1986 to February, 1988.Soil monoliths (15cm x 15cm x 15cm) was randomnly collected by sterile digger in five replicates from each stand of pine (*Pinus kesiya*). The soil samples were throughly mixed in a sterile polythene bag and brought to the laboratory on the same day. All aseptic precautions were taken to avoid contaminations.

The soil samples were further processed to study the microbial population and microbial enzyme activities in the mycorrhizospheric (MR) and non-mycorrhizosphric (NMR) regions of pine.

Enzyme activity:

(i) Dehydrogenase activity

2,3,5-Triphenyl tetrazolium chloride (TTC) reduction technique was adopted for the evolution of dehydrogenase activity of soil (Casida,1977). 0.5 g of fresh soil was taken in the test tube and to it was added 0.1 g of calcium carbonate and mixed properly. One millilitre of 10% TTC solution was added to it. Thereafter,the test tubes were plugged tightly with rubber plug and shaken well for proper mixing of contents.The test tubes were incubated at 30°C for 24h. Five replicates were mantained for each sample. After 24 h. of incubation, slurry was filtered through Whatman filter paper (No.1) and washed with the small amount of methanol. The volume of filtrate was made up to 50ml by adding methanol.The optical density of pink colour was measured on spectrophotometer at 485nm using methanol as blank. Finally,the optional density was calculated with the help of standard curve prepared by known amount of Tri phenyl formagon in methanol. The values of dehydrogenase activity were converted into per gram dry soil taking into consideration the moisture content and expressed in terms of µg formazan per gram dry weight of soil per 24 hours.

(ii) Urease activity

McGarity and Myer's(1967) method was followed to measure the urease activity in soil. Ten grams of fresh soil was taken

into a volumetric flask of 100 ml and 1 ml of toluene solution was added and left for 15 minutes. Thereafter, 10 ml of acetate buffer solution (*p*H-5.2) and 5 ml of urea solution (10%) were added. The flasks were swirled well for proper mixing and incubated at 37° C for 3 h. In control, instead of 5 ml of urea solution, 5 ml distilled water was taken. After incubation, the flasks were taken out and volume was made to 100 ml with sterilized distilled water. The mixture was shaken properly and filtered through Whatman filter paper (No.5). Thereafter, 0.5 ml filtrate was taken into 25 ml volumetric flask and to it 5 ml of distilled water was added. 2 ml of phenolate solution along with 1.5 ml of sodium hypochlorite solution (0.9 % active chlorine) were also mixed. The volume of solution was made 25 ml by adding distilled water. The intensity of blue colour was read out on spectrophotometer at 630 nm. Similar observations were taken for solution without soil as control. The amount of NH_4^+-N released was calculated by the standard calibration curve and expressed in terms of NH_4^+-N per gram dry soil per 3 h.

Preparation of phenolate solution

a) Phenol solution - 62.5 g phenol was dissolved in 20 ml of methanol. After dissolving phenol, 18.5 ml acetone was added and the volume of the mixture was made up to 100 ml with the help of ethyl alcohol.

b) Caustic Soda solution - 27 g of sodium hydroxide was dissolved in 100 ml of distilled water. The phenolate solution

was prepared by mixing phenol, caustic solution and distilled water (20:20:6; v/v/v). The solution was always prepared fresh at the time of use.

iii) Phosphatase activity:

Phosphatase activity was assayed by the method of Tabatabai and Bremner (1969). Soil samples were air dried and sieved (2mm). 0.1 g of soil powder was taken into a 50 ml conical flask , 0.25 ml toluene and 1 ml of 0.115 M *p*-nitrophenyl phosphate (PNP) solutions were added to the flask. The flasks were shaken well to mix the contents and incubated at 37°C. After one hour of incubation, the stoppers were removed and 1 ml of 0.5M calcium chloride and 4 ml of 0.5 M sodium hydroxide solutions were added. The soil suspension was filtered through Whatman filter paper (No.12). The optical density of the yellow colour was taken at 420 nm wave length on spectrophotometer. For the control, similar procedure was followed but 1 ml PNP solution was added after the addition of 0.5 M $CaCl_2$ and 0.5 M NaOH without soil. The concentration of phosphatase activity in terms of *p*-nitrophenyl in each sample was calculated by a standard curve of PNP in water and was expressed as µg *p*-nitrophenol released per gram of dry soil per hour.

Microbial population:

(a) Isolation of soil fungi:

Warcup's soil plate method (Warcup,1950) was followed

for isolation of fungi from the soil. Small amount (0.003 g) of soil was taken from the mycorrhizospheric (MR) and non-mycorrhizospheric (NMR) soil by means of a sterile spatula. The soil was then dispersed in a few drops of sterile water in the sterile Petri dishes. Twenty ml of melted and cooled Martin's rose bengal agar medium supplemented with streptomycin sulphate was poured and the soil particles were dispersed throughout the medium by shaking and rotating the dishes. Five replicates were maintained for each sample. Same method of isolation was followed for all the plantations. The plates were then incubated upside down at a temperature of 25+1^{o} C for 5 days in BOD incubator. The number of colonies forming units (C.F.U.) was counted and population of fungi per gram dry soil was calculated.

Isolation of bacteria

Dilution plate method (Johnson and Curl,1972) was followed for the isolation of bacteria throughout the sampling period. Ten grams of soil from each sample was taken in 250ml sterilized conical flask and a soil solution in sterilized distilled water of 1:10 was prepared. Further dilutions viz., 1:100, 1:1000 and 1:10000 were prepared with sterilized distilled water for each sample. The stock solution was always thoroughly hand shaken for 10-15 minutes before making any dilution. 1:10000 dilution was finally considered suitable for the isolation of bacteria throughout the investigation period. Isolation of bacteria was done on nutrient agar medium

containing beef extract. 0.5 ml of soil suspension from 1:10000 dilution was transferred aseptically to previously poured nutrient agar medium. Separate pipettes were used for transferring the soil solution in each case to avoid contamination. Isolation was carried out in sterilized laminar flow chamber. Five replicate plates were maintained in each case. The Petri plates were shaken gently to disperse the inoculum uniformly over the surface of the agar medium. The plates were incubated upside down at 30+1^{o}C for 24 h. Number of bacterial colonies was counted and their population per gram dry soil was calculated.

Estimation of CO_2 evolution

The CO_2 evolution was measured by the method of MacFadyen (1970). One kilogram of soil was incubated in a glass jar with 50 ml of 0.1N KOH solution and sealed properly. Soil was incubated for 24h at room temperature. A jar without soil was taken as a control. After 24h of exposure the amount of CO_2 evolved and absorbed by KOH solution was measured by titrimetric method using 0.1N HCl and phenolphthalein as an indicator. Three replicates were used in each case. Finally CO_2 evolution was expressed in mg/Kg/24 h.

Results

Bacterial population showed a seasonal variation in the MR and NMR regions during the study period in all the plantations (Figure 2.4). Maximum population was observed

during August in MR regions in all the plantations whereas, it was in April and in August in NMR region and of 2,4,11 year old stands respectively. Minimum population was observed during December in all the stands in MR and NMR regions except in MR region of 4 years old stand where it was in October.

Fungal population also showed a definite seasonal variation (Figure 2.5) in MR and NMR regions in different stands. Maximum and minimum population was observed In 2 and 4 year old pine stands in both MR and NMR regions during August and February respectively. In 11 and 17 years old stand maximum population in MR and NMR regions was observed in April and August respectively. Minimum population was during December in both MR and NMR regions in 11 years old stand and December in MR and February in NMR region in 17 years old stand. A positive correlation between fungal population and urease activity was observed ; Fungal population and organic matter and with CO_2 evolution in 2 and 17 years were also correlated old stand respectively (Table 2.2).

Dehydrogenase activity showed a similar seasonal trend in mycorrhizospheric (MR) and non-mycorrhizospheric (NMR) regions in all the pine stand. Activity was lowest during winter months and highest during rainy season. (Figure 2.1).

Maximum and minimum activity was observed during August and December months in 2 and 4 years old pine stand, in both MR and NMR regions respectively. In 11 and 17 year oled pine stand, activity was maximum during June in both MR and NMR

regions whereas minimum was observed in February in MR regions of both the plantations and in October and December in NMR of 11 and 17 years stand respectively.

Dehydrogenase activity showed a significant variation within the different age plantations($p < 0.01$), sampling periods ($p < 0.01$) and mycorrhizal and non-mycorrhizal region ($p < 0.01$) (Table 2.1)

Urease activity showed a uniform pattern of seasonal variation in MR and NMR regions in all the pine stands. (Figure 2.2). Maximum activity was observed in June and April in MR and NMR regions in 2 year old pine forest respectively. In 4 and 11 year old stand, maximum activity was observed during April in MR and NMR regions. Whereas, it was maximum during June in 17 years old stand. Minimum activity in MR and NMR regions was observed during December.

Urease activity showed a significant variation within the different age plantations ($p < 0.01$), sampling periods ($p < 0.01$), and mycorrhizal and non-mycorrhizal region ($p < 0.01$) (Table 2.1).

Phosphatase activity in the different stands also showed a similar type of variation. The activity being maximum during rainy season and minimum during winter (Figure 2.3). The peak of the activity was observed during October in MR and NMR regions was lowest during February in MR and NMR regions of 2,11 and 17 years old pine stands. Minimum activity was observed in 4 year old stand during December in MR and NMR

regions.

CO_2 evolution showed a similar pattern of variation in MR and NMR regions in various stands (Figure 2.6). In 2 years old stand maximum CO_2 evolution occurred in June and December in MR and NMR regions respectively. Maximum CO_2 evolution was in February, June and August in 4,11 and 17 years old pine stands respectively in both MR and NMR regions and minimum in 2,4 and 11 plantation in MR and NMR regions and in February in 17 years old stand.

Significant positive correlation was observed between bacterial population and CO_2 evolution in 2,4 and 17 years old pine plantations (Table 2.2) and between organic matter and bacteria in 2 and 11 year old pine stands.

DISCUSSION

Dehydrogenase activity was significantly more in the mycorrhizospheric (MR) than in non-mycorrhizospheric (NMR) region in all the pine plantations reflecting the presence of higher population of micro-organisms (Davey,1971; Rambelli et al 1972). The positive correlation between dehydrogenase activity and the bacterial and fungal population suggested that it was the resultant of higher number of microbes and organic carbon. Dehydrogenase activity being maximum in rainy season (June-Aug) and minimum in winter (Dec-Feb) in MR and NMR region was related to the moisture content and temperature of the soil(Ortem and Neuhaus,1970). An increase in soil moisture enhanced the availability of organic carbon to

heterotrophs responsible for the increased microbial activity as indicated by a positive correlation between organic matter and dehydrogenase activity (Ross, 1971). During winter, reduction of soil moisture and temperature caused dryness of soil which became unfavourable for bacterial growth and reduced the dehydrogenase activity (Chendrayan *et al*,1980 and Tiwari *et al*,1987). Higher activity in the older plantations was related to the increase in microbes due to the quality and quantity of root exudates of the pine trees (Bowen,1973). Enhanced urease activity in MR than in NMR region was correlated to the increased excretions of more nitrogenous nutrients by the mycorrhizal root, available for the microbes (Skujins,1967). It was observed that mycorrhizal fungi extend the mycelium to further place in soil to absorb the nitrogen, from litter and humus layer (Trappe,1969a,b; Lindeberg,1970). The increased mycorrhizospheric region due to mycorrhizal fungi may therefore, be stimulatory to nitrogen mineralizing microbes contributing to the enhanced urease activity in the region (Rambelli,1973).

The phosphatase activity was maximum in the late rainy season (October) and minimum in winter (Dec.) in MR and NMR regions. The less activity during winter months might be due to reduced amount of organic carbon, low soil temperature and moisture content compared to the favourable soil temperature and nutrients in the rainy season (Kiss *et al*,1975;Khaziyev,1977; Dormaar *et al*,1984). On the contrary,

Dormaar *et al* (1984) observed a better enzymatic activity during winter season if the availability of water was not a limiting factor. The autolysis of soil microbial cells killed by low temperature during winter accounts for low activity and provides easily accessible source of energy such as free aminoacids and sugars in successive months forcing enhanced activity (Ivarson and Sowden,1970). Comparatively low moisture in soil may reduce the availability of water and other essential elements to microbes needed for phosphatase activity.

Phosphatase activity was significantly enhanced by ectomycorrhizal fungi on the surface of mycorrhizal root and soil than without mycorrhizae in the pine stands and was directly correlated with colonization by mycorrhizae. Mycorrhizal fungi are known to be a good absorbers of phosphate due to high amount of phosphatase in mycelium (Ho and Zak,1987). The increased activity in mycorrhizal roots has also been related to an increase in root surface area (Dodd *et al*,1987). The mantle and mycelium of ectomycorrhizal catalyzed and hydrolysed the complex organic phosphorus more efficiently than non-mycorrhizal root leading to increased available form of phosphorus in mycorrhizospheric region (Ho and Zak,1979).

Higher phosphatase activity in mycorrhizospheric region could also be attributed to the diffusion of phosphatase from mycorrhizal root (Dodd *et al*,1987) which may perhaps be minimal due to absorption, denaturation and proteolysis in

soil (Burns,1983). However, the process of diffusion of enzyme from root to rhizosphere was regulated by physico-chemical properties of the soil. (Headley *et al*,1983).

The phosphatase activity was observed to be more in older stands than the younger ones, which was related to the better colonization and development of mycorrhizae and more organic matter in earlier case than in latter ones. The results of the present study indicate that to get a maximum phosphatase activity, a threshold level of available phosphorus in soil is necessary, contradicting the earlier reports that phosphatase activity was more in phosphorus deficient soil (Mclachlan,1980; Flick, 1984 Bousquet *et al*,1986). Dodd *et al*(1987) have also observed a dependency of phosphatase activity on the concentration and chemical status of phosphorus in the soil.

CO_2 evolution was more during rainy season and minimum during early winter season also reflected the higher number of microorganisms during favourable moisture, temperature and organic matter (Waksman, 1931). CO_2 evolution was higher in mycorrhizosphere than in non-mycorrhizosphere region which was related to the abundance of microbes and to the availability of organic substances to be broken down. Comparison of CO_2 evolution in MR and NMR region was influenced by the age of pine trees (Kshattriya *et al*,1992).

Higher amount of organic carbon added to the

mycorrhizosphere region by sloughing off of root cells, fungal mantle, exudates, secretions, mucigel and lysates appears to be probable cause of the increased respiration and enzymatic activity in the mycorrhizospheric region. Variation in mineralization of nutrients in soil could result due to the changes in aminoacids and sugar contents.

Table 2.1. Analysis of variance (ANOVA) values (f) within the diferent age plantations, sampling periods and mycorrhizal and non-mycorrhizal region of various parameters in the pine plantations.

Source of Variation	D.F.	Parameters					
		Phosphatase activity	Urease activity	Dehydrogenase activity	Bacterial population	Fungal population	CO_2 evolution
1. Row-effects	3	2.96*	9.32**	13.98**	20.2**	22.3**	36.4**
2. column-effects	5	16.60*	8.83**	3.89**	19.17**	25.31**	1.86
3. Layer-effects	1	16.19*	18.51**	6.65**	13.68**	52.84**	17.96**
4. Row-column	15	0.08	0.26	1.13	0.79	0.23	1.42
5. Row-Layer	3	0.09	0.30	0.43	1.05	3.06*	1.54*
6. Column-layer	5	1.33	0.17	0.13	0.89	1.03	0.10
7. Row-Column-Layer	15	0.09	0.11	0.15	0.25	0.15	0.32

Row = Pine plantations
Column = Sampling periods
Layer = Mycorrhizal and non-mycorrhizal regions.
D.F. = Degrees of freedom

* = Significant at $P < 0.05$ level
** = Significant at $P < 0.01$ level.

Table 2.2 : Correlation coefficient (r) values between bacterial population and fungal population with soil enzyme activities, organic carbon, CO_2 evolution and soil moisture in various pine plantations.

	Parameters	Bacterial Population				Fungal Population			
		A	B	C	D	A	B	C	D
1.	Phosphatase	0.30	0.62*	0.74**	0.59*	0.62*	0.56*	0.64*	0.71**
2.	Dehydrogenase	0.49	0.56*	0.63*	0.69**	0.49	0.77**	0.59*	0.89***
3.	Urease	0.38	0.35	0.61*	0.68**	0.52	0.41	0.62*	0.60*
4.	Organic matter	0.66*	0.84**	0.70**	0.87***	0.92***	0.62*	0.86***	0.59*
5.	CO_2 evolution	0.59*	0.63*	0.62*	0.83***	0.57*	0.61*	0.59*	0.62*
6.	Soil moisture	0.57*	0.59*	0.66*	0.74**	0.63*	0.59*	0.88***	0.83***

A = 2 Years old plantation
B = 6 Years old plantation
C = 11 Years old plantation
D = 17 Years old plantation

* = Significant at $P<0.05$
** = Significant at $P<0.01$
*** = Significant at $P<0.001$

Figure 2.1: Bimonthly variation of dehydrogenase activity (µg Formazan released/g dry soil/24h) mycorrhi-zospheric (MR)(-o-) and non-mycorrhizospheric (NMR)(-●-) regions in different pine stands.

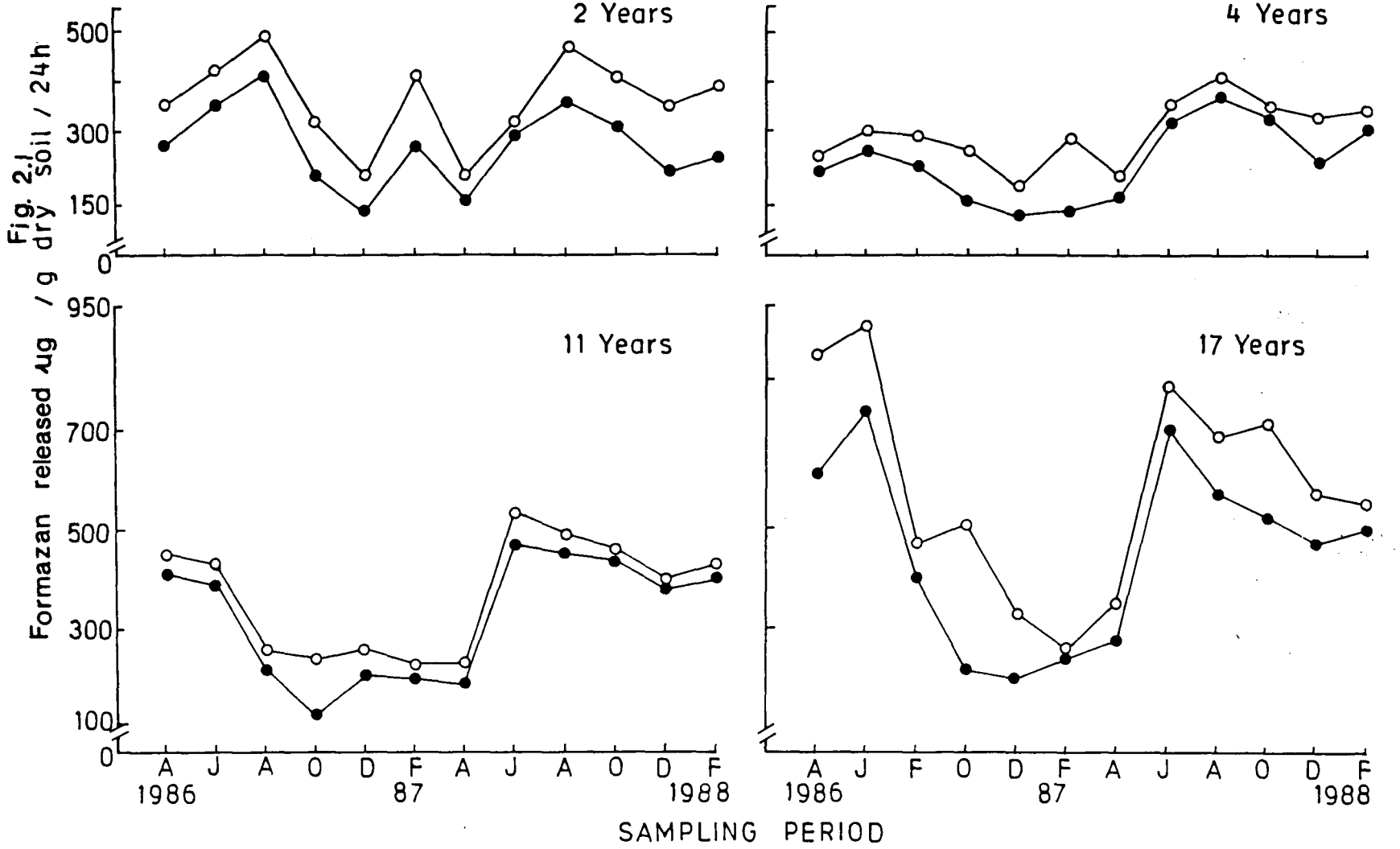

2 Years
4 Years
11 Years
17 Years
500
300
150
0
950
700
500
300
100
0
Formazan released ug / g dry soil / 24h
A J A O D F A J A O D F
1986
87
1988
A J F O D F A J A O D F
1986
87
1988
SAMPLING PERIOD

Fig. 2.1

Figure 2.2: Bimonthly variation of urease activity (µg NH_4-N released/g dry soil/3hrs.) mycorrhizospheric (MR) (-o-) and non-mycorrhizospheric (NMR) (-●-) regions in different pine stands.

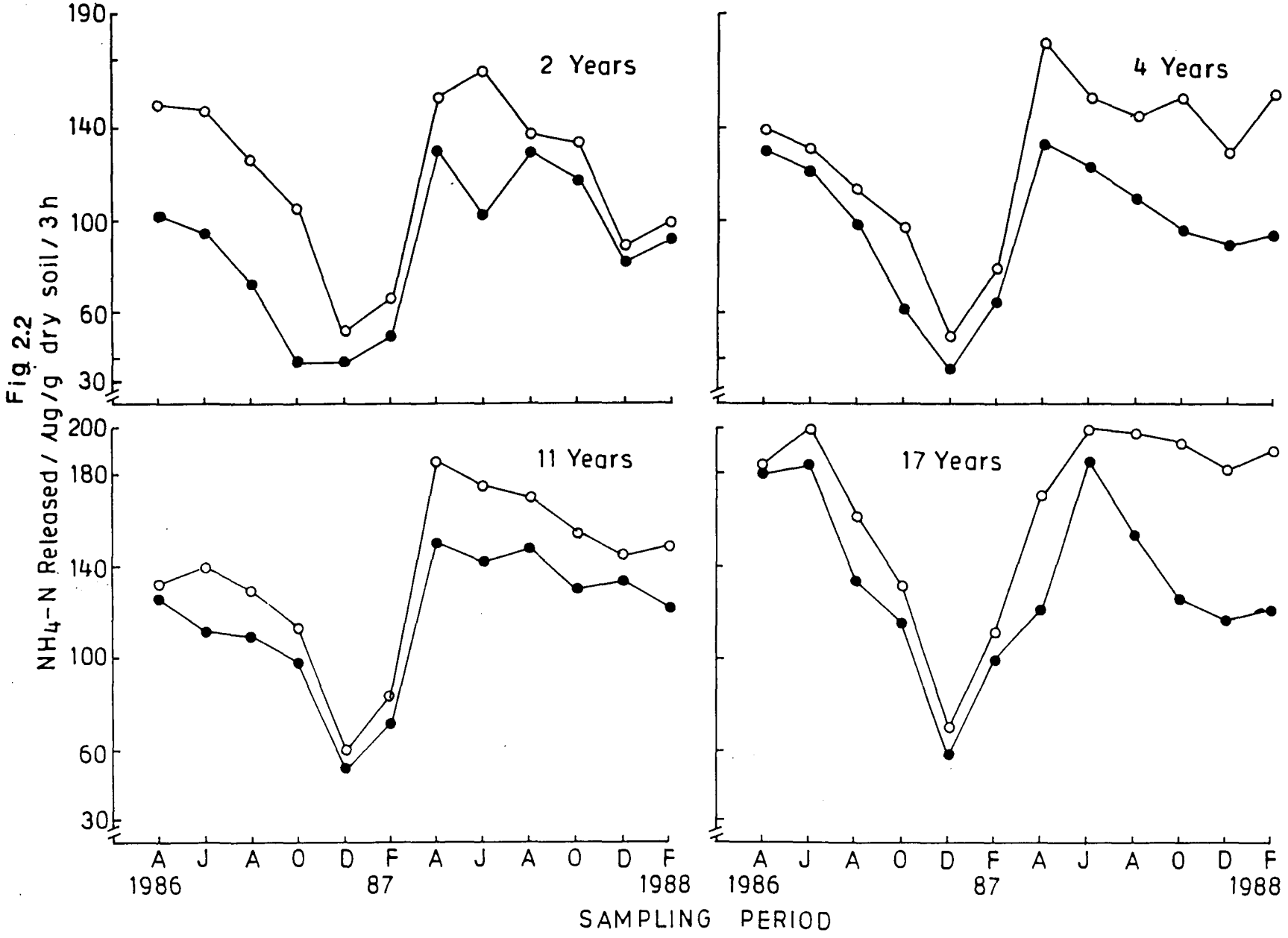

2 Years
4 Years
11 Years
17 Years
190
140
100
60
30
200
180
140
100
60
30
NH4-N Released / Ag / g dry soil / 3 h
A J A O D F A J A O D F
1986
87
1988
SAMPLING PERIOD

Fig 2.2

Figure 2.3: Bimonthly variation of Phosphatase activity(µg P-Nitrophenol released/g dry soil/h) mycorrhizospheric(MR)(-o-) and non-mycorrhizospheric (NMR)(-●-) regions in different pine stands.

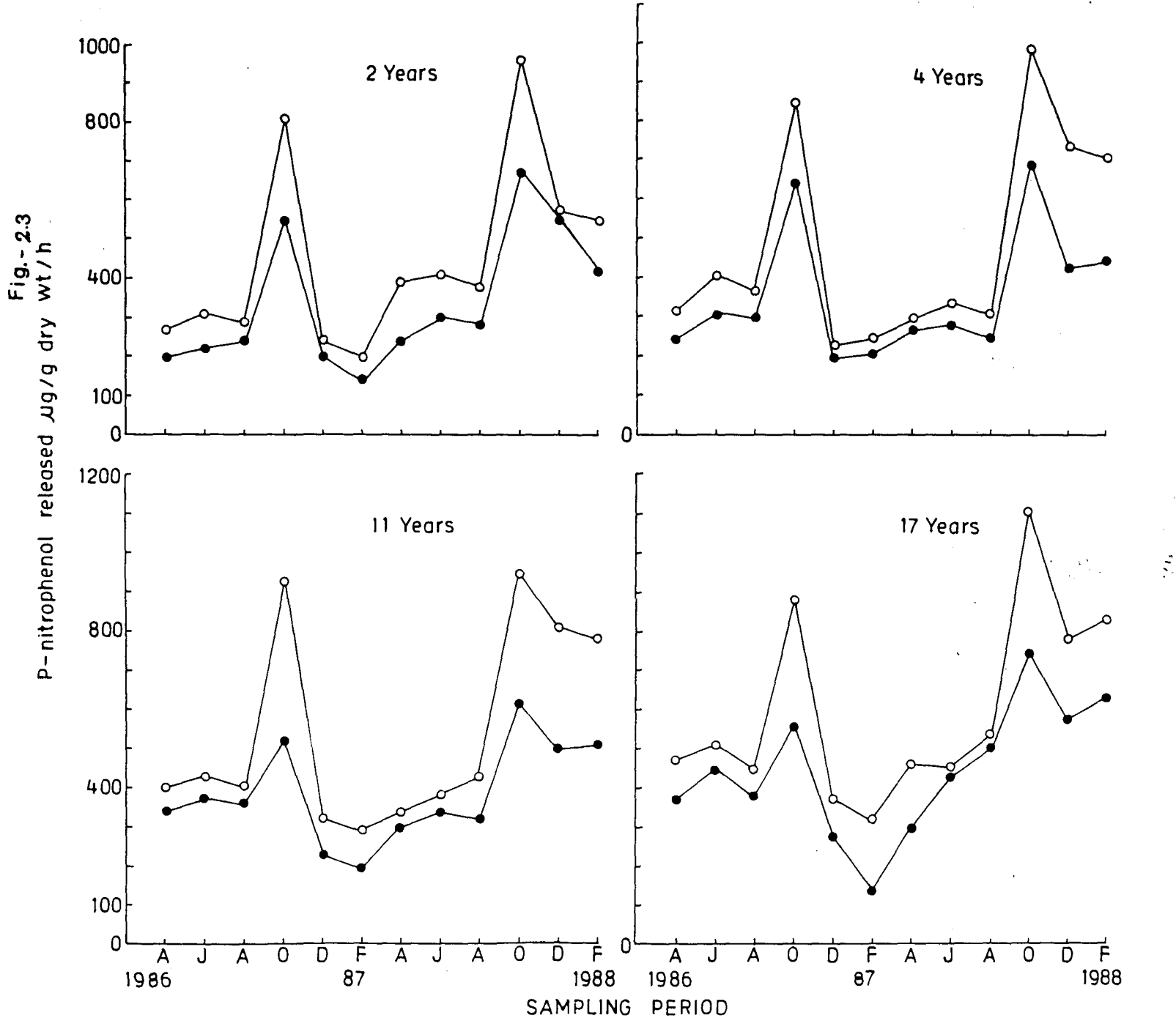


Fig.- 2.3
P-nitrophenol released µg/g dry wt/h
2 Years
4 Years
11 Years
17 Years
1000
800
400
100
0
1200
A J A O D F A J A O D F
1986
87
1988
SAMPLING PERIOD

Figure 2.4: Bimonthly variation of Bacterial population (X 10^5/g dry soil) mycorrhizospheric(MR)(-o-) and non-mycorrhizospheric (NMR)(-●-) regions in different pine stands.

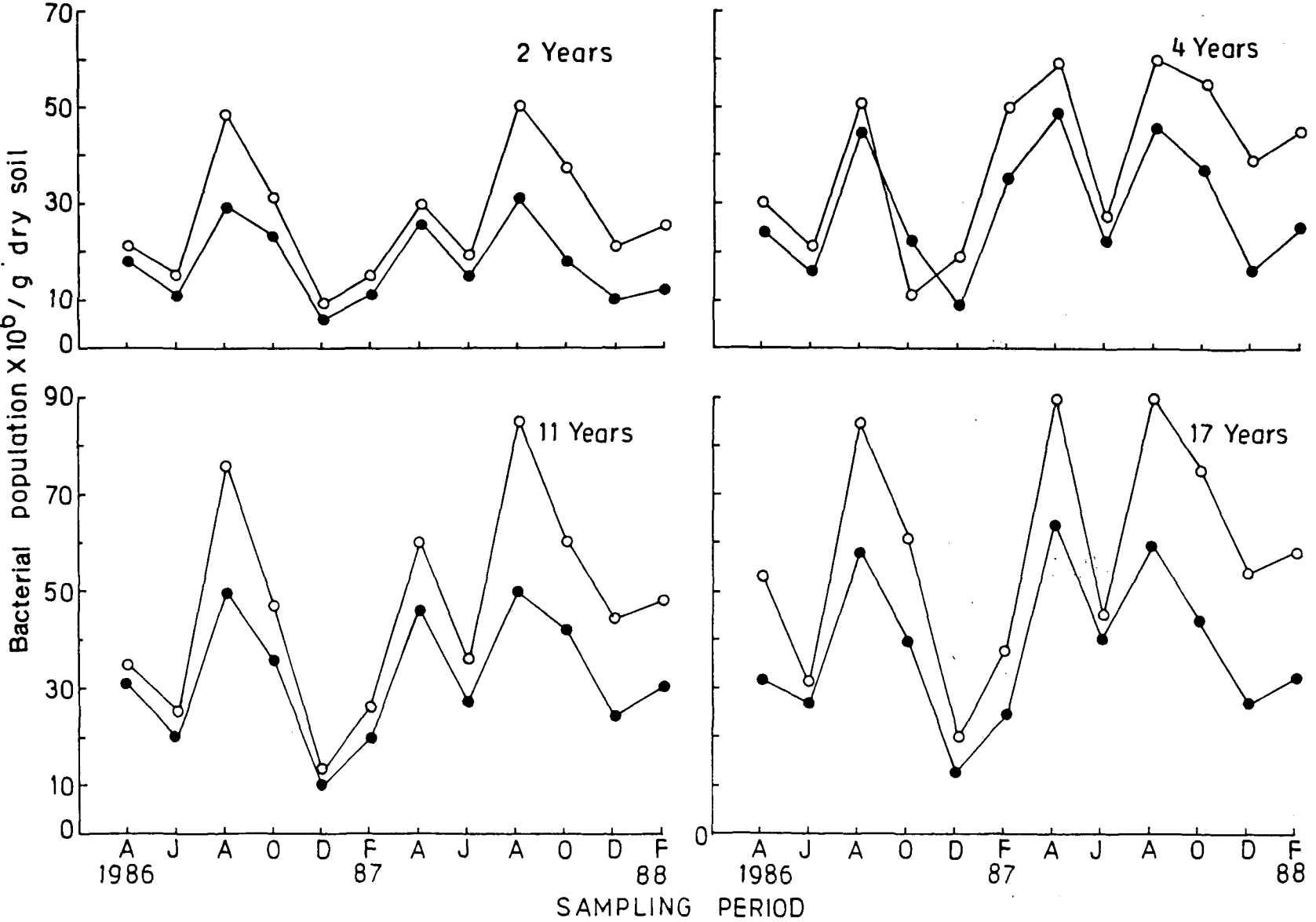

2 Years
4 Years
11 Years
17 Years
Bacterial population X 10^6 / g dry soil
70
50
30
10
0
90
70
50
30
10
0
A J A O D F A J A O D F
1986
87
88
SAMPLING PERIOD

Fig. 2.4

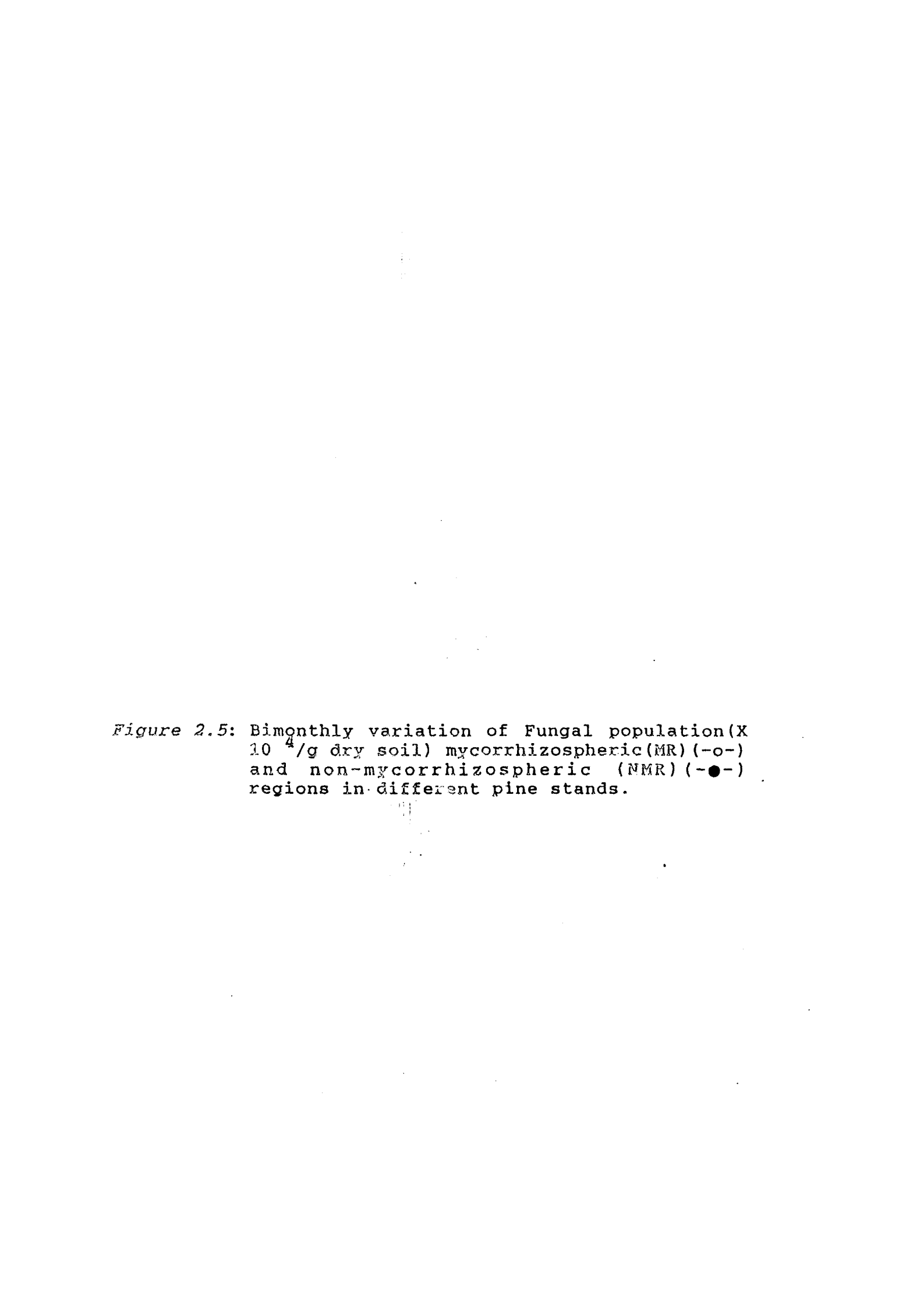


Figure 2.5: Bimonthly variation of Fungal population(X 10^{4}/g dry soil) mycorrhizospheric(MR)(-o-) and non-mycorrhizospheric (NMR)(-●-) regions in different pine stands.

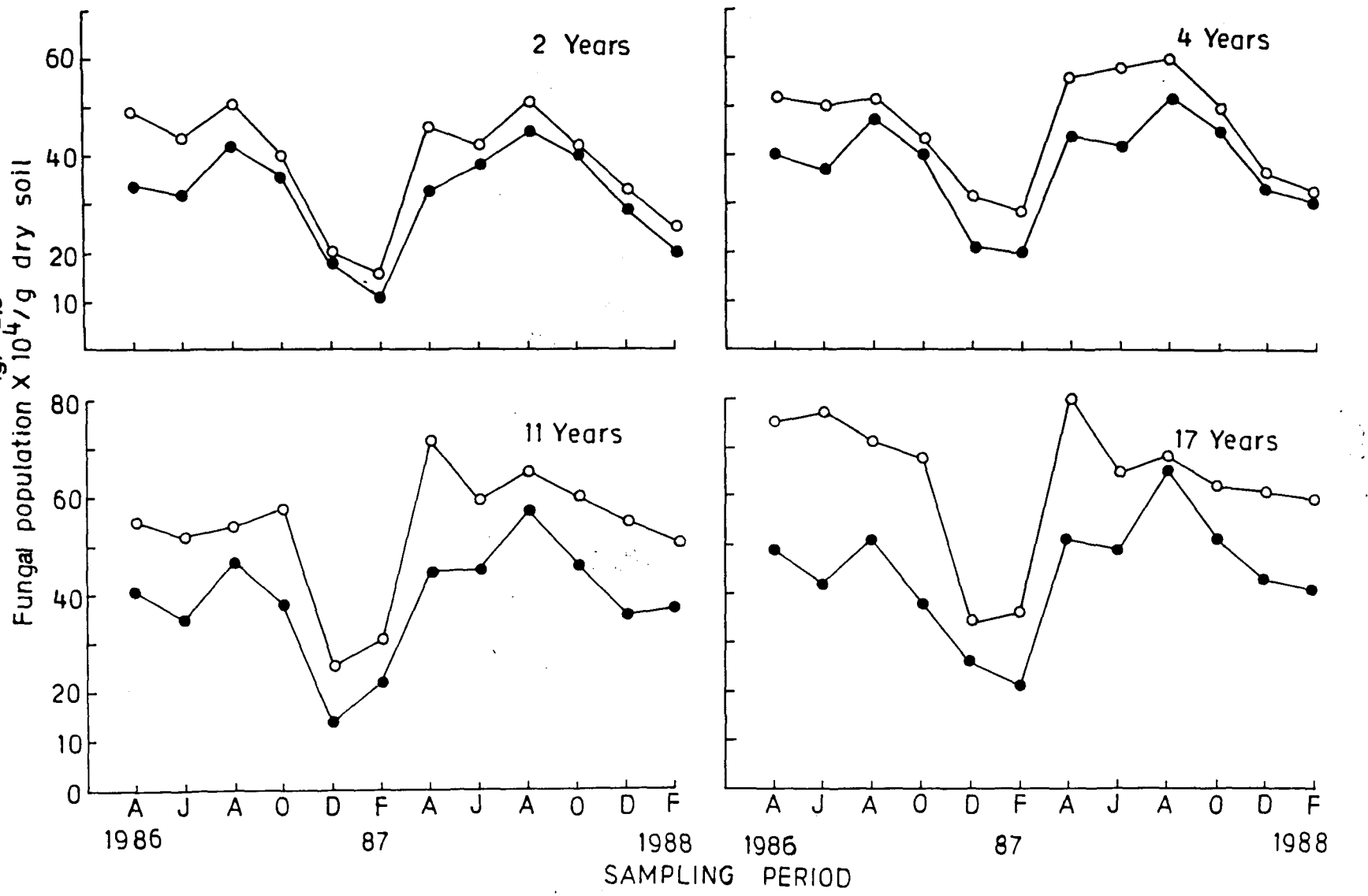

2 Years
4 Years
11 Years
17 Years
Fungal population X 10^4/g dry soil
60
40
20
10
80
60
40
20
10
0
A J A O D F A J A O D F
1986 87 1988
SAMPLING PERIOD

Fig. 2.5

Figure 2.6: Bimonthly variation of CO_2 evolution (X 10^{-3} mg /Kg/24h) mycorrhizospheric(MR)(-o-) and non-mycorrhizospheric (NMR)(-●-) regions in different pine stands.

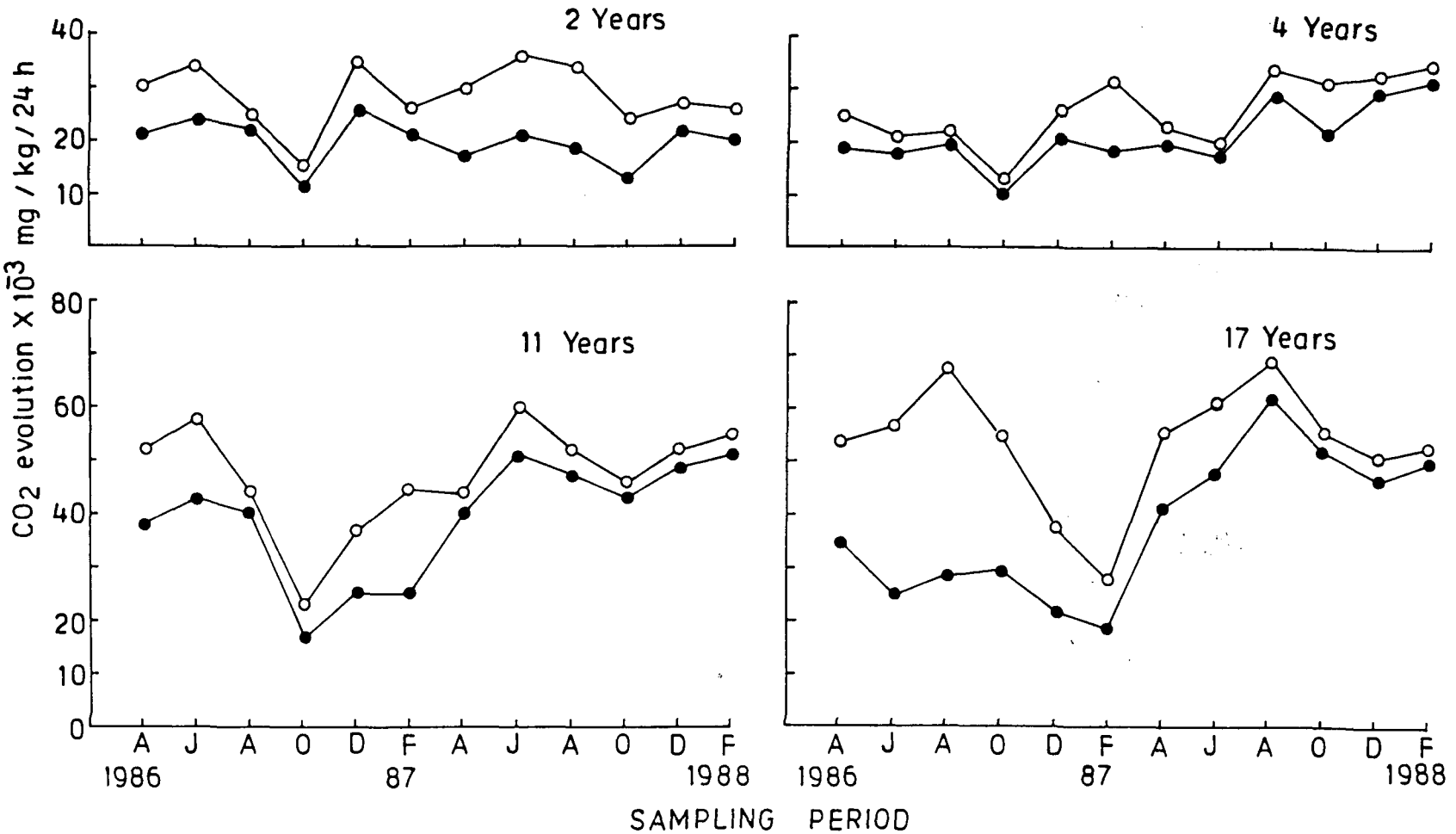

2 Years
4 Years
11 Years
17 Years
40
20
10
80
60
40
20
10
0
A J A O D F A J A O D F
1986
87
1988
SAMPLING PERIOD
CO2 evolution X 10-3 mg / kg / 24 h

Fig. 2.6

CHAPTER 3

PRODUCTION OF ECTOMYCORRHIZAE WITH DIFFERENT MYCORRHIZAL FUNGI AND THEIR INFLUENCE ON PHOSPHATASE ACTIVITY OF ROOTS IN GREENHOUSE CONDITIONS

INTRODUCTION

Ectomycorrhiza improves the nutrient absorbtions of host plants and increases the survival and growth of tree seedlings under different soil conditions (Marx *et al*, 1982). The mycorrhizal fungi are not only structurally efficient in extracting the nutrients, they also produce exogenous enzymes like phosphatases, nitrate reductase etc., which improve nutrient uptake and metabolism (Gianinazzi *et al*, 1979; Dighton,1983). It is well documented that mycorrhizal plants are more competitors for soil nutrients and water than non-mycorrhizal ones. There have been reports in relation to the potential use of mycorrhizae to promote productivity, particularly in terms of uptake of nutrients, those relatively immobile in soil like phosphorus, Copper etc. (Jha *et*

al,1991). The high concentration of some elements in mycorrhizal plants than non-mycorrhizal ones is ascribed to their improved biomass (Jha, 1990).

Artificial inoculation of nursery beds with selected mycorrhizal fungi has been recommended for producing vigorous seedlings (Trappe, 1977). There is much evidence that some mycorrhizal fungi are more effective symbionts than others. (Theodorou and Bowen,1970; Ekwebelam,1979). Seedlings to be outplanted need to be of high quality, preferably with efficient mycorrhizae (Mikola 1969; Marx 1977). Little information is available on the specific mycorrhizal fungi suited for inoculation to a particular host species (Molina and Trappe, 1982).

Root surface phosphatase may be more important in the organic phosphorus mobilization and making it available to the roots of plants (Antibus *et al*, 1992). Some studies on root surface phosphatase activity indicate its relationship with mycorrhial association and *P* uptake (Dodd *et al*,1987).

The number of mycorrhizal rootlets and fungal mycelium associated with them as determined by the specific mycobiont is important. Variation in the efficiency of the mycorrhizae for *P* uptake and phosphatase activity may depend on the specific mycorrhizal fungi. Therefore, the objective of this study was to evaluate the effect of different ectomycorrhizal

fungi on the production of mycorrhizae and their influence on phosphatase activity in Khasi pine seedling in green house condition.

MATERIALS AND METHODS

1. Collection and sterilization of soil

The sandy loam soil was collected from the Botanical garden of School of Life Sciences, North-Eastern Hill University , Shillong (Sand-73%, silt-15%, clay 12%, *p*H 5.2, organic matter- 2.1%, N-0.18%, P-0.07% and K-0.18%). The roots and litter were removed from the soil manually and soil was steam sterilized at 15 psi for 2.5 h continuously for 2 days at the interval of 24 h. The sterilized soil was left for 10 days to regain the microbial activity. 3.5 kg of the sterilized soil was filled in sterilized plastic pots (dia. 16 cm) to be used for various treatments.

2. Raising of Pine seedlings

The seeds of Khasi pine (*Pinus kesiya* Royle Ex.Gordon) were surface sterilized with 0.1% $HgCl_2$ for 5 min., rinsed with sterile distilled water several times, and were germinated in sterilized sand at 30°C in growth chamber. After germination, seedling (3 cm radicle) were transferred to the plastic pots. Six seedlings were maintained in each pot.

3. Preparation of mycobiont inoculum

The sporocarps of *Boletus luteus* and *Scleroderma aurantium* were collected from the pine forest, air dried and put in freezer. The spore suspension, containing 4.8×10^7 spores/ml from sporocarps of each mycorrhizal fungus was prepared with sterilized distilled water. 100 ml of spore suspension of each fungus was inoculated separately in each pot after 15 days of seedling transplantation. Fungal cultures of *Pisolithus tinctorius, Laccaria laccata, Cenococcum graniforme* and *Collybia radiata* were grown in conical flasks of one litre capacity in a mixture of vermiculite and peatmoss (9:1) and moistened to field capacity with modified Melin Norkan's Medium (MMN). After two months, the inoculum culture was rinsed well in distilled water three times to leach out residual sugars. 20 ml of inoculum culture was inoculated near the roots of pine seedlings (Danielson *et al* 1984). Pine seedlings treated as control did not receive any inoculation. Pots were watered regularly to maintain 15-20% moisture in soil. Ten replicates of each treatment were maintained.

4. Harvesting of seedlings

Eight seedlings per treatment were harvested on 45,90,135 and 280 days after transplantation. Seedlings were brought to the laboratory for further studies along with their root system and attached soil.

5. Assessment of mycorrhizal colonization

Roots of seedlings were washed under running tap water and percentage infection of ectomycorrhizal roots was determined (Beckjord *et al*,1984). Approximately 10% of the suspected mycorrhizal roots were sectioned, mounted, and examined under 100x magnification for fungal mantle and Hartig net development.

6. Productivity of ectomycorrhizae

At each harvest date, productivity of ectomycorrhizae was assessed. The entire root system free of adhered soil along with the mycorrhizae was weighed and afterwards the mycorrhiza was removed with help of blade from the root system and weighed again. The weight of mycorrhizae was calculated. The productivity of ectomycorrhizae was measured on dry weight basis of mycorrhizae per seedling and expressed as mg/days (45,90,135,180) unit area (50.28 cm^2).

7. Phosphatase activity

Phosphatase activity of rhizosphere soil and root surface activity of mycorrhizal roots was measured by the method of Dodd *et al* (1987). 100 mg of mycorrhizal root was incubated with 1 ml of 50 mM *p*-Nitro phenyl phosphate (PNP) and 4 ml 0.1M sodium acetate buffer, *p*H 5.2 for 1 h at 25°C in shaking water bath (60 rpm). The reaction was terminated by

adding 5 ml of 0.5 M NaOH and samples were centrifuged at 2500 g for 10 minutes. Thereafter, the optical density of supernatant was measured at 400 nm by U.V. Spectrophotometer. Activity was expressed as the amount of *p*-Nitro phenol released during incubation. The controls were estimated by adding 1 ml PNP to suspension and acetate buffer after 60 minutes immediately before the addition of NaOH. The standard curve was prepared in acetate buffer solution.

Phosphatase activity of rhizosphere soil was estimated by taking 1 g dry weight of soil.

The data was processed by analysis of variance and significant differences among means were identified with Duncan's multiple range test. Correlation coefficient was calculated.

Results

Productivity of ectomycorrhizae increased in all fungual inoculation treatments with time intervals (Fig.3.2) than in control. Control seedlings had only a trace of mycorrhizae in their root system. Production of mycorrhizal roots after 45 days was very less in all the treatments and maximum being in seedlings inoculated with *Boletus* sp (1.96 mg/45/201.1 cm^2). After 90 days, the variation increased in all the treatments significantly. Increase of two folds in *Collybia radiata*, *Laccaria laccata* and *Boletus* sp inoculated seedlings was

observed in comparison to *Pisolithus tinctorius*, *Cenococcum graniforme* and *Scleroderma aurantium* inoculated seedlings which showed a marginal increase (Fig. 3.2).

In 135 days old seedlings it increased about 9 times more than 90 days old seedling in *L.laccata*, 7 times in *B.luteus* sp, 11 times in *S.aurantium* and 5 times in *P.tinctorius* inoculated seedlings.

After 180 days, further increase in the productivity of mycorrhiza was observed in all the treatments. In *P.tinctorius* inoculated seedlings, the productivity was 5 times more than in 135 old seedlings, in *C.graniforme* inoculated seedlings and in others it was marginal. Maximum productivity of ectomycorrhizae was observed in *L.laccata* inoculated seedlings followed by *Boletus* sp. inoculated seedlings (Fig. 3.2).

Phosphatase activity of the roots was higher than in soil. Phosphatase activity in soil and in root increased with the increase in the age of seedlings in all the treatments. Mycorrhizal fungi inoculated seedlings showed higher soil and root phosphatase activity than in the control. Maximum activity was observed in *L.laccata* inoculated seedlings after 180 days of transplantation, whereas root activity was maximum in *S. aurantium* inoculated seedlings. Root phosphatase activity was 11 times more than in the soil in *L. laccata*

inoculated, pine seedlings. Soil phosphatase activity was minimum in *L. laccata* inoculated and minimum being in *P.tinctorius* inoculated pots (Table 3.1).

Maximum mycorrhizal colonization was observed in *S. aurantium* inoculated seedlings (85.3%) and minimum in *C.graniforme* ones after 180 days of growth. *B.luteus* and *L.laccata* showed the similar level of infection (82%). Uninoculated seedlings developed few mycorrhizae. Mycorrhizal colonization increased with the increase in age of the seedlings. At 135 days of seedling growth, maximum mycorrhizal infection was produced by *S.aurantium* followed by *L.laccata* and *B.luteus*. Seedlings inoculated with *C.graniforme* showed a minimum level of mycorrhizal infection (Fig. 3.1). Insignificant correlation was observed between mycorrhizal infection and mycorrhizae productivity.

Root surface phosphatase activity showed a significant positive correlation with mycorrhizal infection with *C.graniforme* ($p < 0.01$), *C.radiata* ($p < 0.01$), *L.laccata* ($p < 0.01$) and *B.luteus*. Mycorrhiza productivity also showed a significant positive correlation with *L.laccata* and *B.luteus* inoculated seedlings only (Table 3.3).

Rhizosphere soil phosphatase activity showed a significant positive correlation with mycorrhizal infection in *B.luteus* inoculated seedlings and with mycorrhizae

productivity with *L.laccata* ($p < 0.001$) and *B.luteus* inoculated ones.($p < 0.01$) (Table 3.3).

DISCUSSION

The results showed that the different mycorrhizal fungi varied in the intensity of mycorrhizal colonization and mycorrhizae productivity. The indigenous mycobiont *S.aurantium* reflected its better compatibility with Khasi pine by colonizing more of the root system than the other mycobionts. Among exotic species *L.laccata* showed a better mycorrhizal infection. The results were supported by Marx (1980) that the physiological compatibility of certain mycorrhizal fungi with host than the others was due to the requirements of specific growth substances (Palmer, 1971).

Ability of ectomycorrhizal fungi to synthesize mycorrhiza may vary in different ecological conditions as was exhibited by an exotic efficient mycorrhizal fungi *P.tinctorius* (Marx,1980) which otherwise has shown better performance than the native mycorrhizal fungi like *S.aurantium* and *B.luteus* (Marx,1980). The greater amount of colonization by the mycobiont resulted in the development of more mycorrhizae thereby increasing nutrient uptake (Kropp and Langlois,1990) and ultimately increasing the mycorrhizal productivity. Increased production of mycorrhiza by the native *B.luteus* and exotic *L.laccata* was due to their better fast

entry and growth in root tissue of pine and in utilizing host's resources better efficiently than the others.

Phosphatase activity was significantly enhanced by ectomycorrhizal fungi on the mycorrhizal roots than in the rhizosphere and was directly correlated with the colonization of mycorrhizae. Phosphatase activity of mycorrhizae has been related to the supply of nutrients to the host plants and in nutrient mineralization (Meyselle *et al*,1991). Mycorrhizal roots form a greater phosphorus sink and the mycorrhizal root surface activity is related to the increased organic contents in the rhizoplane region of the mycorrhizal roots. Ability of mycorrhizal fungi to produce higher phosphatase than the decomposer ones help them in mobilizing more organic phosphorus in rhizosphere region (Dighton,1983). Mycorrhizal fungus with its larger amount of phosphatase, released and hydrolyzed more phosphates (Ho and Zak,1987). Since sheathing mycorrhizas have no intracellular hyphal penetration, PO_4 released by phosphatase activity would only be available if released into the soil solution adjacent to the root surface or into the intercellular spaces of the Hartig net. The increased activity in mycorrhizal roots is also related to the increase in root surface area due to mycorrhizal colonization (Dodd *et al*,1987).

In summary, mycorrhizal infection and the activity of phosphatase of mycorrhizal roots and soil are governed by

complex physico-chemical and biochemical mechanism of different mycorrhizal fungi and the nature of the soil with the Khasi pine seedlings.

Table 3.1 : Phosphatase activity (ug/g dry wt/h) in rhizosphere and root surface region of ● pine seedlings inoculated with different mycorrhizal fungi

Treatments	Rhizosphere Phosphatase activity					Root surface Phosphatase activity				
	Sampling period (days)					Sampling period (days)				
	45	90	135	180	LSD at 5%	45	90	135	180	LSD at 5%
1. Control	14	19	21	33	11.2	140	205	301	372	06.2
2. *P. tinctorius*	19	24	38	55	21.3	310	555	760	890	9.1
3. *C. graniforme*	14	18	23	49	14.6	340	495	590	830	4.9
4. *C. radiata*	41	46	56	79	9.8	570	735	880	1110	8.0
5. *L. laccata*	28	34	61	82	16.5	750	885	1030	1210	11.5
6. *B. luteus*	34	41	53	68	16.2	460	605	705	920	12.2
7. *S. aurantium*	24	31	41	76	17.8	620	915	990	1311	5.8

Table 3.2 : Analysis of Variance (F) of the effect of mycorrhizal treatment and the sampling period of the rhizosphere and root surface phosphatase activity in pine seedlings.

Source of variation	Between treatments (Mycorrhizal fungi)	Between sampling period (days)
Rhizosphere phosphatase activity	11.20**	0.21
Root surface phosphatase activity	5.06**	0.12

** = Significant at p > 0.01 level

Table 3.3 : Correlation Coefficient (r) values of mycorrhizal infection (%) and mycorrhizae productivity with phosphatase activity of root surface and Rhizosphere soil in various mycobionts inoculated pine seedlings.

Treatments (Mycorrhizal fungi)		Rhizosphere Phosphatase activity						Root surface Phosphatase activity					
		MF_1	MF_2	MF_3	MF_4	MF_5	MF_6	MF_1	MF_2	MF_3	MF_4	MF_5	MF_6
MF_1	MI	1.27	-	-	-	-	-	0.97	-	-	-	-	-
	MP	0.89	-	-	-	-	-	2.72	-	-	-	-	-
MF_2	MI	-	1.21	-	-	-	-	-	0.75***	-	-	-	-
	MP	-	0.98	-	-	-	-	-	2.98	-	-	-	-
MF_3	MI	-	-	0.97	-	-	-	-	-	0.75**	-	-	-
	MP	-	-	0.27	-	-	-	-	-	2.44	-	-	-
MF_4	MI	-	-	-	0.89**	-	-	-	-	-	0.59**	-	-
	MP	-	-	-	0.62**	-	-	-	-	-	0.64***	-	-
MF_5	MI	-	-	-	-	0.57**	-	-	-	-	-	0.64**	-
	MP	-	-	-	-	0.77***	-	-	-	-	-	0.50*	-
MF_6	MI	-	-	-	-	-	2.7	-	-	-	-	-	7.23
	MP	-	-	-	-	-	2.1	-	-	-	-	-	0.94

* = Significant at P<0.05
** = Significant at P<0.01
*** = Significant at P<0.001

MI = Mycorrhizal infection
MP = Mycorrhizae productivity

MF_1 = *P. tinctorius*; MF_2 = *C. graniforme*; MF_3 = *C. radiata*;
MF_4 = *L. laccata*; MF_5 = *B. luteus*; MF_6 = *S. aurantium*

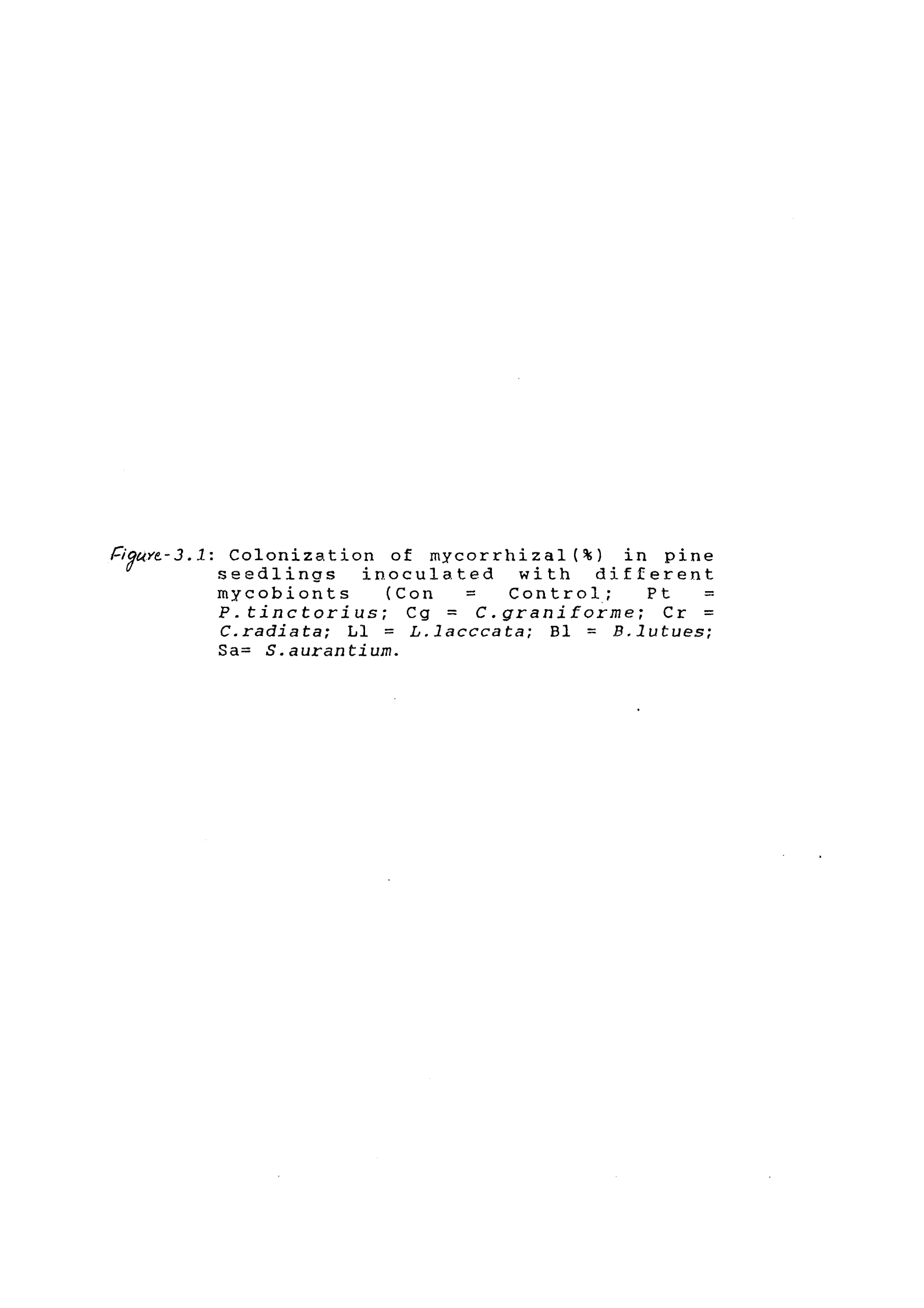


Figure-3.1: Colonization of mycorrhizal(%) in pine seedlings inoculated with different mycobionts (Con = Control; Pt = *P.tinctorius*; Cg = *C.graniforme*; Cr = *C.radiata*; Ll = *L.lacccata*; Bl = *B.lutues*; Sa= *S.aurantium*.

FIG. 3.1

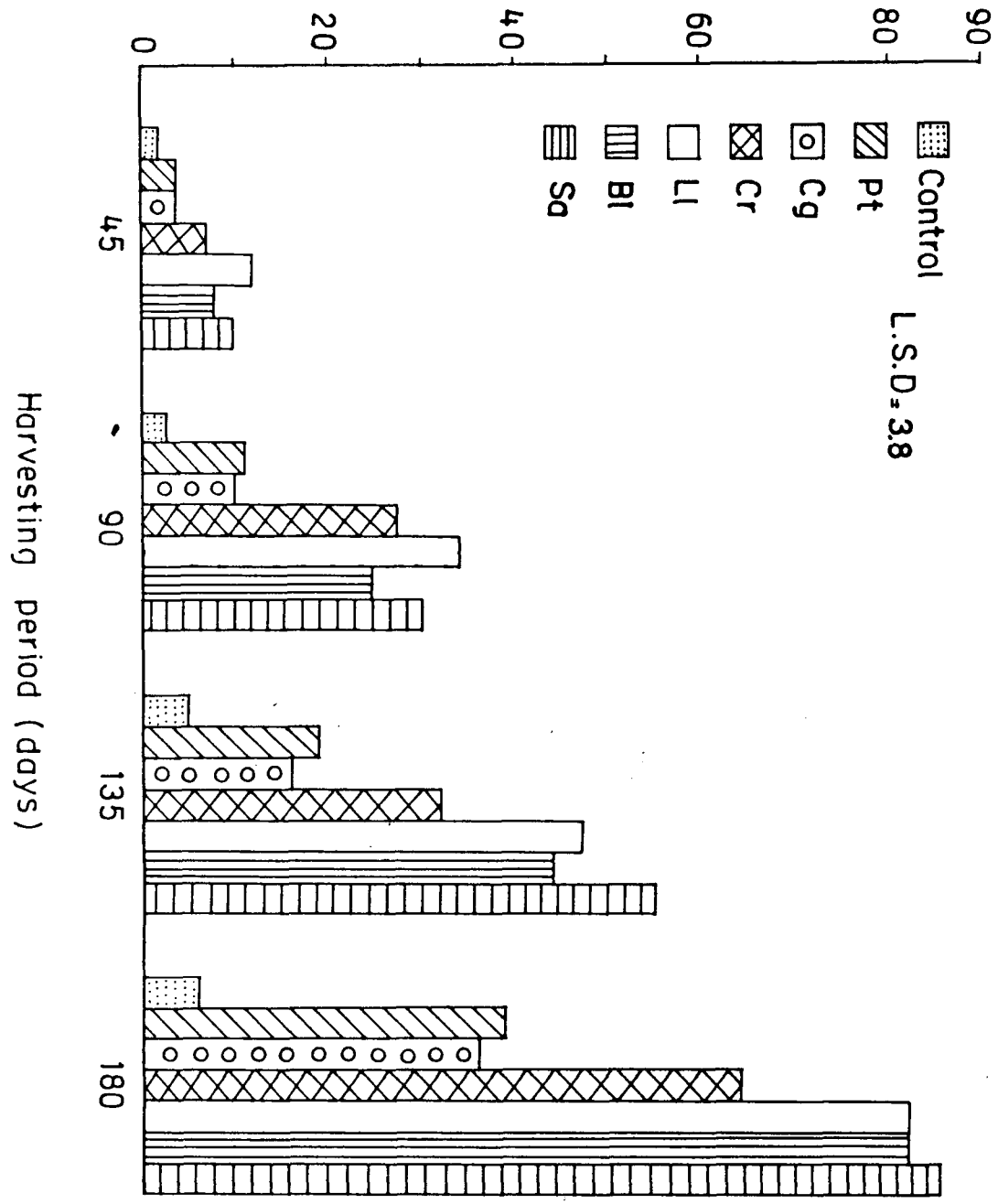
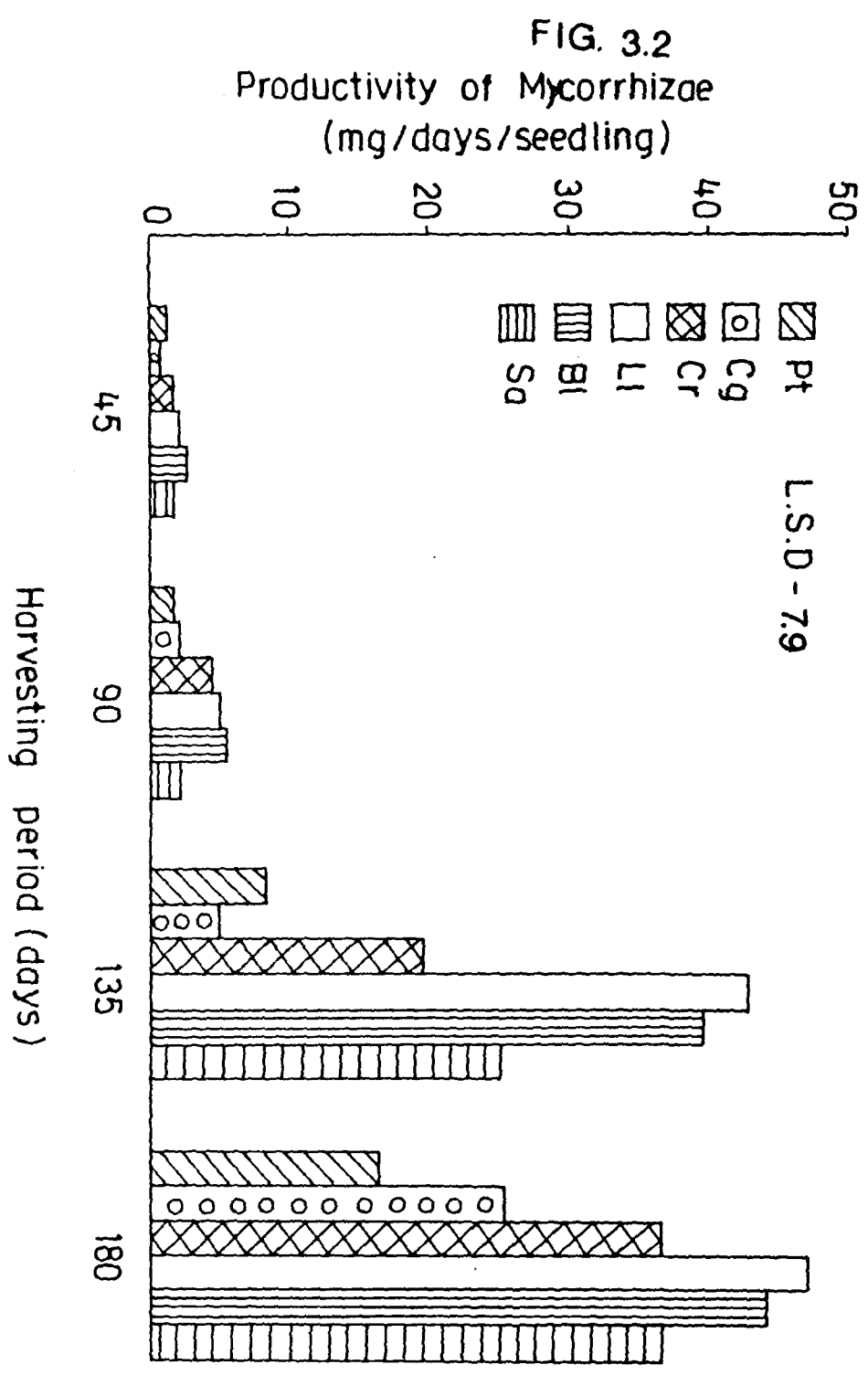

Colonization of Mycorrhizae (%)
0
20
40
60
80
90
Control
Pt
Cg
Cr
LI
BI
Sa
L.S.D=3.8
Harvesting period (days)
45
90
135
180

Figure-3.2: Production of mycorrhizae (mg/days/seedlings) in pine seedlings inoculated with different mycobionts (Con = Control; Pt = *P.tinctorius*; Cg = *C.graniforme*; Cr = *C.radiata*; Ll = *L.lacccata*; Bl = *B.lutues*; Sa= *S.aurantium*.

FIG. 3.2


Productivity of Mycorrhizae
(mg/days/seedling)
0
10
20
30
40
50
Pt
Cg
Cr
Ll
Bl
So
L.S.D - 7.9
45
90
135
180
Harvesting period (days)

CHAPTER 4

EFFICIENCY OF ECTOMYCORRHIZAE FORMED BY DIFFERENT MYCOBIONTS IN IMPROVING GROWTH AND NUTRIENT UPTAKE BY PINE SEEDLINGS ON DEGRADED AND FOREST SOIL

INTRODUCTION:

Ectomycorrhizae are known to increase the survival and growth of the seedlings of tree species (Ruehle and Marx, 1979). Though non-mycorrhizal pine seedlings can be grown in the greenhouse in nutrient rich conditions to minimize stress (Tinus and McDonald,1979). However, such seedlings often fail to survive or grow under natural planting sites, especially during a period of environmental stress (Ruehle, 1982). Artificial inoculation of host plants with specific mycorrhizal fungi has shown that plant growth may depend upon the efficiency of the mycobiont in conifers (Marx *et al*,1982; Browning and Whitney,1993) and that their competitive ability under varied ecological conditions (McAfee and Fortin,1986).

Mycorrhizal inoculation improves plant productivity especially in soils with a low nutrient status. The disinfection often leads to retarded plant growth, reflecting a decrease in soil fertility which can only be overcome by introducing suitable mycorrhizal fungi into soil (Harley and Smith,1983; Gianinazzi-Pearson *et al*,1984). Various soil factors like mineral nutrients especially phosphate may affect the colonization and development of mycorrhizae.

Response of pine seedling to ectomycorrhizal fungi has been demonstrated (Sharma,1981b; Kumar,1990),but mycobiont specific benefit on the Khasi pine seedlings is meagre. Forest soils vary ecologically at different stages of succession. It is essential,therefore,to select a specific ectomycorrhizal association that is adapted to specific aforestation site (Perry *et al*,1987).

In West Khasi hills and adjoining areas, the forest ecosystem is fragile and is difficult to recover them to their original state due to heavy rainfall and sloppy topography (Ramakrishna and Saxena,1984). Large scale deforestation has helped to develop wet deserts at a rapid rate (Ramakrishna,1985).

The present experiment was aimed to study the efficiency of different mycorrhizal fungi in degraded and forest soils in pine seedlings for their survival,growth and nutrient uptake under green house natural conditions.

MATERIALS AND METHODS:

1. Collection and treatment of soil:

Forest and degraded soils (with scanty grasses) were collected from the Nongstoin, West Khasi Hills,Meghalaya. For forest soil, 19 year old pine plantation stand (oldest) was selected. The physico-chemical properties of the soil of this stand are given (Table 4.1).

For degraded soil, and adjacent barren land (with few Caryx sp) was selected. The soil is exposed to marked wet and dry season. The physico-chemical characteristics of the soil are given in table(4.1).

Soils from both the sites were collected from 0-15cm depth in 10 replicates separately and mixed to get homogenous mixture of each type. The soils were steam sterilized at 1 kg cm^{-2} for 1h. Sterilization was repeated twice with an interval of 24h. The soils were allowed to dry and were mixed with autoclaved builders sand in a ratio of 1:1, then 3 kg of the sand-soil mixtures was filled in plastic pots (diameter 16 cm) with a drainage hole.

2. Raising of pine seedlings:

Seeds of pine (*Pinus kesiya* Royle ex Gordon) were collected during December. The seeds were surface sterilized in 0.1% $HgCl_2$ for 5 minutes and washed in sterilized water

several times. They were germinated in sterilized sand at 30°C in growth chamber. Six seedlings (3 cm radicle) were transplanted in each pot. Forty seedlings were used for each treatment.

3. Preparation of mycorrhizal inoculum:

Six ectomycorrhizal fungi, based on their dominance *i.e., Pisolithus tinctorius, Cenococcum graniforme, Collybia radiata, Laccaria laccata, Boletus luteus and Scleroderma aurantium* were selected for the experiment. The sporocarps of *B.luteus and S.aurantium* were collected and a spore suspension was prepared (Chapter 3). 100 ml of each inoculum was inoculated in each pot below 2 cm layer of the soil. The control set received the same amount of autoclaved inoculum. Mycelial slurry of *Pisolithus tinctorius, Cenococcum graniforme Collybia radiata* and *Laccaria laccata* was prepared (Chapter 3) and 10 ml of mycelial slurry was inoculated as described above.

4. Harvesting of seedlings:

Eight seedlings with intact root systems were carefully excavated for each soil type treated with mycobiont at 45,90,135 and 180 days and brought to the laboratory for observations.

5. Assessment of mycorrhizal colonization:

Roots of seedlings were washed under running water.

Percentage infection of ectomycorrhizal short roots was determined by the method Beckord *et al*, (1984).

6. Growth and biomass of seedlings:

Shoot height, root length and root collar diameter of seedlings were measured. Shoot and root dry weight of seedlings were determined by drying them at 60°C for 24 h to determine their biomass. Seedling volume was calculated as [(root collar diameter)^2 x height or D^2H] (Marx,1983).

7. Nutrient contents in seedlings:

Oven-dried shoots and roots of seedling were ground and sieved through 0.2 mm sieve for nutrient analysis. Kjeldahl's digestion method was followed for the determination of total nitrogen in plant tissue as described by Mishra (1968). 0.5 g of the dried plant material was taken in micro-Kjeldahl flask and digested and titrated as described earlier (Chapter I). For the estimation of total phosphorus and potassium in seedlings the wet triacid digestion procedure was followed as suggested by Allen (1974). 0.25 powdered plant material was taken into 100 ml digestion flask. Thereafter, 1 ml of 60 % $HClO_4$, 5 ml HNO_3 and 0.5ml H_2SO_4 were added. The flasks were swirled gently to mix powder with acid solution. The mixture was digested for a period of 40 minutes till the material became colourless. The digested mixture was cooled, diluted to 100 ml with distilled water and filtered through Whatman

filter paper (No.1). The filtrate was used for the estimation of phosphorus and potassium as described earlier for soil samples (Chapter I).

STATISTICAL ANALYSIS:

The data was processed by analysis of variance (ANOVA) and significant differences among means were identified with Duncan's multiple range test at p=0.05 and .01 level. Correlation coefficient was also calculated.

RESULTS:

Mycorrhizal colonization was maximum in *S.aurantium* (85%) inoculated seedlings grown in forest soil and in *B.luteus* inoculated ones in degraded soil. Minimum infection was observed with *C.graniforme* (36%) in forest soil and *C. radiata* in degraded soil. Mycorrhizal infection was better in forest soil where *P.tinctorius* and *C. graniforme* showed less than 50% infection (Table 4.2). A significant correlation was observed between the sampling periods and infection (Table 4.4). A positive correlation coefficient was observed between mycorrhizal infection and root phosphorus and a negative one with shoot:root ratio in forest and degraded soil. A positive correlation was also observed between shoot height with *L.laccata* and *B.luteus* in forest soil and with *L.laccata*, *B.luteus* and *S.aurantium* and seedling volume and root nitrogen

with all the mycobiont inoculations in degraded soil (Table 4.5).

Maximum and minimum number of mycorrhiza was produced with *L.laccata* and *P.tinctorius* respectively in forest soil and with *B.luteus* and *C.graniforme* respectively in degraded soil.It showed a significant variation between various sampling periods (Table 4.4). Positive correlation was observed between seedling volume and root nitrogen.

Mycorrhizal fungi exhibited a promotory effect on the growth of pine seedlings in forest and degraded soils. Stimulation in shoot growth followed a trend *L. laccata* > *S.aurantium* > *C.radiata* > *B.luteus* > *P.tinctorius* > *C. graniforme* in forest soil. Whereas, in degraded soil the order was *B.luteus* > *L.laccata* > *S.aurantium* > *C.radiata* > *P.tinctorius* > *C.graniforme*. Seedling growth was 3 times more in the forest soil than in degraded soil in all the mycobiont treatments (Fig.4.1). There was a significant variation between the shoot height in forest soil than degraded ones. Insignificant variation was observed between the various mycobiont treatments in forest and degraded soil (table 4.4 Plate 2,3

Average root length was more than the shoot height. There was not much significant differences in the shoot:root ratio in forest and degraded soil. Maximum and minimum S/R ratio was observed in C. radiata and C. graniforme in forest

soil and C. graniforme and C.radiata in degraded soils respectively (Fig. 4.1).

Seedling volume (D^2H) was maximum and minimum in *C.radiata* and *P.tinctorius* respectively in forest soil. In degraded soil, it was maximum in *C.graniforme* and minimum in *C.radiata*. Significant difference ($p<0.01$) between the various harvesting periods was observed (Table 4.4).

Biomass of seedlings was more in all the mycorrhizal seedlings than non-mycorrhizal ones in both soil types. Maximum biomass was observed with *L. laccata* and minimum was produced in *P.tinctorius* and *C.graniforme* in forest and degraded soils respectively. Accumulation of seedling biomass increased with the mycobionts than uninoculated ones (Fig 4.2).

Nitrogen, phosphorus and potassium contents in seedlings increased with the inoculations of mycobiont than control. Their content in shoot and roots was more in forest soils than in degraded soil (Tabel 4.3).

Shoot nitrogen was maximum and minimum in *L.laccata* and *S.aurantium* inoculated seedlings grown in forest soil and *B.luteus* and *C.radiata* ones in degraded soil respectively. Insignificant variation was observed between the various mycobiont treatment in forest and degraded soil. Whereas a significant variation was observed between the various

sampling periods in forest soil ($p<0.05$) (Table 4.4).

Root nitrogen was maximum and minimum with *C.graniforme* and *P.tinctorius* in forest soil and in *B.luteus* and *P.tinctorius* in degraded soil respectively. Significant correlation between root nitrogen content between the mycobiont inoculation in forest ($P<0.01$) and degraded ($P<0.01$) soil was observed (Table 4.5).

Shoot phosphorus was maximum and minimum in *L.laccata* and *C.radiata* in forest soil and *B.luteus* and *C.radiata* in degraded soil respectively. A significant relationship was observed between the various mycobiont inoculation ($P<0.01$) and various sampling period ($P<0.05$) in degraded soil. A significant variation was observed between the various sampling periods ($P<0.01$) in forest soil (Table 4.5).

Shoot potassium was maximum and minimum in *B.luteus* and *P.tinctorius* inoculated seedlings in forest and *B.luteus* and *C.graniforme* ones in degraded soil respectively. There was a significant variation between the different mycobiont inoculation ($p<0.01$) and various sampling periods($p<0.01$), (Table 4.4).

Root potassium was maximum and minimum in *L.laccata* and *P.tinctorius* in forest soil and *C.radiata* and *S.aurantium* in degraded soil respectively. There was a significant variation

between the various mycobiont inoculation ($P<0.01$) and sampling periods ($P<0.01$) in degraded soil. Whereas a significant variation was only observed between various sampling periods ($P<0.01$) in forest soil (Table 4.4).

DISCUSSION

Insignificant variation in seedlings growth of pine was observed between inoculated and control treatments at an early stage of seedlings development in forest and degraded soils. Similar reports have been made (Marx and Barnett,1974 and Ruehle and Marx ,1977). Drain of photosynthates from the host to the fungus, may be responsible for invisible enhanced growth by the different symbionts at the early stage of seedling growth. Ga gnon et al (1987) have also reported that mycorrhizal seedlings had less amount of carbohydrates available to seedling growth than uninoculated ones owing to allocation of photosynthates required to the fungal growth. It is, therefore, likelihood that seedling without or with fewer ectomycorrhizae are assumed to little carbohydrate drain and nutrient from rich soil and are able to grow faster than those with abundant ectomycorrhizae (Harley,1978; Marx *et al*, 1982).

However, the enhanced growth of seedlings by ectomycorrhizal fungi in forest and degraded soils at later part of seedling development was attributed to the increase in

root absorbing surface area due to fungal mycelium (Harley and Smith,1983; Langlois,1983). The extensive network of mycorrhizal fungi encountered larger volume of soil than did root hairs alone (Medve,1978).

The growth of 180 days old pine seedlings was observed significantly more in forest soil than in degraded soil, which may be attributed to the high amount of soil organic matter, which favoured better formation of mycorrhizae (Rubtov,1964).

Maximum colonization of pine root by *Boletus luteus* in degraded soil could be related to its affinity with low organic matter content, favourable moisture and aeration levels which favoured the development of mycorrhizae (Maghembe and Redhead,1984; Kumar,1990) compare to in the forest soil where high nutrient concentration in the substrate restricted mycorrhizal infection (Alexander and Fairley,1983). Mycorrhizal infection was minimum with *C. graniforme* in forest and degraded soils which ultimately reflected the reduction in NPK contents, biomass and seedling volume (Ruehle and Wells,1984). Better growth and nutrient contents in seedlings with *Laccaria laccata* than *Cenococcum graniforme* in forest soils, is contradictory to the findings of Hung (1983) who found *C.graniforme* better than *L.laccata.* The difference between these two may be attributed to the host genotype and fertility status of the soils.

Boletus luteus demonstrated its better compatibility with Khasi pine in degraded soil by colonizing more of the root system than the other fungi and by mediating a far superior growth response. Seedlings with shorter root length and shoot height in the degraded soil could be sacrificed to the good development of mycorrhizae and mycorrhizal productivity than the seedlings with better growth characteristics but with less amount of mycorrhizae in forest soil is supported by Marx and Hatchel (1986).

A decrease in shoot root ratio in degraded soil in comparison to the forest soil is similar to that expected by application of fertilizers leading to the high fertility of the substrate (Ruehle and Wells, 1984). It may also be due to a high carbon investment in the fungal symbiont. The difference in seedling growth caused by different mycobionts was attributed to ectomycorrhizae formed by them during the growing season and variation in nutrient and water absorption. Abundant ectomycorrhizae by *L.laccata* improved better growth of seedlings in forest soil similar to that of high fertile soil (Molina and Chamard, 1983). In the low fertile degraded soil, the active fungus of the mycorrhizal seedlings utilized considerably high amount of host photosynthate (Molina,1982;Marx *et al* 1982) . But mycorrhizal root system, increased survival and growth after transplantation.

The differences between the response of different

mycorrhizal fungi in forest and degraded soil is not only due to their edaphic requirement but also their ability to stimulate growth of pine seedlings.

Growth habit of mycorrhizal fungus in soil influenced its nutrient uptake. The differences between mycorrhizal fungi in nourishment of the host were related to overall growth of the host and nutrient content. Best response by *B.luteus* in degraded soil reflected better relative efficiency of the native and naturally occurring mycorrhizal fungus than exotic ones (Bowen,1965). The other indigenous mycorrhizal fungi showed growth promoting ability which were adapted to the local conditions and provided long -lasting benefit from artificial inoculation.

The physiological significance of more mycorrhizal roots per seedlings was related to better growth. The present study clearly indicates that seedlings with more quantity of ectomycorrhizae are able to rapidly regenerate numerous new lateral roots of greater length, form more new ectomycorrhizae, and thereby, utilize available water and nutrients more effectively. The ecological adaptability of ectomycorrhizal fungus hinges on the metabolic pathways it has evolved to contend with environmental variation.

Reclamation and reforestation of the degraded sites in the Khasi hills of Meghalaya can be expedited by using pine

seedlings tailored with mycorrhizae formed by native fungi like Boletus luteus and Scleroderma aurantium, capable of growing under adverse conditions, which were physiologically and ecologically adapted to the adverse conditions.

Eventhough, the effect of a given fungus may only be temporary, its short term influence may make the difference between initial success or failure of seedling establishment.

Table 4.1 : Physico-chemical characteristics of forest and degraded soils.

	Forest soil	Degraded soil
Sand	65.2 %	79.2 %
Silt	19.1 %	10.6 %
Clay	15.7 %	10.2 %
Water holding capacity	73.2	40.7
Total nitrogen (%)	0.096	0.077
Available phosphorus (%)	0.028	0.019
Exchangeable potassium (%)	0.199	0.171
Organic matter (%)	3.6	1.7
Soil pH	5.8	5.5

Table 4.2 : Growth parameters, mycorrhizal infection (%) and mycorrhizae productivity in pine seedlings inoculated with different mycobionts at different time intervals in forest and degraded soils.

Sampling period (days)	Treatments	Forest soil: Root collar diameter (cm)	Shoot/root ratio	Seedling Volume D^2H(cm)^3	MI (%)	MP (mg/day)	Degraded Soil: Root collar diameter (cm)	Shoot/root ratio	Seedling Volume D^2H(cm)^3	MI (%)	MP (mg/day)
45	Control	0.1	1.57	0.064	0	0	0.1	1.66	0.050	0	0
	MF_1	0.1	1.23	0.067	4	6.8	0.1	1.77	0.055	4	10.7
	MF_2	0.1	1.6	0.054	4	8.75	0.1	1.75	0.053	4	10.8
	MF_3	0.1	1.6	0.074	7	16.2	0.1	1.53	0.056	6	12.2
	MF_4	0.1	1.94	0.087	12	17.7	0.1	1.21	0.068	7	15.4
	MF_5	0.1	1.81	0.073	8	20.2	0.1	1.44	0.069	8	20.1
	MF_6	0.1	1.8	0.08	10	17.5	0.1	1.33	0.061	7	19.2
90	Control	0.18	1.89	0.45	3	6	0.11	1.1	0.091	0	0
	MF	0.15	1.65	0.33	11	19	0.11	1.37	0.11	8	22
	MF_1	0.19	2.66	0.53	10	21.8	0.12	1.4	0.133	11	38
	MF_2	0.19	1.96	0.56	27	43.7	0.13	2.03	0.172	18	41
	MF_3	0.15	1.76	0.38	34	48.7	0.14	1.41	0.19	21	49
	MF_4	0.12	1.76	0.23	25	98.4	0.13	1.67	0.17	25	55.3
	MF_5	0.15	1.78	0.36	30	72.2	0.14	1.4	0.19	28	61.1
	MF_6										
135	Control	0.22	0.57	0.93	5	12.2	0.12	0.33	0.12	4	0
	MF	0.16	0.77	0.51	19	38.9	0.12	0.34	0.13	44	89.4
	MF_1	0.2	0.57	0.77	16	59.4	0.15	0.44	0.2	38	71.7
	MF_2	0.2	0.55	0.85	32	119.5	0.13	0.34	0.16	48	96.8
	MF_3	0.22	0.58	1.09	47	141.8	0.15	0.75	0.27	64	124.3
	MF_4	0.22	0.38	1.03	44	138.5	0.15	0.91	0.27	71	142.2
	MF_5	0.18	0.8	0.70	55	125.7	0.15	1.24	0.22	55	116.2
	MF_6										
180	Control	0.22	0.52	0.92	6	20	0.17	0.33	0.26	8	10.2
	MF	0.22	0.46	0.98	39	116.3	0.18	0.38	0.32	69	139.2
	MF_1	0.21	0.48	0.86	36	125.1	0.17	0.54	0.27	55	111.4
	MF_2	0.25	0.60	1.45	64	135	0.19	0.32	0.36	71	141.2
	MF_3	0.21	0.51	1.18	82	146	0.18	0.39	0.4	81	158.1
	MF_4	0.22	0.52	1.03	82	144	0.19	0.4	0.46	85	164
	MF_5	0.23	0.47	1.32	85	136	0.19	0.45	0.38	76	147
	MF_6										

MF_1 = *P. tinctorius*; MF_2 = *C. graniforme*; MF_3 = *C. radiata*; MF_4 = *L. laccata*; MF_5 = *B. luteus*; MF_6 = *S. aurantium*

MI = Mycorrhizal Infection
MP = Mycorrhizae productivity

Table 4.3 : Nutrient contents [Nitrogen (N), Phosphorus (P), Potassium (K)] % in Shoot and root of pine seedlings inoculated with different mycobionts in forest and degraded soils.

Sampling (Days)		Forest Soil						Degraded Soil					
	Treatment	Shoot			Root			Shoot			Root		
		N	P	K	N	P	K	N	P	K	N	P	K
45	Control	1.71	0.24	1.45	0.91	0.24	0.70	1.74	0.28	1.89	0.96	0.24	0.94
	MF_1	1.78	0.29	1.76	0.96	0.25	0.89	1.78	0.27	1.88	0.97	0.32	1.05
	MF_2	1.8	0.27	1.77	1.02	0.22	0.86	1.15	0.32	1.92	0.99	0.28	0.97
	MF_3	1.86	0.28	1.72	1.0	0.27	0.92	1.79	0.32	1.9	0.99	0.29	0.99
	MF_4	1.89	0.35	1.81	1.06	0.32	0.97	1.81	0.29	1.91	1.00	0.31	0.98
	MF_5	1.82	0.33	1.79	0.97	0.26	0.91	1.87	0.36	1.97	1.05	0.33	1.05
	MF_6	1.83	0.35	1.82	1.01	0.30	0.96	1.79	0.32	1.91	1.01	0.29	0.98
90	Control	1.74	0.26	1.55	0.92	0.27	0.8	1.75	0.29	1.91	0.98	0.25	0.95
	MF_1	1.81	0.31	1.79	0.99	0.27	0.92	1.8	0.28	1.89	0.98	0.33	1.07
	MF_2	1.82	0.29	1.81	1.11	0.24	0.89	1.81	0.32	1.80	1.00	0.29	0.99
	MF_3	1.89	0.3	1.77	1.03	0.29	0.96	1.17	0.33	1.96	1.07	0.29	1.02
	MF_4	1.92	0.36	1.89	1.10	0.36	0.99	1.85	0.31	1.92	1.01	0.32	0.99
	MF_5	1.88	0.35	1.82	0.99	0.25	0.93	1.89	0.37	1.99	1.07	0.34	1.09
	MF_6	1.86	0.37	1.86	1.09	0.32	1.0	1.81	0.33	1.92	1.02	0.30	1.00
135	Control	1.81	0.29	1.64	0.98	0.31	0.92	1.77	0.30	1.94	0.99	0.26	0.99
	MF	1.84	0.34	1.87	1.01	0.28	0.95	1.81	0.31	1.91	1.00	0.35	1.10
	MF_1	1.88	0.32	1.87	1.16	0.26	0.91	1.84	0.33	1.92	1.05	0.31	1.01
	MF_2	1.9	0.32	1.81	1.11	0.31	0.99	1.79	0.33	1.99	1.09	0.30	1.11
	MF_3	1.98	0.41	1.92	1.17	0.39	1.02	1.89	0.35	2.00	1.06	0.37	1.03
	MF_4	1.91	0.36	1.86	1.2	0.29	0.98	1.91	0.39	2.01	1.11	0.36	1.11
	MF_5	1.92	0.39	1.89	1.18	0.36	1.04	1.82	0.35	1.95	1.07	0.31	1.01
	MF_6												
180	Control	1.92	0.32	1.73	1.09	0.39	0.99	1.79	0.31	1.96	1.01	0.29	1.0
	MF	2.02	0.38	1.90	1.19	0.36	1.16	1.83	0.33	1.98	1.03	0.37	1.12
	MF_1	2.29	0.47	1.95	1.32	0.36	1.16	1.86	0.35	1.96	1.08	0.36	1.07
	MF_2	2.13	0.38	1.93	1.21	0.34	1.12	1.82	0.34	2.01	1.11	0.31	1.16
	MF_3	2.32	0.54	1.99	1.25	0.42	1.18	1.9	0.39	2.03	1.11	0.38	1.09
	MF_4	2.01	0.39	2.02	1.31	0.35	1.09	1.96	0.41	2.04	1.17	0.39	1.14
	MF_5	1.99	0.44	1.94	1.25	0.38	1.14	1.88	0.38	1.97	1.13	0.32	1.04
	MF_6												

MF_1 = *P. tinctorius*, MF_2 = *C. graniforme*, MF_3 = *C. radiata*,
MF_4 = *L. laccata*, MF_5 = *B. luteus*, MF_6 = *S. aurantium*

Table 4.4: Analysis of variance (F) of various mycobionts and sampling period with various parameters in forest and degraded soils.

Source of variance	Forest soil		Degraded soil	
	Variation Between treatments	Variation Between Sampling periods	Variation Between treatments	Variation Between Sampling periods
Shoot height	NS	108.82**	NS	23.47**
Root length	NS	82.71**	NS	28.1**
Seedling volume	NS	33.32**	NS	38.9**
Mycorrhizal infection	NS	8.89**	NS	65.88**
Shoot Nitrogen	NS	13.35**	NS	NS
Shoot Phosphorus	NS	6.87**	5.67**	3.40*
Shoot Potassium	5.96**	4.03*	NS	6.10**
Root Nitrogen	NS	15.02**	3.3*	6.43**
Root Phosphorus	NS	10.31**	7.08**	3.04*
Root Potassium	NS	12.92**	4.31**	4.74**
Mycorrhizae productivity	NS	NS	NS	52.18**

* = Significant at $p<0.05$ level
** = Significant at $p>0.01$ level

Table 4.5 : Correlation coefficient (r) of mycorrhizal infection(%) and mycorrhizal productivity (mg) with various parameters in forest and degraded soil inoculated with the various mycobionts.

Parameters	Forest Soil												Degraded Soil									
	MF_1		MF_2		MF_3		MF_4		MF_5		MF_6		MF_1		MF_2		MF_3		MF_4		MF_5	
	MI	MP	MI	MP	MI	MP	MI	MP	MI	MP	MI	MP	MI	MP	MI	MP	MI	MP	MI	MP	MI	MP
Shoot height	NS	NS	NS	NS	NS	NS	0.81*	0.94**	0.89**	0.79*	NS	NS	NS	NS	NS	NS	NS	NS	0.74*	0.[illegible]**	0.89*	0.82*
Root length	NS	NS	NS	NS	NS	NS	NS	NS	NSd	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Shoot/root ratio	0.85*	NS	0.86*	NS	0.78*	NS	0.82*	NS	0.89**	NS	0.84*	NS	0.94**	NS	0.88**	NS	0.84*	NS	0.92***	NS	0.88**	NS
Seedling volume	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.85*	0.92**	0.92**	0.84*	0.93**	0.97***	0.84*	0.[illegible]**	0.92**	0.88**
Shoot Nitrogen	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Root Nitrogen	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.86*	0.78*	0.84*	0.84*	0.87**	0.63*	0.86*	0.[illegible]**	0.96**	0.97**
Shoot Phosphorus	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Root Phosphorus	0.87*	0.93**	0.96***	0.71*	0.94**	0.84*	0.86**	0.74*	0.[illegible]*	0.82*	0.78*	0.81	0.86*	0.96***	0.86*	0.78*	0.84*	0.94**	0.84*	0.[illegible]*	0.89**	0.88**
Shoot Potassium	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Root Potassium	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

MF_1 = *P. tinctorius*, MF_2 = *C. granuiforme*, MF_3 = *C. radiata*, MF_4 = *L. laccata*, MF_5 = *B. luteus*, MF_6 = *S. aurantium*

MI = Mycorrhizal infection; MP = Mycorrhizae productivity

* = Significant at P < 0.05 level; ** = Significant at P < 0.01 level; *** = Significant at P < 0.001 level

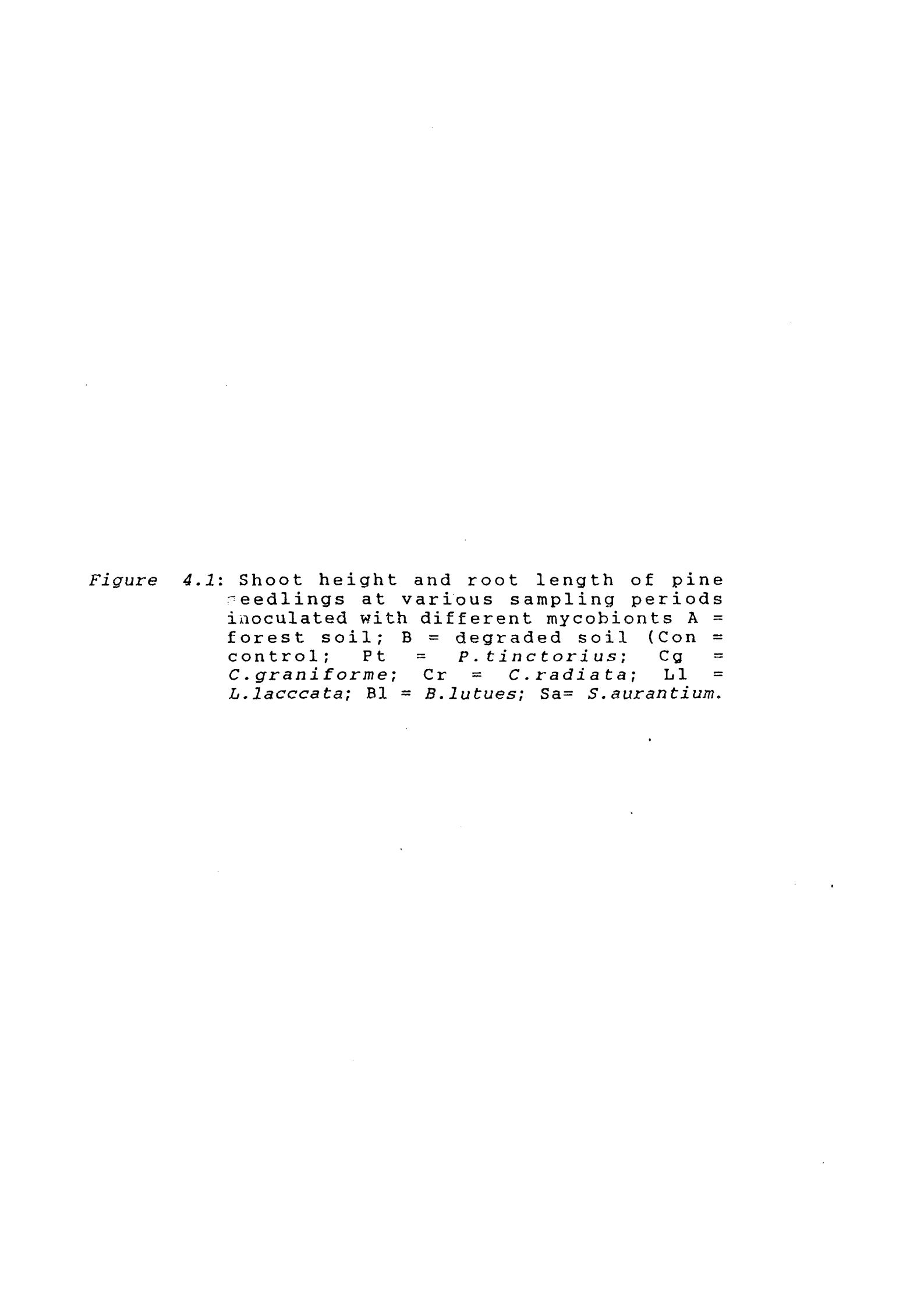


Figure 4.1: Shoot height and root length of pine seedlings at various sampling periods inoculated with different mycobionts A = forest soil; B = degraded soil (Con = control; Pt = *P.tinctorius*; Cg = *C.graniforme*; Cr = *C.radiata*; Ll = *L.lacccata*; Bl = *B.lutues*; Sa= *S.aurantium*.

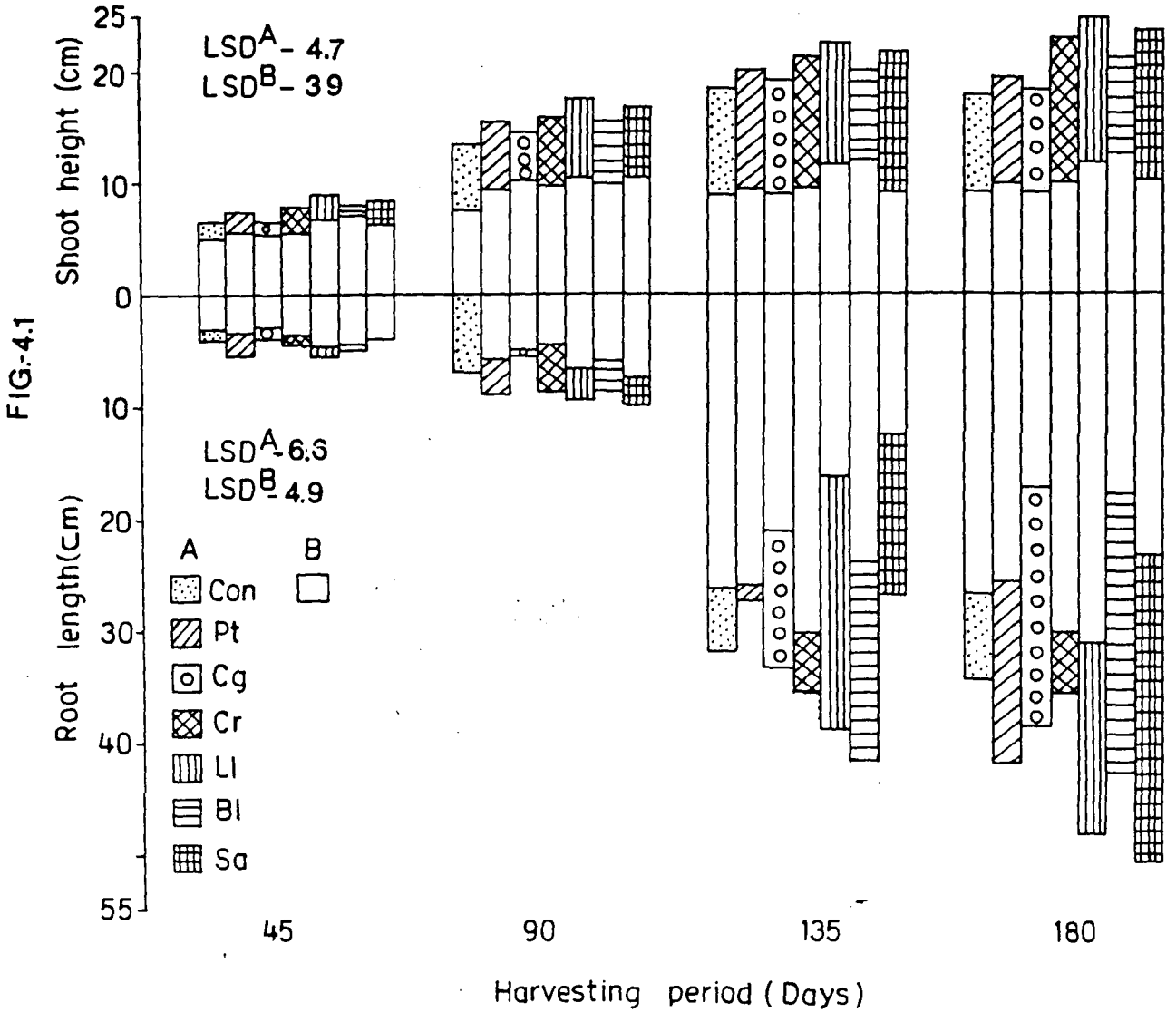


FIG.-4.1
Shoot height (cm)
Root length(cm)
25
20
10
0
10
20
30
40
55
LSD^A - 4.7
LSD^B - 3.9
LSD^A - 6.3
LSD^B - 4.9
A
B
Con
Pt
Cg
Cr
LI
BI
Sa
45
90
135
180
Harvesting period (Days)

Figure 4.2: Biomass (g/seedling) of pine at various sampling periods inoculated with different mycobionts A = forest soil; B = degraded soil (Con = control; Pt = *P.tinctorius*; Cg = *C.graniforme*; Cr = *C.radiata*; Ll = *L.lacccata*; Bl = *B.lutues*; Sa= *S.aurantium*.

FIG.-4.2

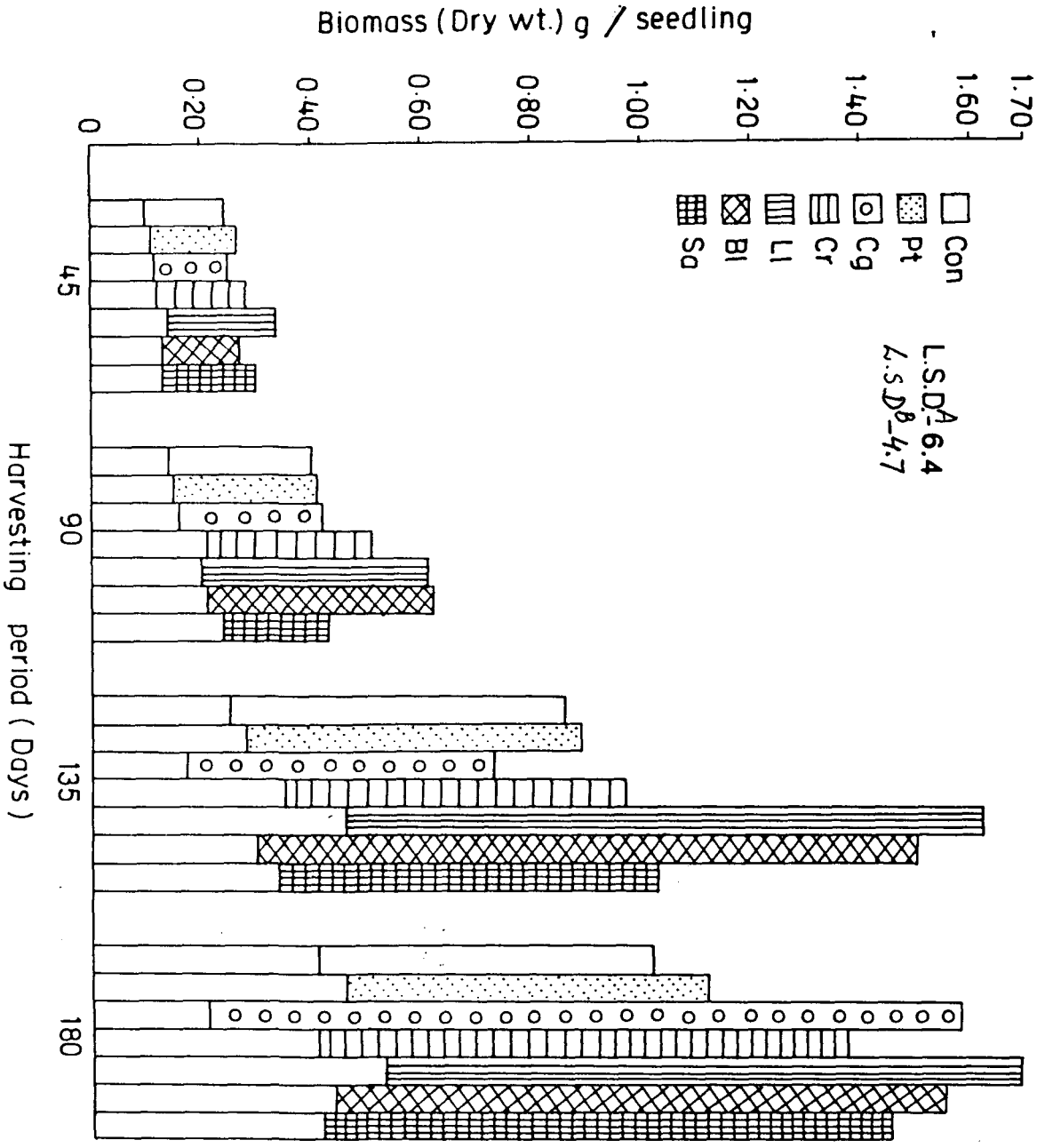
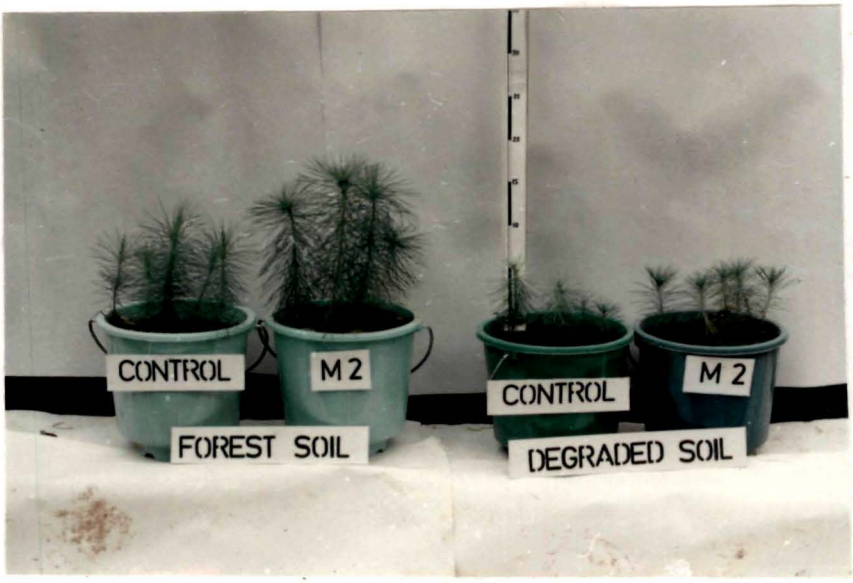

Biomass (Dry wt.) g / seedling
0
0·20
0·40
0·60
0·80
1·00
1·20
1·40
1·60
1·70
Con
Pt
Cg
Cr
Ll
Bl
So
L.S.D.$^{A}_{-}$ 6.4
L.S.D^{B} 4.7
Harvesting period (Days)
45
90
135
180

Plate 2: Comparative study on the efficiency of ectoymocorrhizal fungi on shoot growth of pine seedlings in forest and degraded soil inoculated with various mycobionts (M_1=*P. tinctorius*; M_2=*C. graniforme*; M_3=*C. radiata*)

PLATE 2


CONTROL
M 1
FOREST SOIL
CONTROL
M 1
DEGRADED SOIL


CONTROL
M 2
FOREST SOIL
CONTROL
M 2
DEGRADED SOIL

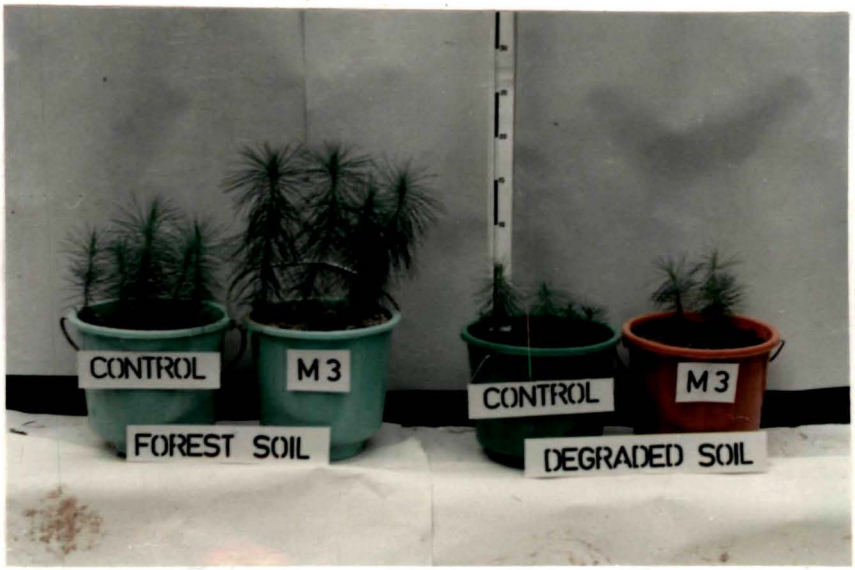
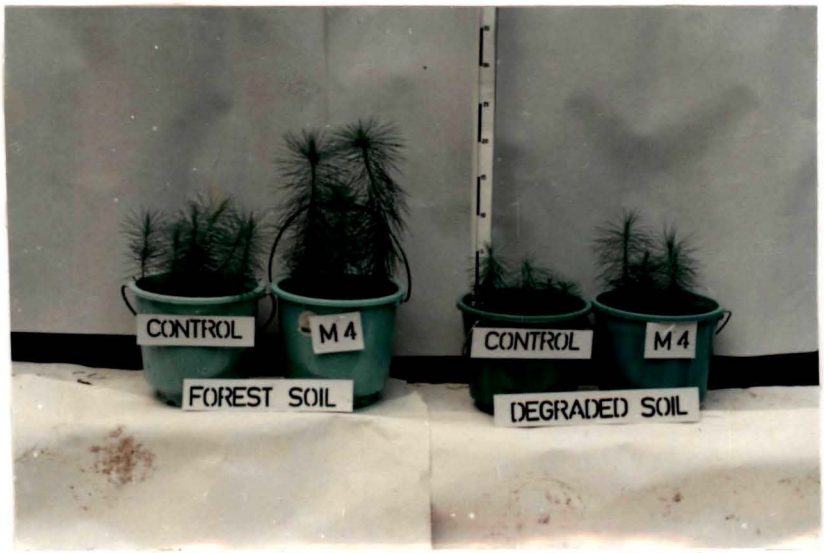

CONTROL
M 3
FOREST SOIL
CONTROL
M 3
DEGRADED SOIL

Plate 3: Comparative study on the efficiency of ectoymocorrhizal fungi on shoot growth of pine seedlings in forest and degraded soil inoculated with various mycobionts (M_4=*L.laccata*; M_5=*B.luteus*; M_6=*S.aurantium*)

PLATE 3


CONTROL
M 4
FOREST SOIL
CONTROL
M 4
DEGRADED SOIL


CONTROL
M 5
FOREST SOIL
CONTROL
M 5
DEGRADED SOIL


CONTROL
M 6
FOREST SOIL
CONTROL
M 6
DEGRADED SOIL

CHAPTER V

EFFECT OF ORGANIC AMENDMENTS ON THE DEVELOPMENT OF ECTOMYCORRHIZAE AND THEIR EFFICIENCY IN P-UPTAKE AND GROWTH OF SEEDLINGS

INTRODUCTION:

Organic matter is the main energy source for the growth and multiplication of microbes. The nutrient release on its decomposition also supports the growth of green plants. Therefore, the level of organic matter in soil determines a multiplicity of micro-organisms and make a system more dynamic (Prescott *et al*,1993). Mycorrhizal fungi depends on their host for the supply of energy source (Harley,1969). However, mineralization of litter may determine the availability of inorganic salts to them and to their hosts. Therefore, litter quality and quantity may regulate the nutrient supply. In such situation, colonization and efficiency of mycorrhizal fungi may be influenced. (Rose *et al*, 1983).

Fungal growth and mycorrhiza formation depends on its genotype, host and also on external factors including, availability of nutrients such as carbohydrates, nitrogen and phosphorus (Duddridge,1986, Gibson and Deacon,1990). Study on the effect of forest degradation on the colonization of mycorrhizal fungi is available (Jha,1990) but information on the quality of litter, which may influence the development of mycorrhiza is not available. Therefore, the objective of study was to evaluate the effect of various levels of organic amendments on the development of ectomycorrhizae and their efficiency in P-uptake and growth of seedlings.

MATERIALS AND METHODS:

1. Collection and treatment of soil:

Sandy loam laterite soil was collected from the Botanical garden of North-Eastern Hill University, Shillong, India. The soil, which had a pH (in soil-water suspension) of 5.2, organic matter 2.1%, nitrogen (total 0.18%, phosphorus (available)-0.021% and potassium 0.16mg g-1, was steam sterilized at 1 kg cm^{-2} for 1 h. Sterilization was repeated twice with an interval of 24 h. The soil was allowed to dry and was mixed with autoclaved builder's sand in a ratio of 1:1, then 3 kg of sand-soil mixture was placed in plastic pots (diameter 16 cm) with a drainage hole.

2. Organic amendments:

The sterilized soil was amended with the various organic materials, which were as (1) Fresh pine litter (2) Pine duff (3) Grass litter and (4) Grass litter + pine duff.

Fresh and duff pine needles were collected from a floor of pine stand (Pinus kesiya Royle) after litter fall in the month of January. The fresh and duff of *Paspalam dilatum, Arundinella bengalensis, Bothriochloa fimbriostris* and *Panicum auritum,* which formed main associative species with pine during early stage of their growth, were also collected. For each amendment, 30 g of fresh pine, pine duff, grass litter and 15 g of pine duff + 15 g of grass litter were mixed separately in the previously sterilized soil kept in the plastic pots. 30 pots were maintained for each amendment. The control set did not receive any litter.

3. Raising of pine seedlings:

Sterilized pine seeds were germinated at 30°C (Chapter-3). Six seedlings (3 cm radicle) were transplanted in each pot and maintained in greenhouse for 6 months. The maximum and minimum temperatures were 21°C and 12°C respectively. The average irradiance was 212 nm. with an average humidity of 78%.

4. Preparation of mycobiont inoculum:

Two mycorrhizal fungi *i.e.*, one indigenous *Boletus luteus* and an exotic *Pisolithus tinctorius* grown previously for 2 months on Modified Melin Norkan's medium (MMN) were used.

10 ml of mycelial slurry of each mycobiont was inoculated 2 cm below the soil surface near the root system of the seedlings in each pot. Ten replicates of each amendments with each treatment were maintained. Control set received the same quantity of autoclaved inoculum which were without amendment.

5. Harvesting of seedlings:

Eight seedlings per amendment per treatment were harvested after 180 days of transplantation. Seedlings along with their root systems were brought to the laboratory for further observations.

6. Assessment of mycorrhizal colonization:

Roots of seedlings were washed under running tap-water. Percentage infection of ectomycorrhizal short roots was determined as suggested by Beckjord *et al* (1984). Approximately 10 percent of the mycorrhizal roots were sectioned, mounted, and examined under 100x magnification for fungal mantle and hartig net development.

7. Growth and biomass of seedlings:

Shoot height, root length and root collar diameter of seedlings were measured. Shoot and root dry weight of seedlings were determined by drying them at 60°C for 72 h in hot air oven. Biomass of the seedlings was calculated on dry weight basis and was expressed as dry weight g^{-1} seedling.

8. Phosphatase activity:

Phosphatase activity of mycorrhizal roots was measured by the method of Dodd *et al* (1987) as described in Chapter (III). Phosphatase activity was expressed as the amount of p-Nitrophenol phosphorus (PNP) released during incubation.

9. Nutrient uptake and phosphorus translocation to the shoot:

Oven-dried seedlings were ground and sieved through a 0.2 mm sieve. Kjeldahl's digestion method was followed for the determination of total nitrogen as described in case of soil (Mishra,1968). 0.5 g dried and powered seedling material was taken in micro-Kjeldahl flask and processed further as described earlier (Chapter-I).

For the estimation of total phosphorus and potassium in seedlings, the wet triacid digestion procedure was followed as suggested by Allen (1974). 0.25 g powdered tissue of seedling was taken into 100 ml digestion flask. Thereafter, 1 ml of 60% $HClO_4$, 5 ml HNO_3 and 0.5 ml H_2SO_4 were added. The flasks were

swirled gently to mix powder with acid solution. The mixture was digested for a period of 40 minutes till the material became colourless. The digested mixture was cooled, diluted to 100 ml with distilled water and filtered through Whatman filter paper (No.1).The filtrate was used for the estimation of phosphorus and potassium as described earlier for soil samples (Chapter I).

Percentage phosphorus translocation to the shoot was calculated as described by Theodorou and Bowen (1993) as follows.

$$\text{PERCENTAGE P-TRANSLOCATION TO SHOOT} = \frac{\text{Shoot P}_{(mg)}}{\text{Total P}_{(mg)}} \times 100$$

Statistical analysis:

The data were processed by analysis of variance and significant differences among means were identified with Duncan's multiple range test at P=0.05. Correlation coefficient was also calculated in many cases.

RESULTS:

Mycorrhizal colonization in seedlings was maximum in *Boletus luteus* inoculated seedlings grown in grass litter amended soil (85%) and minimum in *Pisolithus tinctorius* inoculated once in pine fresh amended soil (61%) (Table 5.1). Seedlings inoculated with *B.luteus* with amendments showed

better colonization than the *P.tinctorius* inoculation with various amendments. Mycorrhizal colonization showed a significant positive correlation with seedling volume. biomass/seedling, root phosphorus, percentage P translocation, phosphatase activity and mycorrhizae productivity (Table 5.3) with *B.luteus* and *P.tinctorius* inoculated seedlings. Insignificant correlation was observed in mycorrhizal infection with phosphatase activity in seedlings grown in unamended soil and biomass of seedling amended with pine duff inoculated with *B.luteus.* However, a significant positive correlation was also observed between mycorrhizal infection with shoot:root ratio and shoot height in grass litter amended soil and in grass + pine duff soil inoculated with *B.luteus* and *P.tinctorius.* Insignificant variation was observed between the various amendments and between the treatments in mycorrhizal infection (Table 5.4).

Correlation with seedling volume, root phosphorus, P-translocation, biomass of seedlings and phosphatase activity except with Biomass in pine fresh amended soil and phosphatase activity in unamended soil inoculated with *B.luteus,* where Insignificant correlation was observed (Table 5.3). A significant positive correlation was also observed with the Biomass of seedling, shoot height and shoot:root ratio amended with grass litter and Grass+pine duff, inoculated with *B.luteus* and *P.tinctorius* (Table 5.3). Insignificant variation was observed between the various amendments and

between the treatments in mycorrhizae productivity. (Table 5.4).

Growth of seedlings was enhanced by mycobiont inoculation than in uninoculated ones. Maximum growth of was shoot obtained with *B.luteus* amended with decomposed grass litter, whereas it produced minimum growth in fresh pine amended soil (Fig 5.1, 5.2). Insignificant variation was observed between the various amendments but a significant positive variation was observed between the various inoculations (Table 5.4).

Root length was maximum in the seedlings with *B.luteus* amended with grass litter. However, it did not differ significantly with other amendments. Maximum shoot:root ratio was observed in *P.tinctorius*, unamended seedlings and minimum in pine duff amended soil inoculated with *P.tinctorius* inoculation (Table 5.1, Plate 4,5). Insignificant variation was observed between the various amendments and between the treatments.

Phosphatase activity was more in mycobiont inoculated and amended soil seedlings than in the control. Maximum activity was in grass litter amended soil with *B.luteus* inoculed seedlings and minimum in pine duff amended soil with *P.tinctorius* inoculation (Table 5.2). A significant variation was observed between the various treatments but not between the various amendments.

Minimum nitrogen content in uninoculated seedlings grown

on pine duff and minimum in uninoculated ones on amended with decomposed grass litter soil was observed. Maximum nitrogen content in seedlings was observed with *B.luteus* on grass litter amended soil and minimum in unamended ones (Table 5.2). Insignificant variation in nitrogen contents of seedlings was observed between the various amendments and treatments (Table 5.4).

Potassium was maximum in shoot and root in *B.luteus* inoculated seedlings grown in grass litter amended soil and minimum in *P.tinctorius* amended with grass+pine duff ones and in uninoculated ones on pine duff amended soil(Table 5.2). Insignificant variation was observed between the various amendments and treatments (Table 5.4).

Phosphorus content of shoot and root was maximum in *B.luteus* inoculated seedlings grown on grass litter amended soil and minimum in *P.tinctorius* ones on fresh pine litter (Table 5.4). Significant variation in P contents of seedlings was observed between treatments (Table 5.4).

Translocation of phosphorus from root to the shoot was more in uninoculated seedlings. Maximum being in unamended soil. Minimum percentage of p-translocation was observed in *B.luteus* inoculated, amended with Grass+pine duff seedlings. No significant relationship was observed between various amendments. However, a significant relationship was observed between the various treatments (Table 5.4).

DISCUSSION

The results showed that mycorrhizal colonization and its productivity was better in the seedlings inoculated with the indigenous mycobiont *B.luteus* than the exotic *P.tinctorius*.

Amendment with grass litter to both the mycobiont inoculated seedlings showed a better mycorrhizal infection and overall better performance than the seedlings amended with pine litter and in unamended soils. Better in performance of growth of seedlings on grass litter amended soil than the pine litter indicated the presence of insoluble and toxic substances.(Berg and Mc-Claugherty, 1989). Release of nitrogen and phosphorus differed between fresh and duff coniferous foliage litter. Among the two litters, more mobilization of nutrients, growth response and mycorrhizal colonization with grass litter may be compared to the poor or absence of leaching phase during decomposition in conifer needles (Berg,1988).

Better deveopment of ectomycorrhiza with *P.tinctorius* and *B.luteus* on pine duff and grass litter than the uninoculated and unamended was related to the improved physicochemical characteristics of the soil(Riffle,1977).

Improvement of growth and accumulation of dry matter in pine seedlings due to mycorrhizal fungi than the uninoculated ones were correlated to the enhanced nutrient uptake by

earlier than the latter ones. (Kropp and Langlois,1990). Improvement in the formation of ectomycorrhizae with *P.tinctorius* and *B.luteus* due to organic amendments was supported by Chou (1979) and Lindeberg (1986). Better shoot and root growth of the mycorrhizal seedlings on various organic amendments was attributed to the improved mycelial strands production by the mycobiont. It conferred high penetration of large inter root distances and had a positional advantage for competition with other micro-organisms for both inorganic and organic nutrients. Lyr (1963) found cellulolytic enzymes in *B.luteus, B.varieagatus and B.subtomentosus* which were able to attack hemicelluloses of the litter, and other naturally occurring complex carbohydrates and obtain their required carbon compounds (Hacskaylo,1973). Poor growth of seedlings with fresh pine litter was due to the inhibitory phenols and organic acids which suppressed the growth of *C.graniforme and L. forninos* (Mikola,1948).

Increased growth and dry matter in inoculated seedlings with grass litter was related to the presence of stimulatory easily available nutrients like vitamins and amino acids which increased the growth of mycorrhizal fungi (Lindeberg,1944;Melin,1946). The enhanced growth of conifer seedlings amended with litter than unamended ones was attributed to biological rather than nutritional factors (Parke *et al*,1983). Presence of more phosphorus in the mycorrhizal seedlings than the non-mycorrhizal ones was

supported by Stribley *et al* (1980). Higher nutrient uptake by mycorrhizal plants was due to improved hyphal growth, better exploitation of the soil volume and the competitive ability of *B.luteus* over the *P.tinctorius*.

Seedlings amended with grass litter and inoculated with *B.luteus* exhibited the maximum growth and was supported by the view of Schisler and Linderman,(1989) humic substances released from grass litter were in turn responsible for the increased microbial growth and activity.

Higher phosphate uptake was correlated to higher rate of phosphatase activity in mycorrhizal than non-mycorrhizal plants. (Meyselle *et al*,1991).

The results have suggested that the inoculation of the indegenous mycobiont *B.luteus* along with organic amendments to the soil with grass litter during the early stage of seedling growth of pine is better than the introduction of exotic mycobiont *P.tinctorius* in unamended soil. Addition of pine litter to the pine nursery may have detrimental effect on the development of mycorrhiza as well as on the growth of the seedlings.

Table No. 5.1

Growth Parameters, Dry matter Production and Productivity of Mycorrhizae (fresh wt) in Pine seeding inoculated with different mycobionts under different organic amendments.

Treatments (Mycobiont type)	Parameters	Organic Amendments					
		Unamended	Pine Fresh	Pine Duff	Grass litter	Grass + Pine(Duff)	L.S.D
Control	Shoot/Root ratio	0.42	0.41	0.64	0.42	0.33	2.96
	Root Collar Diameter(cm)	0.23	0.25	0.22	0.24	0.23	3.16
	Seeding Volume $D^2H(cm)^3$	0.576	0.687	0.561	0.714	0.65	5.1
	Drymatter/Seeding(gm)	0.46	0.46	0.42	0.47	0.55	4.81
	Mycorrhizal infection(%)	3	5	5	8	7	4.01
	Productivity of Mycorrhizae (fresh weight mg)	-	-	-	-	-	
P.tinctorius	Shoot/Root ratio	0.92	0.47	0.46	0.54	0.48	5.2
	Root Collar Diameter(cm)	0.29	0.28	0.26	0.27	0.24	3.86
	Seeding Volume $D^2H(cm)^3$	1.27	1.12	0.92	1.37	0.84	2.9
	Drymatter/seeding(gm)	0.6	0.67	0.46	0.96	0.64	4.4
	Mycorrhizal infection(%)	72	61.0	66.0	69	64.0	4.9
	Productivity of Mycorrhizai (fresh wt/gm)	75	82.0	88.2	181.6	100.1	3.1
B.luteus	Shoot/Root Ratio	0.44	0.51	0.58	0.54	0.50	3.8
	Root Collar Diameter	0.26	0.25	0.23	0.28	0.24	5.3
	Seeding Volume $D^2H(cm)^3$	1.00	0.86	0.81	1.74	1.11	3.0
	Dry matter/seeding (gm)	0.57	0.51	0.53	1.08	0.98	4.3
	Mycorrhizal infection (%)	76.0	69.0	74.0	85.0	82.0	2.99
	Productivity of Mycorrhizae (fresh wt/gm)	110.0	93.6	88.9	255.0	186.0	3.4

Table 5.2

Nutrient Concentration (NPK), percentage P translocation to the shoot and Phosphatase activity in pine seeding inoculated with different mycobionts under different organic amendments.

Parameters	Treatments														
	Control					*P.tinctorius*					*B.luteus*				
	Amendments					Amendments					Amendments				
	UA	PF	PD	GL	GP	UA	PF	PD	GL	GP	UA	PF	PD	GL	GP
Shoot Nitrogen(%)	2.44	2.98	2.77	1.91	1.94	2.74	2.61	2.55	2.81	2.71	2.02	2.18	2.47	2.88	2.82
Root Nitrogen(%)	2.20	1.99	1.88	0.94	0.96	1.15	1.21	1.19	1.28	1.29	1.01	1.19	1.30	1.30	1.29
Shoot Potassium(%)	2.03	1.87	1.85	1.89	1.74	2.42	2.40	2.44	2.13	1.21	2.28	2.5	2.6	2.71	2.68
Root Potassium(%)	0.94	0.88	0.79	0.82	0.85	0.96	0.92	0.89	0.98	0.96	1.01	1.11	1.16	1.20	1.18
Shoot Potassium(%)	0.32	0.3	0.29	0.31	0.32	0.34	0.29	0.31	0.30	0.33	0.36	0.32	0.34	0.39	0.38
Root Phosphorus(%)	0.22	0.27	0.25	0.23	0.21	0.26	0.21	0.28	0.23	0.32	0.36	0.31	0.42	0.47	0.41
Amount of P in shoot (mg)	115.2	83.7	87.0	113.77	80.0	125.8	107.7	93.0	222.0	132.0	129.5	115.2	137.36	288.6	231.8
Amount of P in Seeding (mg)	137.2	135.8	118.5	138.38	152.0	184.04	172.8	140.32	274.21	209.76	205.1	163.56	190.7	448.4	424.5
Percentage P translocation to the shoot = shoot P / total p X 100	83.96	61.63	73.41	82.22	64.63	68.3	62.32	66.27	80.95	62.9	63.1	70.4	70.02	64.36	54.66
Acid phosphatase (ug/g[illegible] wt/h)	369.0	356.0	343.0	387.0	366.0	892.0	888.0	819.0	898.0	868.0	833.0	874.0	889.0	902.0	896.0

UA - Unamended; PF Pine fresh; PD-Pine duff; GL - Grass litter; GP - Grass + Pine duff.

Table 5.3 Corrrelation coefficient (r) of mycorrhizal infection (%) and mycorrhizae productivity (fresh wt/gm) with various parameters within the amendments.

Parameters	Pisolithus tinctorius										Boletus luteus									
	UA		PF		PD		GL		GP		UA		PF		PD		GL		GP	
	MI	MP	MI	MP	MI	MP	MI	MP	MI	MP	MI	MP	MI	MP	M1	MP	MI	MP	MI	MP
1. Shoot length	NS	NS	NS	NS	NS	NS	0.88^{b}	0.89^{b}	0.81^{b}	NS	NS	NS	NS	NS	NS	NS	0.87^{b}	0.83^{b}	0.88^{b}	0.91^{b}
2. Root length	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
3. Shoot/Root ratio	NS	NS	NS	NS	NS	NS	0.88^{b}	0.84^{b}	0.78^{a}	0.81^{b}	NS	NS	NS	NS	NS	NS	0.92^{b}	0.94^{c}	0.86^{b}	0.93^{c}
4. Seedling volume	0.94^{c}	0.83^{a}	0.9^{b}	0.94^{c}	0.93^{c}	0.81^{a}	0.97^{c}	0.84^{a}	0.88^{b}	0.97^{c}	0.93^{c}	0.81^{b}	0.95^{c}	0.93^{c}	0.8^{a}	0.88^{c}	0.97^{c}	0.96^{c}	0.95^{c}	0.95
5. Biomass/seedling	0.85^{b}	0.91^{b}	0.86^{b}	0.84^{a}	0.92^{b}	0.91^{c}	0.82^{b}	0.8^{a}	0.86^{b}	0.83^{b}	0.83^{b}	0.91^{b}	0.89^{b}	NS	0.87^{b}	0.94^{c}	0.92^{c}	0.86^{b}	0.93^{c}	0.81^{b}
6. Shoot nitrogen	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
7. Root nitrogen	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
8. Shoot potassium	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
9. Root potassium	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
10.Shoot phosphorus	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
11.Root phosphorus	0.84^{b}	0.93^{c}	0.94^{c}	0.86^{b}	NS	0.94^{c}	0.88^{b}	0.91^{b}	0.85^{b}	0.94^{c}	0.91^{b}	0.87^{b}	NS	0.82^{b}	0.85^{b}	0.87^{b}	0.83^{b}	0.91^{b}	0.97^{c}	0.89^{b}
12.% P-translocation to shoot	NS	NS	NS	NS	0.92^{b}	0.88^{b}	0.92^{c}	0.89^{b}	0.95^{c}	0.84^{b}	0.92^{c}	0.86^{b}	0.93^{c}	0.92^{c}	NS	0.93^{c}	0.87^{b}	0.94^{c}	NS	NS
13.Root phosphatase activity	NS	0.82^{b}	0.78^{a}	0.84^{b}	NS	NS	0.72^{a}	0.8^{a}	0.84^{b}	0.92^{c}	NS	NS	0.91^{b}	0.94^{c}	0.89^{b}	0.91^{b}	0.84^{b}	0.79^{a}	0.91^{b}	0.92c
14.Mycorrhiza productivity	0.88^{b}	-	0.92^{c}	-	0.89^{b}	-	0.79^{a}	-	0.91^{b}	-	0.92^{c}	-	0.95^{c}	-	0.91^{b}	-	0.79^{a}	-	0.84^{b}	-

UA = unamended; PF = Pine fresh; PD = Pine duff; GL = Grass litter; GP = Grass + Pine duff

MI = Mycorrhizal infection; MP = Mycorrhizae productivity;

a = Significant at < 0.05 level.
b = Significant at < 0.01 level.
c = Significant at < 0.001 level.

Table 5.4

Analysis of Variance (F) of various amendments and treatments with various parameters.

Parameters	Between Amendments	Between Treatments
1. Shoot height	NS	9.16**
2. Root Length	NS	NS
3. Shoot/Root + Ratio	NS	NS
4.Seeding Volume	NS	4.36*
5.Drymatter/seeding	NS	NS
6.Shoot Nitrogen	NS	NS
7. Root Nirotrogen	NS	NS
8. Shoot Potassium	NS	5.60*
9. Root Potassium	NS	28.71**
10. Shoot Phosphhorus	NS	NS
11. Root Phosphorus	NS	5.99*
12. Percentage P translocation to shoot	NS	NS
13. Root Phosphatase activity	NS	3.89*
14. Mycorrhizal productivity	NS	NS
15. Mycorrhizal intution	NS	NS

NS - Not significant.

* = Significant at P = 0.05

** = Significant at p .0.01.

Figure 5.1: Shoot height and root length of pine seedlings inoculated with different mycobionts (Con = control; Pt = *P.tinctorius*; Bl = *B.lutues*) with various soil amendments UA = unamended; PF = Pine fresh; PD= Pine duff; GD = Grass litter; G+P = Grass + Pine duff)

FIG.-5.1

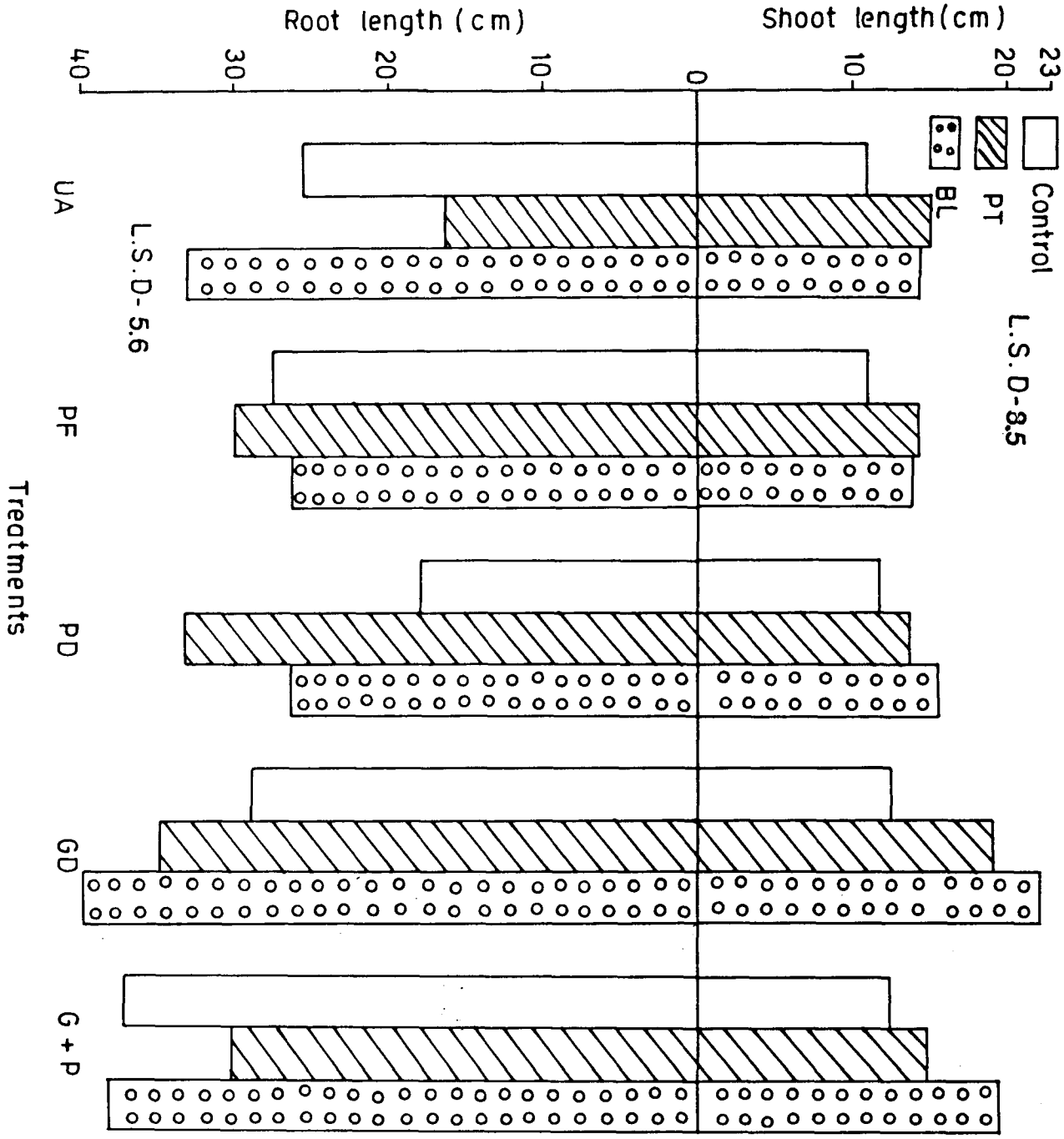

Root length (cm)
Shoot length (cm)
40
30
20
10
0
10
20
23
Control
PT
BL
L.S.D-8.5
L.S.D-5.6
UA
PF
PD
GD
G + P
Treatments

Figure 5.2: Biomass (g/seedling) inoculated with different mycobionts (Con = control; Pt = *P.tinctorius*; Bl = *B.lutues*) with various soil amendments UA = unamended; PF = Pine fresh; PD= Pine duff; GD = Grass litter; G+P = Grass + Pine duff)

FIG.-5.2

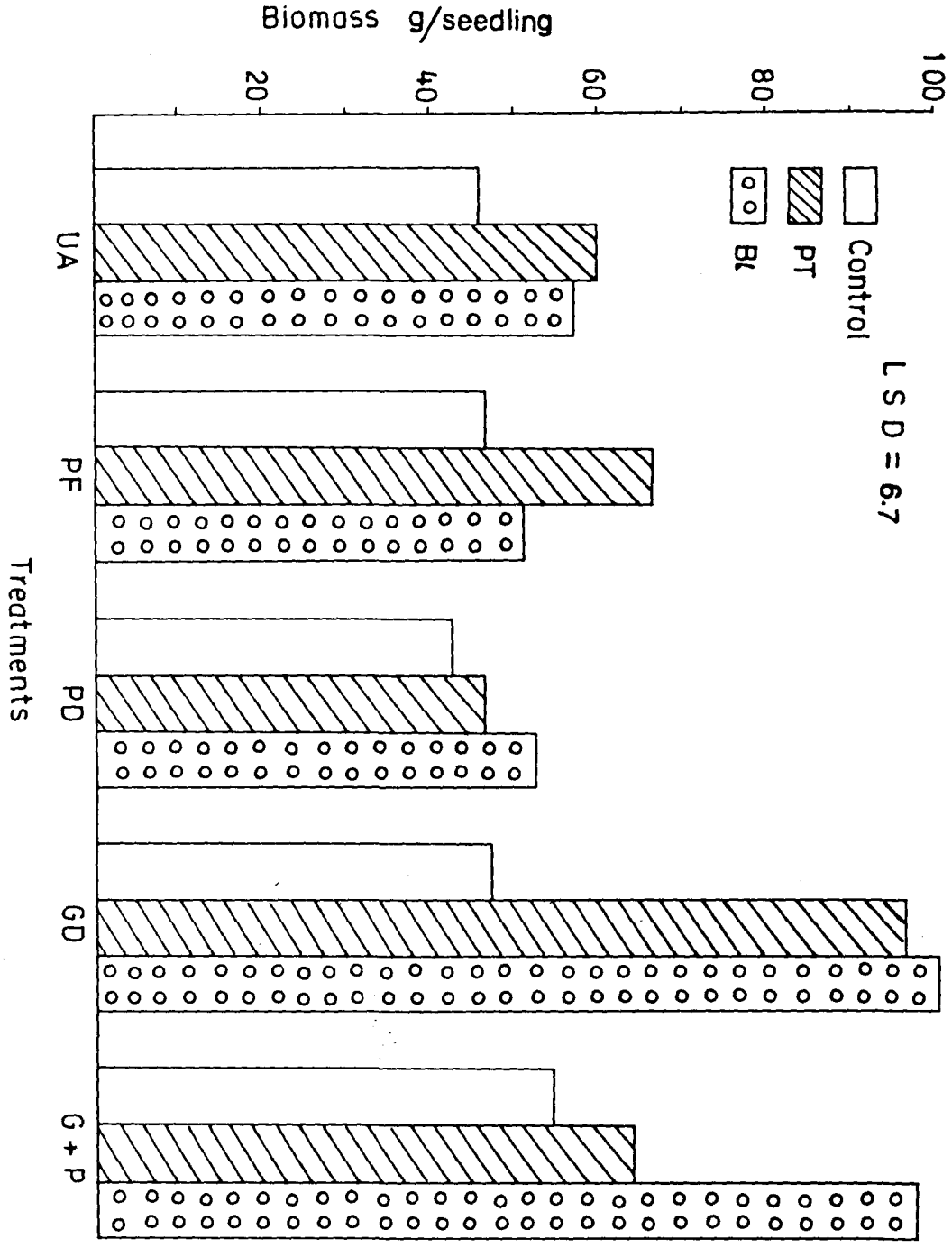
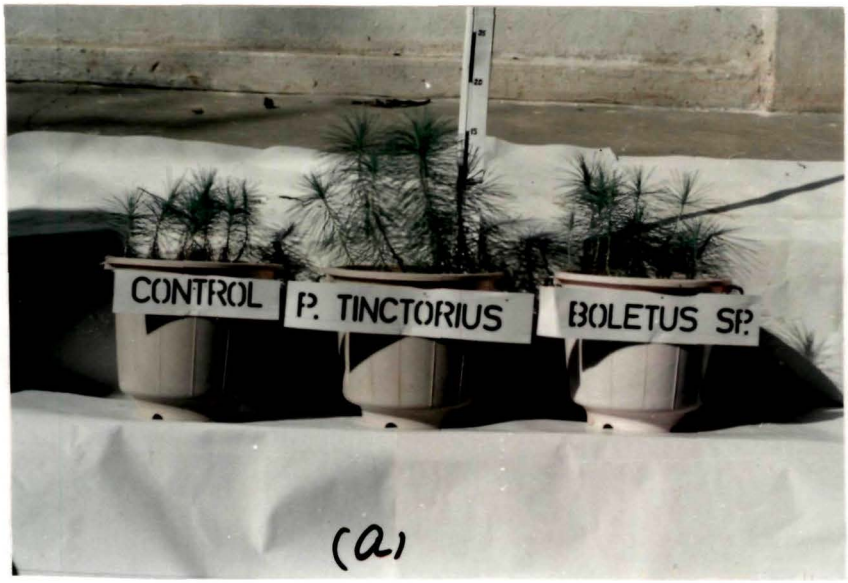

Biomass g/seedling
20
40
60
80
100
Control
PT
Bt
L S D = 6.7
UA
PF
PD
GD
G + P
Treatments

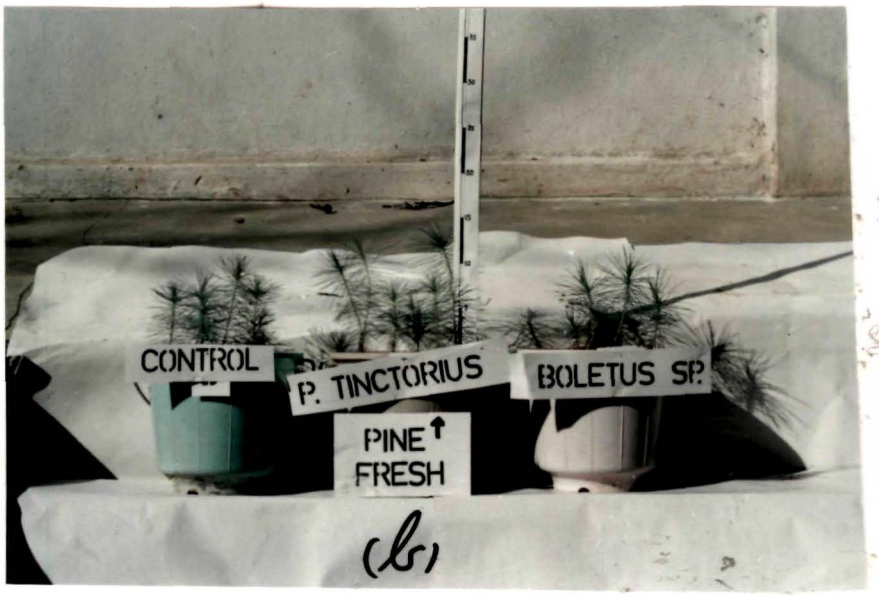
Plate 4: Effect of organic amendments on the growth of pine seedlings inoculated with different ectomycorrhizal fungi.

(a) Unamended soil,
(b) pine fresh amended,
(c) pine duff amended

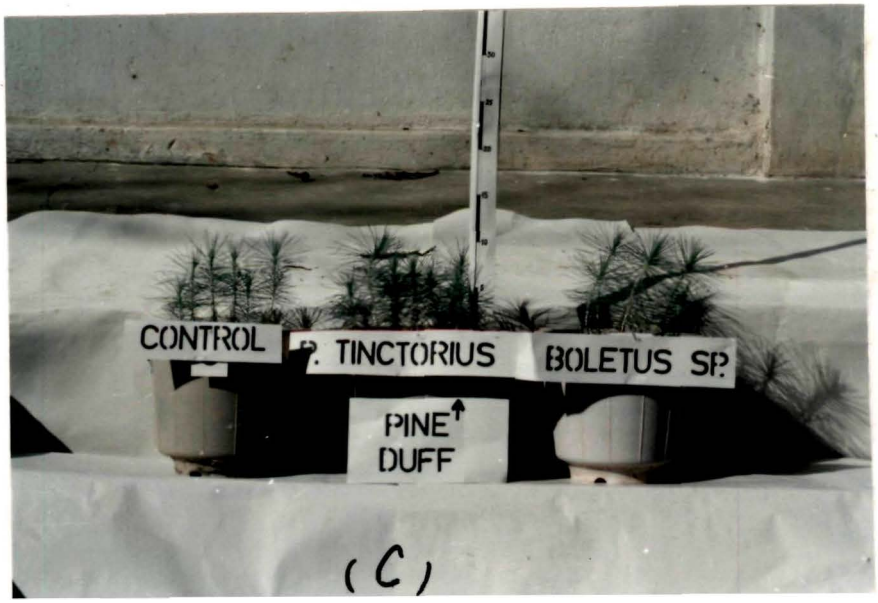
PLATE 4


CONTROL
P. TINCTORIUS
BOLETUS SP.

(a)


CONTROL
P. TINCTORIUS
BOLETUS SP.
PINE
FRESH

(b)

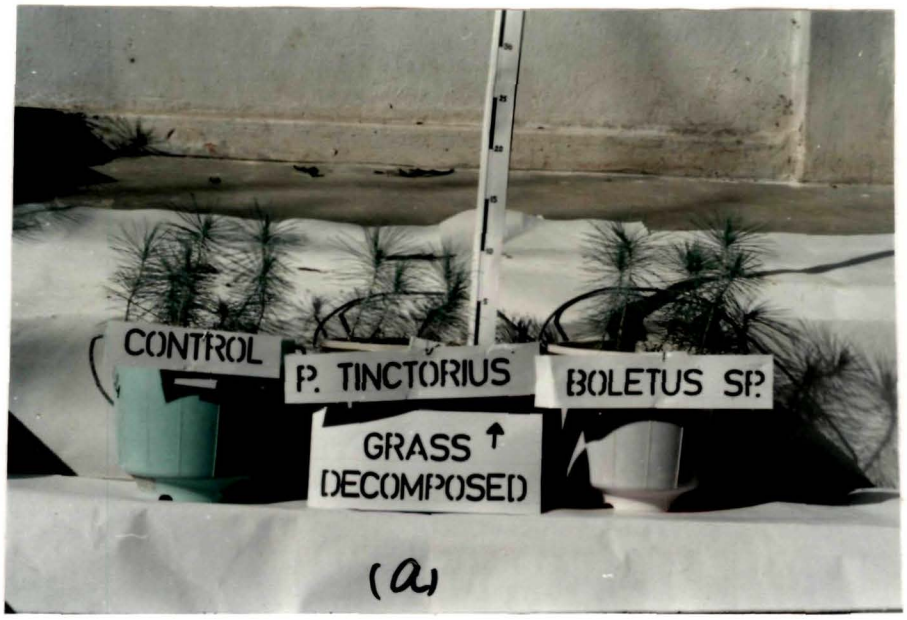

CONTROL
P. TINCTORIUS
BOLETUS SP.
PINE
DUFF

(c)

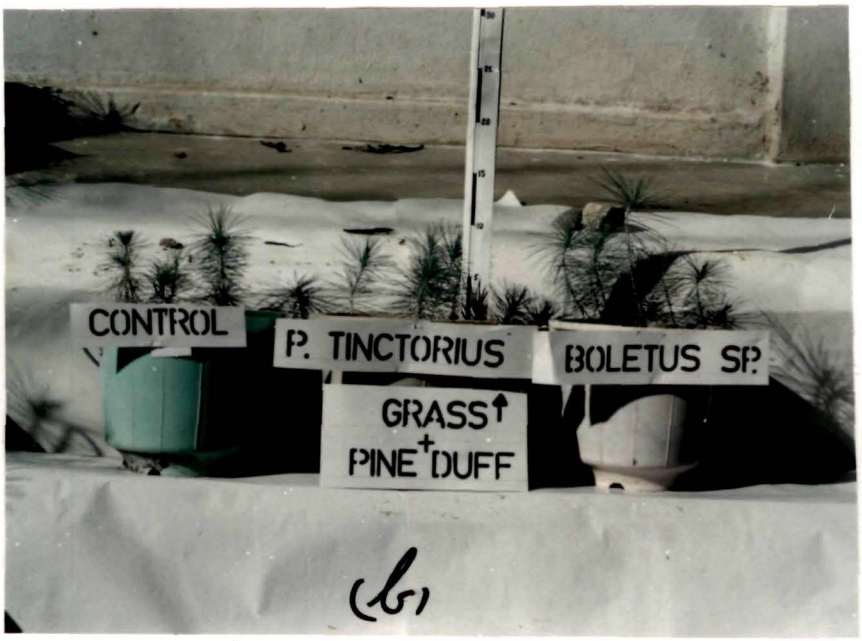
Plate 5: Effect of organic amendments on the growth of pine seedlings inoculated with different ectomycorrhizal fungi.

(a) Decomposed grass litter amended soil
(b) In grass + pine duff amended soil

PLATE 5


CONTROL
P. TINCTORIUS
BOLETUS SP?
GRASS ↑
DECOMPOSED

(a)


CONTROL
P. TINCTORIUS
BOLETUS SP?
GRASS↑
+
PINE DUFF

(b)

GENERAL DISCUSSION

Thirteen species of ectomycorrhizal fungi were recorded from the different age groups of pine stands. The low number of sporocarps during winter months was attributed to the low temperature and moisture of the soil (Sharma,1981b). Needle fall during winter months also influenced adversely the supply of nutrients to sheathing mycorrhizal fungi and also their growth (Hacskaylo,1965). The population of ectomycorrhizal fungi was positively correlated with the soil moisture, temperature, rainfall, *p*H, available phosphorus, total nitrogen and organic matter. Increased acidity in soil during winter months due to litter fall along with low soil moisture and temperature was mainly responsbile to affect the sporocarp production.

Relative abundance of sheathing mycorrhizal fungi was high in older pine stands during rainy season than young stands which was attributed to better soil moisture, optimum tempearture, organic carbon, N,P and K level in soil. Thick layer of pine litter in old plantation might also have helped in conserving the moisture and water evaporation from the surface of soil and thus maintaining nutrient supply to the mycorrhizal fungi from organic matter. (Mason and Last,1986). An optimum *p*H, moisture and nutrient equilibrium in soil were important regulatory factors in the production of fruiting

bodies of the ectotrophs. Species belonging to *Boletus luteus, Scleroderma aurantium, Tricholoma saponacum and Hygrophorus limacinus* were recognised as an early colonizing fungi in the young pine stands. However, in older plantations, species of *Boletus, Amanita, Russula and Cenococcum* were observed as late stage fungi (Last and Fleming, 1985). The sclerotia of *Cenococcum graniforme* were observed during late rainy season when the moisture content of soil was moderate and *p*H was slightly less in contrast to its adaptation to dried soils sites (Trappe,1962), indicating a variation.

Results have shown that *B.luteus* and *S.granulatus* had the characteristics of both early and late stage fungi which exhibited their broad ecological amplitude. Colonization of mycorrhizal fungi with pine roots was significantly correlated to soil moisture, *p*H, temperature, organic matter and available phosphorus which stimulated the colonization of fungi (Herman *et al*,1977).

Increased nitrogen in mycorrhizosphere and non-mycorrhizal region during the rainy season may be due to rapid microbial litter decomposition due to the temperature and moisture. Death of mycorrhizal roots and hyphal strands of mycorrhizae and other microbes due to low temperature and moisture in the winter months decreased the availability of nitrogen in both the regions (Gagnon *et al*,1988).

Phosphorus content in MR and NMR regions was significantly higher during rainy season, which was positively correlated with mycorrhizal colonization, indicating their increased solubilizing capability, diffusion surface area and volume of root in soil. Significant increase in phosphorus in MR than NMR region in all the plantations was due to the mycorrhizal symbiosis (Heap and Newman,1980; Marx *et al*,1985; Heinrich and Patrick,1986). Better utilizing efficiency of the fixed phosphate in adverse environmental conditions by the mycorrhizae has been assigned to their high amount of phosphatase activity. Amount of exchangeable potassium in both MR and NMR regions followed the similar trend like of phosphorus. The increased K uptake by mycorrhiza is mainly associated with the improved growth of the plants. (Maghembe and Redhead, 1984).

Quantitatively, the bacterial population was more numerous than the fungal population in MR and NMR regions in all the stands. Bacterial and fungal populations showed a similar trend of seasonal variation in MR and NMR regions in all the pine stands. Maximum bacterial and fungal population was observed during rainy season and minimum during winter months.Bacterial and fungal populations showed a significant positive correlation with organic matter, soil moisture, CO_2 evolution and enzyme activities in all the plantations. Higher amounts of organic carbon added to the mycorrhizal region by

sloughing off of root cells, fungal mantle exudates, secretions, mucilages and lysates appeared to be the probable cause of the increase in the population of bacteria and fungi.

Dehydrogenase activity was significantly more in the MR than in NMR regions in all the plantations reflecting the presence of higher population of microbes. The positive correlation between dehydrgenase activity and the bacterial and fungal population suggested that it was the resultant of higher number of microbes and organic carbon. Higher activity in the older plantations was related to the increase in microbes due to the quality and quantity of root exudates of the pine trees.

Enhanced urease activity in MR than in NMR region was correlated to the increased excretions of more nitrogenous nutrients by the mycorrhizal root, available for the microbes. (Skujins,1967).

Phosphatase activity was significantly enhanced by ectomycorrhizal fungi on the surface of mycorrhizal root and soil than without it and was directly corrleated with the colonization by mycorrhizae. The increased activity in mycorrhizal roots has also been related to the increase in root surface area. (Dodd *et al*,1987). The phosphatase activity was more in older stands than younger ones which was related

to the better colonization and development of mycorrhizae and more organic carbon in earlier case than in latter ones. The results of the present study indicated that to get a maximum phosphatase activity , a threshold level of available phosphorus in soil is necessary, contradicting the earlier reports that phosphatase activity was more in phosphorus deficient soil. (Bousquet *et al*,1986; Antibus *et al*,1992).

CO_2 evolution was higher in mycorrhizosphere than in non-mycorrhizosphere region which was related to the abundance of microbes and to the availability of organic substances to them. CO_2 evolution in MR and NMR regions was influenced by the age of pine trees.

Experimental results under greenhouse conditions have shown that the different mycorrhizal fungi vary in their intensity of mycorrhizal colonization and mycorrhizal productivity due to their varied growth pattern and nutrient requirements. The indigenous mycobiont *S.aurantium* Colonized better on the roots of Khasi pine colonized than the other mycobionts. Ability of ectomycorrhizal fungi to synthesize mycorrhizae may vary in different ecological conditions as was exhibited by exotic, efficient mycorrhizal fungi, *P.tinctorius* which otherwise has shown better performance than the native mycorrhizal fungi (Marx,1980). Increased production of mycorrhizae by the native *B.luteus* and exotic *L.laccata* was

due to their fast entry and growth in root tissue of pine and better efficency in utilizing nutrients than the others.

Phosphatase activity was significantly enhanced by ectomycorrhizal fungi on the mycorrhizal roots than in the rhizosphere. It was directly correlated with the colonization of mycorrhizae. Phosphatase activity of mycorrhizae has been related to the phosphorus demand by the host plants during nutrient mineralization (Meyselle *et al*,1991). Ability of mycorrhizal fungi to produce higher phosphatase than the decomposer ones helped them in mobilizing more organic phosphorus in rhizosphere region (Dighton,1983). Mycorrhizal fungus with its larger amount of phosphatase, released and hydrolyzed more phosphates. (Ho and Zak,1987).

Insignificant variation in seedlings growth was observed between inoculated and control treatments at an early stage of seedlings development in forest and degraded soils. Drain of photosynthates from the host to the fungus, may be responsbile for invisible enhanced growth by the different symbionts at the early stage of seedling growth. However, the enhanced growth of seedlings by mycobionts in forest and degraded soils at a later part of seedlings development was attributed to the increase in root absorbing surface area due to fungal mycelium (Langlois, 1983).

The growth of 180 days old pine seedlings was

significantly more in forest soil than in degraded soil and was attributed to the high amount of soil organic matter, which favoured better formation of mycorrhizae (Rubtov,1964). Better colonization of pine roots by *B.luteus* in degraded soil could be related to its affinity with low organic matter, moisture and aeration levels which favoured the development of mycorrhizae (Kumar,1990), compare to the forest soil where high nutrient concentration in the substrate restricted mycorrhizal infection (Alexander and Fairley,1983). Improvement of growth and nutrient contents in seedlings with *L.laccata* than *C.graniforme* in forest soils is contradictory to the findings of Hung (1983) who found *C.graniforme* better than *L.laccata*. The difference betwen the two may be assigned to the host genotype and fertility status of the soils.

The difference between the responses of different mycorrhizal fungi in forest and degraded soil is not only due to their edaphic requirements but also their ability to stimulate growth of pine seedlings. Growth habit of mycorrhizal fungus in soil influenced its nutrient uptake. The differences between mycorrhizal fungi in nourishment of host were related to overall growth of the host and nutrient contents. Improved growth response of seedling by *B.luteus* in degraded soil was due to its better relative efficiency as a native and naturally occuring species than the exotic ones. Variation in seedlings growth mycorrhizal infection,

mycorrhizae productivity due to harvesting time was related to the age of the seedlings and its effect on the host-mycobiont relationship.

A significant positive correlation between mycorrhizal colonization and seedling volume, biomass, root phosphorus,P-translocation, phosphatase activity, nutrient contents of seedlings and mycorrhizae productivity with various amendments reflected the more absorpiton of during their nutrient release from litter decompostion. Heterogenicity of microorganisms during organic litter decay made the system more dynamic (Prescott *et al*,1993). The results have showed that colonization of mycorrhizae and its productivity was better in the seedlings inoculated with the indigenous mycobiont *B.luteus* than the exotic *P. tinctorius*. Amendment with grass litter to both the mycobiont inoculated seedlings showed a better mycorrhizal infection and overall better performance than the seedlings amended with pine litter and in unamended soil than the pine littter indicating the presence of stimulatory compounds in earlier one and insoluble and toxic substances in the later ones (Berg and McClaugherty,1989). Release of nitrogen and phosphorus differed between fresh and duff coniferous foliage litters. Among the two litters, more mobilization of nutrients, growth response and mycorrhizal colonization with grass litter may be compared to the poor or absence of leaching phase during decompostion in conifer

needles (Berg,1988). Improvement of growth and accumulation of dry matter in pine seedlings due to mycorrhizal fungi than the uninoculated ones were correlated to the enhanced nutreint uptake by the earlier than the latter ones (Kropp and Langlois,1990). Poor growth of seedlings with fresh pine litter was due to the inhibitory phenols and organic acids which suppressed the growth of mycobionts. Presence of more phosphorus in the mycorrhizal seedlings than the non-mycorrhizal ones was supported by Stribley *et al* (1980). Higher nutrient uptake by mycorrhizal plants was due to improved hyphal growth, better exploitation of the soil volume and the competitive ability of *B.luteus* over the *P.tinctorius*. Seedlings amended with grass litter and inoculated with *B.luteus* exhibited the maximum growth and was supported by Schisler and Linderman (1989). The humic substances released from grass litter were in turn responsbile for the increased microbial growth and activity. Higher phosphate uptake by seedlings was correlated to higher rate of phosphatase activity in mycorrhizal than non-mycorrhizal ones.

The results have revealed that mycorrhizae are important for the growth and survival of khasi pine. The mycorrhizal colonization and productivity is related to the various physico-chemical characteristics of the soil, climatic conditions, age of the host and specificity of mycobiont. The better performance of indegenous mycorrhizal fungus *B.luteus*

to improve the growth of pine seedlings may be exploited in regenerating the degraded and phosphorus poor soils of the khasi hills of Meghalaya.

SUMMARY

Seasonal study on ectomycorrhizae and mycorrhizal fungi of khasi pine (*Pinus kesiya* Royle ex. Gordon) in 2, 4, 11 and 17 years old pine plantations was carried out in West Khasi Hills of Meghalaya.

Thirteen mycorrhizal fungi forming ectomycorrhiza with khasi pine were observed. Diversity of index of mycorrhizal fungi was directly proportional to the age of the pine stand. Maximum number of fungal species was observed in oldest stand. Evenness of the sheathing mycorrhizal fungi was also increased with the increase in age of pine.

The sporocarps of *Boletus luteus, Scleroderma aurantium, Tricholoma saponaceum* and *Hygrophorus limacinus* were observed as an early colonizing fungi with P. kesiya. However, in older platations *Russula lepida* and *Amanita phalloides* were observed as late stage fungi. *B.luteus* and *S. aurantium* were dominant species in all the pine stands. Sporocarps of mycorrhizal fungi were maximum during rainy season and minimum during winter months.

A positive correlation was observed between the colonization of myocrrhizae and population of mycorrhizae with soil moisture, soil pH, total nitrogen, available phoshphorus, exchangeable potassium and organic matter of soil.

Colonization and population of mycorrhizae showed a uniform pattern of seasonality in all the pine plantations. Maximum colonization and population of mycorrhizae was observed during rainy (Aug-Oct) season and minimum during winter (Feb) season. Positive correlation was observed between colonization of mycorrhizae and population of ectomycorrhizae in all the pine plantations.

Mycorrhizospheric region had more of N,P,K and organic matter than non-mycorrhizospheric ones.

Microbial enzyme activities (dehydrogenase, urease and acid phosphatase) were higher during rainy season than in winter. All the enzyme activities were positively correlated with population of bacteria and fungi. Microbial enzyme activity and microbial population of bacteria and fungi was higher in mycorhizosphere (MR) than in non-mycorrhizospheric region.Population of bacterial, and fungi, and CO_2 evolution were maximum during rainy season and minimum during winter months. A positive correlation was observed between CO_2 evolution and microbial population, dehydrogenase activity and CO_2 evolution, phosphatase activity and microbial population.

A significant variation was observed in number of microbes and enzyme activities within different age plantations, sampling periods and mycorrhizospheric and non-mycorrhizospheric regions.

Different mycorrhizal fungi varied in their colonizing ability of roots of pine and production of ectomycorrhizae. Ectomycorrhizal productivity was increased with inoculation of various mycobionts. Marginal increase productivity of mycorrhizae was observed after 45 days in all treatments. Maximum productivity was observed in *Laccaria laccata* inoculated seedlings followed by *Boletus luteus* inoculated ones. Minimum productivity was observed in the seedlings with *P.tinctorius* inoculation.

Root surface phosphatase activity was always higher than in soil. Root activity was maximum in *L.laccata* inoculated seedlings, which was 11 times more than in the soil. Minimum activity was observed with *P.tinctorius* inoculation. A significant positive correlation was observed between the root surface phophatase activity and mycorrhizal productivity and mycorrhizal infection.

Insignificant variation in seedling and biomass and mycorrhizal infection was observed within the various mycobiont inoculated pine seedlings. After 180 days, colonization of mycorrhiza was maximum in *S.aurantium* (85%) inoculated seedlings in forest soil and in *B.luteus* inoculated ones in degraded soil. Productivity of mycorrhizae was maximum with *L.laccata* and minimum with *P.tinctorius* inoculated seedlings grown on forest soil.

Whereas, in degraded soil, maximum and minimum productivity was observed in *B.luteus* and *C.graniforme* inoculated seedlings respectively. All the mycorrhizal symbionts exhibited a promotory effect on the growth of pine seedlings in forest and degraded soils. Significantly highest stimulation in growth of seedlings was observed by *L.laccata* .It followed a definite trend of growth stimution by *S.aurantium* > *C.radiata* > *B.luteus* > *P.tinctorius* > *C.graniforme* in forest soil. Whereas in degraded soil the order was *B.luteus* > *L.laccata* > *S.aurantium* > *C.radiata* > *P.tinctorius* > *C.graniforme*. Seedling growth was 3 times more in forest soil than in degraded soil in all the mycobiont treatments. Maximum productivity of mycorrhizae was observed in degraded soil inoculated with *B.luteus* than in forest soil with *L.laccata* . Similarly mycorrhizal infection was highest in degraded soil (85%)in seedling inoculated with *B.luteus*. Shoot height, root length, seedling volume and biomass and nutrients contents in shoot and roots of seedlings grown in forest soil with various mycobionts showed comparatively better improvement than those grown in degraded soil.

Colonization of mycorrhizae of pine seedlings was maximum by *B.luteus* fungus on grass litter amended soil (85%) and minimum by *P.tinctorius* on pine fresh amended soil (61%).It showed a significant positive correlation with

seedling volume, biomass, root phosphorus, P transolocation, phosphatase activity and mycorrhizal productivity with the various litter amendments. Productivity of mycorrhizae was maximum in *B.luteus* inoculated with grass-litter amended soil and minimum in those inoculated seedlings with *P.tinctorius*, grown on unamended soil .Productivity of mycorrhiza showed a significant positive correlation with seedling volume, root phosphorus, P-translocation, biomass and phosphatase activity in all the treatments except with biomass in pine fresh amended soil and phosphatase activity in unamended soil inoculated with *B.luteus*.

Maximum phosphatase activity of root was observed in grass litter amended soil having *B.luteus* inoculated seedlings.

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* Original not seen.