

ECOLOGICAL STUDIES ON MYCORRHIZAE OF PINE (PINUS KESIYA ROYLE EX. GORDON)

ABSTRACT

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**SUBMITTED IN FULFILMENT OF THE REQUIREMENT OF
THE DEGREE OF**

DOCTOR OF PHILOSOPHY

To



**NORTH-EASTERN HILL UNIVERSITY
SHILLONG, INDIA**

JANUARY, 1981

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Pinus Kesiya Royle ex Gordon is an early colonizer among the tree species at higher altitude (1100 - 1600 M) of Meghalaya. Forests are rapidly vanishing due to shifting agricultural practice prevailed in north-eastern India. Long and continuous harshment of site, in slash and burnt agricultural practice may initiate the colonization of nonmycorrhizal grasses and other plant species, which may inhibit the germination and propagation of mycorrhizal fungi and tree species. Furthermore, it may thus slow down the process of succession and may alter the pattern of species composition (Reeves et al., 1979, Miller, 1979). North-eastern India receives maximum precipitation (Mawmai - Cherrapunji) in world but it is provoked towards desertification due to less mycorrhizal symbionts population (Mishra et al. unpublished). On the other hand, fast growing demand for energy and timber due to increasing human population pressure, provided an opportunity to work on this topic to serve two broad ideas. I - Analysis of fungal symbiont (Mycorrhizal fungi) with pine tree, which may be used in afforestation, reclamation and silvicultural programmes on waste land. II - To improve the growth of tree, wood quality and protect them from fungal pathogen by exploiting certain effective mycorrhizal fungi. Thirtythree mycorrhizal fungi were observed with pine (Pinus Kesiya Royle) in nature. Among basidiomycetes, agaricales were maximum in number to form mycorrhizal association. Age of pine tree influenced the

population of sporocarp of fungal symbionts. Middle rainy season i.e. June to September was most conducive for the development of sporophores of mycorrhizal fungi. No sporocarp was observed during winter months. Boletus, Clitocybe, Collybia, Cortinarius and Tricholoma were first to produce their fruiting bodies. Boletus edulis, B.luteus, Lactarius deliciosus, L. Sp, Russula delica and Scleroderma geaster were dominant mycorrhizal fungi. Different kind of mycorrhizae were observed in nature but bifurcate to coralloid forms occurred frequently. Mycorrhizal fungi were found to influence significantly the bacterial, actinomycetes and fungal population in rhizosphere of pine. Significant variation in higher fungal species in pines of different age groups was also obtained.

Pure culture studies on agar with pine and mycorrhizal fungi confirmed their symbiotic relationship. Different mycorrhizal fungi induced different kind of mycorrhiza, however, bifurcate type was most common. Variation in time for mycorrhizae establishment with different forms was also observed. Intensity of mycorrhizae varied from species to species. Variation in mantle, Hartig net and colour of mycelium was also recorded.

Studies on mycorrhizae in pot culture exhibited that mycosymbionts reduce mortality and induce robust growth

in pine seedlings. Efficiency of mycorrhizae development varied from symbiont to symbiont. Mycorrhizal inoculation also stimulated stem growth, root length, leaf production and leaf size. Uninoculated pine seedlings grew weak and exhibited the yellowish leaves and symptoms of stunting. Photosynthetic pigment (Chlorophyll) was greater in inoculated seedlings than uninoculated ones. Nutrient status analysis revealed that uptake of almost all the cations and anions is enhanced in mycorrhizal seedlings than non-mycorrhizal ones. Phosphorus uptake was found four times greater in inoculated seedlings than uninoculated ones. Bifurcate to coralloid types of mycorrhizae were found dominant. Chromatographical and Colorimetric studies on sugar and amino-acid flux of roots of mycorrhizal inoculated and uninoculated ^{seedlings} indicated that mycorrhizal roots have different kind of sugars and aminoacids and also possessed more sugars and aminoacids than nonmycorrhizal root system. Allocation of higher biomass in above ground and below ground parts was observed in seedlings inoculated with mycorrhizal fungi. Significant variation in dry matter production and mycorrhizae development was observed.

Nintynine fungal species were isolated from nonrhizosphere (NR), rhizosphere (RS) and rhizoplane (RP) regions of mycorrhizal roots of pine. Highest number of fungal species were recorded in rhizosphere region. Asidia,

Mucor, Trichoderma, Penicillium, Cladosporium and Fusarium dominated nonrhizosphere while in rhizosphere region Trichoderma, Penicillium and Cladosporium were dominant. Rhizoplane was surrounded by basidiomycetes throughout the study. Trichoderma and Penicillium were also dominantly associated in this region. Highest microbial population was observed during rainy season in all the three regions of mycorrhizal roots. Edaphic and climatic condition significantly influenced the microbial population. Nitrogen, Phosphorus and Potassium content varied seasonally in mycorrhizal roots. Sugars and aminoacids status was also different in different months of mycorrhizal roots. Nutrient status of mycorrhizal roots influenced significantly the microbial population.

Present study emphasised that a variety of mycorrhizal forms are associated with pine which increase absorptive surface area of roots and enhance nutrient uptake and survivality of pines. Mycorrhizae also influence the microbial complex in rhizosphere region.

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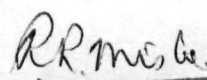
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I certify that the thesis entitled "ECOLOGICAL STUDIES ON MYCORRHIZAE OF PINE (Pinus kesiya Royle Ex. Gordon) submitted by Shri Gauri Dutt Sharma, M.Sc. for the degree of Doctor of Philosophy of the North-Eastern Hill University, Shillong embodies 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.

Dated : January 28, 1981
Place : Shillong


Signature of the
Supervisor.

ACKNOWLEDGEMENTS

I express my deep sense of gratitude to my teacher Professor R.R. Mishra, Head, Botany Department and Dean, School of Life Sciences, NEHU, Shillong for providing an opportunity to work on such an interesting problem. His constant guidance, encouragement and valuable suggestions throughout the course of the investigation are sincerely acknowledged. My heart felt thanks are also extended to him for his keen interest in giving the training in other aspects of microbiology.

I am sincerely grateful to Dr. R.S. Tripathi, Reader in Botany for his helpful discussion and healthy comments from time to time during the course of study. My thanks go to Dr. Donald H. Marx, Director, Mycorrhizal Res. USDA, Georgia, Dr. E. HacsKaylo, U.S.A., Dr. J.M. Trappe, U.S.A., Dr. I.R. Hall, New Zealand, Dr. D.S. Hayman, England and Dr. G.D. Bowen, Australia for answering my various queries. I would also like to thank the Directors of Commonwealth Mycological Institute and Royal Botanical Garden, Kew, England for confirmation of identity of fungi.

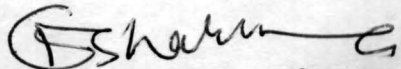
Thanks go to Shri B.K. Tiwari, for useful discussions and kindly reading the manuscript. Thanks

are also due to Shri H.K. Deka, Shri P.P. Kharbuli, Shri N.K. Verma, Shri A.R. Gathphoh, Mrs Indira Bhattarai, Shri P. Chakravarty, Miss M. Baruah and Miss M.S. Dkhar research scholars of microbial ecology laboratory for group discussion and to all other research scholars for their help in various ways.

Thanks are extended to Mrs Sisley, Mr. B.K. Das, Mr. M.H. Burbhuya and Mr. N.P Goel of this University for their help in typing, photography, drawing and statistical analyses of data.

I also wish to acknowledge the generous cooperation of my colleagues of Botany and Zoology Departments and authorities of the University for providing all kind of help and facilities during the course of investigation. I extend my thanks to my revered parents who have been a constant source of encouragement during the course of present study.

Last but not the least I acknowledge the cooperation rendered by my wife, who permitted me to abstain from many household duties during the course of the present investigation.


G. D. Sharma 20.1.81

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I N T R O D U C T I O N

The term mycorrhiza was first coined by Frank (1885) to denote the association between fungus and root (mykes; fungus; rhiza: root). Mycorrhiza based on its morphological characters has been delineated in two major groups i.e. ectomycorrhiza (where fungus does not enter inside the cortical cells and lies intercellularly and may form mantle and Hartig net) and endomycorrhiza (where fungus enters inside the cortical cells and grows both within and between the cortical cells, but does not form any fungal sheath or Hartig net). A third group: ectendomycorrhiza is also recognised (where fungus forms Hartig net and penetrates inside the cortical cells). Many beneficial fungi form association with roots of higher plants. It has now been well established that such association is more or less a rule and not the exception. In certain cases, however, like *Centrop^sermae* (Cactaceae exception), Plumbaginales, Cruciferae, Helobiae, Farinosae, Cyperales, Droseraceae, Denotheraceae and Helorrhagaceae, mycorrhizae generally are not reported. Certain nonmycorrhizal plants on the other hand when transferred from marshy to dry habitat develop mycorrhizae.

Most of the tree species particularly the members of the families : Pinaceae, Fag^aceae, Batulaceae, Silicaceae and Tiliaceae possess ectomycorrhizal association. Ectomycorrhizae are generally formed by higher basidio-

mycetes but the members of the ascomycetes, deuteromycetes and some zygosporic species of phycomycetes have also been reported to form this type of association (Gerde mann, 1974; Trappe, 1962, 1977).

Endomycorrhiza is mainly formed by two types of fungi i.e. those with septate and nonseptate hyphae. Fungi of the former group are generally associated with the roots of orchids and the members of Ericales group. Among endomycorrhiza, vesicular arbuscular mycorrhiza (VAM) is widely distributed and is formed by phycomycetous fungi belonging to the family Endogonaceae. Vesicles function as a storage or reproductive organs while the arbuscules serve primarily as an organ for nutrient exchange between plant root and the fungus. Arbuscules have very short life and may collapse within few hours after discharging the nutrients. The hypha may re-enter in the same cell and may again form the arbuscules. Versicular-arbuscular mycorrhiza does not induce morphological changes in the roots of host plant ✓ and they play very little role in protection of roots from pathogens.

Ectomycorrhiza helps in the establishment and survival of forest tree species through absorption of inorganic nutrients, production and supply of growth regulators, deterrent to root pathogens, decreasing soil toxicity

and increasing resistance to extreme soil temperatures. In absence of mycorrhiza, tree species become stunted and yellow or they may even die. Mycorrhizal fungi generally are not host specific and may have wide range of host spectrum. A tree species may harbor more than one fungal symbiont at a given time. The mycorrhizal fungi depend on host plant for supply of simple carbohydrates, a photosynthate of green plants and cannot compete with saprophytic microorganisms in utilizing energy from litter decomposition. Ectomycorrhize modify the morphology of roots and this change is correlated with the production of cytokinins and other growth regulators which increase the physiological process of the roots.

Mycorrhizal association develops when either a spore or mycelium of mycorrhizal fungi encounters the roots of planted seedlings. The spore germinates and mycelium surrounds the roots and enters inside it. If the process is delayed the seedlings either die or even if survive becomes stunted. To overcome this practical problem in forestry, inoculation of the seedlings with suitable mycorrhizal fungi before or at the time of planting is recommended. Supply of fertilizer and water does not help the recovery of the stunted seedlings until the suitable mycorrhizal fungi are inoculated. This phenomenon becomes more clear when an exotic tree species fails to flourish if planted in absence of the mycorrhizal fungi.

Though studies on ectomycorrhizae have received enough attention during the past few years, yet the nature of mycorrhizal formation is not properly understood. The factors affecting the host and the fungus and their interaction in relation to environmental and edaphic factors need careful investigation.

It has been found that pine seedlings grow better in presence of Boletus felleus and Hebeloma meso-phaeum (Rosendahl, 1943). However, their efficiency at species level for nutrient uptake and plant growth may vary in different environmental conditions. Some of them may tolerate a wide range of environmental conditions while few others may not.

A large quantum of information is available on phosphorus uptake by mycorrhizal fungi but the knowledge on uptake of other nutrients is fragmentary. Some indications, however, exist about the uptake of sulphur (Rhodes and Gardemann, 1978^{ab}), nitrogen, (Melin, 1952; Richard, 1964), Calcium, magnesium, iron and other elements.

After the introduction of term rhizosphere by Hiltner (1904) it is now well established that maximum biological activity lies in root region than in soil away from root. Mycorrhizal and rhizospheric microbes play an

indispensable role in nutrient cycling, converting nutrients from unavailable to available form, releasing some enzymes and chemicals which regulate nutrient absorption by root and maintaining a balance between pathogenic and nonpathogenic population of soil ecosystem. Some of the rhizospheric microbes may be selective in their effect on the growth of mycorrhizal fungi and their metabolic products regulate the association of a particular fungus with host plant(Nye,1968). In mycorrhizal roots the nature of the exudates which regulate the root microflora may be different. [Root exudates generally have the selective effects on the root microflora and this nature of selectivity of ectomycorrhiza is not understood properly. The studies on the interaction of rhizospheric microflora and mycorrhizal fungi may provide better information regarding the understanding of synergism, satelism and antagonism processes in a forested ecosystem. The study will provide clues regarding the nature of diversity and ecotypic variation on effectiveness of certain ectomycorrhizal fungi and will offer the immense opportunities for management of forests(Marks and Kozlowski, 1973).

It is obvious that rhizosphere region exerts a preferential effect on symbiotic or parasitic associations. A detailed investigation of the microbiology of the root region in this light is therefore, warranted.

Soil temperature, pH and nutrient status have great impact on the proper functioning of the mycorrhiza. Laboratory studies on the effect of these factors are available. Not much is, however, known about the field conditions where numerous other factors also operate simultaneously.

The present work was undertaken to understand the ecology of mycorrhizal association of pine (Pinus kesiya Royle) and also its effect on rhizosphere population and nutrient cycling in a subtropical pine forest ecosystem. Mycorrhizal fungi and rhizosphere microbial complexes were studied at regular monthly interval for three years. Pure cultures of fungal symbionts were obtained by using different techniques. To confirm their symbiotic relationship with pine, axenic mycorrhizal synthesis in agar was also conducted. Their role in nutrient uptake was assessed in pot culture studies.

HISTORICAL

Frank (1885) coined the term mycorrhiza for a fungus-root organ and later on in 1887 he distinguished two types of mycorrhiza i.e. ectotrophic and endotrophic, according to the presence of fungal hyphae outside (inter) or inside (intra) the cortical cells of root. He assumed that fungal association is purely a symbiotic one. Masui (1927) opined that mycorrhiza is a form of parasite and he compared the hyphal invasion with the damping off of seedlings inoculated with the fungus Tricholoma matsutake. Melin and Nilson (1950) using radioactive techniques confirmed the uptake of phosphorus by the mycorrhizal hyphae of pine. Melin (1952) demonstrated that mycorrhizal fungi do not fix atmospheric nitrogen but transport the ammonium nitrogen to the host.

Pontana (1962) in a survey of mycorrhizal association noticed that 14 tree species from plain, hill and mountains of Italy possessed ectotrophic mycorrhiza. In some of the localities, however, he also observed a phycomycetous type of mycorrhizae along with ectotrophic mycorrhizae.

Richard and Wilson (1963) noticed the changes during mycorrhizal development in Pinus caribaea, after addition of nutrients, but they did not observe any direct correlation between concentration of carbohydrate and mycorrhizal development. They also suggested that

carbon/nitrogen status should be taken into consideration while relating the mycorrhizal development in plant.

Miller (1967) isolated and identified three different kinds of cytokinins produced by Rhizopogon reseolus an ectomycorrhizal fungus and suggested that the cytokinins may be responsible for the change in morphology of ectomycorrhizal root.

Theodorou (1968) reported phytase acticity in mycorrhizal fungi and found that they effect sodium and calcium phytate but not ferric phytate.

Theodorou and Bowen (1969) studied the effect of pH and nitrate on the mycorrhizal development in Pinus radiata. They observed that at pH 6.2 growth of pine was about 60-80% higher than at pH 8 and mycorrhizal development was 60% lower at pH 8 than at pH 6.2. At pH 5, the nitrate (575 ppm) had no effect on the growth of pine while at pH 8 it affected the growth adversely.

Theodorou (1971) inoculated seeds of Pinus radiata with spores of Rhizopogon luteolus. 48% and 36% enhancement was noted in height of fumigated and unfumigated soils respectively. In fumigated soil rhizomorph population was higher. He (1978) further observed that different mycorrhizal fungi respond to different soil water potential. The maximum mycorrhizal infection was at 13% - 20% and it

decreased at 27%.

Theodorou and Bowen (1971) found that non-host plants have inhibitory effect on mycorrhizal fungi of Pinus radiata either by producing phytotoxins or by lowering the nutrient status.

Daft and Nicolson (1969a) observed that the effect of endogone on growth of plants depends upon the inoculum density of the former. They (1969b) further reported that Vesicular arbuscular mycorrhiza and phosphate nutrition are interrelated processes. They observed that endophytase activity was depressed when soluble PO_4 was applied over increasing period or when it is applied at an early phase of growth of host plant. According to them mycorrhizal fungi are more efficient in extracting phosphorus from podosphere and are very important in cycling of the key metabolic element like nitrogen.

Daft and Okusanya (1973) observed that mycorrhizal infection increased the amount of vascular tissue in tomato, Petunia and maize plants. Besides this it also stimulated flower production in Petunia, formation of fruits in strawberry and development of pollens in maize. The stimulation of ontogeny and senescence in tomato leaves may be due to production of some hormones.

Daft and HacsKaylo (1977) inoculated the red maple

in sand and anthrocite waste with Glomus macrocarpus var-geosporus or Gigaspora gigantea and found that mycorrhizal plants had greater phosphate and nitrate uptake than nonmycorrhizal ones. They correlated this nutrient status to the leaf size, stem anatomy and chemical composition of plant tissues.

Marx (1969) studied the antagonism of ectotrophic mycorrhizal fungi to Phytophthora cinnamomi and other root pathogens. He found that the growth of fortyeight root pathogens was nearly half with Laccaria laccata, Lactarius deliciosus, Leucopaxillus cerealis var-piceina, Pisolithus tinctorius and Suillus luteus. Maximum antibiotic activity was observed at rapid growth phase in liquid culture. Age of culture, incubation and temperature strongly influenced the production of inhibitory substances. He identified antibacterial substances produced by Leucopaxillus cerealis var-piceina, as diatretyne nitrite. He observed 20% inhibition in Phytophthora cinnamomi by 50-70ppb and suggested that it also has fungistatic property. Higher concentration of this antibiotic compound was found lethal to P. cinnamomi and bacterial growth was inhibited by 12ppm. He also remarked that this antibiotic does not have any effect on pine germination in aseptic conditions upto a concentration of 40ppm. In aseptic conditions 10ppm concentration, however, has phytotoxic effect on the growth of seedlings.

Marx and Bryan (1969) reported that Scleroderma bovista, an ectomycorrhizal fungus produces antibiotics which are very effective against several pythiaceus fungi. Besides its function as biological deterrent it also increases the absorbing capacity of pecan.

Marx (1970) reported that ectomycorrhizae of short leaf pine are resistant to infection by pathogenic fungi. He also stated that fungal mantle functions as mechanical barrier and Hartig net checks the spreading of pathogenic fungi. He did not observe the production of antibiotic by an ectomycorrhizal fungus-Psilothus tinctorius and thus biological control in this system is mainly attributed to mechanical barrier only.

Marx and Bryan (1971) indicated that genetic constitution of Pinus elliotti may regulate the ectomycorrhizal development and benefit may vary from one progeny to other.

Marx et al (1971) reported that endomycorrhizae (Endogone mossea) increases the growth of rough lemon (Citrus lemon) but not of the sour orange (C. aurantium).

Marx (1972) concluded that ectomycorrhizae help in biological control of root pathogens by their complex defence mechanism. Ectomycorrhizae depending upon the conditions may act as physical, mechanical and chemical

barrier to the root pathogens. He (1973) further suggested that mycorrhizae could be better exploited in root disease control than physiological processes and their ecological significance in biological control should be given proper attention.

In another study, Marx (1975) found that tree species having ectomycorrhizae with Pisolithus tinctorius could survive and grow better than nonmycorrhizal plants. He advised that on a strip mined lands the ectomycorrhizal fungi could be exploited potentially for reclamations.

Marx (1975) further reported that all exotic species of pine possess ectomycorrhizae, while Cupressus macrocarpa has vesicular-arbuscular mycorrhiza. The mycorrhizal pines grew better than nonmycorrhizal. The main fungal symbionts were Pisolithus tinctorius, Thelaphora terrestris and Cenococcum graniforme.

Marx et al (1976) inoculated three pine species with vegetative hyphae and basidiospores of Pisolithus tinctorius in three nurseries in Georgia, Florida and North Carolina. In North Carolina 140 percent growth stimulation was noticed by vegetative inoculation of P. tinctorius on seedlings of Pinus taeda and 100 percent in P. virginiana and P. strobus.

Marx et al (1977) correlated that N and P level in soil decreases sucrose content of short roots of loblolly pine and the susceptibility of pines to ectomycorrhizae development by Pisolithus tinctorius also decreased.

Mejstrik and Benecke (1969) studied the ectotrophic mycorrhizae of Alnus viridis (chaix) and distinguished three distinct types of mycorrhizae. They found that 60% of mycorrhizal rootlets absorb phosphate five times more rapidly than non-mycorrhizal roots. The root nodules of the host plant do not take any active part in phosphorus absorption.

Mejstrik (1970) observed two main types of mycorrhizal infection which were classified as transitive between phytophagous and to tytophagous mycorrhizae. He (1972) further studied the intensity and frequency of vesicular mycorrhizal infection in species of Molinietum coerulescens L. in relation to the fluctuation in ground water table and found that it does not exert a strong direct influence on the intensity of mycorrhizal infection during its vegetative period.

Trappe and Strand (1969) observed that long agricultural practices result in ectomycorrhizal destruction and seedlings transplanted on such sites do not survive until ectomycorrhizal fungi are inoculated. They further noticed that if some fertilizer is added with ectomycorrhizal fungi,

seedling development is improved.

Trappe (1977) attempted to provide a detailed account on selection of ectomycorrhizal fungi for inoculation in forestry nursery practices. He concluded that inoculum potentiality of mycorrhizal fungi should be determined to see how effective a given fungus is in host vigor. A continuous series of experiments in an organized way is required to cope with such a diverse problem. The screened fungi should be refined with laboratory tests and followed by experimental inoculation of seedlings and planting them subsequently to a variety of sites.

Bevege (1970) concluded that mycorrhiza in hoppel pine are beneficial in phosphorus and nitrogen uptake only when available nutrient level in soil is high, whereas at low level the effect is not favourable.

Palenzona and Pontana (1970) found that the type of soil also has effect on the development and morphology of mycorrhizae and they supported the Slinkis-hypothesis of nitrogen availability in soil and mycorrhiza development.

Hadley and Williamson (1971) observed linear increase in length and breadth of protocorm of Dactylorhiza purpurella just after the infection by symbiotic fungi, whereas the growth of uninfected protocorm was negligible.

They suggested that in mycorrhizal plants the growth may be stimulated just after the entry of hyphae and before intracellular lysis of the hyphae. They opined that "digestion" of endophyte is only defensive mechanism not a prerequisite for a growth stimulant, and growth stimulation takes place by transferring nutrient from fungus to host.

Hayman and Mosse (1971) found in onion a large increase in shoot dry weight upto eighteen fold and with Coprosma nineteen fold by adding phosphate. Mycorrhiza also had similar type of effect (fifteen times in Coprosma and twelve fold in onion) in absence of phosphate fertilizer. Mycorrhizal root could make available phosphorus to plants, while nonmycorrhizal roots failed to do so.

Krupa and Pries (1971) showed that the production of volatiles is associated with the active mycelial growth of Boletus variegatus, a mycorrhizal fungus of pine. Some of these volatile substances were found to have fungistatic property. They observed six times higher concentration of these volatile substances in mycorrhizal roots than the nonmycorrhizal. They suggested that production of the fungistatic volatile organic compounds may be correlated with the disease resistance of mycorrhizal root.

Mosse and Phillips (1971) studied the effect of phosphate and other nutrients on the establishment



mycorrhiza in Trifolium parviflorum inoculated with Endogone spores. They observed that Caphytate, Camonohydrogen phosphate, Na-phytate, Fe-phytate, Phytin, Lecithin and DNA were good sources of PO_4 for the plants and the symbionts both.

Mosse and Hepper (1975) established vesicular-arbuscular mycorrhiza in clover root organ cultures. They found that pH of the medium is a critical factor for determination of initiation and spread of mycorrhizal infection.

Mosse et al (1976) studied the interaction between vesicular-arbuscular mycorrhiza, utilization of rock phosphate and nitrogen fixing organism in three legumes- and in onions. They observed that in neutral and alkaline soil, rock phosphate was unavailable to mycorrhizal roots.

Mosse (1977) observed that vesicular-arbuscular mycorrhizae stimulate the nodulation by rhizobia due to increase in utilization of more phosphate from non-available rock phosphate but she did not observe any relationship between phosphorus and nodulation in irradiated soil.

Safir et al (1971) noticed that mycorrhiza produced by Endogone mosseae, decreases the resistance to water transport in Soybeans (Glycine max L.). The decrease

was associated with an increase in the growth of shoot.

Khan (1972) found that vesicular-arbuscular mycorrhiza increases growth, dry matter, grains number per ear and grain weight in maize. It also helps in greater uptake of phosphorus.

Khan (1974) investigated the Endogone spores in rhizosphere soil and also incidence of mycorrhizae in halophytes, xerophytes and hydrophytes. He suggested that except in a few, vesicular-arbuscular mycorrhiza frequently occur in almost all the plants.

Khan and Saif (1975) studied seasonal variation in Endogone population in wheat field. Number of Endogone spores in wheat field soil was almost similar during the vegetative growth and tillering stage of wheat plants. The extent of mycorrhizal infection was related to Endogone population.

Rambelli et al (1972) confirmed the nitrogen fixing activity in the mycorrhizosphere of Pinus radiata by gas chromatograph technique.

Hattingh et al (1973) demonstrated the movement of P³²-labelled phosphate from soil to mycorrhizal plants and concluded that the mycelial network of endomycorrhizal fungi enables plants to remove phosphate from larger soil

volume, extending hyphae beyond the immediate vicinity of the root surface.

Mexal and Reid (1973) studied the effect of water stress on three ectomycorrhizal fungi. Genococcum graniforme was quite tolerant and exhibited maximum growth at 15 bars water potential, while Suillus luteus and Thelephora terrestris grow well at -5 bars.

Zak (1973) provided a meaningful and practical classification to ectomycorrhiza in nature. The system provides a better understanding of the frequency, density and the variation of the symbionts.

Zak (1975) concluded that the mycorrhizae benefit the trees in phosphorus uptake and in protection from pathogens, root aphids and nematodes. The association also helps the plants from phytotoxins produced by soil micro-organisms.

Zak and Larsen (1978) recognized three new ectomycorrhizae of Pseudo-tsuga menziesii formed by Byssoporia terrestris vars. lilacinorosea, parksii and sublutea.

Baylis (1974) reviewed the evolutionary significance of phycomycetous mycorrhiza in land flora and concluded that fungi have become an unseperable symbiont due to long coevolutionary processes.

Bowen et al (1974) demonstrated that Zn absorption was higher in ecto and endomycorrhizae of Pinus radiata and Araucaria cunninghamii respectively than nonmycorrhizal roots.

Crush (1974) reported that in phosphate deficient soils vesicular-arbuscular mycorrhiza strongly stimulates the nodulation and growth of Controseta pubescens, Stylosanthes guyanensis, Trifolium repens and Lotus pedunculatus. He found that tropical legumes are much more dependent on mycorrhiza than temperate and suggested that when leguminous and nonleguminous plants are grown together mycorrhizas preferentially stimulate the growth of legumes and also the nodule formation.

Crush (1976) found that mycorrhizal plants of Trifolium repens and T. pratense grew better than non-mycorrhizal. He observed that some mycorrhizal fungi were parasitic to T. hybridum and Medicago sativa and suggested that the endophyte-host relationship in symbionts changes from mutualism to parasitism on increasing of phosphorus availability.

Hayman (1974) studied the effect of light and temperature on development of vesicular-arbuscular mycorrhiza and growth of onions in phosphate deficient soil. Maximum

number of arbuscules and the best growth of the host was observed in the presence of 25000 lux at 23°C while no growth stimulation was noted at 14°C in light intensity of 13000 lux.

Lamb and Richard (1974) observed that in a soil lacking mycorrhizal fungi, addition of phosphate @ 40kg/ha enhances the mycorrhiza formation in Pinus radiata. Besidiospores inoculation was better effective than chlamydospores.

Strzemska (1974) studied 37 species of Gramineae and Papilionaceae and found that all the species have endotrophic mycorrhizal association. He observed that variation in p-H and soil had no effect on mycorrhizal development. The presence of endomycorrhiza was an essential and constant feature of these plants.

Thoen (1974) reported seven subtypes of mycorrhiza classified on "Dominik classification System" of Pinus insularis and observed several mushrooms under pines and Eucalyptus canopy. Suillus granulatus was more frequent. He found that introduction of symbiont from the litter of old plantation has more beneficial effect on growth of seedlings in nursery than those of young plantations.

Vozzo and HacsKaylo (1974) identified both ecto

and endomycorrhiza in five species of *Populus* and on the basis of their histochemical studies suggested that protein synthesis in mycorrhizal roots was more in mycorrhizal roots than in case of non-mycorrhizal ones.

Dehne and Schonbeck (1975) observed that degree of wilting caused by *Fusarium oxysporum* var. *lycopersici* decreased significantly in tomato plants when inoculated with *Glomus mosseae*. They found higher chlorophyll content ion flux in leaves of mycorrhizal plants than in case of nonmycorrhizal ones.

Gadgil and Gadgil (1975) concluded that mycorrhizal fungi suppress the litter decomposition in *Pinus radiata* forest. The actual mechanism of suppression, however, is unknown. They stated that moisture and pH do not play a significant role in relation to function of mycorrhiza.

Gaie (1975) synthesized ectomycorrhizae with *Pisolithus tinctorius* and *Betula pendula* in laboratory conditions. In a subsequent study he (1977) successfully synthesized mycorrhiza in cuttings of woody plant with *Paxillus involutus* and *Pisolithus arhizus* under axenic conditions.

Harley (1975) critically reviewed the problems in mycotrophy. He remarked that studies on mycorrhiza have gathered quite good information regarding the effect of the

association on plant growth and the uptake of mineral nutrients. He further suggested that carbon nutrition studies should now be given importance. The selectivity and permeability of fungal symbiont and movement of nutrients within symbiotic system are the problems to be solved in future.

Harley et al (1977) studied the effect of potassium phosphate, 24DNP, KCN and MCLAM on the respiration of beech mycorrhiza during ageing. They observed that cyanide and DNP applied together induced considerable inhibition and further addition of MCLAM caused additional inhibition.

Ogawa (1975a) studied the effect of seasonal changes and ageing of pine in relation to development of mycorrhiza in a forest ecosystem and tried to investigate the ecological niche as a basal study for artificial culture.

Powell (1975) found that in natural field the rushes and sedges possessed less infection and had only mycelium in epidermal cells. In pot culture studies he noticed that the nonmycorrhizal plants produced more dry matter than mycorrhizal and in P-deficient soil they rejected mycorrhizal infection.

Powell (1976) studied development of mycorrhizal infection by root segments and Endogone spores by agar coated

slide technique, buried in soil. He found that *Endogone* spores germinate after 16 days and give rise to a fan like hyphae, and infection in onion roots takes place in proximity of these spores. He also observed that indigenous mycorrhizal fungi enhanced dry matter of shoot 130% (in steep-land soil) to 37% in silt loam over nonmycorrhizal plants. He revealed that E_3 endomycorrhizal spore was more effective than indigenous fungi. He did not obtain any correlation between phosphorus uptake efficiency of mycorrhizal fungi and frequency of their hyphal connection in soil and the root system. In a later study he (1977a) further observed 90% more growth in shoot of clover inoculated with E_3 than indigenous spores in silt loam and 50% more in south aspect Waingaro soil but 42% less in north aspect Waingaro soil. Rate of uptake of phosphate was 108% greater in E_3 than indigenous mycorrhizal fungi. He suggested that E_3 should be introduced in hill country soil to increase uptake of residual and fertilizer phosphorus from soil. He (1977b) found that mycorrhizal fungi stimulate shoot growth in rye grass (*Lolium perenne* L.). *Gigaspora margarita* stimulated from 8% less to 213% more phosphorus uptake than indigenous mycorrhizal fungi in first crop of four hilly country soils. After four successive crops uptake ranged from 59% to 550% in four soils. In depleted soils after successive crops nonmycorrhizal and less effective mycorrhizal fungi were not able to help in uptake of phosphorus properly.

Powell and Sithamparanathan (1977) studied the rate of infection in grass and legume species under field conditions by indigenous mycorrhizal fungi and revealed that infection occurred quickly throughout the year, except in late winter due to water logging. Mycorrhizal fungi stimulated the nodule formation.

Powell and Daniel (1978) demonstrated that Nauru rock phosphate could be utilized by mycorrhizal plants but is unavailable to nonmycorrhizal plants. Plants infected with Glomus tenuis and indigenous mycorrhizal fungi utilized 10-27% of the phosphate fertilizer while 0.4-13% could be utilized by nonmycorrhizal ones.

Scannerini et al (1975) suggested three successive stages of fungal symbiont in a gradual digestion of micro-organisms. Transition and the continuity between haustoria, coils and arbuscules and the existence of bacteria like organisms give explanation for the continuous flow of materials to the host cells. This cytological relationship helps in understanding of morphological display for the increased metabolites consumption by host cells and function as predator.

Schenek et al (1975) studied the effect of temperature and light on the germination of the spores of endomycorrhizal fungi. Highest germination in Glomus mossaeae

occurred at 20°C while in Gigaspora coralloides and G. heterogama it was obtained at 34°C. Darkness had significantly superior role in spore germination.

Stribley et al (1975) observed that relative growth rate in mycorrhizal plants increased due to more absorbing area.

Ashton (1976) detected 18 basidiomycetes intimately associated with Eucalyptus regnans and they formed mycorrhiza ranging from simple to coralloid type. The colour of mycorrhiza largely depends on type of the fungal symbiont. He observed that mycorrhizal development was enhanced by light intensity, diminished by high level of nitrogen and was unaffected by high level of phosphate. Inoculation of mycorrhizal fungi in rich loamy soil enhanced plant growth better than other soil.

Azcon et al (1976) observed that Endogone and two bacteria are capable of solubilizing rock phosphate in vitro. They noted that when Lanandula spica was inoculated with Endogone and bacteria, phosphorus uptake was more than in plants inoculated either by Endogone or by bacteria alone.

Guycox and Tinker (1976) concluded that transfer of phosphate through arbuscular digestion is not so significant as compared to the transfer through the membrane of living

fungus and this is more related to the mechanism of phosphate uptake.

^{et al}
Harvey (1976) emphasized that in forest management, prescribed burning and soil organic matter should be given proper attention to save the ectomycorrhizal association as most of the active mycorrhizae live in the organic fraction layer (66 percent in humus, 21 percent in decayed wood and 8 percent in the charcoal).

Hepper (1976) developed a colorimetric method for measuring glucosamine a resultant of conversion of chitin and correlated this with the intensity of vesicular-arbuscular mycorrhiza in roots.

Purves and Hadley (1976) found that Goodyera repens could grow on 1% glucose and potato extract as asymbiotically but it grew best with symbiotic fungi on a cellulose carbon source. He stated that protocorms acquire high level of starch in asymbiotic conditions and grow slowly while in association with the symbiont growth was rapid and no starch was observed. Their studies in plantlets revealed a greater incorporation of insoluble substances in mycorrhizal roots than that of nonmycorrhizal ones.

Sanni (1976) collected the spore forming vesicular-arbuscular mycorrhizal fungi and studied their effect on the

growth of Vigna unguiculata, Lycopersicon esculentum and Zea mays. He observed that vesicular-arbuscular mycorrhiza has a positive correlation with P and N amount in tissue.

Schoknecht and Hattinck (1976) examined onion roots with vesicular-arbuscular mycorrhizal fungi Glomus fasciculatus and G. mosseae by scanning electron microscopy (SEM) and energy dispersive x-ray microanalysis techniques and found that the cells having arbuscules possessed more phosphorus and those lacking arbuscules did not possess measurable amount of phosphorus. Other chemicals (S, Cl, K and Ca) were also present in more quantity in mycorrhizal cells than in those of nonmycorrhizal ones.

Alvarez and Cobb (1977) studied the mycorrhizae of Abies concolor and they recognised nine different types of mycorrhizae occurring in naturally growing white fir seedlings in North Central Sierra Nevada. Cenococcum graniforme was also observed. They found that none of the naturally occurring mycorrhizal types were found on nursery seedlings.

Barrows and Roncadori (1977) found that Gigaspora margarita enhances the rootings in Euphorbia pulcherrima and the mycorrhizal plants grow better under low fertility and moisture stress. Addition of proper quantity of phosphate

increased the mycorrhizal development but a high and low quantity inhibited the growth.

Becker and Gerdemann (1977) suggested a colorimetric method for quantification of vesicular-arbuscular mycorrhiza in onions inoculated with Glomus fasciculatus. They found that mycorrhizal infection determined by Chitin assay or staining procedure had significant correlation with percentage of yellow roots by weight and the colorimetric method also had significant correlation with root/shoot ratios.

Blaich (1977) reported a yeast Aureobasidium pullulans to form mycorrhizae with Vitis repens under sterilized conditions. The fungus produced Hartignet, plectenchymatic layer and induced inter nodal elongations of rootlets.

Clement et al (1977) observed that ectomycorrhiza improves growth of Pinus nigra. The association also eliminates chlorosis, reduces cations level in foliar tissues and corrects the pathways of aminoacids and protein synthesis. Pinus nigra seedlings could be grown with mycorrhiza on soil having more calcium carbonate, while it failed to survive without mycorrhizal fungi and exhibited the symptoms of chlorosis, unbalanced nitrogen metabolism and high cations in tissues.

Furlan and Fortin (1977) studied the effect of different light intensities on formation, reproduction and influence of vesicular-arbuscular mycorrhiza on onion plants. They found that lower light intensity enhances mycorrhizal infection. They further observed that spore production was directly proportional to light intensity and the maximum growth was observed under 10 KLUX light intensity.

Homola and Mistrella (1977) listed the species of Boletaceae which were supposed to form ectomycorrhizae with the tree species in Maize. They found that except one or two fungal species other occur with most of the tree and do not have any specificity with the host plant.

Johnson (1977) found that in New Zealand a typical vesicular-arbuscular mycorrhiza is dominant in liverworts, monocots, dicots and in coniferous plants. He observed nine different types of sporocarpic species and their distribution was influenced by soil types but not by plant species. He concluded that Rhizophagus tenuis is a pioneer endophytic mycorrhizal fungus.

Kormanik et al (1977) revealed that many of the hardwood species require mycorrhizal infection in the production of seedlings. Late association of fungi caused inferior grade of seedlings.

Roncadori and Hussey (1977) found that when

an endophytic fungus Gigaspora margarita was jointly inoculated with a nematode Meroidagynes incognita, stunting caused by this nematode was reduced by the development of mycorrhizae. Azygospores were produced in joint culture, while G. margarita did not affect the production of the eggs of nematode but increased the total number of eggs as mycorrhizal root system is generally larger than the nonmycorrhizal ones.

Smith and Daft (1977) observed that mycorrhiza enhanced the nodulation, nitrogenase activity and nitrogen and phosphate content of Medicago sativa. They suggested that tripartite symbiosis i.e. legume, rhizobium and mycorrhizal fungus results in better growth than uni or bipartite symbiosis.

Von (1977) observed that vesicular-arbuscular mycorrhiza develops not only in roots but also in the pegs of Arachis hypogaea, when grown in insoluble phosphate. He assumed that phosphate content is enhanced in these pegs also.

Bagyaraj and Menge (1978) studied interaction between vesicular-arbuscular mycorrhiza and Azotobacter and the effect of the association on rhizosphere and plant growth. They found maximum bacterial and actinomycetes population in the rhizosphere of mycorrhizal and Azotobacter

chroococcum than noninoculated one. Fungal population was effected by V.A. mycorrhiza (Glomus fasciculatus) and Azotobacter, chroococcum. Dry weight of mycorrhizal and Azotobacter inoculated plants was significantly higher than dry weight of noninoculated plants.

Bagyaraj and Manjunath (1980) have reported that mycorrhizal fungi significantly increased the root and shoot dry weights and phosphorus uptake in crop plants under unsterile soil conditions.

Cooper and Lossel (1978) observed that mycorrhizal roots have more lipid and triglyceride than nonmycorrhizal roots. External mycelium possessed more neutral lipids (Triglyceride, diglyceride and free fatty acids).

Daft and Elghiami (1978) demonstrated the stimulation in growth of the host by Endogone species. They reported that phosphorus concentration and inoculum density are responsible for mycorrhizal action. They suggested that mycorrhiza may not be so important in agriculture where good fertility level exists but on the other hand inoculation would be of ecological importance in natural communities where fertility level is poor.

Hall (1978a) studied the effect of vesicular-arbuscular mycorrhiza and of five different phosphorus levels

on the growth of Trifolium repens and Lolium perenne when grown in pure and mixed cultures. Vesicular-arbuscular mycorrhizal clover was found to have better inter and intra specific competitive ability for phosphorus with rye grass. Rye grass develops less vesicular-arbuscular mycorrhiza and had poor phosphorus responses due to nitrogen deficiency.

Hirrel et al (1978) surveyed Chenopodiaceae and Cruciferae families for vesicular-arbuscular mycorrhiza and concluded that when plants are grown in natural condition this association develops. The association, is, however, not seen in pure culture.

Macdonald and Lewis (1978) cytochemically demonstrated the acid phosphatases and dehydrogenases in vesicular-arbuscular mycorrhizal fungus Glomus mosseae. Acid phosphatase activity occurred in lysing and growing fungal hyphae and they interpreted that fungus has EMP (Embden Meyerhof Parnes) system, a tricarboxylic acid cycle and a hexose monophosphate shunt.

Pearson and Gianinazi (1978) in enzyme extracts of onion roots inoculated with Glomus mosseae demonstrated the presence of soluble alkaline phosphatase specific to vesicular-arbuscular mycorrhizae. They observed a close correlation between mycorrhiza-specific phosphatase activity

and development of symbiont and host. Maximum activity occurs at 100% arbuscular stage. This activity they related to assimilation of phosphorus in vesicular-arbuscular mycorrhizal system.

Schonbeck (1978) observed that mycorrhizal roots were more resistant to Fusarial wilt of tomato and root rot in tobacco and cotton than nonmycorrhizal plants. He also found that mycorrhizal roots had adverse effect on the development and establishment of a root nematode Meloidogyne incognita.

Wills and Cole (1978) studied by scanning electron microscopy (SEM) the vesicular bodies in mycorrhizal roots of Pinus mugo (Turra) and concluded that their nature of relationship with Hartig net is unsolved.

Gianinazi et al (1979) studied the localization and distribution of acid and alkaline phosphatase in onion roots infected with a vesicular-arbuscular mycorrhizal fungi, i.e. Glomus mosseae (Nicol and Gerd.). They found a strong acid phosphatase activity in terminals of immature arbuscules and alkaline phosphatase in mature arbuscule and intercellular hyphae.

Nicolson and Schenck (1979) reported 21 species of endogonaceae from Florida. The species were found to

form vesicular-arbuscular mycorrhiza.

Malloch et al (1980) provided a critical review on ecological and evolutionary significance of mycorrhizal symbiosis. They emphasised that endomycorrhizal plants usually occur in forests of high species richness, whereas ectomycorrhizal ones occur in forest of low species richness.

M A T E R I A L S A N D
M E T H O D S

Selection of Site and Sampling

Riat Khwan pine forest Mawlai, Shillong (Meghalaya) was selected for the present study. The forest had different age groups of plantations starting from those raised in 1955 to 1979. A comparative study of the different plantations, viz. 1955, 1965 and 1975 was done for the fungi during the year 1975-76. As no significant variation in the micro-fungal flora was observed amongst the different plantations, 1965 pine plantation was selected for the detailed investigation. The sites are represented by black dots in Plate 1 and 2. Also this plantation is least affected by biotic agencies which also accounted for the selection. The site is situated at an altitude of 1200 M from sea level. The Khasi Hills get the maximum precipitation in the world. Average rainfall per year in the area is 200 cm. The soil is loamy sandy. Sampling of soil, mycorrhizal roots and mycorrhizal fungi was done at monthly interval from October 1976 to September 1978. Macrofungi were collected from May to November only in each year. During the other part of the year the macrofungi were not available.

Dynamics of higher fungi in pine forest community under natural conditions :

Observations for the occurrence of macrofungi in the pine forest was carried out during the years 1976-1978.

In addition to pure stands of pine (P. Kesiya Royle) as dominant tree species other undergrowth vegetation comprised of Lantana camera, Imperata cylindrica, Eupatorium adenophorum, Smilex sp. and a few trees of Schiema wallichii.

Collection and observations of sporophores of higher fungi which could be expected to be associated with Pinus Kesiya Royle were done in a uniform forest site. The mutual relationship was determined by tracing the hyphal connections between fruiting bodies and mantle of mycorrhizal roots of pine tree. The observations were made at a regular monthly interval from May - November each year. The sporophores were collected in sterilized container and brought to the laboratory for identification and isolation in pure cultures. The occurrence and abundance of higher fungi were determined on the basis of the appearance of the sporocarps in pine forest during the period of investigation. Sporophores of higher fungi were identified as suggested by Singer (1962).

For quantitative study of sporophores ten pine trees of same age were selected randomly. Observations were made on monthly interval from May to November of 1978 and 1979. Distance of the sporocarps from the tree trunk was measured and plotted out on graph. Percentage relative abundance of macrofungi was calculated from the

following formula :-

$$\text{Percentage relative abundance} = \frac{\text{Total number of individual species} \times 100}{\text{Total number of fungal species}}$$

After calculating percentage relative abundance the macrofungi were grouped in different classes, viz. dominant (81 - 100%), common (61 - 80%), frequent (41 - 60%), occasional (21 - 40) and rare (1 - 20%) Vittal, 1976).

Isolation of Mycorrhizal fungi from sporophores :-

For the isolation of the fungi from closely associated mushrooms, the young immature or developing fruit bodies, free from insect attack were collected from pine forest community in a sterilized polythene bags and were brought to the laboratory. The dorsal surface of the cap of these sporocarps was sterilized with 90% alcohol and were then kept in sterilized Petriplates. To minimise the contamination, the sporophores were aseptically cut into pieces and small pieces of tissue taken from inside were inoculated to the nutrient medium. Only one piece was inoculated in each plate and ten plates were used in each case. The inoculated plates were incubated at 20°C for 25 days. The plates were checked at a regular interval

and the contaminated ones were discarded. Isolates were then examined and those with clamp connections were sorted out and purified by hyphal cut method. Pure cultures were maintained in a refrigerator at 10°C throughout the period of investigation.

Isolation of mycorrhizal fungi from roots :-

Roots from different trees were randomly collected at monthly interval from October 1976 to September 1978. For the collection of mycorrhizal roots a sharp sterilized knife was used and the roots of about 10 - 15 cm. size were collected in a sterilized specimen tubes and brought to the laboratory and washed thoroughly with water. The roots were then examined under binocular microscope and young active ones with fungal mantle were sorted out. Twenty pieces of approx. 5 mm size were finally prepared from such roots and were transferred to another test tube containing a few drops of liquid detergent (Teepol, BDH) and shaken vigorously for 1 - 2 minutes. The detergent was then poured off. The root segments were later transferred to a 250 ml conical flask which had 150 ml sterilized water. The flask with root segments was shaken vigorously to wash the roots and this process was repeated 5 - 6 times. After washing, root segments were surface sterilized with 1 percent solution of sodium hypochlorite

for 10 minutes and were transferred to another sterilized conical flask (250 ml). The root segments were given about 10 washings with sterilized water. After proper washing, the segments were removed and transferred to sterilized Petridishes.

Different nutrient media were tried (a list is given separately) but Hagem agar medium (Modess, 1941) with 80 ppm streptomycin sulphate proved best. Five root segments (approx. 5 mm) were inoculated in each plate. The segments were tested under aseptic conditions before plating. All the inoculations were done in four plates which had twenty replicates. The inoculated Petridishes were incubated at 20°C in dark in a BOD incubator for 25 days. After the incubation period, the plates were taken out and examined for the growth of mycorrhizal fungi. The cultures thus obtained were transferred to culture tubes which had slants of malt extract or Hagem agar medium. These culture tubes were incubated for about 6 - 7 days and were stored in freeze at 10°C.

Isolation of rhizosphere microflora :-

For the study of rhizosphere microflora young root system with mycorrhizal association was dug out carefully with a sterilized spatula and brought to the laboratory

in sterilized polythene bags. The root system was tapped gently to remove the extra soil and cut into 1 cm segments by sterilized scissors. The roots with closely adhering soil particles were aseptically transferred to a conical flask containing 100 ml of distilled sterilized water. The flasks were wrist shaken for about 10 minutes keeping mouth closed with sterilized cotton plug to get a homogeneous soil suspension which was used for assessment of bacteria and actinomycetes. One ml suspension was pipetted out with sterilized pipette on nutrient plates containing suitable medium (described separately). In each case separate sterilized pipette was used. Inoculation was done in five replicates for each sample. The root segments were taken out from this rhizospheric suspension and were washed several times with sterilized water to remove the adhering soil particles in another sterilized conical flask. The rhizospheric suspension in conical flask was dried in hot air oven at 105°C for 18 hours, cooled at room temperature and weighed. The dry weight of rhizospheric soil was also determined by evaporating the suspension left after the inoculation separately on a hot water bath and finally keeping them in a hot air oven at 105°C for 18 hours. Thereafter, the soil was cooled to room temperature and was weighed. Microbial population (Actinomycetes and bacteria) per g dry soil and root was calculated separately by estimating the soil and root present in one ml of suspension.

Simultaneously the corresponding nonrhizospheric soil samples were sampled away from root regions of Pinus kesiya Royle. The upper few mm soil surface was discarded and below it a pit of $10 \times 10 \text{ cm}^2$ was made to collect the soil from five different places and a composite sample was made to minimize the sample variation. The samples were brought to the laboratory in sterilized polythenebags.

10 g of nonrhizospheric soil was weighed in aseptic conditions and the suspension and dilution series were prepared in 250 ml sterilized conical flasks which had 90 ml of distilled sterilized water. The 1 : 10000 dilution series was finally selected for bacterial and actinomycetes inoculation. 1 ml/plate of soil suspension from this series was inoculated on nutrient media, for bacteria (beaf extract) and actinomycetes(starch case in agar medium supplemented with nystatin of 50 mg/litre). Ten replicates were used for each samples and separate sterilized pipettes were used for different sets. The suspension was uniformly spread over the entire surface of the media. All inoculations were done aseptically in a laminar flow chamber.

For the isolation of fungi, Warcup's (1950) method was used. 0.003 g of soil was transferred to each

plate by means of the flat end of a fine spatula. The method for collection of rhizospheric and nonrhizospheric soil was same as discussed earlier in case of bacteria and actinomycetes isolation. For inoculation of rhizospheric soil the mycorrhizal root system was taken and tapped in a sterilized Petridish and was homogenised with the help of small sterilized glass rod. In case of nonrhizospheric soil about 5 g of soil was taken into sterilized Petriplate and was homogenized with a sterilized glass rod. With the help of fine flat spatula, soil (0.003 g) was taken and transferred to a sterilized Petriplate. The soil was spread over the plates by putting few drops of distilled sterilized water and rotating the plate. About 15 ml autoclaved nutrient medium (Martin rose bengal agar medium supplemented with streptomycin sulphate 30 mg/litre) and the soil particles were dispersed in medium by rotating the plate thoroughly. Each sample was replicated in ten plates and all steps were conducted in aseptic conditions.

For the isolation of rhizoplane fungi, the remaining part of the mycorrhizal roots collected in case of rhizosphere study was aseptically transferred to separate conical flask containing 100 ml sterilized distilled water. The conical flask was hand shaken vigorously. These roots were given about ten washings and were transferred to sterilized Petridish. The water from outer surface of the

roots was soaked with sterilized Whatman filter paper. Thereafter, the roots were aseptically cut into small segments (5 mm approx.). The pieces were then teased with the help of sterilized needles and inoculated in sterilized Petriplate containing Martin's rose bengal agar medium (15 ml.) supplemented with streptomycene sulphate (30 mg/litre). Four segments were plated out in each plate and ten plates were inoculated in each case.

Inoculated plates were incubated at $25 \pm 1^{\circ}\text{C}$ in BOD incubator for five days for fungi. In case of bacteria and actinomycetes the incubation was done at $30 \pm 1^{\circ}\text{C}$ in bacteriological incubator for 24 hours and seven days respectively. After the incubation period the plates were taken out for quantitative determination. The bacterial, actinomycetes and fungal colonies were counted with the help of automatic colony counter. Fungal colonies were identified upto generic and specific level (Gilman, 1959; Subramaniam, 1971; Barnett and Hunter, 1972). Slow growing forms were transferred to fresh nutrient plates. Isolation and purification of fungi for detailed study were done either by single spore method or by transferring a portion of hyphal mass to fresh media in 5 cm plates. Pure cultures were maintained by transferring the isolates to the slants of malt extract and Czapek's dox agar media.

After two to three days of incubation of the tubes at $25 \pm 1^{\circ}\text{C}$, all the cultures were kept in freeze and maintained at 5°C .

Quantitative assessment of bacterial and actinomycetes population was done by multiplying the dilution factor to the average number of colonies per plate after correcting the figures by taking moisture content into consideration. Total fungal population was calculated as per g of dry weight of soil. Rhizoplane fungi were calculated as propagules per segment (5 mm) of mycorrhizal roots.

Percentage relative abundance of fungi was calculated as detailed below :-

$$\begin{array}{l} \text{Percentage} \\ \text{relative} \\ \text{abundance} \end{array} = \frac{\text{Total number of individuals of fungus}}{\text{Total number of all fungal species}} \times 100$$

After calculating % relative abundance of the fungal species they were grouped in different classes, viz. dominant (81 - 100%), common (61 - 80%), frequent (41 - 60%), occasional (21 - 40%) and rare (1 - 20%) as suggested by Vittal, (1976).

Studies on synthesis of mycorrhizae in pure cultures :-

The seeds of Pinus kesiya Royle were collected from the Riat Khwan forest during the month of January 1978 and stored in refrigerator at 10°C. Sufficient quantity of seeds were surface sterilized in 5% Sodium hypochlorite solution for about 3 minutes and washed repeatedly with sterilized water in 250 ml conical flasks. After washing, the seeds were transferred to sterilized Petridishes. The germination of the seeds was carried out in the moist chamber which were made by putting cotton and filter paper, in the Petriplates. Sufficient water was added to keep the chamber humid. The chambers were sterilized in an autoclave for 15 minutes at 15 lb/inch pressure. The surface sterilized seeds were transferred aseptically to the moist chambers. Ten pine seeds were transferred to each chamber, which were then kept in BOD incubator with light arrangements (20000 lux). Every alternate day the moist chambers were checked for water and wherever necessary sterilized water was given with the help of sterilized pipettes. Seedlings with about 2 cm long radicles were transferred to test tubes with proper media (described separately).

One week before starting seed germination experiments cultures of mycorrhizal fungi were inoculated

on M N (Melin Norkman's medium, described separately) medium from stem cultures maintained in culture tubes at low temperature (5°C in freeze). These plates were incubated at 20°C in dark for one month.

For mycorrhizae synthesis the culture tubes of 19.5×2.2 cm size were selected. These tubes were washed with liquid soap (Teepol BDH) and water, cotton plugged and dried. The culture tubes were sterilized in an autoclave under 15 lb/inch pressure for 15 minutes. At the same time A.T. medium (Agar thiamine described on separate sheet) was prepared and sterilized. 100 mg. of sterilized thiamine (dissolved in 19 ml of distilled water) after filtering through bacteriological filter paper was added to the autoclaved cooled medium (40°C). The pH of medium was adjusted to 5.5 - 6.0 after autoclaving.

20 days old seedlings were transplanted in these culture tubes. After ten days, the tubes were inoculated with pure cultures of different mycorrhizal fungi separately. Fungal inoculum (0.8 cm^3) from each culture was placed near the roots of pine seedlings on the surface of medium. 20 tubes were used for each set. The culture tubes were kept in a BOD incubator which had light arrangements. To maintain 70 - 90% humidity the water was placed in plates at

lowest column of the incubator. A constant temperature (20°C) was maintained throughout the study.

The mycorrhizal establishment was observed (ten replicates) regularly at the interval of 15 days and after twelve months of mycorrhizal establishment, twenty culture tubes at a time were taken out. The tubes were kept for about one hour on hot water bath for liquifying the culture medium and the whole synthesis set was flooded in water. The mycorrhizae were observed under binocular microscope for morphological characters. The mycorrhizal roots were then preserved in F.A.A. (5 + 5 + 90 ml V/V, formalin (4%), acetic acid glacial and ethyl alcohol (70%) respectively). The mycorrhizae were passed through a series of alcohol and embedded in paraffin (Johnson, 1940). Paraffin blocks of mycorrhizal roots were prepared and cut (12 - 15 μ thick) with rotary microtome. The ribbons were fixed and spread over the slides (glass) and passed through the xylol-alcohol series and stained in safranin and fast green. The stained slides were mounted in canada balsam and observed under microscope for mantle structure, Hartig net and penetration of fungal mycelium in cortical region of the seedlings.

Studies on Synthesis of Mycorrhizae in pot cultures in natural conditions

For the study of mycorrhizae synthesis in pot cultures, the soil was collected from botanical garden of North Eastern Hill University, Shillong (Meghalaya). The soil was sterilized in autoclave at 20 lb/inch pressure for 2.5 hours two times. About 1 kg. (moisture content 18% and pH 5.7 org. matter 1.8%) sterilized soil was filled in plastic pots (11.5 x 11 cm) with drain holes covered by a layer of steam sterilized small stones. Healthy mature sporophores collected from the pine stand under study were dried in freeze at 5°C with silica gel for about three months and were used for further experimentation. Five dominant mycorrhizal fungi viz. Scleroderma aurantium, Russula lutea, Lactarius deliciosus, Boletus edulis and Amanita sp. were selected. After drying the sporocarps were squeezed with hand and were screened through 125 μ m mesh sieve. Experiment was randomizedly designed. 12 pots were used for each treatment. Treatments were used as follows.

- I. Uninoculated sterilized soil.
- II. 2 gm of fridge dried basidio carps of different species, viz. Amanita sp., Boletus edulis, Lactarius deliciosus, Russula lutea, and Scleroderma aurantium

were mixed to each pot which had 1 kg of sterilized soil.

12 sets were maintained in each case. To decrease the effect of detoxication by steam sterilization and for the establishment of mycorrhizal fungi, the pots were left for 3 months in glass house.

In the month of May, seeds of pine collected from the Riat Khwan forest in the months of December and January, 1978 and stored at 10°C, were taken out and soaked for 24 hours in sterilized water. After soaking, surface sterilization of seeds was done in 30% Hydrogen peroxide for 30 minutes and the seeds were then given 10 - 12 washings with sterilized water. Thoroughly washed seeds were transferred to sterilized Petriplates.

About 2 mm deep holes were made in soil with the help of sterilized needle and one pine seed was introduced in each hole with the help of sterilized forceps. The seeds were covered with the soil of the pot. 20 seeds were put in each pot.

Number of germinated seedlings was counted at monthly interval for six months. Seedlings were observed for survivality by counting their number in each month and percentage of survival of pine seedlings in each

treatment was determined by the following formula :

$$\text{Percentage survivality of pine seedlings} = \frac{\text{Total number of pine seedlings at the time of harvesting}}{\text{Total number of pine seedlings at the time of germination}} \times 100$$

Mycorrhizal assessment was done by harvesting the seedlings and observing the intensity of infection. Percentage mycorrhizal association was obtained by counting dichotomously forked rootlets at a regular interval of one month after germination and the process continued for six months.

$$\text{Percentage of mycorrhizae establishment} = \frac{\text{Total number of dichotomously forked rootlets}}{\text{Total rootlets number}} \times 100$$

The growth of plants was assessed in terms of the height of plant and number and length of leaves at monthly interval for six months. 20 seedlings were harvested every time for the assessment. After six months fresh and dry weight of above ground stem and leaves was recorded. Simultaneously fresh and dry weight of underground roots was also calculated. Dry weight was calculated by oven dry method.

After harvesting mycorrhizal roots were fixed in F.A.A. These roots were dehydrated, embedded and fixed in paraffin (Johnsen, 1940). Sections of the paraffin fixed mycorrhizal roots (12-15 μ m thickness) were cut by rotary microtome. Microscopic examination was done after staining and mounting the sections. Chlorophyll content of fresh leaves of six months old plants was determined (method given separately). Total nitrogen, phosphorus and potassium (methods are given separately) was also determined.

Determination of pH and moisture contents of soil samples:-

pH of mycorrhizospheric and non-mycorrhizospheric soil was determined by digital pH meter. 20 g of soil was suspended in 100 ml of double glass distilled water. The soil suspension was stirred for 15 minutes on magnetic stirrer before taking the pH. The moisture content was determined by drying 10 g freshly collected soil in a hot air oven at 105°C for 24 hours. The dried soil was cooled at room temperature in a desiccator and finally the samples were weighed. Percentage of moisture content was calculated from the formula given below.

$$\text{Percentage of moisture content} = \frac{\text{Loss in weight on drying(g)}}{\text{Initial sample weight(g)}} \times 100$$

Detection of amino acids in mycorrhizal roots:-

The qualitative and quantitative determination of amino acids was done by unidirectional paper chromatography and colorimetric methods (Smith, 1960a). 1 g of fresh mycorrhizal roots was grinded in 5 ml of 95% ethyl alcohol. The extract was filtered through filter paper. The filtrate (0.02 ml) was pipetted with the help of micropipette and spotted on chromatography sheets (Whatman No.1). After spotting the sheets were kept in a developer (n - butanol, glacial acetic acid and distilled water in the ratio of 60:15:25 respectively) for 12-14 hours. The chromatograms were air dried. The location reagent; ninhydranin (0.2 percent in acetone w/v) was sprayed over the chromatograms by glass atomiser, air dried at room temperature and finally dried at 60°C in oven for about 2 minutes. The spots developed were marked with 2B lead pencil. The R_f values of amino acid of root extracts were compared with the R_f value of known amino acids. Total free amino acid content present in mycorrhizal root extract was estimated by the method described by Peach and Tracey (1955). The values were expressed in $\mu\text{g}/100\text{mg}$ dry roots.

Detection of sugars in mycorrhizal roots:-

Qualitative and quantitative determination of sugars was carried out by unidirectional paper chromatography

and colorimetric methods (Smith, 1960b and Peach and Tracey, 1955). The extract was obtained as in case of amino acids and 0.02 ml of the former was spotted with the help of micropipettes on 30 x 30 cm Whatman (No.1) chromatographic sheet. After spotting the sheets were kept in a saturated chamber of developer (n - Butanol, Acetic acid glacial and distilled water in the ratio of 60:15:25 ml respectively). After running the chromatograms for 12-14 hours they were air dried and sprayed with p anisidine (0.2% in Hcl w/v). Standard chromatograms were also run side by side and spots developed were identified (Smith, 1960b). The total sugar of the sample was determined by Anthrone reagent method and optical density was measured on spectronic-20. The amount of sugar was expressed as $\mu\text{g}/100\text{mg}$ fresh weight of the mycorrhizal roots.

Quantitative determination of chlorophyll:-

Acetone (80%) method was used for the determination of chlorophyll (Allen, 1974). 10g fresh pine leaves were grinded in 80% acetone with few pinch of sodium sulphate salt. The grinded material was filtered and centrifuged at 15000 rpm for 5 minutes. The supernatant was used for photometric purpose. For total chlorophyll, reading was taken at 652 nm against 80% acetone as blank and calculated by the following formula:

$$= (\text{OD}652) (29)$$

For chlorophyll a and chlorophyll b reading was taken at 663 nm and at 645 nm respectively against 80% acetone as blank and the amount was calculated with the following formulae:

$$\mu\text{g chl a/ml} = 12.7 (\text{OD } 663) - 2.69 (\text{OD}645)$$

$$\mu\text{g chl b/ml} = 22.9 (\text{OD } 645) - 4.68 (\text{OD}663)$$

Determination of organic matter:

Available organic matter of rhizospheric and nonrhizospheric soil was determined by Walkley-Black(1934) method (Allen, 1974). 500 mg powder of dried soil was sieved through 0.2 mm mesh placed in 500 ml conical flask which contained 10 ml of 1N $\text{K}_2\text{Cr}_2\text{O}_7$ solution. The mixture was gently mixed and left for about 30 minutes. To the flask was added 200 ml of water, 10 ml of 85% phosphoric acid and 0.2 g NaF. Few drops of Diphenyl amine were added as an indicator. The solution was titrated with 0.5 N Ferrous sulphate solution and calculations were done according to the following formula:

$$\text{Percentage of organic matter (OM)} = 10 \left(1 - \frac{T}{S} \right) \times 1.03$$

Where T = Sample titration, ml Ferrous sulphate solution.

S = Standardization blank titration, ml Ferrous sulphate solution.

Determination of total N₂ in soil and plant tissue:

The total nitrogen in soil and plant tissue was determined by Kjeldahl method (Outlined by Mishra, 1968). 5.0g powdered and sieved (through 0.15 mm mesh/inch) soil and 0.5g dried, sieved plant tissue (through 0.4 mm mesh/inch) were rapped in a qualitative 11 cm Whatman filter paper and dropped in 800 ml Kjeldahl digestion flask. 0.20g of Na₂SO₄ plus catalyst digestion mixture was added. 50 ml of water was added to each digestion flask and left for 30 minutes. 35 ml of concentrated H₂SO₄ was added later to each flask and mixed by wirling the flask and kept for digestion in a Kjeldahl digestion unit. For the determination of nitrates 35 ml of conc. H₂SO₄ containing 1 g of Salycyclic acid was also added and left for 30 minutes. Then 5 g Na₂S₂O₃ · 5H₂O and 50 ml of water were added to the mixture and digestion continued. The digestion was continued for 1-1.30 hour and temperature was adjusted between 362-410°C. At the end of digestion, the heating was stopped but fume exhaustion was continued until fuming stopped. 300 ml of NH₄ free water was added to cooled flask.

In 500 ml conical flask 25 ml of 4% Boric acid was pipetted and 4 drops of Bromcresol green methyl red indicator solution was added. A glass receiver tube was attached to the still and placed in the flask were mixed and placed in distillation stand at about 45° angle. About 1.25 ml of 40%

NaOH was poured. Heating was switched on and solution was mixed thoroughly. After about 50 ml of distillation, receiver flask and tubes were disconnected.

The boric acid was titrated with $\frac{N}{14}$ HCl. At the end point blue colour disappeared and a single drop in excess changed the solution to pink colour. The percentage of Nitrogen was calculated by the following formula.

$$\text{Percentage of N} = (T-B) \times N \times \frac{1.4}{S}$$

Where T = Sample titration, ml standard acid

B = Blank titration, ml standard acid

S = Weight of sample in g.

Determination of Potassium:

Potassium was determined by Flamephotometer Bray method (outlined in Allen, 1974). 30.0g of freshly collected soil passed through a 0.5 mm sieve was transferred to 500 ml conical flask. 300 ml of 1N NH_4OAC solution was added. The flasks were shaken for about 30 minutes. Suspension was filtered. The filtrate was collected in a beaker and evaporated to dryness on a hot plate. The sides were washed with NH_4OAC and evaporation was allowed to continue till dryness. 5 ml of 30 percent H_2O_2 was added and the solution was digested for about 30 minutes. The solution was finally evaporated to dryness.

The sides of beaker were washed and two drops of

1 percent Phenolphthalein were added. 10% Na OH (0.5 ml) was also added. The same quantity was added to blank. The solution was evaporated to dryness on a hot plate and subsequently 2 ml of NH_4 free 1N HCl was added. The HCl was dried. After cooling the beaker 15 ml of 1N NH_4OAc was added and solution was filtered. It was then placed in the flame photometer for flame emission. The potassium in ppm was determined by the following formula

$$\text{ppm of K} = \frac{\text{mg mk} \times 2000}{\text{weight of sample (g)}}$$

mg mk was determined with the help of standard known curve.

Determination of Phosphorus, Calcium and Magnesium:

Dried plant material was ground in mortar and pestle to fine powder and passed through sieve (0.05 mm). Further analysis was carried out following the procedures given by Allen (1974). Total phosphorus was measured colorimetrically after saturating of dry ash with $\text{Mg}(\text{NO}_3)_2$ by the Molybdenum Blue method. Calcium and Magnesium were analysed by EDTA titration method.

Statistical analysis of data:

Data obtained under present investigation were statistically analysed by applying analysis of variance and correlation coefficient using standard method (Smith, 1969).

List of culture media used during the course of investigation.

1. Peptone Dextrose Rose Bengal Agar (Martin, 1950)

Agar, 20 g; KH_2PO_4 , 1 g; $\text{Mg SO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g; Peptone, 5 g; Dextrose, 10 g; Rose Bengal (1%), 2.3 ml, distilled water, 1000 ml Streptomycin, 30 mg.

2. Nutrient Agar (Difco Manual, 1955).

Agar, 15; Beef extract, 5 g; Peptone, 5 g; NaCl, 8 g; distilled water, 1000 ml. After autoclaving pH adjusted to 7.3

3. Starch Casein Agar (Russett and Williams, 1964)

Agar, 18 g; Starch, 10 g; Casein (Vitamin free), 0.3 g; KNO_3 , 2 g; NaCl 2 g; K_2HPO_4 , 2 g; $\text{Mg SO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g; CaCO_3 , 0.02 g; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g; distilled water, 1000 ml, Nystatin and actidione, 50 ug/ml of each. After autoclaving pH was adjusted to 7-7.2

4. Hagen Agar (Modified by Modess, 1941)

Agar, 20 g, Glucose, 5 g; Malt extract, 5 g; KH_2PO_4 , 0.5 g; $\text{Mg SO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g; NH_4Cl , 0.5 g; FeCl_3 , 0.1 ml (1%); distilled water 1000 ml. pH adjusted to 5.5 - 6 after autoclaving.

5. Melin-Norkrans' nutrient solution (Modified, Marx, 1969a)

Agar, 15 g; CaCl_2 , 0.05 g; NaCl, 0.02g; KH_2PO_4 , 0.5 g;

$(\text{NH}_4)_2\text{HPO}_4$, 0.25 g; $\text{Mg SO}_4 \cdot 7\text{H}_2\text{O}$, 0.15 g; FeCl_3 (1%) 1.2 ml; Thiamine HCl, 100 ug; Malt extract (paste) 3 g; distilled water, 1000 ml. After autoclaving pH was adjusted to 5.5 - 5.7.

6. Malt extract agar medium (Raper and Thom, 1949)

Malt extract, 20 g; Dextrose, 20 g; Peptone, 1 g; Agar 20 g; distilled water, 1000 ml. Medium autoclaved at 15 lb/inch for 15 minutes.

7. Czapeg's Dox agar medium (Raper & Thom, 1949)

Agar, 15 g; NaNO_3 , 3 g; K_2HPO_4 , 1 g; $\text{Mg SO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g; KCl, 0.5 g; FeSO_4 , 0.01 g; Sucrose 30 g; Yeast powder, 1 g; distilled water, 1000 ml. After sterilization pH was adjusted to 7.0

8. Pp agar medium (Pachlawska and Pachlewski, 1974).

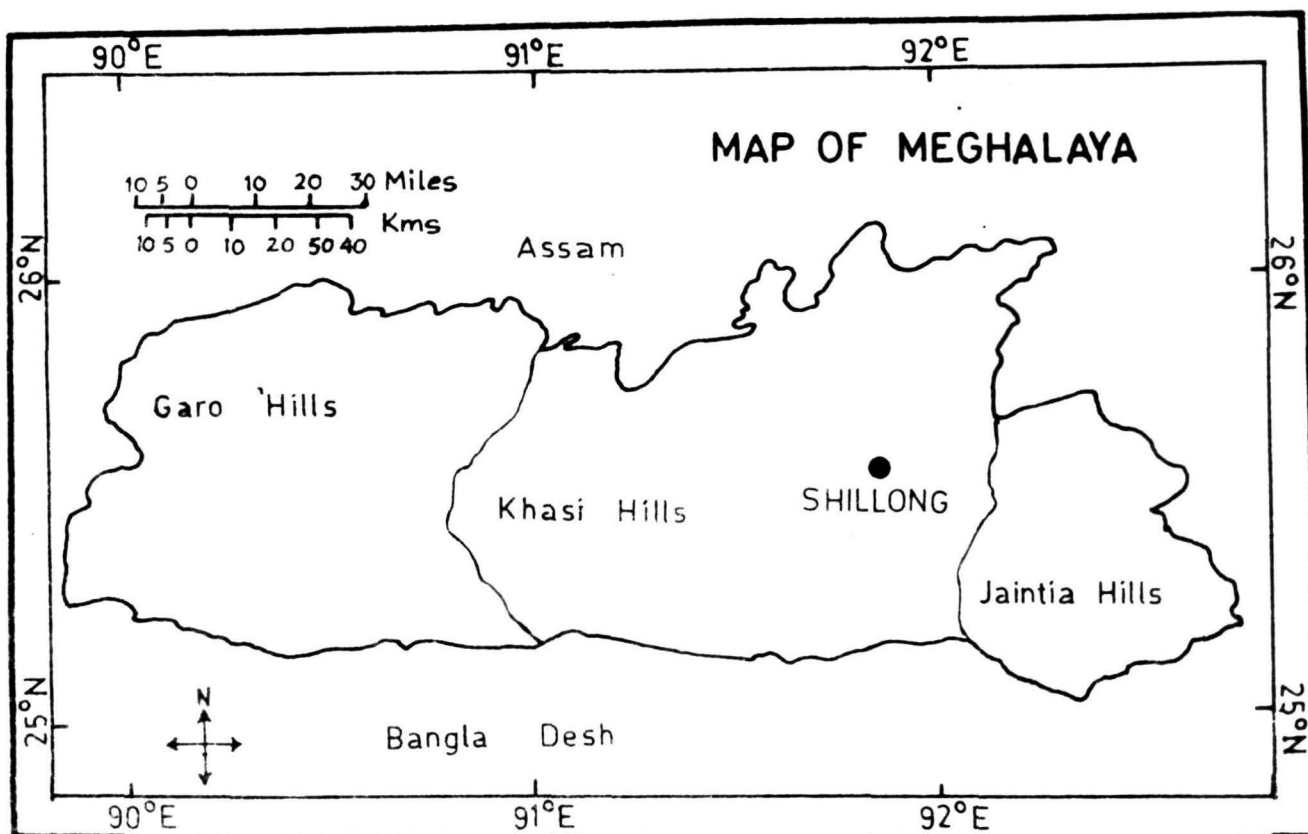
Agar, 15 g; Glucose, 20 g; Maltose, 5 g; $(\text{NH}_4)_2\text{C}_4\text{H}_4\text{O}_6$, 0.5 g; KH_2PO_4 , 1 g; $\text{Mg SO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g; $\text{C}_3\text{H}_4(\text{OH})(\text{COO})_3$ $\text{Fe} \cdot \text{H}_2\text{O}$ (1%) 0.5 ml; Zn SO_4 (0.2%) 0.5 ml; Thiamine 50 ug; distilled water 1000 ml.

9. AT agar medium (Pachlawska and Pachlewski, 1974).

Agar, 15 g; Thiamine, 100 ug; distilled water, 1000 ml. Following to autoclave the pH of medium amounted to 5.5 - 6.0.

Explanation of Plate 1.

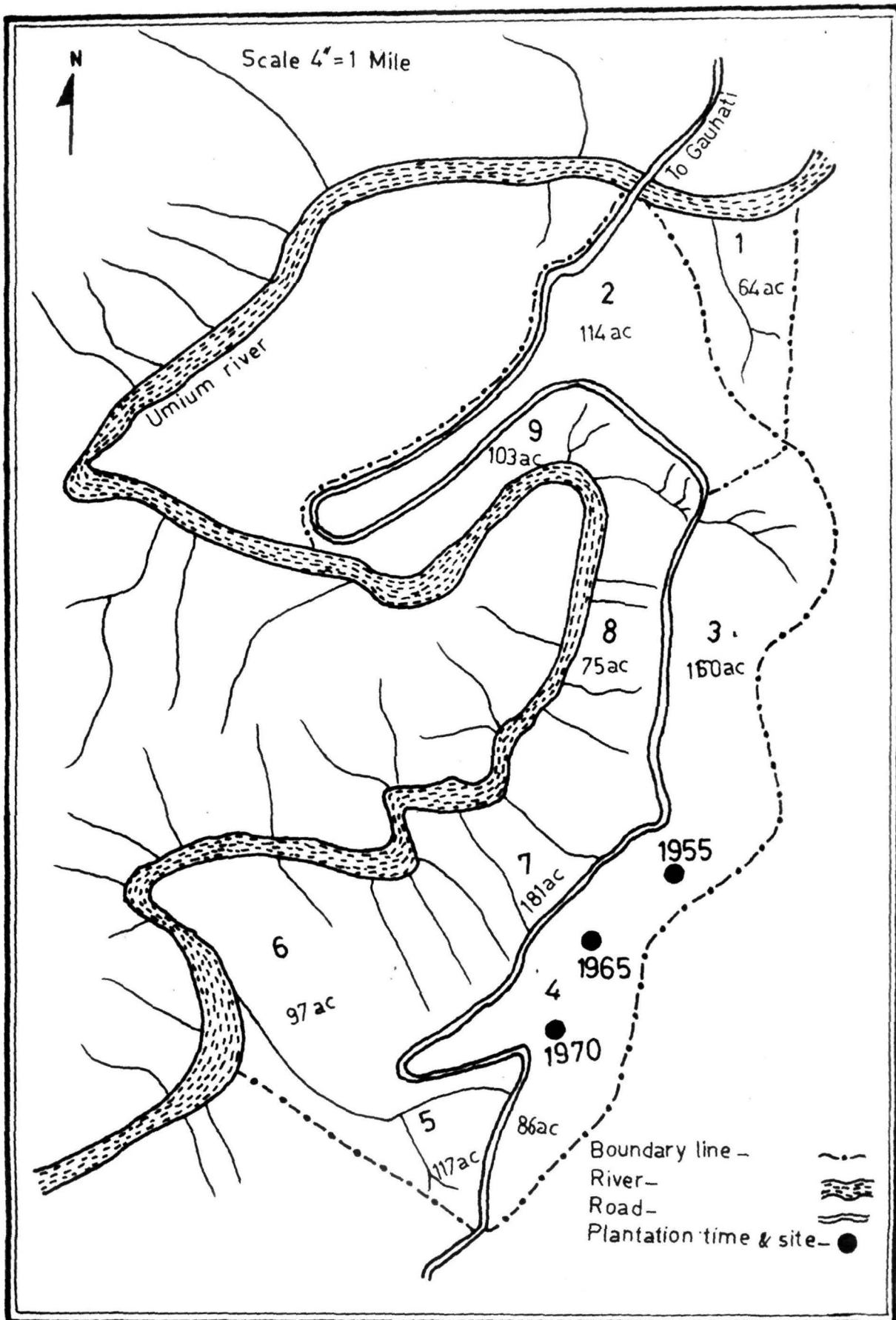
Map of Meghalaya. Site of study is shown by closed circles.



Map-1, Location of Shillong in Meghalaya.

Explanation of Plate 2.

Enlarged map of study site. Plantations are shown by closed circles.



Map-2. Riakwan reserve forest, Shillong.

TOPOGRAHY, SOIL TYPE
AND CLIMATE OF SHILLONG

Shillong lies between 25.34°N latitude and 92.47°E longitude. Physiographically the area exhibits a remnant of an ancient plateau of Pre Cambrian Peninsular belt uplifted to its present form. The Kernel of the Plateau is exposed Archaen gneisses and phyllites intruded later by younger granites and basic suits. The clear marked preserved cycles of denudation of this ancient plateau are still prominent. It is hidden beneath the mesozoic traps along the central southern fringe and Cretaceous Tertiary and post Tertiary sediments. The present form of physiographic configuration of plateau is the result of change in geological events since Mesozoic era to present day as evident from polycyclic erosional surface at various levels. Shillong plateau in its present form of an altitude varying from 610-1544 M above sea level has been blocked uplifted since Jurassic time and its historic structural geology starts with the effusion of plateau basalts (Sylhet traps) ~~th~~ough fractures and faults in the basement and upliftment and subsidence in adjacent basement blocks. This tectonic force has been vertically dominated and controlled by differential movement along these basements fractures.

The soils of the district is red laterite under red loam or brown loam soil type. The texture varies from very light (sand upto 90 percent insome parts of eastern part of the district) and very heavy (high clay contents in low lying area of the district). The fertility status of soil

represent that Nitrogen contents in term of organic carbon percentage is very high (above 70-75%) whereas phosphorus is very low (less than 20 kg./acre) or medium in certain soil (20-50 kg./acre). Soil of Shillong is acidic in reaction and pH varies between 4-6.5.

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

1. Rainy season: It starts from the second week of April and ends in last week of October. From middle of April to middle of May the precipitation is slightly less, whereas from middle of May to July the highest rainfall could be recorded. Again August, September and October receives comparatively less rainfall than previous months, thus the rainy season may further be splitted into early, middle and later rainy periods. The maximum rainfall (approx. 80 percent) occurs during middle rainy period. The mean relative humidity during rainy season is about 78 and 86 percent at 8.30 a.m. and 5.30 p.m. respectively. The average rainfall is 31.0.3 cm. and mean maximum and minimum temperatures are 24° and 16.5° respectively.
2. Winter season: It extends from November to the middle of February. The period is characterised with low temperature and less rainfall. The average maximum and minimum temperatures are recorded as 16.1°C and 7.5°C respectively with an average

rainfall of 1.15 cm. The mean relative humidity is approximately 59 and 64 percent at 8.30 a.m. and 5.30 p.m. respectively.

3. Spring season: It extends from second week of February to the middle of April. The period is characterised with high velocity of wind and less humidity with moderate temperature. The average rainfall is accounted to 3.15 cm in this season. The maximum and minimum temperatures are 20.5° and 12.3°C along with 54 and 73 percent average relative humidity at 8.30 a.m. and 5.30 p.m. respectively.

Practically the typical summer as prevalent in other parts of the country does not exist but during rainy season (June-August) mean maximum and minimum temperature rises to 24° and 19°C respectively and one can enjoy the summer of this hill station.

The climatological data for a whole period (October 1976 - September 1978) of investigation are presented in Plate 3.

Explanation of Plate 3.

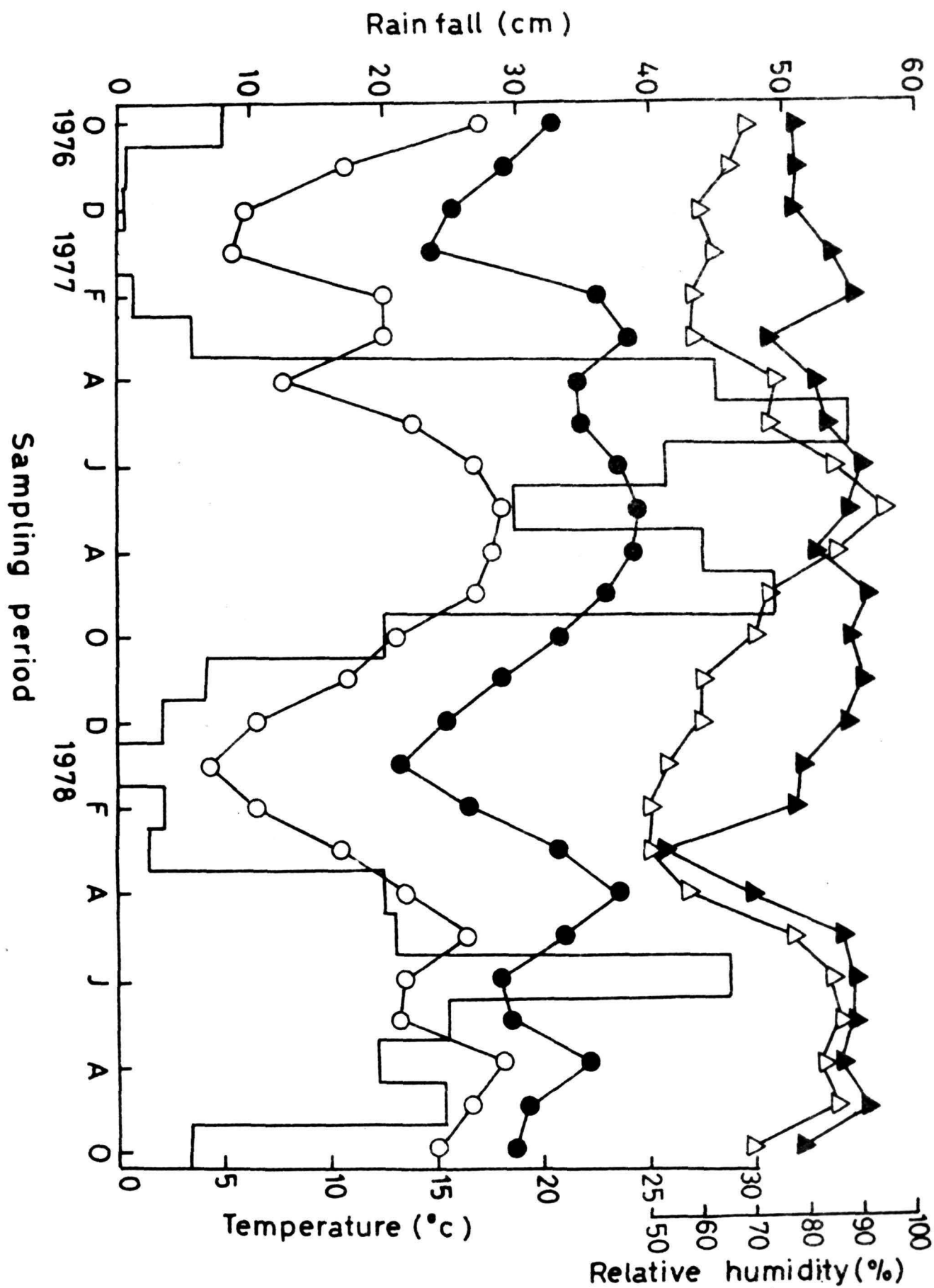
Climatological data of the study area.

Rainfall; Histogram, Maximum temperature:

Closed circle, Minimum temperature: Open circle;

Relative humidity at 5.30 p.m.: Closed triangle,

Relative humidity at 8.30 a.m.: Open triangle.



RESULTS

Distribution pattern of mycorrhizal fungi in natural pine stand.

During the course of present investigation thirty three mycorrhizal fungi were observed from three different age groups of pine (Pinus kesiya Royle) plantation (Appendix 1 & 2). The maximum fungal species were noted in oldest plantations and the minimum in the youngest ones. In 1977, 20, 22 and 25 fungal species were recorded from 1970, 1955 and 1955 plantations respectively while in the second year of investigation (1978), the number observed was 24, 25 and 32 in above plantations respectively. It was found that more than one mycorrhizal fungi were associated with the same tree, (Plate 7a). The sporocarps of the higher fungi started appearing after few showers, in the month of May and disappeared at the beginning of winter season i.e. in November. May to November months were quite favourable for the growth and development of sporocarps of mycorrhizal fungi in pine stands of Meghalaya.

The sporocarps of Boletus spp., Clitocybe spp., and Collybia spp. appeared first in May of the year 1977 while in the following year (May 1978), Boletus spp., Clitocybe spp., Cortinarius spp. and Tricholoma were first seen with fruiting bodies. The maximum production of sporocarps was observed during June to September each

year which declined in the following months (Plate 7a). Boletus spp., Clitocybe spp., Russula spp. and Scleroderma spp. occurred frequently during the course of investigation. Amanita spp. and Inocybe spp. were also observed frequently in 1977 and 1978 respectively (Plate 7 a & b). All fungi belonged to Basidiomycetes except Cenococcum graniforme and an unidentified member of fungi imperfectii. Among the dominant species, Boletus edulis, B. luteus, Lactarius deliciosus, L. sp., Russula delica and Scleroderma geaster were observed in different months of investigation in all three plantations, (Plate 6). These dominant and common mycorrhizal fungal species mainly occurred from June to September but few species like Boletus sp. and L. deliciosus were commonly found in the month of October and May in 1955 and 1965 years of pine plantation respectively, (Plate 6). It was observed that the maximum fungal species appeared in oldest pine plantations i.e. 1955 than other plantations except in July (1978) when higher number of fungal species were observed in 1965 plantation than the others (Plate 8 and 9).

The quantitative data revealed that June and July months favoured the maximum production of

sporocarps per tree in 1978 and 1977 respectively (Plate 4 and 5). In November the minimum number of sporocarps per tree was recorded. The highest sporocarps per tree were observed in 1965 plantation in July and June (1977 and 1978) but this trend was not followed in other months and in some months like August, September, October and November (1978) the maximum production of sporocarps was found in the oldest plantation i.e. 1955 (Plate 5).

The statistical analysis of variance exhibited a pattern that age of pine plantation influenced the sporocarp production and species occurrence significantly influenced by age of pine and sampling period (Table 3-6). A correlation between mycorrhizal fungi, actinomycete and fungal population was also observed (Table 32). It indicated that fungi in rhizoplane and actinomycetes in rhizosphere might influence the sporocarp production of mycorrhizal fungi (Table 33).

Different macroscopic characters of different mycorrhizae were observed (Table 1). The colour reactions of sporophore, rhizomorph and mycorrhizae were observed, however not much difference could be obtained (Table 2). The internal structure varied in association with fungal symbiont (Table 7).

Table 1. Macroscopic characters of some common Mycorrhizae of Pinus kesiva

Mycorrhizal species	Type of mycorrhizae	Colour	Texture
<u>Boletus</u> spp.	Bifurcate to coralloid	Yellowish white	Smooth
<u>Scleroderma</u> spp.	Bifurcate	White and Yellow	Smooth
<u>Lactarius</u> spp.	Bifurcate	Greenish white	Smooth
<u>Amanita</u> spp.	Bifurcate	Reddish white	Smooth
<u>Lycoperdon</u> spp.	Coralloid	White	Smooth
<u>Cortinarius</u> spp.	Digitate	White	Rough
<u>Russula</u> spp.	Corralloid	White	Smooth
<u>Cenococcum graniforme</u>	Digitate	Black	Rough

Table 2. Colour reaction of some common sporocarp, rhizomorph and mycorrhiza of Pinus kesiya to different chemical reagent.

Fungal species		Fecl ³ Soln.	H ₂ SO ₄ Conc.	NH ₄ OH Conc.	KOH (15%)	Melzer soln.
<u>Boletus</u> sp.	S	Brown (Blackish)	Pinkish purple	Black	Black	Reddish purple
	R	Red	Black	Brown	Light brown	Light yellow
	M	Red	Dark brown	Light brown	Red	Light green
<u>Scleroderma</u> sp.	S	Light brown	Reddish brown	Pink	Light pink	Light green
	R	Red(crystal shaped)	Black	Light Red	Olive yellow	Light yellow
	M	White	Black	Pink	Whitish yellow	Yellow
<u>Lycoperdon</u> sp.	S	Brown	Black	Olive green	Dark green	Yellowish brown
	R	Yellow	Brownish violet	No colour	Light red	Yellow
	M	White	Brown	Light yellow	Olive brown	Light yellow
<u>Amanita</u> sp.	S	Light brown	Bronish red	Brown	Black	Brown
	R	Pinkish red	Brown	White	Brown	No colour change
	M	Light red	Brown	No colour	No colour	Light yellow
<u>Russula</u> sp.	S	Red	Bronish black	No colour	Pink	Pink
	R	No colour	Pink, Black	No colour	Green	Brown
	M	Red	Light black	Brown	Light yellow	Light green
<u>Lactarius</u> sp.	S	Brown	Light brown	No colour change	Light pink	No colour change
	R	Red(crystalization)	Brown	No colour change	Pink	Light pink
	M	Red(Oily crystal)	Dark brown	Light brown	Green	Light green
<u>Cortinarius</u> sp.	S	Light red	Pink	No colour change	No colour change	No colour change
	R	Red	Bronish black	No colour change	Brown	Light green
	M	Red	Dark brown	White	Light brown	No colour change

S = SPOROCARP
R = RHIZOMORPH
M = MYCORRHIZA

Table 3. Analysis of variance of total higher fungal species in different plantations (1977).

Source of variation	DF	SS	MSS	Calculated value	Table value	
					5%	1%
Sampling period	6	64.67	10.7	0.73	3.00*	4.82**
Different age plantations	2	104.96	52.48	3.59	3.68*	6.93**
Error	12	175.33	14.6			
Total	20					

* and ** significant at 5 and 1 percent level respectively.

Table 4. Analysis of variance of total higher fungal species in different plantations (1978).

Source of variation	DF	SS	MSS	Calculated value	Table value	
					5%	1%
Sampling period	6	29.39	4.89	0.84	3.00*	4.82**
Different age plantations	2	98.96	49.48	8.53	3.88	6.93
Error	12	69.61	5.8			
Total	20					

* and ** significant at 5 and 1 percent respectively.

Table 5. Analysis of variance of total higher fungi in different plant age group (1977).

Source of variation	DF	SS	MSS	Calculated value	Table value	
					5%	1%
Sampling period	6	4.27	0.71	.44	3.00*	4.82**
Different age groups	2	19.85	9.9	6.18	3.88	6.92**
Error	12	19.33	1.6			
Total	20					

* and ** significant at 5 and 1 percent respectively.

Table 6. Analysis of variance for total higher fungi in different age groups of pine plantations (1978).

Source of variation	DF	SS	MSS	Calculated value	Table value	
					5%	1%
Sampling period	6	15.77	2.29	1.19	3.00*	4.82**
Different age groups	2	24.57	12.28	6.39	3.88	6.93**
Error	12	23.14	1.92			
Total	20					

* and ** significant at 5 and 1 percent level respectively.

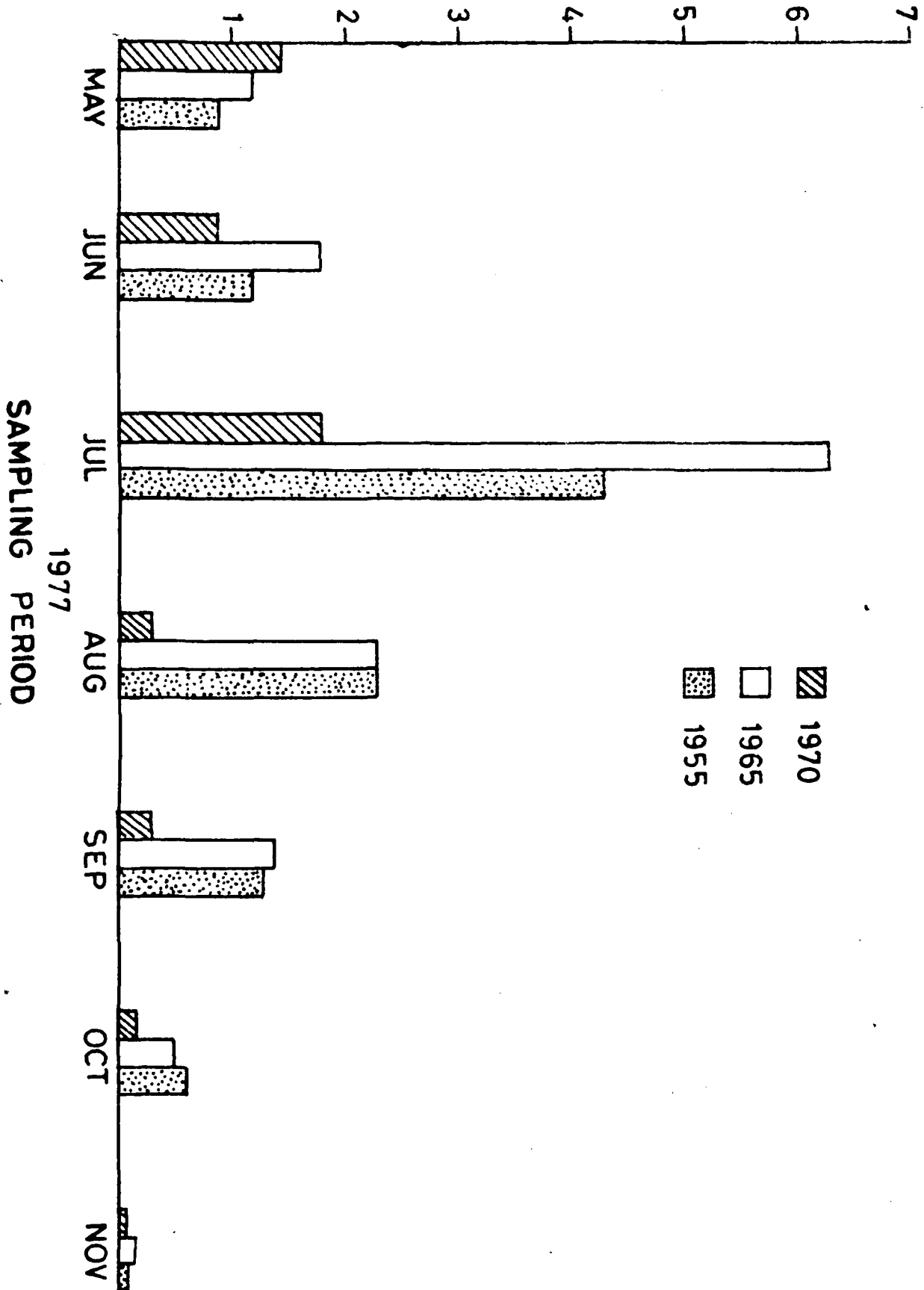
Table 7. Internal morphological characters of different ectomycorrhizae.

Fungal species	Mantle thickness (u)	Hartig net	Cell area (u)	Mycellium extension from root surface
<u>Amanita</u> sp	42	++	1422	+
<u>Boletus edulis</u>	53	+++	1025	+++
<u>B.</u> sp	58	+++	1120	+++
<u>Cenococcum graniforme</u>	52	+	1230	+
<u>Cortinarius mucosus</u>	42	++	1230	+
<u>Lactarius deliciosus</u>	43	++	1236	+++
<u>Russula delica</u>	46	++	896	+++
<u>Scleroderma parentium</u>	58	+++	1152	++
<u>Tricholoma</u> sp	52	+++	1140	++
<u>Pungi imperfecti</u>	40	+	896	+

Explanation of Plate 4.

Showing the pattern of mycorrhizal fungi (per pine tree) in pine plantations of different age groups in 1977 (= 1970, = 1965 and = 1955).

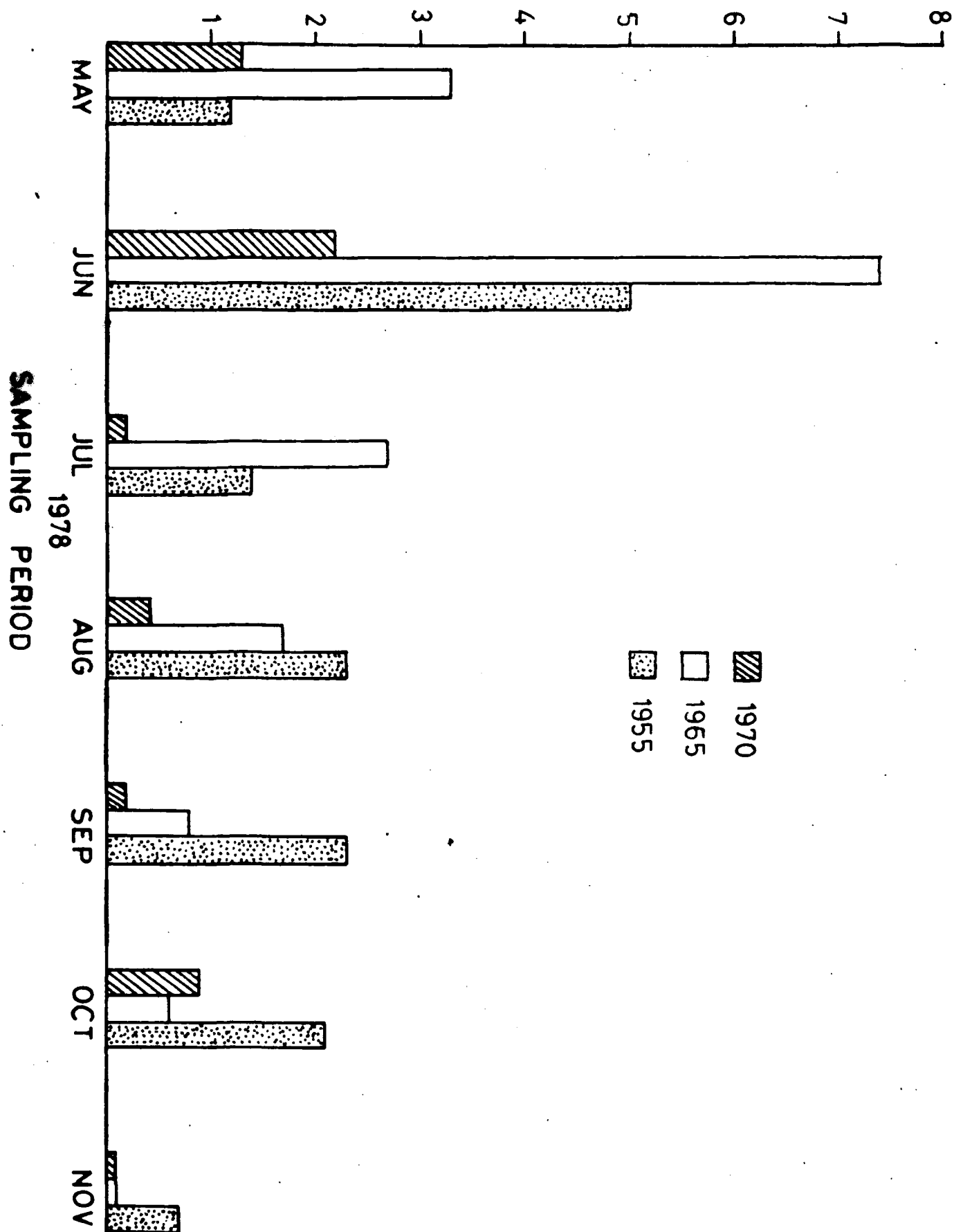
POPULATION OF MYCORRHIZAL FUNGI (PER PINE TREE)
IN PINE PLANTATION OF DIFFERENT AGE GROUPS



Explanation of Plate 5.

Showing the pattern of mycorrhizal fungi (per pine tree) in pine plantations of different age groups in 1978 (= 1970, = 1965 and = 1955).

POPULATION OF MYCORRHIZAL FUNGI (PER PINE TREE)
IN PINE PLANTATIONS OF DIFFERENT AGE GROUPS



Explanation of Plate 6.

Plate 6 is showing the distribution pattern of higher fungal species in pine plantations of different age groups (1977 and 1978)

(	= 1970,	= 1965,	= 1955 year
of plantations;	= Dominant,	= Common	
in 1977 and	= Dominant and	= Common	
in 1978).			

DISTRIBUTION OF MYCORRHIZAL FUNGI IN PINE PLANTATIONS
OF DIFFERENT AGE GROUPS

Amanita sp.

A. verna

Boletus sp.

B. edulis

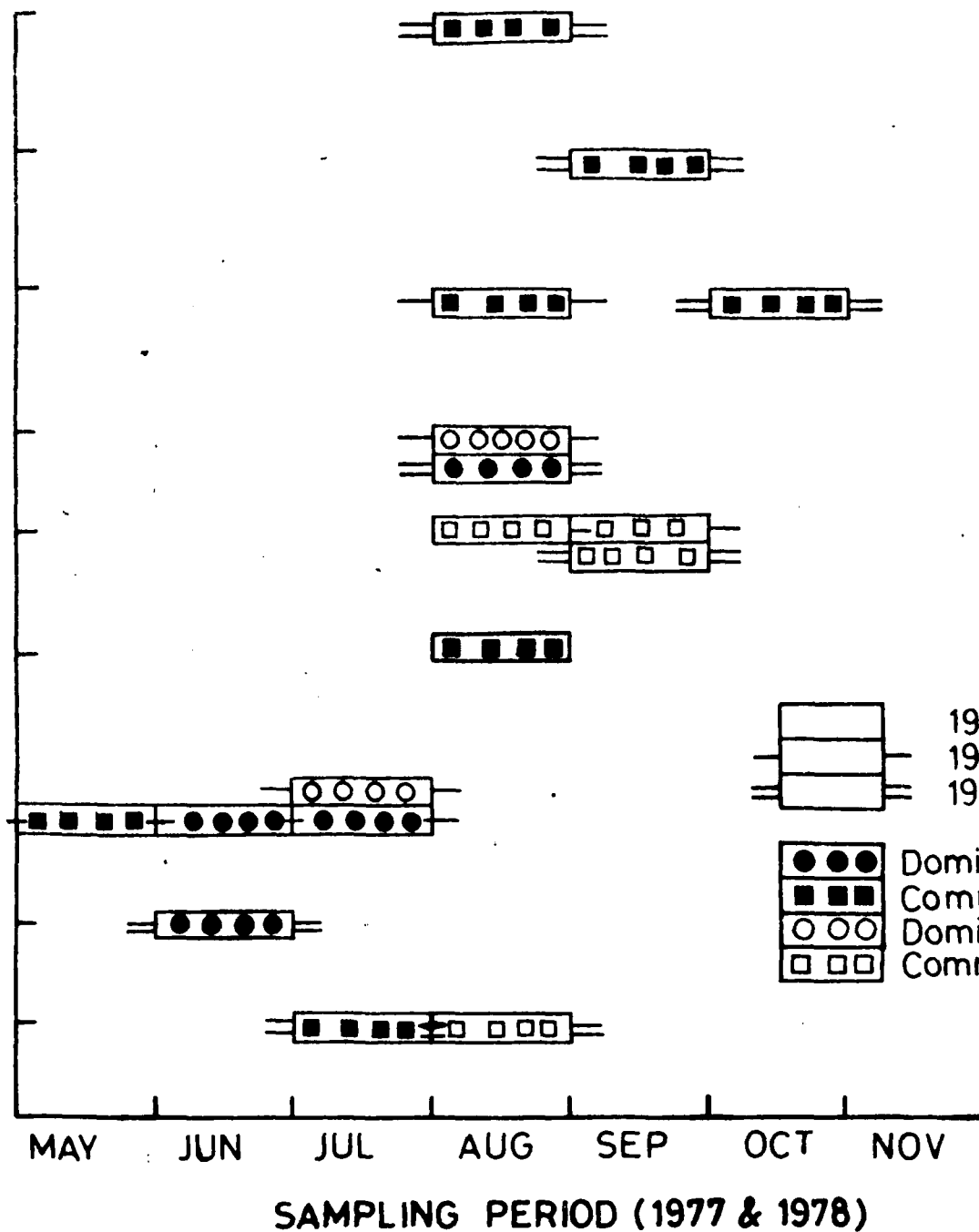
B. luteus

Lactarius affinis

L. deliciosus

Russula delica

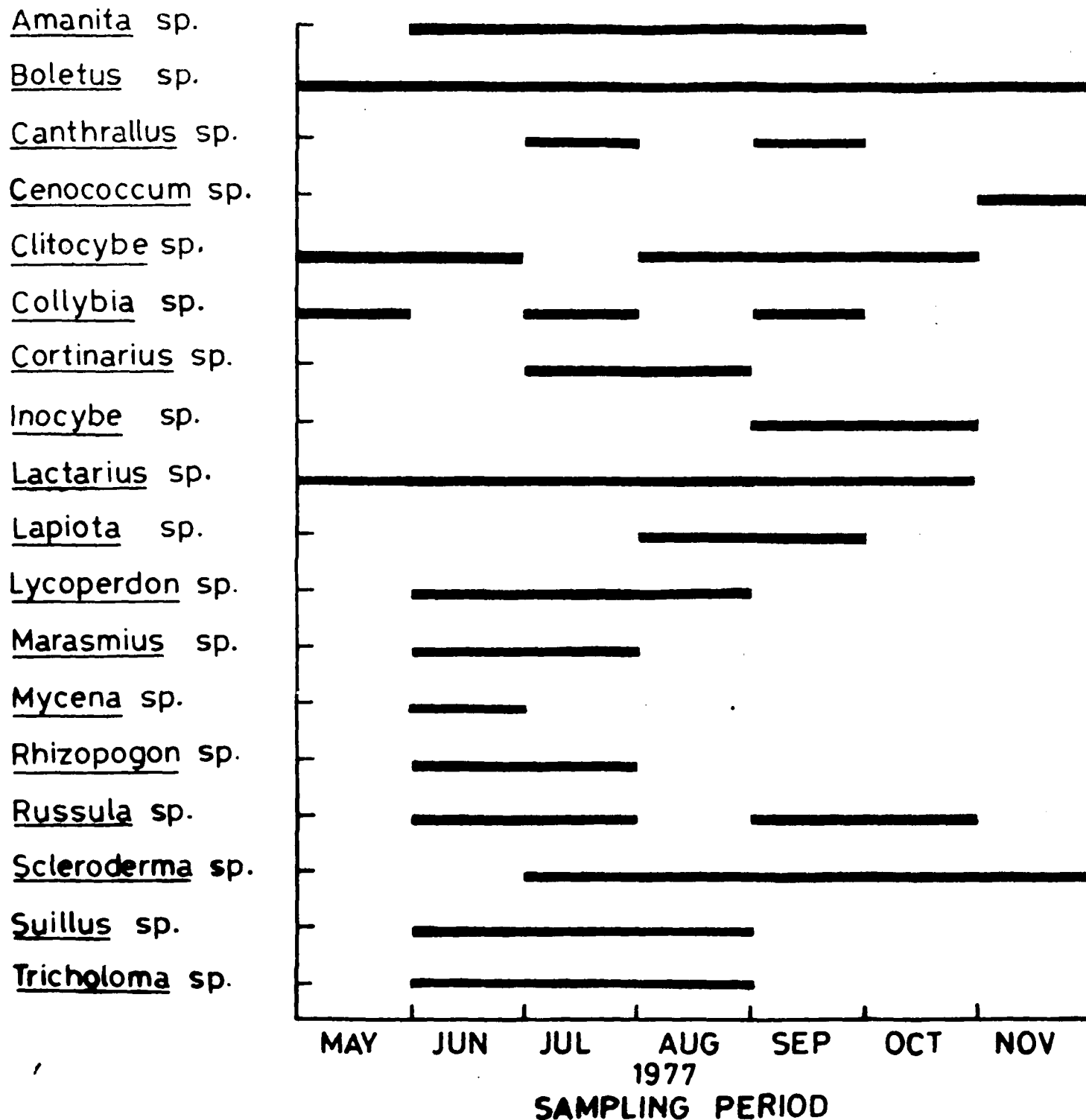
Scleroderma geaster



Explanation of Plate 7a.

**Seasonal variation in occurrence of higher fungi
forming mycorrhizal association with pine (1977).**

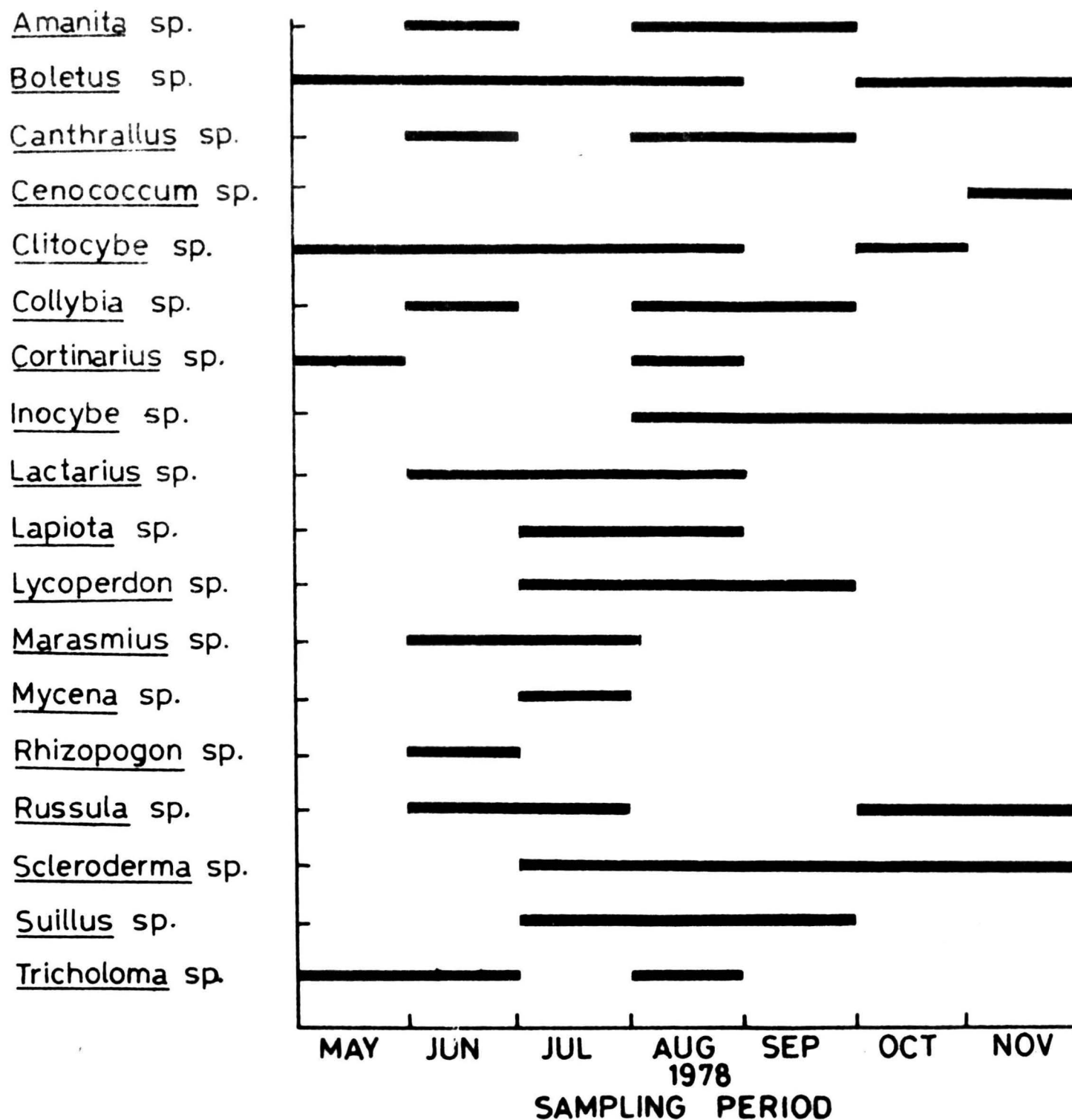
OCURRENCE OF MYCORRHIZAL FUNGI



Explanation of Plate 7b.

Seasonal variation in occurrence of higher fungi
forming mycorrhizal association with pine (1978).

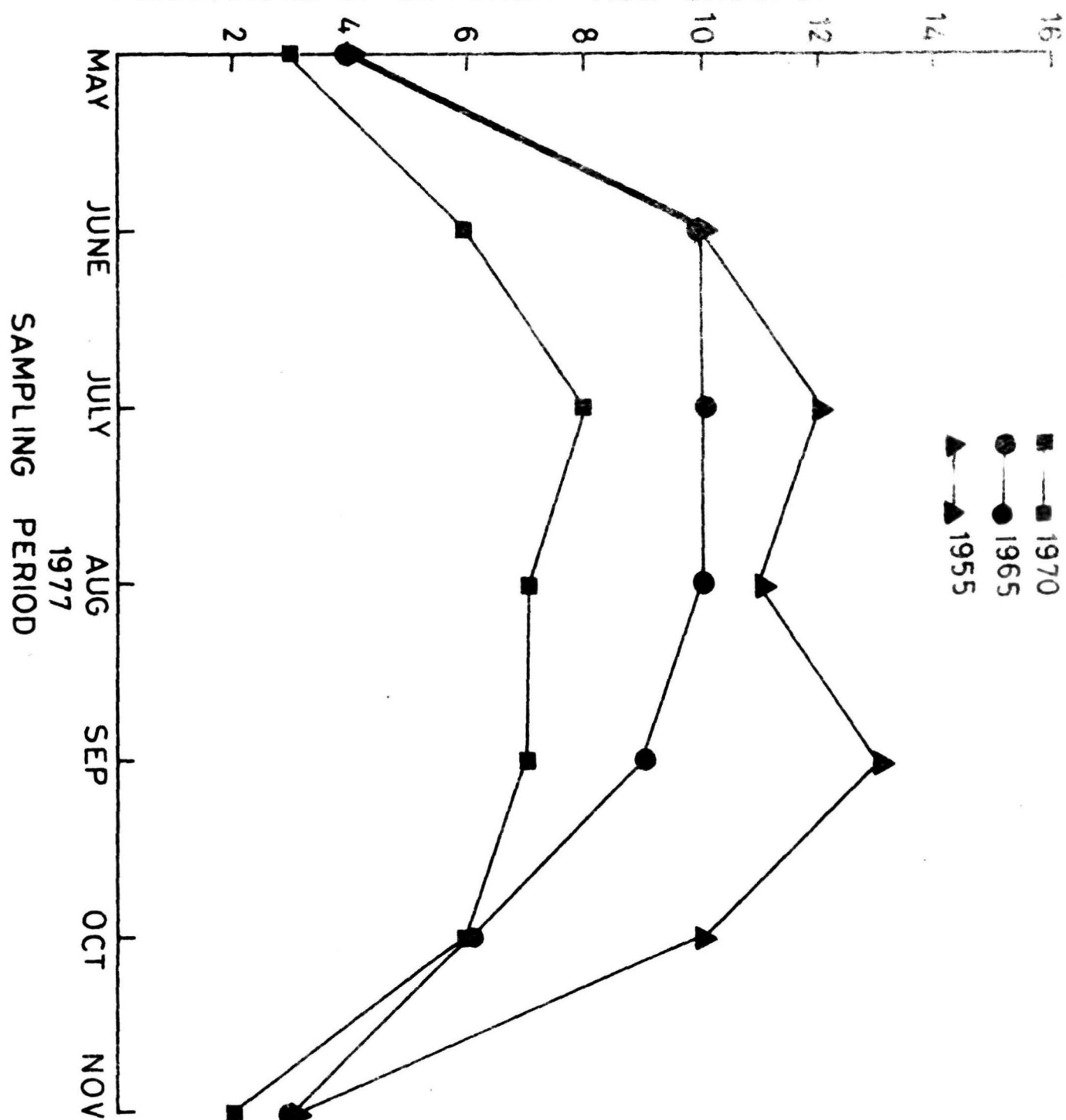
OCCURRENCE OF MYCORRHIZAL FUNGI



Explanation of Plate 8.

Plate 8 is exhibiting the monthly variation in total number of mycorrhizal fungal species in pine plantations of different age groups (1977).

NUMBER OF SPECIES OF MYCORRHIZAL FUNGI IN PINE PLANTATIONS OF DIFFERENT AGE GROUPS.



Explanation of Plate 9.

Plate 9 is exhibiting the monthly variation in total number of mycorrhizal fungal species in pine plantations of different age groups (1978).

The graph displays the number of eggs per female for three species over a six-month period. The y-axis represents the number of eggs, ranging from 0 to 14. The x-axis represents the months from May to November. The three species are represented by different symbols: circles, squares, and triangles. All species show a similar trend, with a peak in August and a decline in November.

Month	Species 1 (Circles)	Species 2 (Squares)	Species 3 (Triangles)
MAY	2	2	4
JUN	10	5	11
JUL	11	10	9
AUG	12	10	13
SEP	7	6	10
OCT	4	4	5
NOV	2	2	3

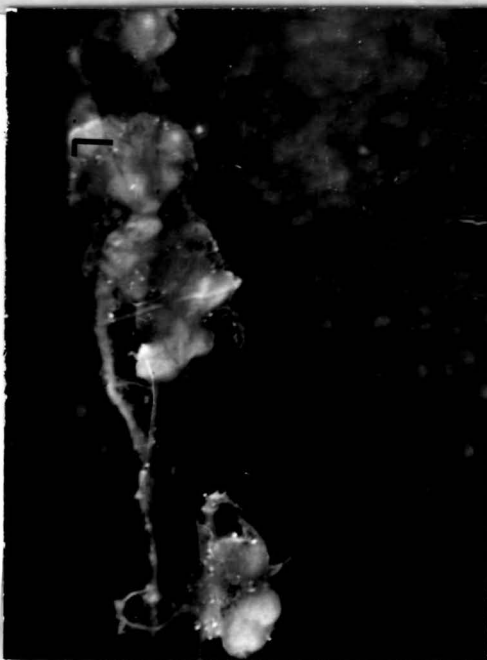
SAMPLING PERIOD

Explanation of Plate 10A.

Mycorrhizal association with pine under natural conditions.

Fig.1 = Russula delica, Fig.2 = Boletus sp., Fig.3 = Amanita
verna, Fig.4 = Scleroderma geaster with pine seedling,
Fig.5 = S. geaster associated with pine roots,
Fig.6 = Basidiomyceteous hyphae (B. edulis).

PLATE -10A



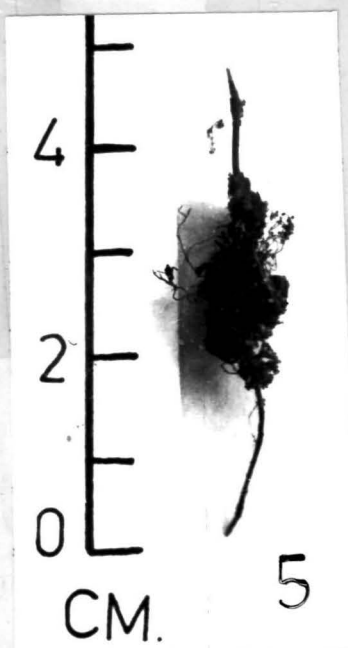
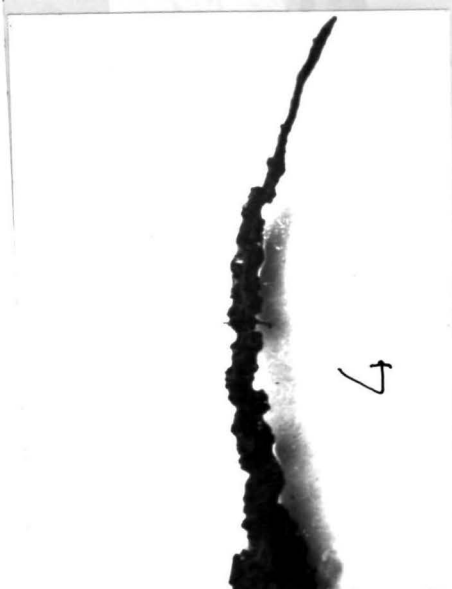
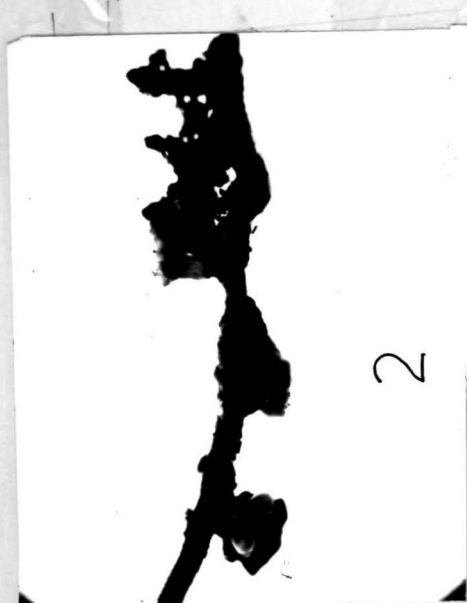
Explanation of Plate 10B.

Different types of mycorrhizae of pine in nature.

Fig.1 = Dichotomous with apical swelling (Lactarius deliciosus), Fig.2 = Small dichotomy proceeding towards tuberculate type of mycorrhizae (Boletus sp),

Fig.3 = Enlarged dichotomy (x30x), Fig.4 = Tuberculate mycorrhizae enclosed in fungal mycellium (B. edulis),

Fig.5 = Coralloid mycorrhizae with rhizomorph (Lycoperdon pyriforme), Fig.6 = Simple small dichotomy (Amanita sp) and Fig.7 = Corralloid mycorrhizae enclosed in fungal mycellium and sclerotia of unidentified fungi imperfecti.



Explanation of Plate 11A.

Variation in internal structure of mycorrhiza.

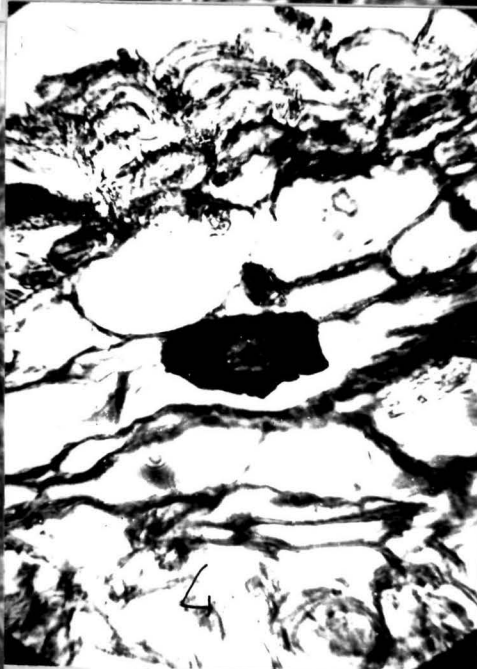
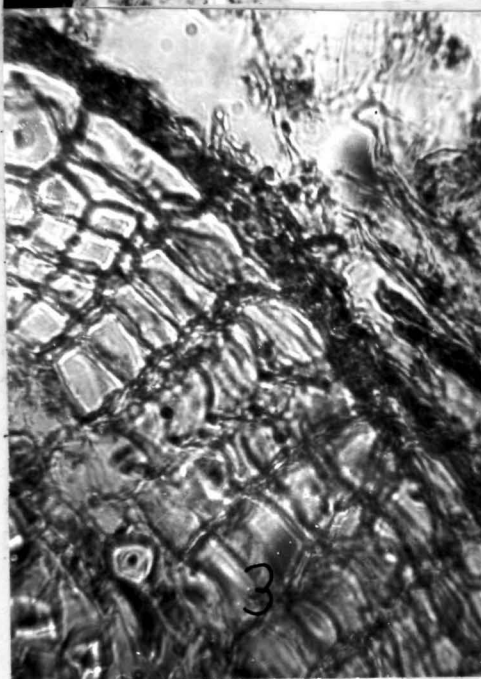
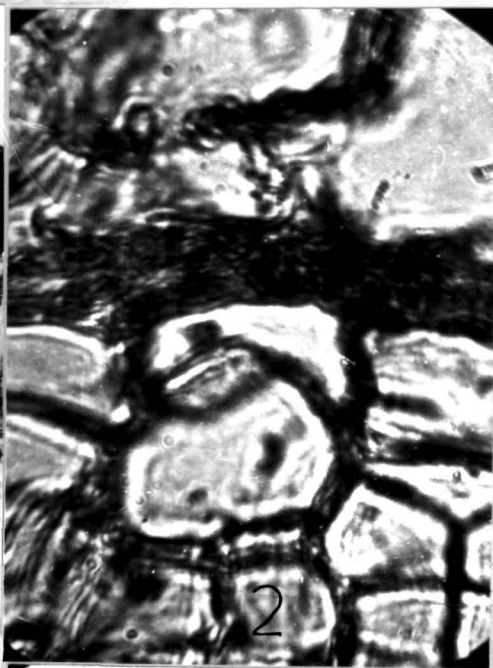
Fig.1 = Loose fungal mantle (M) (Cenococcum graniforme),

Fig.2 = Compact fungal sheath (Lactarius deliciosus),

Fig.3 = Hartignet and mantle of Boletus edulis and

Fig.4 = Loose mantle and nutrient discharge (Amanita verna).

Magnification x 400.

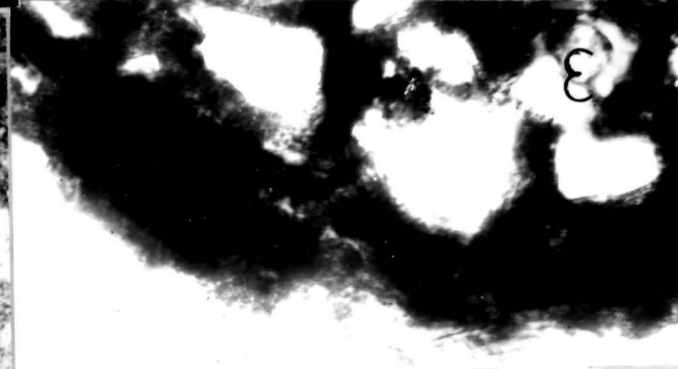
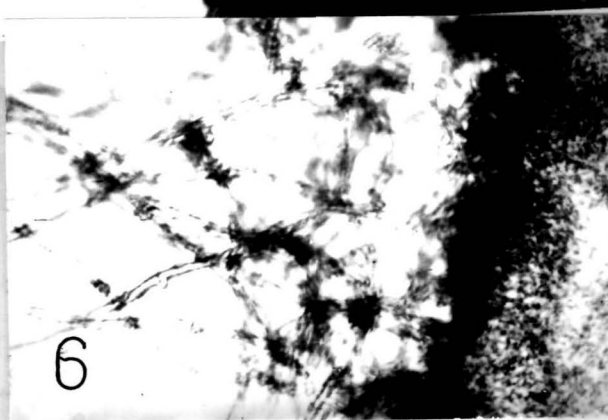
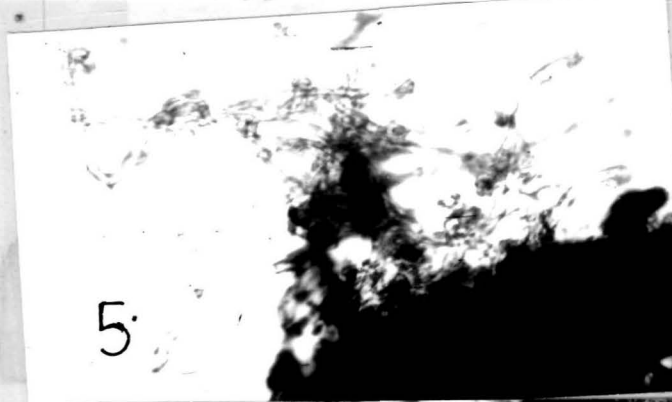
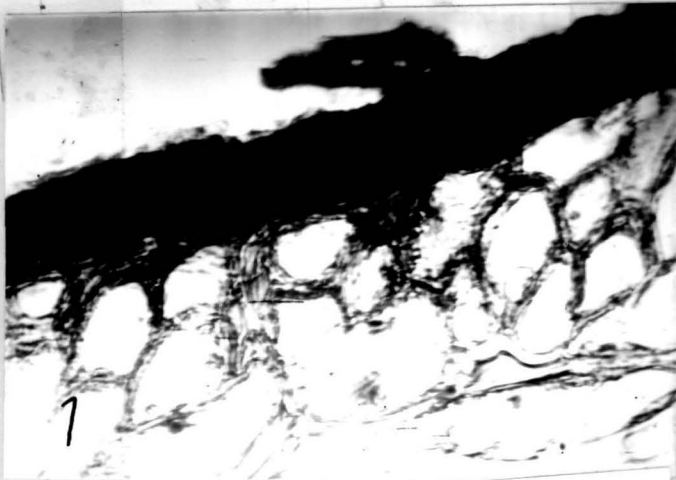


Explanation of Plate 11B.

Variation in internal morphology of mycorrhizae.

Fig.1 = Compact, smooth mantle (fungi imperfecti, unidentified), Fig.2 = Thick mantle and Hartig net, Fig.3 = Routh mantle (Amanita sp), Fig.4 = Loosely arranged mantle (Tricholoma sp), Fig.5 = Thick black, compact with extending basidiomycetes mycellium (Inocybe sp) Fig.6 = Loosely interwoven mantle with extending basidiomycetous mycellium (Scleroderma geaster). Magnification x 400.

PLATE -11B



Synthesis of mycorrhizae of Pinus kesiya Royle in pot cultures under natural conditions

Amanita sp., Boletus edulis and Scleroderma aurantium were found to synthesize mycorrhizal association after one month of germination. Lactarius deliciosus and Russula lutea synthesized mycorrhiza after two months of seed germination. After establishment of mycorrhizal symbiont with the roots of pine a steady increase in the fungi was observed. Scleroderma aurantium and Boletus edulis induced the maximum mycorrhizal roots (Table 11). After six months of harvesting of the seedlings 78, 81 and 82 percent of mycorrhizae were synthesized with Lactarius deliciosus, Amanita sp. and Boletus edulis respectively. Russula lutea and Scleroderma aurantium stimulated 83 and 86 percent of mycorrhizal association with the seedlings (Table 8). All the fungal species produced ectomycorrhizae. In spite of best precautions some of the roots in case of control set (uninoculated) also exhibited mycorrhizal development. The percentage infection in control, however, was only 8%. Uninoculated seedlings, exhibited very poor type of ectomycorrhiza (Table 8).

Different mycorrhizal fungi were responsible for different degree of survivality of the seedlings

(Table 9). Highest survivality of pine seedlings was observed in the post inoculated with Scleroderma aurantium. As compared to uninoculated (control) seedlings, mycorrhizal seedlings exhibited very low degree of mortality.

Inoculation of mycorrhizal fungi affected the growth of pine seedlings. Some of the fungi i.e. Russula lutea and Amanita sp. stimulated the stem growth which was almost double to that of uninoculated (control) seedlings. In all the sets mycorrhiza favourably affected the growth of stem (Table 10). It was found that the production of mycorrhizal roots also was much higher in inoculated seedlings than that of corresponding uninoculated control sets (Table 11). The maximum mycorrhizal roots per seedlings were observed in Scleroderma aurantium followed by Lactarius deliciosus. In uninoculated seedlings very few mycorrhizal roots were observed (Table 11).

Besides stem and roots, leaf production, size of leaf and photosynthatate pigments were also affected by the mycorrhizal fungi (Table 12, Plate 27, 28 and 29). Also the inoculated seedlings possessed higher number of needles. The maximum needles were produced in the sets inoculated with Lactarius deliciosus and

Russula lutea. Uninoculated (control) seedlings generally possessed less number of needles, and they were small in size and yellow in colour. Boletus edulis stimulated the maximum amount of total chlorophyll production and it was followed by Russula lutea (Plate 29). Uninoculated sets could synthesize the lowest amount of chlorophyll a, chlorophyll b and total chlorophyll (Plate 27 and 29). The maximum production of photosynthetic pigments i.e. chlorophyll a and chlorophyll b was obtained in the seedlings inoculated with Scleroderma aurantium and Boletus edulis. In comparison to other mycorrhizal fungi Lactarius deliciosus could stimulate the synthesis of chlorophyll a and chlorophyll b least.

Biomass production was also influenced by mycorrhizal fungi. Above ground and below ground dry matter production was higher in inoculated sets. The maximum above ground biomass was noticed in the sets inoculated with Amanita sp. and it was followed by Lactarius deliciosus. Below ground biomass was maximum in the seedlings inoculated with Russula lutea. The maximum total biomass was observed in case of Lactarius deliciosus set and it was followed by Boletus edulis. Uninoculated sets exhibited nearly half total biomass production in comparison to Lactarius deliciosus production of above

ground and below ground biomass was very poor in uninoculated seedlings (Table 13).

Amongst the type of ectomycorrhizal association, bifurcate and coralloid types were found to be dominant with the seedlings of Pinus kesiya Royle (Table 14). In all the inoculated sets development of mantle was common which was conspicuously absent from noninoculated plants. The colour of mantle varied in case of different fungal symbionts, i.e., yellowish white, white, brownish white, and white type of mantle was produced with Boletus edulis, Scleroderma aurantium, Lactarius deliciosus, Russula lutea and Amanita sp. respectively. Lactarius deliciosus and Russula lutea harboured smooth mantle, whereas Boletus edulis exhibited both i.e. smooth and rough types of mantle. Mycelium was observed around the mycorrhizae in inoculated sets while uninoculated sets lacked the development of any mycelium in root region (Table 14).

Statistical analysis of data for analysis of variance exhibited that mycorrhizae development and dry matter production was significantly influenced by fungal symbiont treatment (Table 16 and 18).

Table 8. Development of fungal symbiotic association in pine seedlings with inoculated mycorrhizal fungi in pots.

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Mycorrhizal fungi	Percentage of symbiotic association after germination						Kind of symbiotic association
	1	2	3	4	5	6	
<u>Amanita</u> sp.	13	27	41	54	67	81	Ectomycorrhizae
<u>Boletus edulis</u>	15	24	39	56	71	82	"
<u>Lactarius deliciosus</u>	-	13	30	53	64	78	"
<u>Russula lutea</u>	-	14	29	51	68	83	"
<u>Scleroderma aurantium</u>	16	28	41	56	70	86	"
Control	-	-	-	3	5	8	

Table 9. Survival of pine seedling in response to mycorrhizal infection (after six months).

Mycorrhizal fungi	Survival (%)	Mortality (%)
<u>Amanita</u> sp.	95.0	5.0
<u>Boletus</u> <u>edulis</u>	97.3	2.7
<u>Lactarius</u> <u>deliciosus</u>	96.2	3.8
<u>Russula</u> <u>lutea</u>	95.6	4.4
<u>Scleroderma</u> <u>surfantius</u>	98.1	1.9
Control	76.7	23.3

Table 10. Mean height (cm.) of pine seedlings (P. kesiva) in response to inoculation of mycorrhizal fungi.

Mycorrhizal fungi	Months after germination					
	1	2	3	4	5	6
<u>Amanita</u> sp.	3.2	3.5	4.5	5.4	6.3	7.98
<u>Boletus</u> <u>lutea</u>	2.7	3.3	4.0	4.9	6.0	7.2
<u>Lactarius</u> <u>deliciosus</u>	2.9	3.2	4.1	5.1	6.3	7.61
<u>Russula</u> <u>lutea</u>	3.1	3.6	4.3	5.2	6.4	8.33
<u>Scleroderma</u> <u>aurantium</u>	2.8	3.2	4.2	5.1	6.2	7.44
Control	2.8	3.3	3.7	3.9	4.1	4.55

Table 11. Mean main root length (cm) and total mycorrhizal roots/plant after harvest in relation to inoculation of mycorrhizal fungi.

Mycorrhizal fungi	Root length (cm.)	Mycorrhizal roots/seedlings	Ratio of mycorrhizal roots and main root
<u>Amanita</u> sp.	38	68	0.56
<u>Boletus edulis</u>	39	88	0.44
<u>Lactarius deliciosus</u>	41	91	0.45
<u>Russula lutea</u>	40	70	0.57
<u>Scleroderma aurantium</u>	42	132	0.32
Control	31	8	3.87

Table 12. Mean leaf number per pine seedlings in response to inoculation of mycorrhizal fungi.

Mycorrhizal fungi	Months after inoculation					
	1	2	3	4	5	6
<u>Amanita</u> sp.	19	33	46	61	89	129
<u>Boletus edulis</u>	17	33	50	63	84	120
<u>Lactarius deliciosus</u>	13	32	63	82	105	135
<u>Russula lutea</u>	14	34	49	64	98	134
<u>Scleroderma aurantium</u>	16	36	51	73	107	132
Control	14	31	48	67	77	84

Table 13. Effect of mycorrhizal fungi on dry matter production of P. kesiva
(after six months of harvesting).

Mycorrhizal fungi	Dry matter		
	Above ground (mg/seedlings)	Below ground	Total
<u>Ananita</u> sp.	154.21	93.7	247.91
<u>Boletus edulis</u>	139.09	148.8	287.89
<u>Lactarius deliciosus</u>	142.08	149.5	291.58
<u>Russula lutea</u>	124.28	152.0	276.28
<u>Scleroderma aurantium</u>	124.61	90.6	215.2
Control	94.23	66.9	160.13

Table 14. Ectomycorrhizal characteristics in response to mycorrhizal fungi inoculated in pine seedlings in pot cultures.

Mycorrhizal fungi	Mantle	Colour	Surface	Type of mycorrhiza	Mycelium/Rhizomorp	Colour
<u>Amanita</u> sp.	+	White	Smooth	Bifurcate	+	White
<u>Boletus edulis</u>	+	Yellowish	Smooth	Bifurcate long few sur- rounded with mycelium	+	Yellowish
<u>Lactarius deliciosus</u>	+	White	Smooth	Bifurcate small 1-2 corralloid	+	White
<u>Russula lutea</u>	+	Brownish	Smooth	Bifurcate & racemose	+	White
<u>Scleroderma aurantium</u>	+	Yellowish	Rough	Bifurcate, corralloid	+	Yellowish
Control	-	-	Rough	Bifurcate	-	-

Table 15. Analysis of variance for stem height of pine seedlings.

Source of variation	DF	SS	MSS	Calculated value	Table value	
					5%	1%
Sampling period	5	80.46	16.09	94.6	2.60	3.86
Treatments	5	8.21	1.64	9.64	2.60	3.86
Error	25	4.38	0.17			
Total	35					

Table 16. Analysis of variation for mycorrhizal development in pine seedlings.

Source of variation	DF	SS	MSS	Calculated value	Table value	
					5%	1%
Sampling period	5	61265.33	1225.3	33.78	2.60	3.86
Treatments	5	3186.45	637.29	1.75	2.60*	3.86**
Error	25	9066.55	362.66			
Total	35					

* Significant at 5% level.

** Significant at 1% level.

Table 17. Analysis of variation for leaf production in pine seedlings in response to mycorrhizal fungi inoculations.

Source of variation	DF	SS	MSS	Calculated value	Table value 5%	Table value 1%
Sampling period	5	1227.47	245.49	3.19	2.60	3.86**
Treatments	5	45924.47	9184.89	119.4	2.60	3.86
Error	25	1923.70				
Total	35					

** Significant at 1% level.

Table 18. **Analysis of variance for dry matter production in relation to mycorrhizal fungi inoculation to pine seedlings.**

Source of variation	DF	SS	MSS	Calculated value	Table value	
					5%	1%
Sampling period	2	-119538.71	119538.71	2.41	6.61*	16.26**
Treatments	5	-127625.11	2545.0	0.05	2.60*	3.86**
Error	5	-247165.82	49432.76			
Total	11					

* and ** significant at 5% level and at 1% level respectively.

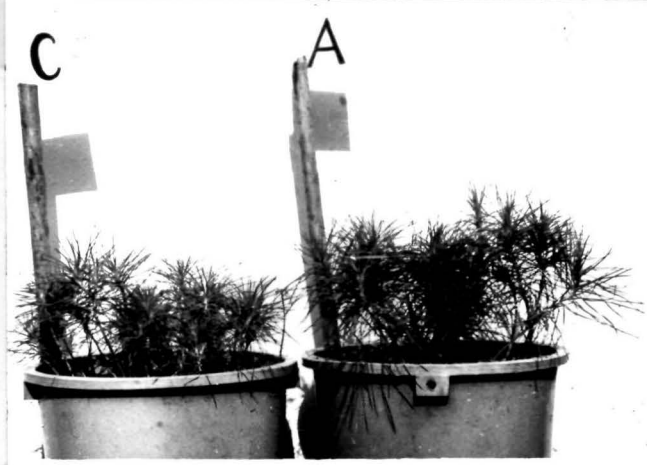
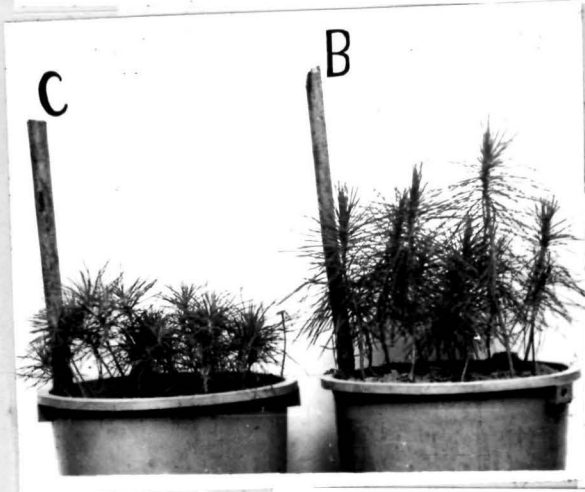
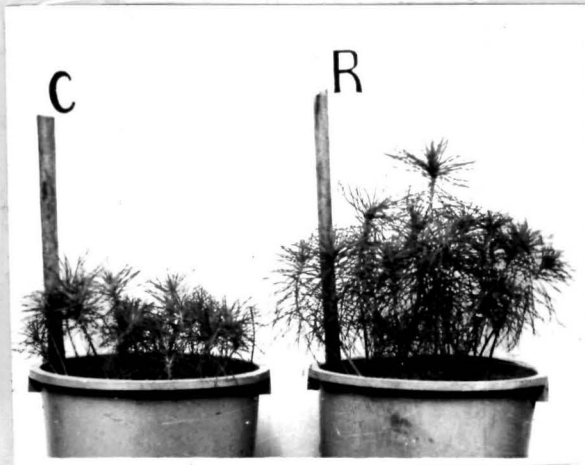
Table 19. Analysis of variation for leaf production in pine seedlings in response to mycorrhizal fungi inoculations.

Source of variation	DF	SS	MSS	Calculated value	Table value 5%	Table value 1%
Sampling period	5	1227.47	245.49	3.19	2.60	3.86**
Treatments	5	45924.47	9184.89	119.4	2.60	3.86
Error	25	1923.70				
Total	35					

** Significant at both 5% and 1% level.

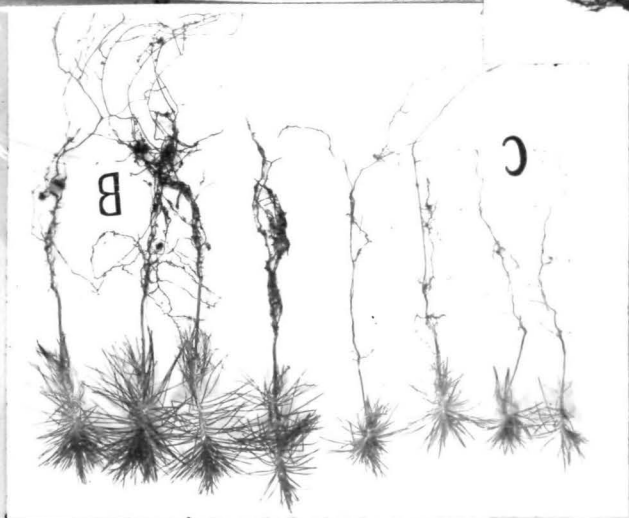
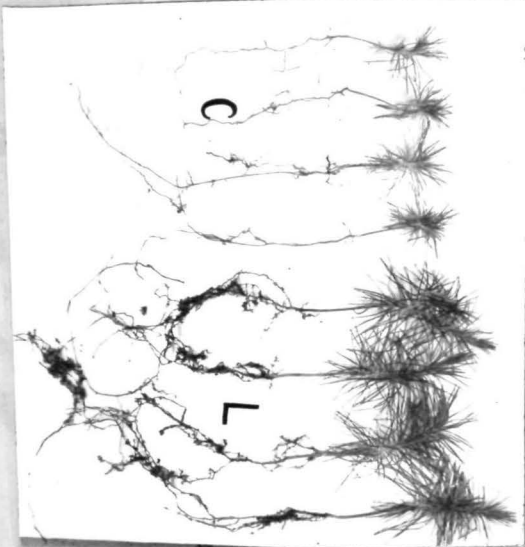
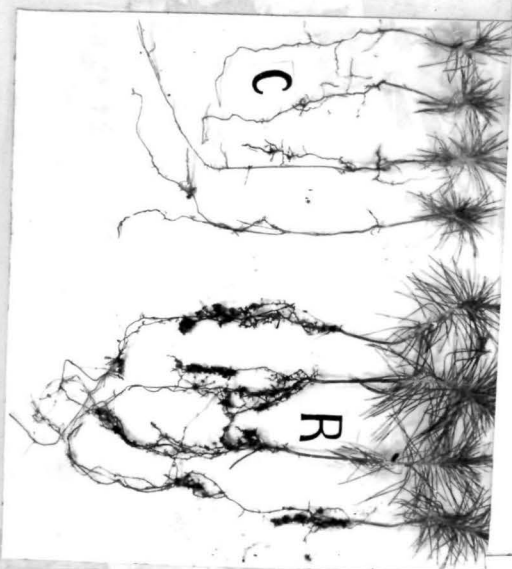
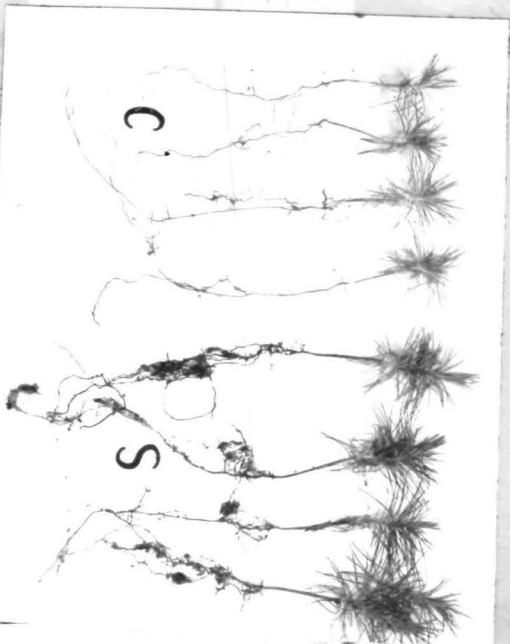
Explanation of Plate 12.

Effect of mycorrhizal fungi on growth of pine seedlings in pot cultures. (A = Amanita sp, B = Boletus edulis, L = Lactarius deliciosus, R = Sussula lutea, S = Scleroderma aurentium and C = Control).



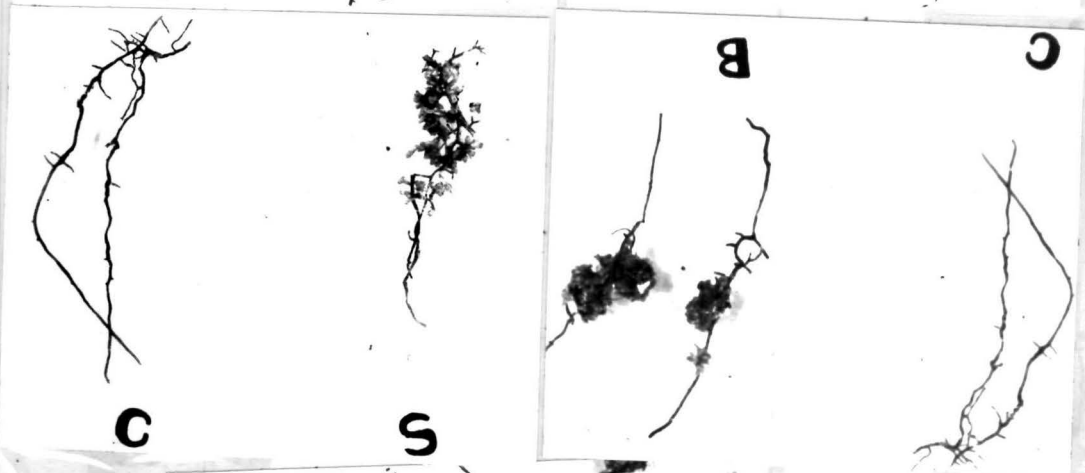
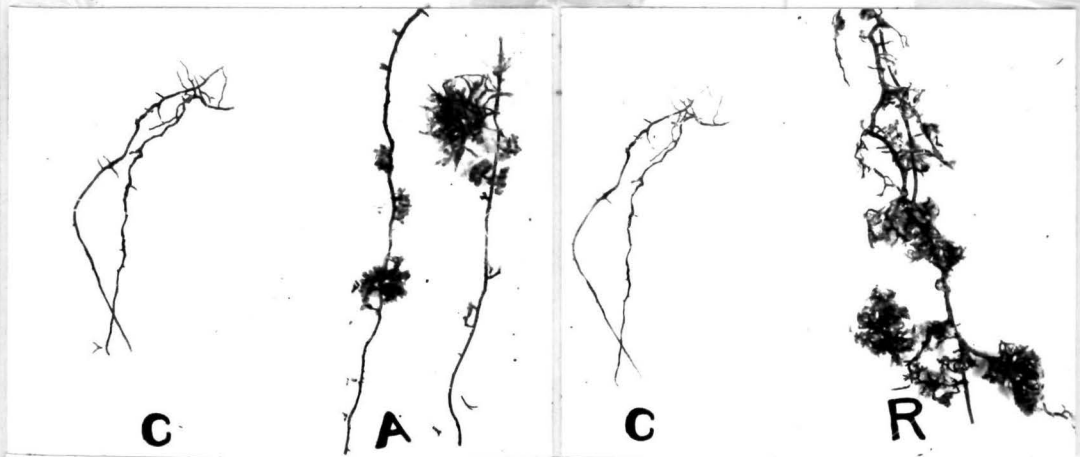
Explanation of Plate 13.

Showing the effect of mycorrhizal fungi inoculation on root production. (Abbreviations are explained in Fig. 12).



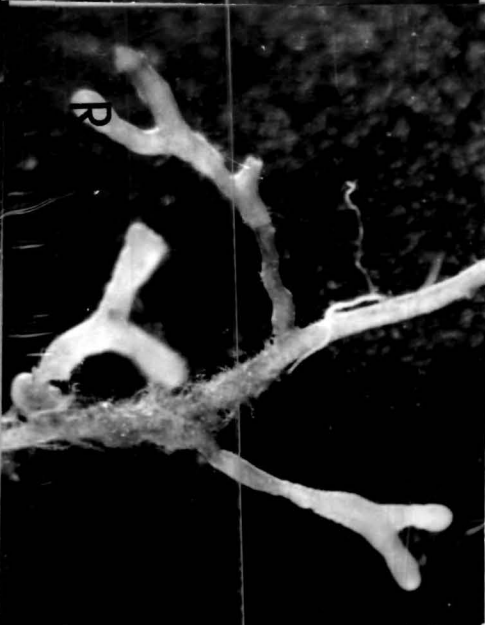
Explanation of Plate 14.

Showing the root morphology caused by different
fungal symbiont inoculation in pot culture
studies (Abbreviations are explained in Fig. 12).



Explanation of Plate 15.

Showing different mycorrhizal roots produced by
different fungal symbionts in pots. (Abbreviations
are explained in Plate 12).



Synthesis of mycorrhizae in pure culture on agar.

Ectomycorrhizae of Amanita sp.

Appearance of first ectomycorrhiza was recorded after three months of the inoculation. The number of mycorrhizae formed was low. Mainly monopodial branches developed. Swollen tip of mycorrhiza was covered with white fungal mantle. The mantle was short, continuous and compact. At the end of experiment, the average mycorrhiza per seedling was 20 (Fig.1, Plate 16).

Ectomycorrhizae of A. verna.

The first mycorrhizal formation was observed after two and a half months since inoculation. The development of mycorrhiza in this set was slightly faster and at the end of experiment an average of 42 mycorrhiza per seedling was recorded. Mycorrhizae were dichotomously branched. Bifurcation started at short distance of rootlet and was simple. The whole mycorrhizal rootlet was enclosed in loosely inter-woven fungal sheath. The mantle was white in colour and provided smooth appearance to mycorrhiza. The mycorrhizae were produced both above and within the substrate (Fig. 2, Plate 16).

Ectomycorrhizae of A. mappa.

The appearance of first mycorrhiza was recorded after three months since inoculation and it

successively increased reaching to an average number of 60 per seedling at the end of the experiment.

Mycorrhizae were very simple, with short bifurcations. The branching occurred slightly away from the main root. The mantle was brownish in colour (Fig. 3, Plate 16).

Ectomycorrhizae of Boletus edulis.

First mycorrhiza was noted after one month since inoculation and the number of mycorrhiza increased rapidly. The repeated bifurcate dichotomous branching resulted in a coral like forms. At the end of experiment number of mycorrhizae amounted upto 120 per seedling. The mantle was white in the beginning which changed to cream colour subsequently. The thin rhizomorph type of structures were also observed. The coral forms mycorrhizae were enclosed in loosely fine mycelium (Fig.4, Plate 16).

Ectomycorrhizae of B. luteus.

The first occurrence of mycorrhiza was noted after one month of the inoculation. The production of mycorrhiza increased rapidly towards the end of experiment. The mycorrhizal rootlets previously grew without any coverage of mantle but at later stage whole rootlet system was covered with light creamy coloured

mantle. Mycorrhizae were mostly produced aeri-ally but few were also observed in the substrate. No regular branching was followed at its initial stage but later on dichotomous branches were observed. At the liquidation of experiment the number of mycorrhiza amounted to 120 per seedling (Fig.1, Plate 17).

Ectomycorrhizae of Cenococcum graniforme

The appearance of first mycorrhiza was noticed after one and a half months since inoculation. The number gradually increased and reached the maximum at the time of liquidation of the experiment. The mycorrhizae were simple generally unbranched but few dichotomous bifurcate branches were also observed. These were formed throughout the root system. The black characteristic mantle with radially extended hyphae covered the rough surfaced mycorrhiza. Sometimes this mantle covered only apical portions but reached upto the base of mycorrhiza. The total number at the end of experiment was 210 per seedling (Fig. 2, Plate 17).

Ectomycorrhizae of Clitocybe gigantea

The appearance of first mycorrhiza was noticed after two and a half months since inoculation. Gradual increase and development of mycorrhiza amounted

to 80 per seedling at the final phase of experiment. Lack of dichotomous branches in this set was a characteristic feature. Mycorrhizal roots were simple, small in length, and appeared without disruption in mantle. It was also noticed that long lateral branches got converted into mycorrhiza maintaining the continuity of mantle. The mantle was white in colour. Mycorrhizae were distributed on the whole root system (Fig. 3, Plate 17).

Ectomycorrhizae of *Cortinarius mucosus*.

The first mycorrhiza appeared after three months since inoculation. Mycorrhizae were produced both in agar substrate and in aerial portion. The number at the end of experiment reached upto 70 per seedling. Irregular branching was characteristic feature and pure dichotomy was lacking. The simple, elongated mycorrhizae were slightly thick with yellowish mantle (Fig. 4, Plate 17).

Ectomycorrhizae of *Inocybe rimosa*.

The appearance of first mycorrhiza was observed after three and a half months since inoculation. The growth and development of mycorrhiza was gradual. The characteristic long dichotomously bifurcated mycorrhizal

rootlets provided a coral like form (Fig. 1, Plate 3). The tip of rootlets was covered with thin loose white yellowish mycelium. These mycorrhizae were more on middle portion of root and at the end of the experiment total average number was accounted to 70 per seedling. The mantle was smooth (Fig. 1 and 2, Plate 18).

Ectomycorrhizae of *Lactarius* sp.

Mycorrhizal formation was first observed after three and a half months since inoculation. The number of mycorrhiza amounted on average to 90 per seedling at the end of the experiment. Both type of mycorrhizae i.e. unbranched and dichotomously branched were observed (Fig. 3, Plate 3) (Fig. 4, Plate 3). The tips of these rootlets were swollen and enclosed into a mantle. The colour of the mycorrhizal roots was light brown and the mycorrhizae were distributed on the whole root system. The mycelium was attached at base and also enveloped to short dichotomous branches (Fig. 3 and 4, Plate 18).

Ectomycorrhizae of *L. deliciosus*.

The first mycorrhiza appeared after four months since inoculation. Mycorrhizae were formed mostly on aerial portion along with some in agar substrate. Long simple

dichotomous branches with broad bifurcation along with swelling apical portions of the branches were observed. The basal portion of rootlets was surrounded by a mat of mycelium of whitish colour. Mantle surface was smooth and glossy (Fig. 1, Plate 19). The average number of mycorrhiza at the time of liquidation of experiment amounted to 90 per seedling.

Ectomycorrhizae of Rhizopogon luteolus

The appearance of first mycorrhiza was noted after one and a half months of the inoculation. The mycorrhizae which at the beginning were noticed at upper portion spread later on the entire root system. These were dichotomously bifurcated and simple monopodial. Multiple bifurcation was not observed. Mycorrhizae were short, covered with compact white mantle which provided thin rhizomorph like structures upto the base of rootlets (Fig. 2, Plate 19). Brown droplets were also observed at the tip of well developed mycorrhiza. The total number amounted to 115 per seedling at the end of investigation.

Ectomycorrhizae of Russula sp.

The first mycorrhiza was recorded after four months since the inoculation. The mycorrhizae were simple unbranched elongated and light brown in colour. The white

coloured mantle covered the whole rootlet. The mycorrhizal production increased steadily and accounted to 42 per seedling at the end of experiment. The thin white mycelium extended from rootlets provided somewhat glossy appearance to it. These were mostly produced in upper and middle part of root system (Fig. 3, Plate 19).

Ecotomycorrhizae of R. delica.

The appearance of first mycorrhiza was noted after four and a half months of inoculation. The gradual increase in mycorrhizae attained an average of 30 per seedling at the time of liquidation of experiment. The production of mycorrhiza was mainly confined to upper aerial portion of root system. Mycorrhizae were long elongated, simple, dichotomously branched. The apices of long dichotomy were again bifurcated into shortened thin segments. At juvenile stage of mycorrhiza the colour of mantle was white turning to light brown in old ones. Loose attached mycelium was also observed at the base of rootlets (Fig. 4, Plate 19).

Ecotomycorrhizae of Suillus bovinus.

The first formation of mycorrhiza was noticed after one month since inoculation. Simple shortened

dichotomous, bifurcated mycorrhizae were formed. These were covered with massy creamy coloured white mycelium (Fig. 1, Plate 20). The bifurcated branches possessed the tendency of multiple branching and due to the swelling of apical tips they gave an appearance of bulbous forms. The mantle was light brownish at later stage. The mycorrhizae were distributed on all over the root system and attained an average number to 180 per seedling.

Ectomycorrhizae of *Scleroderma aurentium*.

The appearance of first mycorrhiza was recorded after two months since inoculation. The mycorrhizae increased gradually and reached upto an average of 160 per seedling at the end of experiment. The mycorrhizae were simple, dichotomously bifurcated with short depression of apical end which was slightly swollen (Fig. 2, Plate 20). The rootlets possessed a light yellowish mantle and frequently occurred at aerially and also in the substrate throughout the entire root system.

Ectomycorrhizae of *Tricholoma* sp.

First mycorrhiza was observed after one and half months since inoculation. Occurrence of mycorrhizae

was noticed throughout the entire root system. The mycorrhizae were mainly dichotomously branched with short lobe enclosed in cottony white mycelium (Fig. 3, Plate 20). The frequent repeated branching of rootlets provided an appearance of coral like structure. The colour of mantle was white. The average number of mycorrhiza was accounted to 140 per seedling at the last phase of the experiment.

Ectomycorrhizae of unidentified fungi imperfecti.

The first mycorrhizal appearance was noticed after one month of inoculation. The number of mycorrhiza increased gradually and attained an average number of 130 per seedling. Both monopodial to dichotomous rootlets were observed (Fig. 4, and 5, Plate 5). The dichotomous mycorrhizae with frequent division provided a structure like coral forms (Fig. 4, Plate 20). The mycelium was loosely attached to the rootlets. The colour of mantle and mycorrhiza was brown.

Table 20. Symbiotic properties of fungi forming ectomycorrhizae with Pinus kesiva Royle in pure culture on agar.

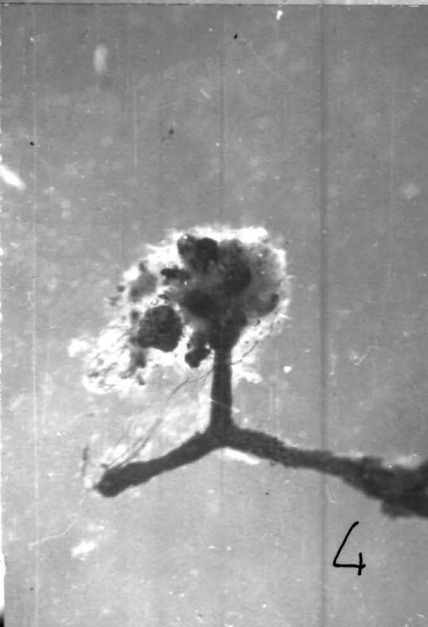
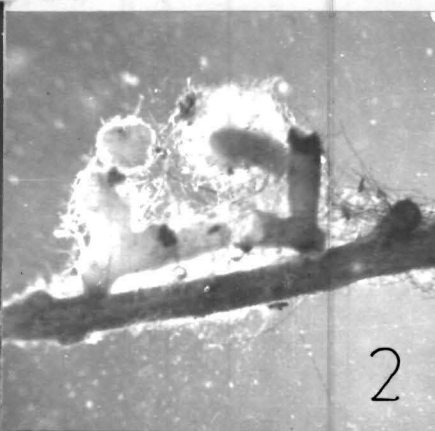
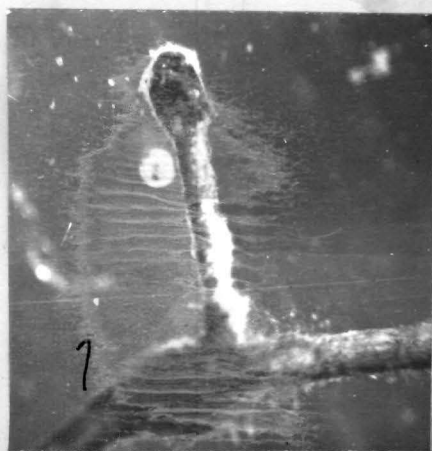
Fungal species	Mycorrhizal activity		Mycorrhizal virulence	Effect on seedling development		Morphological effect on ectomycorrhizae			
				Stem	Root	Decomposed	Shortened	Elongated	Segmented
<u>Amanita</u> sp	++		++	-	S	+	+	-	-
<u>A. verna</u>	++		++	-	-	+	-	+	-
<u>A. mappa</u>	++		++	S	S	+	-	+	-
<u>Boletus edulis</u>	+++		++	S	S	++	+	-	-
<u>B. luteolus</u>	+++		++	S	S	+++	+	-	-
<u>Cenococcum graniforme</u>	+++		++	S	S	-	-	++	-
<u>Clitocybe gigantea</u>	++		++	S	S	+	+	-	-
<u>Cortinarius mucosus</u>	++		++	S	S	+	+	-	-
<u>Inocybe rimosa</u>	+++		++	-	-	+	-	+	-
<u>Lactarius</u> sp	++		++	S	S	++	+	-	-
<u>L. deliciosus</u>	++		++	S	S	++	+	-	-
<u>Rhizopogon luteolus</u>	+++		++	S	S	++	+	-	-
<u>Russula</u> sp	+		++	S	S	+	+	-	-
<u>R. delica</u>	+		++	S	S	++	+	-	-
<u>Suillus bovinus</u>	+++		+++	S	S	++	+	-	-
<u>Scleroderma aurentium</u>	+++		++	S	S	++	+	-	-
<u>Tricholoma</u> sp	+++		++	S	-	++	+	-	-
<u>Fungi imperfecti</u>	+++		++	S	S	-	+	-	-

Table 21. Results of mycorrhizal synthesis of *Pinus kesiya* with fungal symbionts.

Fungal species	Developments of components in synthesis sets (mean values)				
	Fungus mycelium aerial	Sub- strate	Stem height (cm)	Leaf produc- tion	Seedlings Length of root (cm) Mycorrhizae
<i>Amanita</i> sp	-	+	13.5	90	24.2
<i>A. verna</i>	+	+	13.0	94	23.0
<i>A. mapae</i>	-	+	14.0	90	24.0
<i>Boletus edulis</i>	+	+	15.2	105	26.0
<i>B. luteolus</i>			14.2	102	24.6
<i>Cenococcum graniforme</i>	+	+	13.6	96	24.1
<i>Clitocybe gigantea</i>	+	+	14.6	103	24.6
<i>Cortinarius mucosus</i>	+	+	13.8	95	22.8
<i>Inocybe rimosa</i>	-	+	15.2	103	25.0
<i>Lactarius</i> sp	+	-	15.0	107	26.3
<i>L. deliciosus</i>	+	-	13.6	96	25.6
<i>Rhizopogon luteolus</i>	+	+	16.0	108	24.3
<i>Russula</i> sp	+	+	15.8	102	26.0
<i>R. delica</i>	+	-	15.4	106	25.6
<i>Suillus bovinus</i>	+	+	14.8	96	26.2
<i>Scleroderma aurentium</i>	+	+	13.2	95	23.8
<i>Tricholoma</i> sp.	+	-	14.6	95	23.0
<i>Pungi imperfecti</i>	+	-	15.0	103	24.8

Explanation of Plate 16.

- Fig. 1 : Ectomycorrhiza of Pinus kesiya with Amanita sp.
in Vivo - above the substrate - monopodial
branch with swollen tip.
- Fig. 2 : Ectomycorrhiza of P. kesiya with A. mapps in
Vivo - following preservation.
- Fig. 3 : Ectomycorrhiza of P. kesiya with A. verna in
Vivo - mycelium enclosed dichotomous branches
and also mother branch - following preservation.



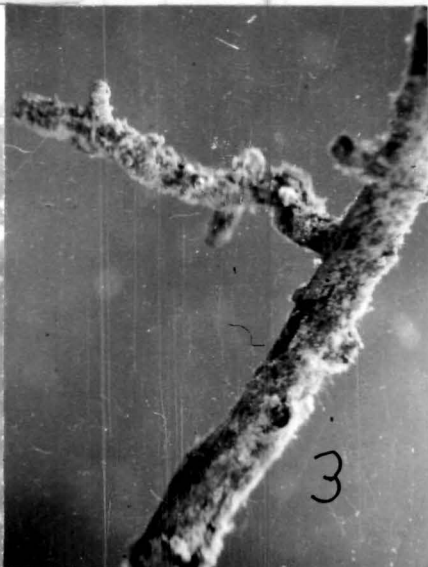
Explanation of Plate 17.

Fig. 1 : Ectomycorrhizae of Pinus kesiya with B. luteus in Vivo - following preservation.

Fig. 2 : Ectomycorrhizae of P. kesiya with Cenococcum graniforme in Vivo - following preservation surrounded with black characteristic mycelium.

Fig. 3 : Ectomycorrhizae of P. kesiya with Clitocybe gigantea in Vivo - following preservation monopodial branching.

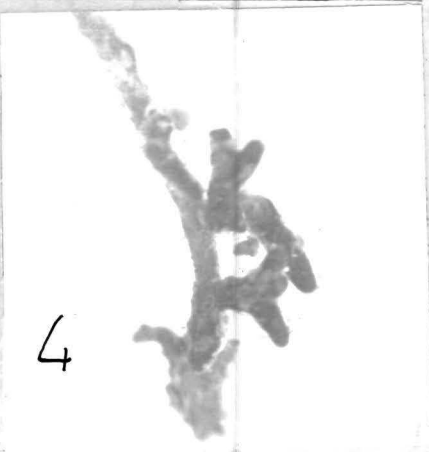
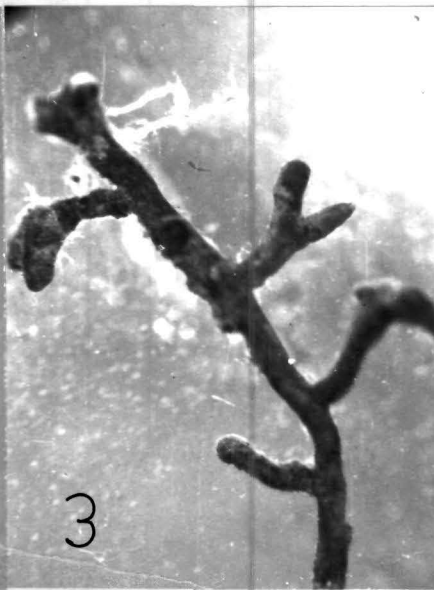
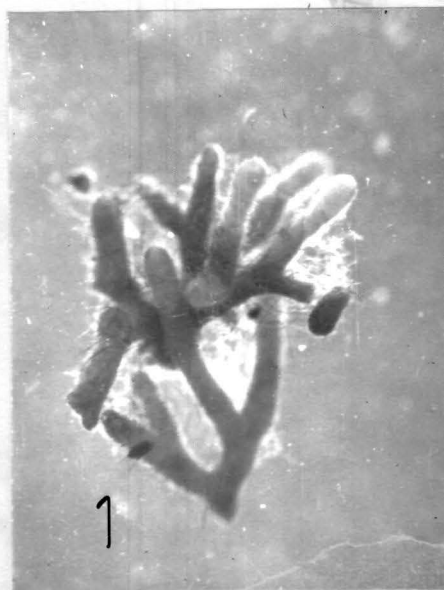
Fig. 4 : Ectomycorrhizae of P. kesiya with Cortinarius mucosus in Vivo - following preservation.



Explanation of Plate 18.

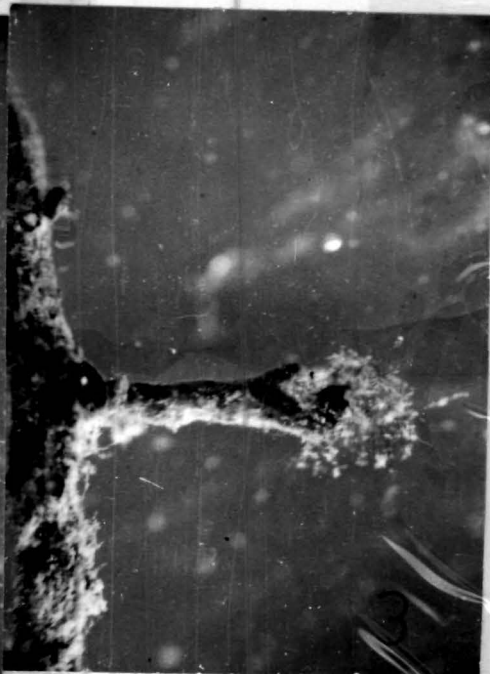
Fig. 1 & 2 : Ectomycorrhizae of Pinus kesiya with Inocybe rimose in Vivo - above the substrate (1) and following the preservation (2), mycelium attached to dichotomy.

Fig. 3 & 4 : Ectomycorrhizae of P. kesiya with Lactarius sp. in Vivo - in substrate (3) and following to preservation (4).



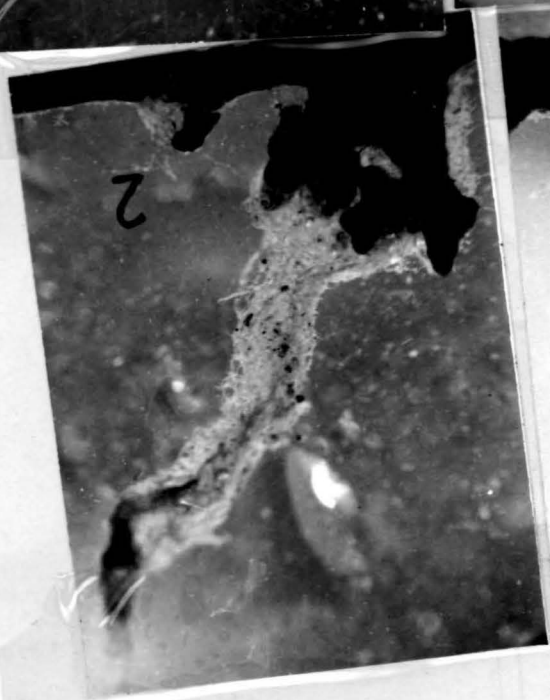
Explanation of Plate 19.

- Fig. 1 : Ectomycorrhizae of Pinus kesiya with Lactarius deliciosus in Vivo - dichotomous branches with mycelium attachment following preservation.
- Fig. 2 : Ectomycorrhizae of P. kesiya with Rhizopogon luteolus in Vivo - following to preservation, both mono and bipodial branches.
- Fig. 3 : Ectomycorrhizae of P. kesiya with Russula sp. in Vivo - above the substrate.
- Fig. 4 : Ectomycorrhizae of P. kesiya with R. delica in Vivo - large and young dichotomy.



Explanation of Plate 20.

- Fig.1 : Ectomycorrhizae of Pinis kesiya with Scleroderma aurentium in Vivo - repeated dichotomous branching.
- Fig.2 : Ectomycorrhizae of P. kesiya with Suillus bovinus in Vivo - young dichotomy with long mycelium mass.
- Fig.3 : Ectomycorrhizae of P. kesiya with Tricholoma sp. in Vivo - Coral like form enclosed in mycelium.
- Fig.4 & 5: Ectomycorrhizae of P. kesiya with an unidentified fungi imperfecti in Vivo - Coral like form (1) and monopodial branch (2) in later case (2) mother branch transforming into mycorrhizal branch.



DISTRIBUTION OF FUNGI IN NONRHIZOSPHERE (NR),
RHIZOSPHERE (RS) AND RHIZOPLANE (RP) REGION OF
MYCORRHIZAL ROOTS.

Ninety nine fungal species were isolated from all three regions of mycorrhizal roots. Nonrhizosphere, rhizosphere and rhizoplane regions harboured seventy eight, eighty two and forty four fungal species respectively (Table 24). Nonrhizosphere region comprised of thirteen phycomycetes, four ascomycetes, fifty eight deuteromycetes and three sterile forms. From rhizosphere region, fifteen, five, sixty and two fungal species belonging to phycomycetes, ascomycetes, deuteromycetes and sterile fungi respectively were isolated. Eleven, one, thirty, one and one fungal species belonging to phycomycetes, ascomycetes, deuteromycetes, sterile form and basidiomycetes were recorded from rhizosphere region (Table 22). Members of deuteromycetes, however, were always highest in occurrence.

The maximum number of species was recorded from rhizosphere region. Rhizoplane region harboured the lowest number which was primarily because of the fall in the number of deuteromycetes (Table 24).

Nonrhizosphere was dominated by Absidia, Mucor, Trichoderma, Penicillium, Cladosporium and

Fusarium, Absidia, while Trichoderma, Penicillium and Cladosporium appeared dominantly in rhizosphere region also. Rhizoplane region was dominantly associated with Trichoderma and Penicillium species (Table 24).

Certain fungal species, viz. Absidia cylindrospora, Mucor hiemalis, Pythium sp., Trichoderma viride, Penicillium lilacinum, P. chrysogenum, Acremonium sp. and Verticillium sp. were frequently recorded from nonrhizosphere region (Table 24).

Absidia corymbifera, A. cylindrophora, Cunninghamella elegans, Ascomycete (?), Phoma sorghina, Trichoderma viride, Aspergillus niger, A. tamarii, Penicillium brefeldianum, P. chrysogenum, P. citrinum, P. cyclopium var. echinulatum, P. fellutianum, P. frequentans, P. implicatum, P. lanthenellum, P. lilacinum, P. oxalicum, P. rubrum, P. verrucosum var. cyclopium, Acremonium sp., Verticillium sp., Cladosporium oxysporum, Fusarium sp. and white sterile form were isolated from all the three, i.e. NR, RS and RP regions of mycorrhizal roots (Table 24). The highest fungal species in non-rhizosphere (NR), rhizosphere (RH) was recorded in July while in rhizoplane (RP) highest number was observed in October (Table 25).



Few fungal species i.e. Ulocladium charturum, Memnoniella echinata, Phialophora fastigiata, Metarhizium anisopliae, Curvularia lunata, Trichurus spiralis, Coniothyrium sp. and yellow sterile forms were restricted to nonrhizosphere only. Neurospora sitophila, Aspergillus aculeatus, A. nidulans, Penicillium fumosoroseus, Eupenicillium sp, Mycogone perniciosa, Cladosporium herbarum, Bauveria bassiana and Epicoecum purpurascens could be recorded only from the rhizosphere region. Aspergillus (glaucus group), Cladosporium verticillatum, Pleochaeta sp, Fusarium arthrosporioides, Nectria haematococca and basidiomycete (?) forms confined to rhizoplane only (Table 24).

The highest fungal population in nonrhizosphere, rhizosphere and rhizoplane regions was recorded in the month of June (1977) and in July in following year (1978), (Plate 21 and Plate 22).

A definite pattern of variation in bacterial population (expressed in per g dry weight of soil in NR and R²S regions and per segment for RP region) was observed in three regions of mycorrhizal roots. Bacterial population in rhizosphere was generally higher than nonrhizosphere and rhizoplane regions except in May (1977) when nonrhizosphere harboured larger bacterial population

than rhizosphere (Plate 23 and 24). The highest bacterial population in NR, RS and RP regions was observed during August (1977) and July (1978), (Plate 23 and 24).

The maximum actinomycetes population was obtained in July from all the three regions (NR, RS and RP) during the course of investigation (Plate 25 and 26).

Statistical analysis of data revealed a significant variance between total fungal actinomycetes and bacteria with sampling period (Table 26, 27, 29, 30, 31 and 32). Significant analysis of variance due to nonrhizosphere, rhizosphere and rhizoplane regions and different taxonomic groups and actinomycetes population was also observed. A positive significant correlation between soil available phosphorus, available potassium and moisture content with fungi of NR, RS and RP regions was obtained (Table 33), while soil organic carbon showed a negative correlation with the bacterial population of NR region. Soil pH, moisture content, available potassium, available phosphorus, temperature and rainfall exhibited a positive correlation with bacteria of NR, RS and RP regions. Significant correlation between actinomycetes and temperature, rainfall, soil pH, moisture content and

available potassium was observed (Table 33). Among nutrient contents of mycorrhizal roots, Nitrogen, Potassium and sugar contents of roots significantly influenced the microbial population (Table 33).

Table 22. **Distribution of total taxonomic groups of fungi in nearrhizosphere (NR)**
rhizosphere (RS) and rhizoplane (RP), (1976-78).

Taxonomic groups	NR	RS	RP
Phycomycetes	25(43)	33(32)	16(15)
Ascomycetes	6(8)	10(14)	- (1)
Basidiomycetes	-	-	- (1)
Deuteromycetes	97(73)	94(94)	45(33)
Mycelia sterila	1(6)	4(4)	3(1)
Total	129(130)	141(144)	64(51)

Figs. outside the bracket for 1976-77 and inside the brackets for 1977-78.

Table 23. Distribution of fungi of different taxonomic groups in nonrhizosphere, rhizosphere and rhizoplane regions.

Taxonomic groups	NR	RS	RP
<u>Phycomycetes</u>			
<u>Absidia</u> spp.	3	3	3
<u>Mucor</u> spp.	3	4	3
<u>Cunninghamella</u> spp.	2	3	2
Other Phycomycetes	5	5	3
<u>Ascomycetes</u>	4	5	1
<u>Deuteromycetes</u>			
<u>Trichoderma</u> spp.	6	6	3
<u>Aspergillus</u> spp.	6	6	4
<u>Penicillium</u> spp.	20	22	12
<u>Cladosporium</u> spp.	3	5	3
<u>Fusarium</u> spp.	5	5	2
Other Deuteromycetes	18	16	6
<u>Mycelia sterilia</u>	0	2	1
<u>Basidiomycetes</u>	-	-	1
	78	82	44

Table 24. Distribution of different fungal species in nonrhizosphere (NR), rhizosphere (RS) and rhizoplane(RP) regions.

Fungal species	NR	RS	RP
<u>Phycomycetes</u>			
<u>Absidia corvmifera</u>	+	+	+
<u>A. cylindrospora</u>	+	+++	+
<u>A. heterospora</u>	+++	+++	+
<u>Rhizopus nigricans</u>	+	+	+
<u>Mucor circinelloides</u>	+	+	+
<u>M. hiemalis</u>	+	+	-
<u>M. racemosus</u>	+++	+	+
<u>M. sp. (racemosus section)</u>	+	+	+
<u>Mortierella sp.</u>	-	+	+
<u>Cunninghamella echinulata</u>	+	+	-
<u>C. elegans</u>	-	+	+
<u>C. sp.</u>	+	+++	-
<u>Syncephalastrum sp.</u>	+	+	-
<u>Pythium sp.</u>	++	+	+
<u>Phycomycete (?)</u>	+	+	+
<u>Ascomycete</u>			
<u>Ghaetomium globosum</u>	+	+	-
<u>Neurospora sitophila</u>	-	+	-
<u>Ascomycetes (?)</u>	+	+	-
<u>Deuteromycetes</u>			
<u>Phomopsis sp.</u>	+	+	-
<u>Phoma sorghina</u>	+	+	+
<u>Petalotiopsis clissliminata</u>	+	+	-
<u>Geotrichum sp.</u>	-	+	+

Fungal species	NR	RS	RP
<u>Trichoderma hamatum</u>	+	+	+
<u>T. harazinum</u>	+	+	-
<u>T. longibrachiatum</u>	+	+	-
<u>T. koningii</u>	+	+++	+++
<u>T. viride</u>	+++	+++	+
<u>T. sp.</u>	+	+	-
<u>Aspergillus sp. (glaucus group)</u>	-	-	+
<u>A. aculeatus</u>	-	+	-
<u>A. fischeri</u>	+	+	-
<u>A. flavus</u>	-	+	-
<u>A. nidulans</u>	+	-	-
<u>A. niger</u>	+	+	+
<u>A. sulphureus</u>	+	-	+
<u>A. tamarii</u>	+	+	+
<u>A. versicolor</u>	+	+	-
<u>Penicillium brefeldianum</u>	+	+++	+
<u>P. brevicompactum</u>	+	+	-
<u>P. chrysogenum</u>	+++	+++	+
<u>P. citrinum</u>	+	+	+
<u>P. corvophilum</u>	+	+	-
<u>P. crustosum</u>	+	+	-
<u>P. cyclopium</u> var. <u>echinulatum</u>	+	+	+
<u>P. daleae</u>	+	+	-
<u>P. fellutianum</u>	+	+	+
<u>P. frequentans</u>	+	+	+
<u>P. fumosoroseus</u>	-	+	-
<u>P. helicum</u>	+	+	-
<u>P. implicatum</u>	+	+	+
<u>P. islandicum</u>	+	+	-

Fungal species	NR	RS	RP
<u>P. janthinellum</u>	+	+	+
<u>P. liliacinum</u>	+++	+	+
<u>P. nigricans</u>	+	+	-
<u>P. oxalicum</u>	+	+	+
<u>P. rubrum</u>	+	+	+
<u>P. variotii</u>	+	+	-
<u>P. verrucosum</u> var. <u>cyclopium</u>	+	+	+
<u>Eupenicillium</u> sp.	+	+	-
<u>Scopulariopsis brevicaulis</u>	+	+	-
<u>Gliocladium roseum</u>	+	-	+
<u>G.</u> sp.	+	+	-
<u>Ulocladium chartarum</u>	+	-	-
<u>Acremonium</u> sp.	++	+	+
<u>Sclerotinia ricini</u> (Botrytis state)	+	+	-
<u>Verticillium</u> sp.	+	+	+
<u>Trichothecium roseum</u>	++	+	-
<u>Mycogone perniciosa</u>	-	+	-
<u>Papularia pullulans</u>	+	+	-
<u>Stachybotrys</u> sp.	-	+	-
<u>Humicola</u> sp.	+	+	-
<u>Mammoniella echinata</u>	+	-	-
<u>Cladosporium cladosporioides</u>	+	+++	-
<u>C. herbarum</u>	-	+	-
<u>C. oxysporum</u>	+	+	+
<u>C. sphaerospermum</u>	+	+	-
<u>C. tenuissimum</u>	-	-	-
<u>C. verticillatum</u>	-	-	+
<u>Phialophora fastigiata</u>	+	-	-
<u>Metarhizium anisopliae</u>	+	-	-
<u>Pleochaeta</u> sp.	-	-	+

Fungal species	NR	RS	RP
<u>Curvularia lunata</u>	+	-	-
<u>Alternaria alternata</u>	+	+	-
<u>Trichurus spiralis</u>	+	-	-
<u>Fusarium acuminatum</u>	+	+	-
<u>F. arthrosporioides</u>	-	-	+
<u>F. moniliiforme</u>	+	+	-
<u>F. oxysporum</u>	+	+	-
<u>F. solani</u>	+	+	-
<u>F. sp.</u>	+	+	+
<u>Coniothyrium sp.</u>	+	-	-
<u>Nectria haematococca</u>	-	-	+
<u>Bauveria bassiana</u>	-	+	-
<u>Epicoccum purpurascens</u>	-	+	-
<u>Mycelia sterilia</u>			
Sterile dem. taceous			
Sterile septate (white)	+	+	+
Sterile (yellow)	+	-	-
Basidiomycetes	-	-	+

+++ Dominant
 ++ Sub dominant
 + Rare
 - Absent

Table 25. Distribution of total number of fungi in the nonrhizosphere (NR) rhizosphere (RS) and rhisoplane (RP) of Pinus kesava (1976-78).

Months	NR	RS	RP
October 1976	8	11	8
November 1976	8	14	7
December 1976	7	7	6
January 1977	12	9	5
February 1977	8	9	5
March 1977	7	11	4
April 1977	8	7	6
May 1977	15	13	4
June 1977	16	15	4
July 1977	16	17	6
August 1977	12	14	5
September 1977	12	14	4
October 1977	13	15	4
November 1977	12	15	4
December 1977	13	14	5
January 1978	11	14	5
February 1978	12	8	6
March 1978	11	13	4
April 1978	13	12	3
May 1978	12	10	3
June 1978	16	11	6
July 1978	18	9	5
August 1978	11	10	4
September 1978	11	11	3

Table 26. Analysis of variance for fungal population in nonrhizosphere (NR) rhizosphere (RS) and rhizoplane (RP) regions (1976-1977).

Source of variation	DF	SS	MSS	Calculated value	Table value	
					5%	1%
Due to (NR, RS & RP) treatments	2	286.06	143.03	22.27	3.44	5.72
Due to sampling Period	11	129.89	11.80	1.83	2.27*	3.20**
Error	22	141.28	6.42			
Total	35					

* and ** significant at 5 and 1 percent level respectively.

Table 27. Analysis of variance of fungal population in nonrhizosphere (NR), rhizosphere (RS) and rhizoplane (RP) and sampling period (1977-1978).

Source of variation	DF	SS	MSS	Calculated value	Table value 5%	Table value 1%
Due to (NR, RS & RP) treatments	2	2912.10	1456.05	62.17	3.44	5.72
Due to sampling period	11	777.68	70.69	3.01	2.40-2.25	3.45-3.12 **
Error	22	515.33	23.42			
Total	35					

** = Significant at 1% level.

Table 23. Analysis of variance in taxonomic group and treatments i.e. non-rhizosphere (NR), rhizosphere (RS) and rhizoplane (RP) regions (1976-1978).

Sources of variances	DF	SS	MSS	Calculated value	Table value 5%	Table value 1%
Due to (NR, RS & RP) treatments	2	686.54	343.27	2.25	4.46*	8.65*
Due to Taxonomic group	4	13060.2	3265.05	21.4	3.84	7.01
Error	8	1219.2	152.4			
Total	14					

* and ** significant at 5 and 1 percent level respectively.

Table 29. Analysis of variance of actinomycetes population in nonrhizosphere (NR), rhizosphere (NR) and rhizoplane (RP) regions and sampling period (1977-78).

Source of variation	DF	SS	MSS	Calculated value	Table value 5%	Table value 1%
Due to (NR, RS & RP) treatments	2	1703.51	851.75	41.04	3.44	5.72
Due to sampling period	11	108.34	9.84	0.47	2.40-2.25*	3.45-3.12**
Error	22	456.67	20.75			
Total	35					

* and ** significant at 5% and 1% level respectively.

Table 30. Analysis of variance for actinomycetes population in nonrhizosphere (NR), rhizosphere (RS) and rhizoplane (RP) regions and sampling period (1977-78).

Source of variation	DF	SS	MSS	Calculated value	Table value	
					5%	1%
Due to (NR, RS & RP) treatments	2	2312.38	1156.19	64.77	3.44	5.72
Due to sampling period	11	218.72	19.97	1.11	2.27*	3.20**
Error	22	592.88	17.85			
Total	35					

* and ** significant at 5% and 1% level respectively.

Table 31. Analysis of variance for bacteria in nonrhizosphere (NR), rhizosphere (RS) and rhizoplane (RP) regions and sampling period (1976-1977).

Source of variation	DF	SS	MSS	Calculated value	Table value 5%	Table value 1%
Due to treatments (NR, RS & RP)	2	204.3	102.15	34.43	3.44	5.72
Due to sampling period	11	34.9	3.17	1.04	2.4-2.23*	3.45-3.12**
Error	22	67.0	3.05			
Total	35					

* and ** significant at 5 and 1 percent level respectively.

Table 32. Analysis of variance for bacteria in nearrhizosphere (NR), rhizosphere (RS) and rhizoplane (RP) regions and sampling period (1977-78).

Source of variation	DF	SS	MSS	Calculated value	5	Table value 1
Due to treatments (NR, RS and RP)	2	196.27	98.13	32.71	3.44	5.72
Due to sampling period	11	31.92	2.90	0.98	2.40-2.23*	3.45-3.12**
Error	22	66.0	3.0			
Total	35					

* and ** significant at 5 and 1 level respectively.

Table 33a. Correlation and regression coefficient among different variables and microbial population in nonrhizosphere, rhizosphere and rhizoplane regions.

Variables/Microbial Population		Edaphic characters					Climatic characters			
		PH	MC	OC	AVP	AVK	Min. temp.	Max. temp.	Rain-fall	Humi-dity
F 1976-77	NR	-.13	.17	.21	.09	.23	-.04	-.22	-.04	.29
	RS	-.98	-.4	-.24	.01	.69*	.09	.18	-.37	-.37
	RP	-.10	-.2	.03	.24	.21	-.18	-.17	-.47	-.17
F 1977-78	NR	-.05	-.558*	-.16	-.52	-.76*	-.3	-.45	-.42	-.19
	RS	-.14	.10	.49	.60*	.60*	.17	.18	-.015	-.09
	RP	-.38	-.056*	.45	.54	.25	-.058	.18	-.20	-.17
B 1976-77	NR	.37	.27	-.80*	.10	-.46	.35	.2	.62*	.42
	RS	.52	.67*	-.19	.23	-.44	.47	.23	.63*	-.32
	RP	-.12	.17	.23	.75*	-.34	-.007	-.32	-.10	.024
B 1977-78	NR	.57*	.74*	-.41	.42	.66*	.58*	.58*	.53	.64*
	RS	.17	.26	-.32	-.08	.32	.42	.48	.22	.64*
	RP	.20	.51	-.07	-.54	.34	.01	-.12	.24	.71*
A 1976-77	NR	.82*	.68*	-.45	-.13	.19	.78*	.45	.47	.73*
	RS	.67*	.69*	-.26	-.20	-.024	.72*	.61*	.60*	-.05
	RP	-.28	.15	.24	.58*	.012	.11	.25	.63*	-.39
A 1977-78	NR	.037	.37	.18	.42	.60*	-.073*	.045	.18	.25
	RS	-.49	-.053	.14	.034	-.20	-.26	-.009	.03	.1
	RP	-.66*	-.60*	.50	-.29	-.23	-.83*	-.85*	-.68*	.39

F = Fungi; B = Bacteria; A = Actinomycetes; MC = Moisture contents; OC = Organic carbon; AVP = Available Phosphorus; AVK = Available Potassium; Min.temp.= Minimum Temperature; Max.temp.= Maximum temperature and * = Significant.

Table 33b. Correlation coefficient between microbial population of rhizosphere, rhizoplane and nutrient contents of root.

Variable/Microbial Population		Nutrient contents of root				
		Nitrogen	Phosphorus	Potassium	Amino acids	Sugar
F						
1976-77	RS	-.23	-.15	-.24	-.054	-.26
	RP	.10	-.29	.26	-.27	-.37
F						
1977-78	RS	-.54	-.25	.30	-.28	-.20
	RP	-.37	-.44	.22	-.37	-.16
B						
1976-77	RS	.48	.06	.47	-.11	.46
	RP	.27	.27	.39	-.13	-.016
B						
1977-78	RS	-.33	.16	.071	.023	.60*
	RP	-.40	.53	-.037	.39	.55*
A						
1976-77	RS	.67*	.0031	.75*	-.002	.33
	RP	.068	-.10	.18	-.12	-.16
A						
1977-78	RS	-.07	-.17	.46	.33	.36
	RP	.063	-.17	-.6	-.03	.42

F = Fungi; B = Bacteria; A = Actinomycetes and * = Significant.

Table 33c. Correlation coefficient between higher fungi and microbial populations in different environments.

Environments	B/HP	A/HP	F/HP
1977			
NR	.027	.49	.44
RS	.084	.70*	.36
RP	.40	.11	
1978			
NR	.077	.13	-.51
RS	-.15	.17	.105
RP	-.42	-.42	.055

* = Significant; NR = Nonrhizosphere; RS = Rhizosphere; RR = Rhizoplane;
B = Bacteria; A = Actinomycetes; F = Fungi and HP = Higher Fungi.

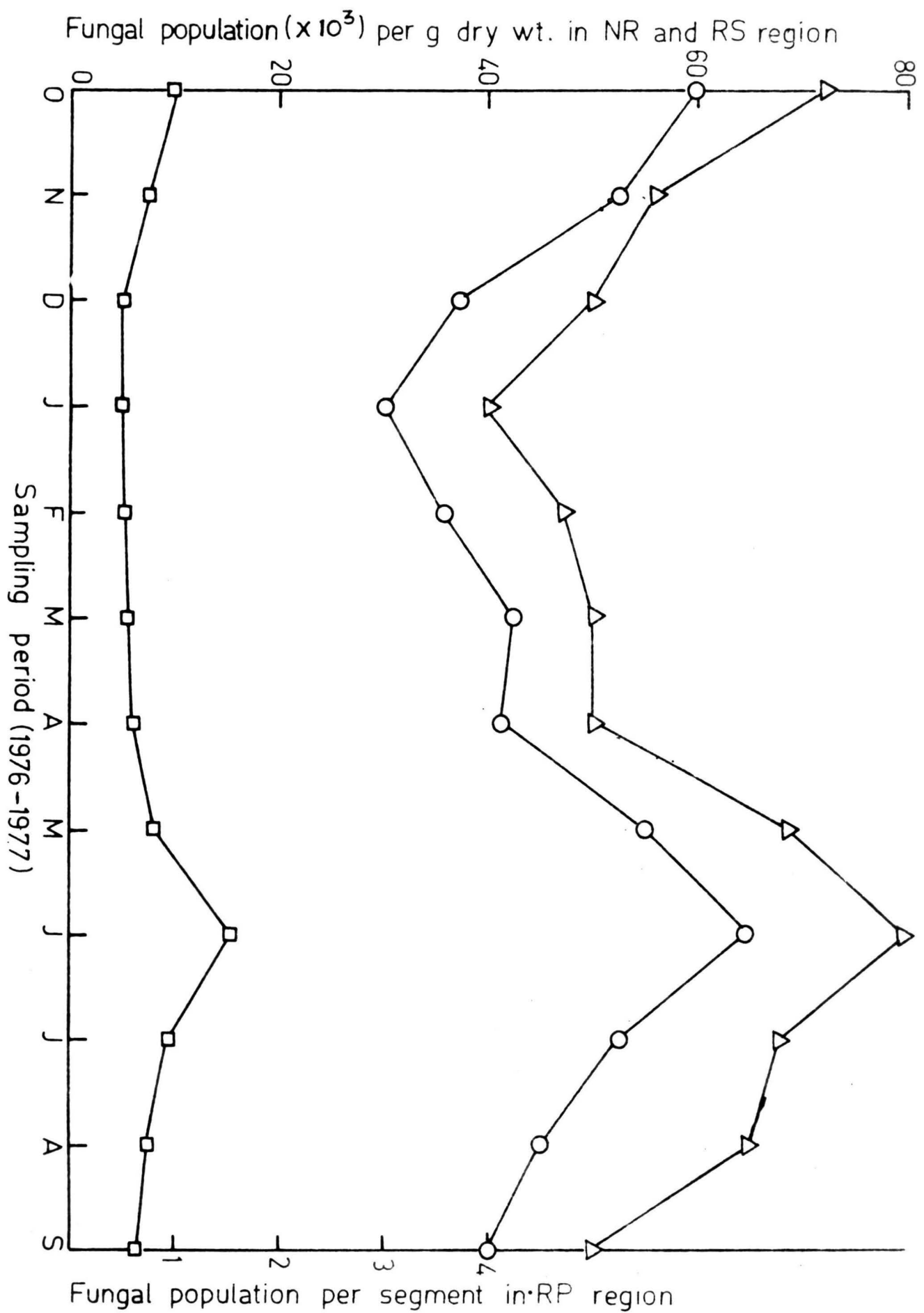
Table 33d. Correlation coefficient between higher fungi and nutrient status of root.

Variable/ Sampling period	Soil nutrients/Higher fungi					Root nutrients/Higher fungi				
	pH	MC	OC	AVP	AVK	N	P	K	AA	Su
1977	-.24	.73*	-.1	-.02	.051	.20	.43	.37	.56*	.7*
1978	-.36	.37	.23	.1	-.27	.28	.52	.78*	.93*	.63*

MC = Moisture content; OC = Organic Carbon; AVP = Available Phosphorus;
 AVK = Available Potassium; N = Nitrogen; P = Phosphorus; K = Potassium;
 AA = Amino acids and Su = Sugar.

Explanation of Plate 21.

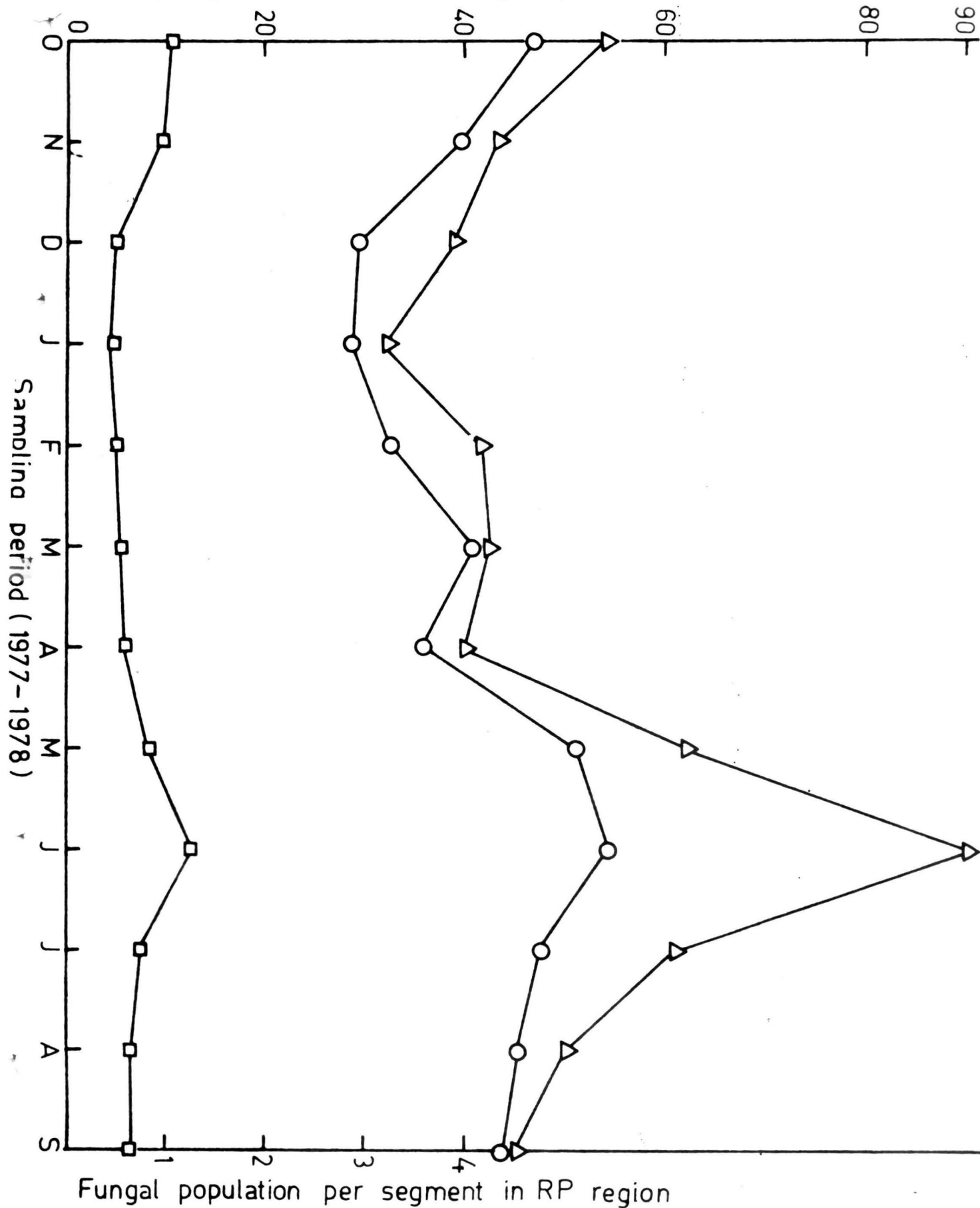
Monthly variation in fungal population in different regions of pine (1976-1977), (NR = nonrhizosphere, RH = rhizosphere and RP = rhizoplane).



Explanation of Plate 22.

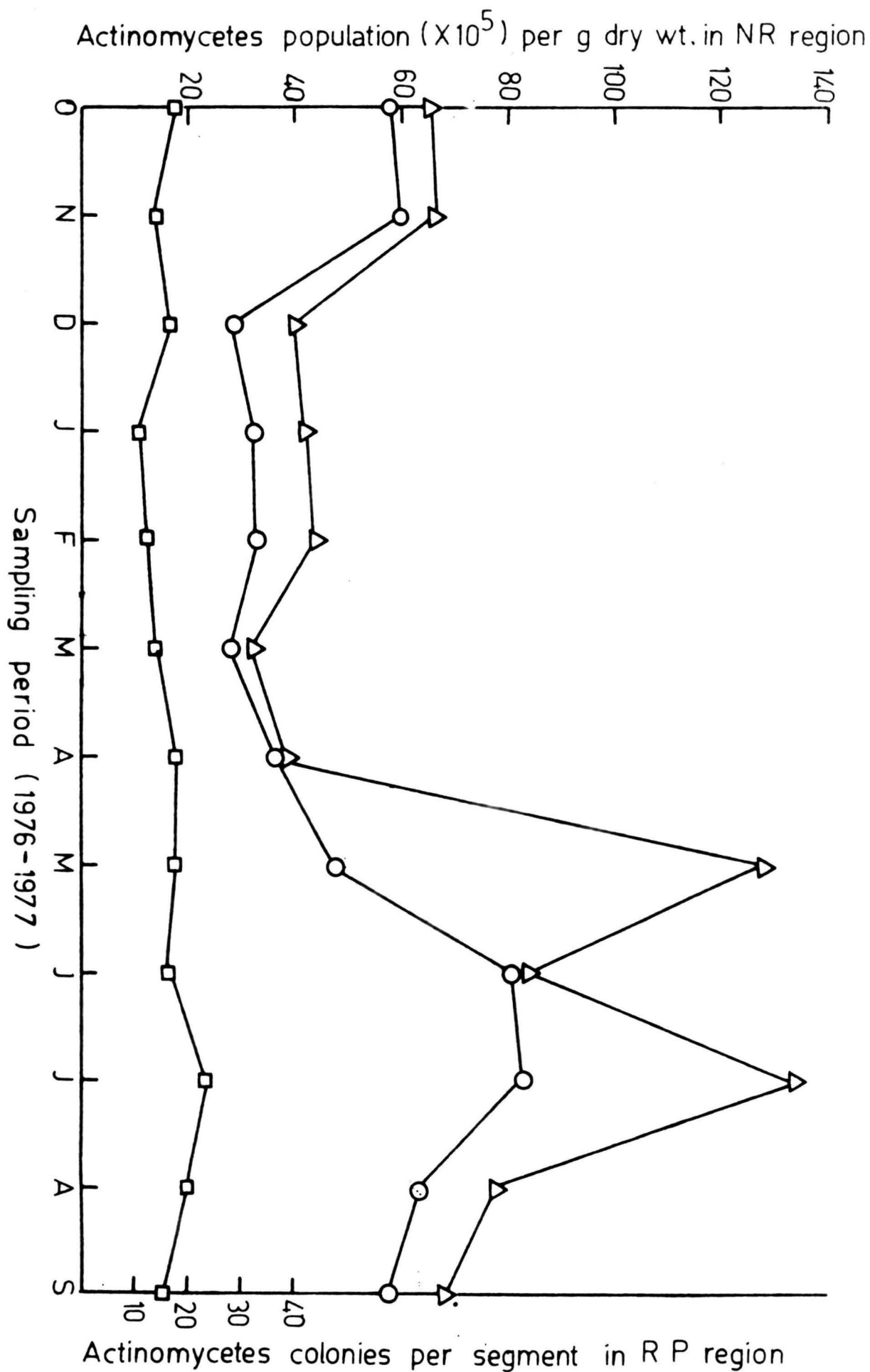
Monthly variation in fungal population in different regions of pine (1977-1978), (RH = rhizosphere, NR = nonrhizosphere, and RP = rhizoplane).

Fungal population ($\times 10^3$) per g dry wt. in NR and region



Explanation of Plate 23.

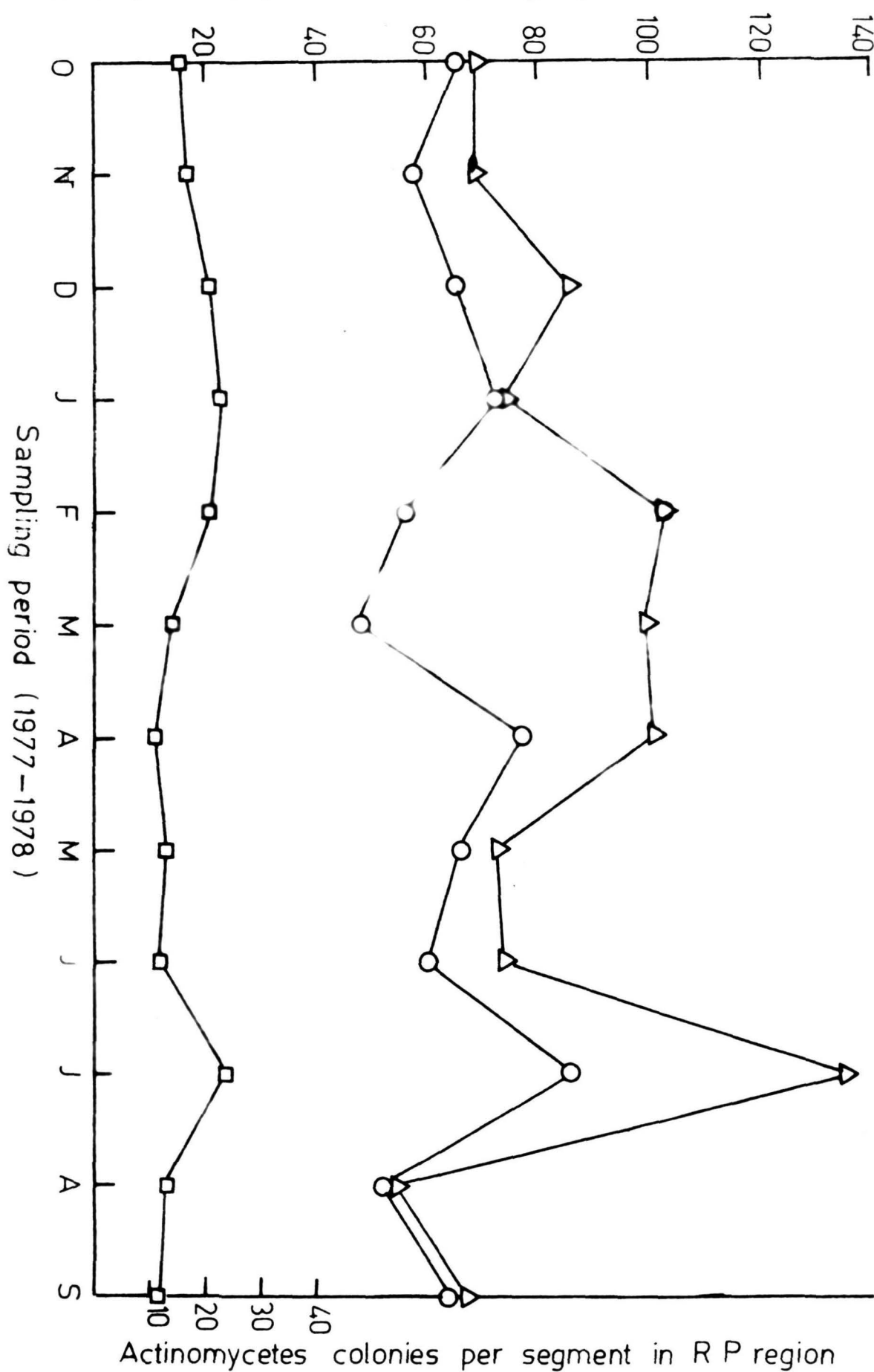
**Monthly variation of actinomycetes population in
nonrhizosphere (NR), rhizosphere (RH) and rhizoplane (RP)
regions of pine (1976-1977).**



Explanation of Plate 24.

Monthly variation of actinomycetes population in
nonrhizosphere (NR), rhizosphere (RH) and
rhizoplane (RP) regions of pine (1977-1978).

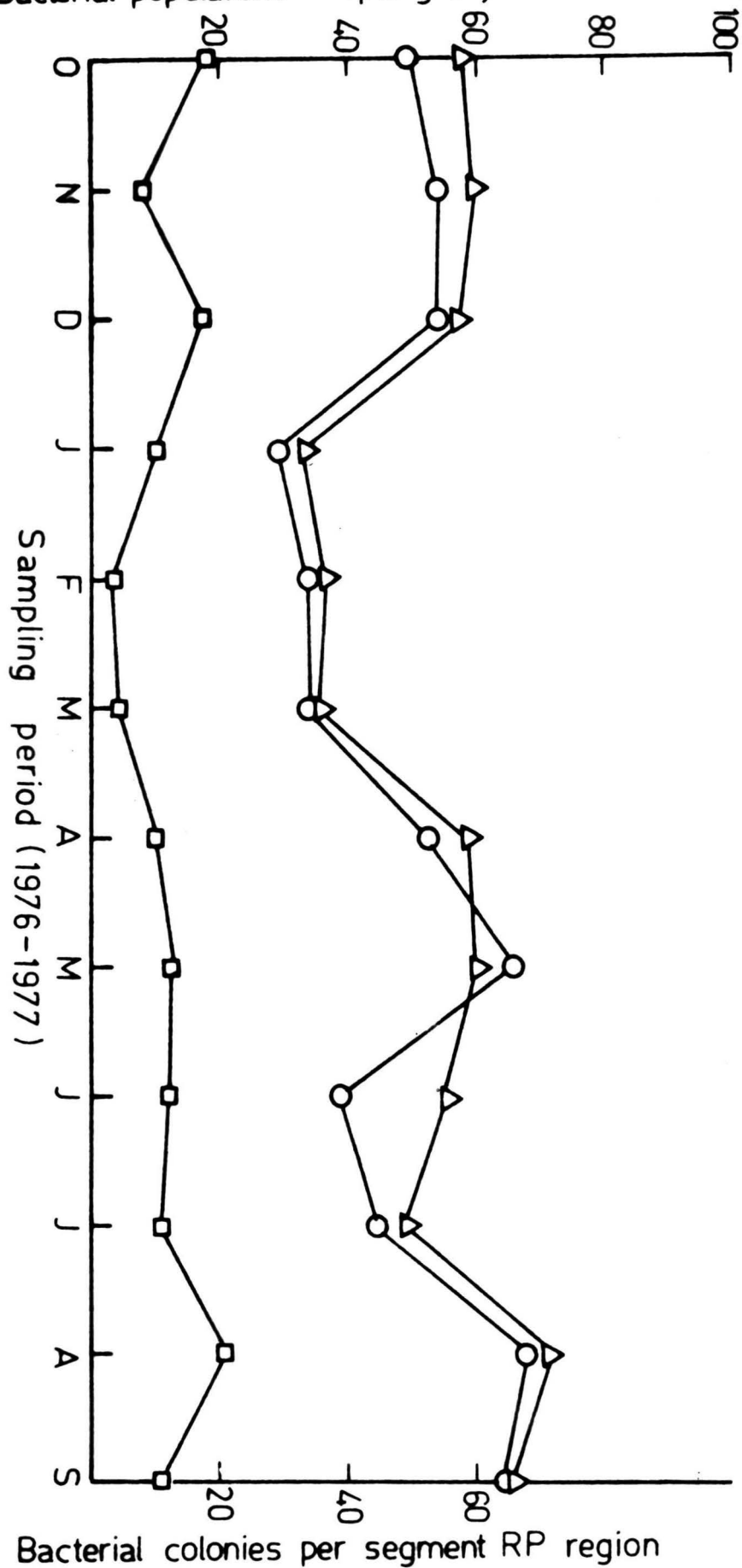
Actinomycetes population ($\times 10^5$) per g dry wt. in NR and RS region



Explanation of Plate 25.

**Monthly variation in bacterial population in
nonrhizosphere (NR), rhizosphere (RH) and
rhizoplane (RP) regions of pine (1976-1977).**

Bacterial population ($\times 10^6$) per g dry wt. in NR & RS region



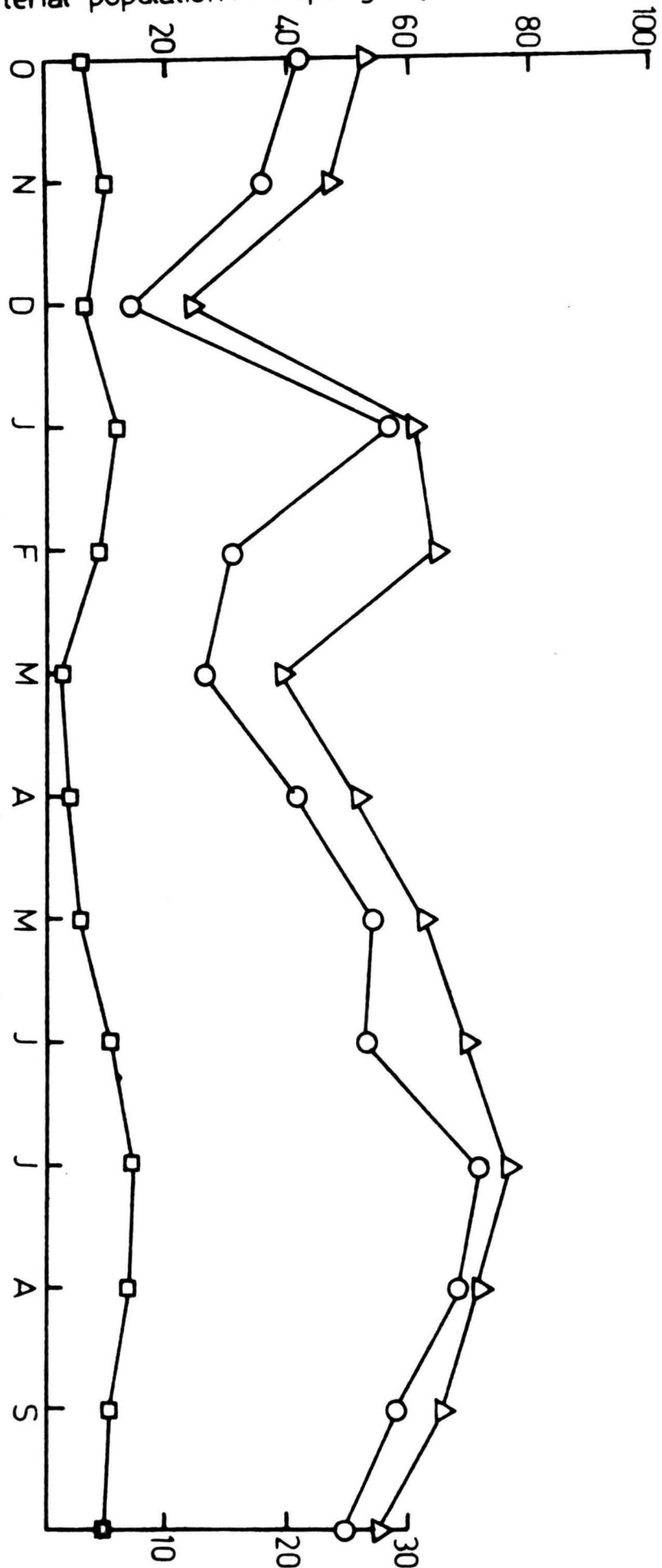
Explanation of Plate 26.

Monthly variation in bacterial population in
nonrhizosphere (NR), rhizosphere (RH) and
rhizoplane (RP) regions of pine (1977-1978).

Bacterial population ($\times 10^6$) per g dry wt. in NR & RS region

Sampling period (1977-1978)

Bacterial colonies per segment in RP region



Biochemical Analysis:

Amino Acids contents in the root extracts:- Eighteen amino acids were chromatogramed from the extracts of mycorrhizal roots of Pinus kesiya during 1976-1978. Leucine, methionine, Alanine, Phenylalanine, Diphenylalanine, Aminobutyric acid, Glutamic acid and Tyrosine were frequently observed while Arginine, Histidine, Cystine, Proline and Serine were of infrequent occurrence. Valine and Threonine were detected only in September (1977) and May (1978). Two unidentified amino acids (Rf value 0.59 and 0.80) were also detected (Table 39-40).

The amount of total free amino acids ($\mu\text{g}/100\text{mg}$ dry root) exhibited almost a similar variation pattern during the course of study. The maximum total free amino acids during both the years was observed in June. The second maxima was obtained in January, whereas the minimum amount was recorded in October (1976) and November (1977) (Plate 31).

Sugars in the root extracts:- Eleven sugars were detected from the mycorrhizal roots during the course of investigation (Table 37-38). Glucose, Sucrose, Raffinose and Ribose sugars were detected frequently whereas Galactose, Fructose,

maltose and xylose were less frequent. Two unidentified sugars content ($\mu\text{g}/100 \text{ mg}$ dry root) was always observed in June. Another maxima was observed in January. November (1976) and December (1977) months yielded the minimum amount of sugars (Plate 31).

Physico-chemical analysis of soil samples of nonrhizosphere (NR), and rhizosphere (RS) regions of mycorrhizal roots.

Variation in pH of the soil samples of nonrhizosphere and rhizosphere regions was not much. Same was the case for the samples collected at different time intervals. The samples always exhibited acidic nature (Table 34). Moisture content was always maximum during the rainy months (July, August) and it dropped considerably towards the end of the winter months (March) (Table 34 and 35).

The organic carbon was maximum in January in both nonrhizosphere and rhizosphere regions and the minimum was recorded during the rainy season. The available phosphorus varied from 0.01 - 0.08 percent in rhizosphere and 0.01 - 0.05 percent in nonrhizosphere (Table 34 and 35). The available potassium was determined in the range 0.09 - 0.32 percent in rhizosphere and 0.10 - 0.33 percent in nonrhizosphere regions during 1976-1977

and 0.08 - 0.37 percent in RS and 0.09 - 0.37 percent in NR regions during 1977-1978 (Table 34 and 35).

Nitrogen, phosphorus and potassium contents in mycorrhizal roots:

The nitrogen contents of mycorrhizal roots varied from 0.56 - 1.7 percent (1976-1977) and 0.58 - 1.60 percent (1977-1978). The highest nitrogen was determined in June. The phosphorus was also maximum during June and July. The phosphorus amount varied between 0.1 - 0.35 percent. Phosphorus content in mycorrhizal roots was less during the winter. The potassium content varied between 0.32 - 0.92 percent during the period of investigation (Table 36).

Nutrient content of above ground and below ground parts of pine seedlings with mycorrhizal fungi inoculated:

All mycorrhizal fungi induced a significant increase in nutrient status of the seedlings (Plate 32). The nitrogen content in above ground parts of the seedlings inoculated with Russula lutea was almost double than the uninoculated ones. Other mycorrhizal fungi also enhanced nitrogen accumulation in ground parts of the seedlings (Plate 32). The phosphorus content in above ground parts of the seedlings inoculated with Amanita sp showed an

increase of about five times over the control sets. Likewise the pine seedlings inoculated with other mycorrhizal fungi also exhibited an increase in phosphorus content of above ground parts (Plate 32). The potassium, calcium and magnesium uptake in the seedlings was also stimulated by the mycorrhizal fungi.

In below ground parts also nutrient level was enhanced by mycorrhizal fungi. The nutrients like nitrogen, phosphorus, potassium, calcium and magnesium in roots were favourably influenced in inoculated seedlings (Plate 32).

Amino acids contents in pine seedlings inoculated with mycorrhizal fungi:

Eleven amino acids were isolated in above and below ground parts of the seedlings. Leucine, Aspartic acid, Aminobutyric acid were quite frequent in below ground parts whereas Histidine, Proline and Tyrosine were infrequently detected from above ground part of the seedlings. Qualitatively amino acids were more in inoculated seedlings than that of noninoculated ones. In above ground parts Leucine and Aminobutyric acid were dominant whereas Phenylalanine, Cystine, Arginine and Histidine were infrequently detected. The

total free amino acids ~~were~~ more in above and below ground parts of seedlings inoculated with mycorrhizal fungi than those of noninoculated ones (Table 42 and Plate 31).

Sugar contents in pine seedlings inoculated with mycorrhizal fungi:

Ten sugars were detected from above and below ground parts of the seedlings. Both quantitatively and qualitatively more sugars were observed in pine seedlings inoculated with mycorrhizal fungi (Table 41 and Plate 31). In noninoculated seedlings, Sucrose, Glucose, Xylose, and Sorbose sugars were detected from above and below ground parts of seedlings while in case of inoculated sets in addition to the above sugars Fructose, Raffinose, Galactose and rhamnose sugars ^{were} also observed.

Table 34. Physico chemical characteristics of soil in nonrhizosphere and rhizosphere regions (1976-77).

Sampling period	NR					RS				
	pH	MC %	Org. Carbon %	Av. P %	Av. K %	pH	MC %	Org. Carbon %	Av. P %	Av. K %
Oct.	5.30	22	3.10	0.04	0.18	5.25	21	3.18	0.07	0.17
Nov.	4.95	20.2	3.20	0.02	0.10	4.95	20	3.47	0.05	0.29
Dec.	4.30	22	3.70	0.04	0.24	4.40	21.5	3.76	0.08	0.18
Jan.	4.60	20	4.10	0.06	0.23	4.55	19.0	4.06	0.03	0.32
Feb.	4.70	18	3.6	0.01	0.21	4.75	17	3.12	0.02	0.23
Mar.	5.10	13.0	3.15	0.03	0.34	5.10	14	2.65	0.04	0.45
Apr.	5.40	15.5	2.80	0.04	0.14	5.45	15	2.88	0.03	0.16
May	5.55	28	2.7	0.06	0.18	5.50	27	3.53	0.05	0.32
June	5.90	28	3.32	0.03	0.38	5.05	28	2.83	0.05	0.17
July	5.95	30	2.90	0.02	0.27	5.90	24	2.47	0.01	0.10
Aug.	5.80	29	2.60	0.02	0.25	5.75	31	2.64	0.02	0.22
Sept.	5.60	27	2.75	0.04	0.20	5.55	28	2.95	0.04	0.20

Table 35. Physico chemical characteristics of soil in nonrhizosphere (NR) and rhizosphere (RS) regions (1977-1978).

Sampling period	NR				RS					
	pH	MC %	Org. Carbon %	Av. P %	Av. K %	pH	MC %	Org. Carbon %	Av. P %	Av. K %
Oct.	5.40	21.0	2.6	0.02	0.24	5.35	22.0	2.6	0.03	0.25
Nov.	5.20	20.5	2.9	0.01	0.20	5.20	20.0	2.8	0.07	0.24
Dec.	4.9	17.5	3.8	0.02	0.12	4.9	18.0	3.8	0.03	0.21
Jan.	4.4	16.0	4.0	0.04	0.23	4.45	17.0	4.2	0.04	0.23
Feb.	4.7	11.5	3.1	0.03	0.18	4.65	12.0	3.7	0.03	0.21
March	4.9	8.0	3.0	0.03	0.10	4.95	8.5	3.20	0.06	0.20
Apr.	5.05	23.0	3.0	0.05	0.32	5.0	22.0	2.9	0.05	0.10
May	5.0	28.0	3.5	0.02	0.30	5.05	26.2	3.9	0.08	0.42
June	5.4	28.5	3.9	0.06	0.22	5.35	28.8	3.9	0.07	0.24
July	5.75	29.0	2.6	0.05	0.26	5.00	30.5	2.7	0.04	0.29
Aug.	5.85	29.0	1.47	0.03	0.29	5.9	30.0	2.1	0.02	0.27
Sept.	5.6	26	1.9	0.03	0.21	5.65	25.0	1.7	0.02	0.38

Table 36. Nitrogen, phosphorus and potassium contents of mycorrhizal roots of *Pinus kesiya* (1976-1978).

Ions	Year	S A M P L I N G P E R I O D											
		O	N	D	J	F	M	A	M	J	J	A	S
Nitrogen (%)	1976-77	1.1	0.75	0.87	0.66	0.56	0.58	0.68	0.98	1.7	1.4	1.2	1.1
Phosphorus (%)	"	0.26	0.11	0.17	0.20	0.17	0.20	0.21	0.14	0.34	0.20	0.22	0.20
Potassium (%)	"	0.60	0.50	0.55	0.34	0.35	0.32	0.46	0.60	0.68	0.78	0.50	0.45
Nitrogen (%)	1977-78	1.08	0.75	0.67	0.53	0.70	0.90	0.80	0.20	0.60	0.40	0.35	1.03
Phosphorus (%)	"	0.28	0.30	0.30	0.28	0.25	0.16	0.17	0.20	0.35	0.35	0.32	0.25
Potassium (%)	"	0.65	0.55	0.52	0.58	0.58	0.58	0.52	0.80	0.92	0.84	0.68	0.50

Table 37. Distribution pattern of sugars in mycorrhizal roots of pine (1976-77).

Sugars	Months											
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
Fructose	-	-	-	-	-	-	-	-	-	-	-	-
Glucose	-	+	-	+	+	+	+	-	+	+	+	+
Galactose	-	-	-	-	-	-	+	-	-	-	-	-
Maltose	-	-	-	-	-	-	-	-	-	+	+	+
Raffinose	+	+	+	+	-	+	-	+	-	-	-	-
Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-
Ribose	+	+	-	+	+	+	+	+	+	-	+	+
Sucrose	+	+	+	-	+	-	+	-	-	-	-	-
Xylose	-	-	+	-	-	-	-	-	-	+	-	-
Unidentified (?)	-	-	-	-	+	+	+	+	-	-	-	-

+ = Present and - = Absent.

Table 58. Distribution pattern of sugars in mycorrhizal roots of pine (1977-78).

Sugars	Months											
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
Fructose	-	-	+	+	-	-	-	-	-	-	-	-
Galactose	-	-	-	-	-	-	-	-	-	-	+	-
Glucose	+	+	-	+	+	+	+	+	+	+	+	+
Maltose	+	-	-	-	-	-	-	-	-	-	-	-
Raffinose	-	+	-	+	+	+	+	+	+	+	-	-
Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-
Ribose	-	-	+	-	-	+	+	+	+	+	+	+
Sucrose	-	-	+	-	-	-	-	-	-	-	+	+
Xylose	+	+	-	-	+	-	-	-	-	-	-	-
Unidentified(?)	-	-	+	-	-	-	-	-	-	-	-	-

+ = Present and - = Absent.

Table 39. Distribution of amino acids in mycorrhizal roots of pine (1976-1977).

Amino acids	Months											
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
Alanine	-	+	-	-	-	-	-	-	+	-	-	+
Arginine	-	+	-	-	-	-	-	-	-	-	-	-
Amino butyric acid	-	-	+	-	+	+	-	-	-	-	-	-
Cystine	-	-	-	+	-	-	-	-	-	-	-	-
Diphenylalanine	-	-	-	-	+	-	-	+	-	-	-	-
Glutamic acid	-	-	-	-	-	-	+	-	+	+	-	-
Histidine	-	-	-	+	-	-	-	-	-	-	-	+
Leucine	-	-	-	-	-	-	-	-	-	-	-	-
Methionine	-	-	-	-	-	-	-	-	+	-	-	-
Proline	-	-	-	+	+	+	+	+	-	-	+	-
Serine DL	-	-	+	-	-	+	-	-	-	-	-	-
Threonine	+	+	-	-	-	-	+	-	-	+	-	-
Tyrosine	-	-	+	-	-	-	-	+	-	-	-	+
Valine	-	-	-	-	-	-	+	-	-	-	-	-
Unidentified	+	+	+	+	+	+	+	+	+	+	+	+

+ = Present and - = Absent.

Table 40. Distribution pattern of amino acids in mycorrhizal roots of pine (1977-78).

Amino acids	Months											
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
Aminobutyric acid	-	-	-	+	-	-	-	-	+	+	+	-
Alanine	-	-	-	-	-	-	+	+	-	-	-	-
Arginine	-	+	-	+	-	-	-	-	-	-	-	-
Aspartic acid	-	-	-	-	-	-	-	-	-	-	-	+
Cystine	-	-	-	+	-	-	-	-	-	-	-	-
Diphenylalanine	-	-	-	-	-	+	-	-	-	-	-	-
Histidine	-	+	-	-	-	-	-	-	-	-	-	-
Glutamic acid	+	-	+	+	-	-	-	+	+	-	-	-
Leucine	-	+	+	-	+	-	-	-	+	+	-	+
Methyonine	+	-	-	-	+	+	-	-	-	-	-	-
Serine	-	-	-	-	-	+	-	-	-	-	-	-
Threonine	+	-	-	-	-	-	-	-	-	-	-	-
Tyrosine	-	-	-	-	+	-	+	-	-	+	+	+
Unidentified (1)	-	-	-	-	-	-	+	+	+	+	-	+
Unidentified (2)	+	+	-	-	-	-	-	-	+	+	+	-

+ = Present and - = Absent.

Table 41. Allocation of different sugars in mycorrhizal and nonmycorrhizal seedlings.

Sugars	Above ground					Below ground				
	A	B	R	S	C	A	B	R	S	C
Sucrose	+	+	+	-	+	+	+	+	+	+
Glucose	-	-	+	+	+	-	-	-	-	+
Galactose	+	-	-	-	-	-	-	-	-	-
Fructose	+	+	-	+	-	-	+	+	+	-
Sorbose	-	+	-	-	-	+	+	+	-	-
Xylose	-	-	+	-	-	+	-	-	-	+
Raffinose	-	-	-	+	-	-	-	+	+	-
Maltose	-	-	-	-	-	+	-	-	-	-
Rhamnose	-	-	-	-	-	-	+	-	+	-
Ribose	-	-	-	-	-	-	-	+	+	-

A = Amanita sp.; B = Boletus edulis; L = Lactarius deliciosus;

R = Russula delica; S = Scleroderma aurentium and C = Control.

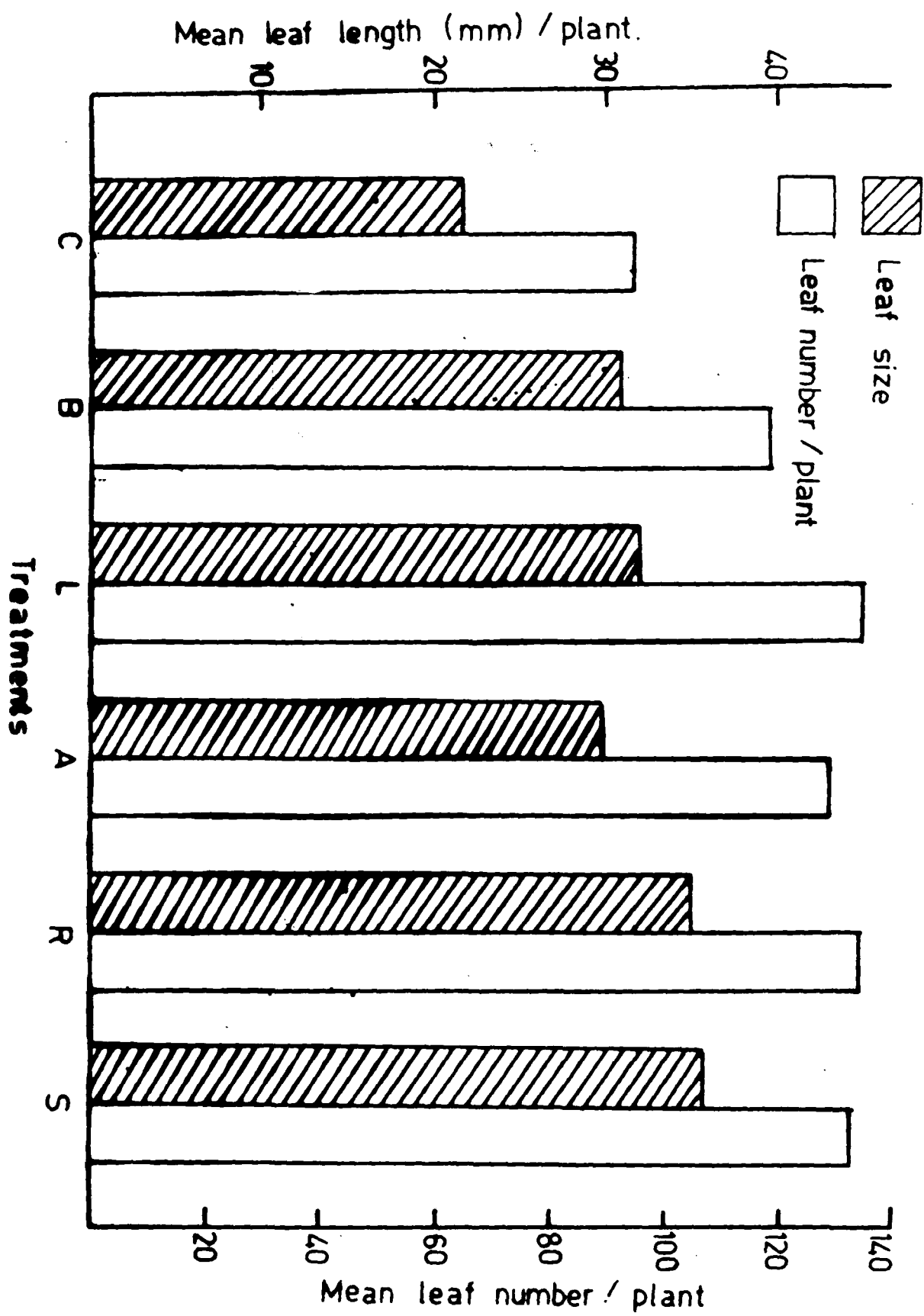
Table 42. Allocation of different amino acids in mycorrhizal and nonmycorrhizal seedlings.

Amino Acids	Above ground						Below ground					
	A	B	L	R	S	C	A	B	L	R	S	C
Amino butyric acid	+	+	+	+	-	-	+	+	-	+	+	+
Aspartic Acid	-	+	+	-	+	-	+	-	+	+	+	+
Arginine	-	+	+	-	-	-	-	-	-	-	-	-
Cystine	-	-	-	+	+	-	+	+	+	-	-	+
Diphenylalanine	-	-	-	+	+	-	-	-	-	-	-	-
Histidine	+	-	-	-	-	+	-	-	-	+	-	-
Leucine	+	+	+	+	-	+	+	+	+	+	+	-
Phenylalanine	-	-	-	+	+	-	-	-	-	-	-	-
Proline	-	-	-	-	-	-	-	-	+	-	+	-
Serine	+	+	-	-	-	+	-	-	-	-	-	-
Tyrosine	-	-	-	-	-	-	+	+	-	-	-	-

A = Amanita sp; B = Boletus edulis; L = Lactarius deliciosus; R = Russula delica;S = Scleroderma aurentium; C = Control; + = Present and - = Absent

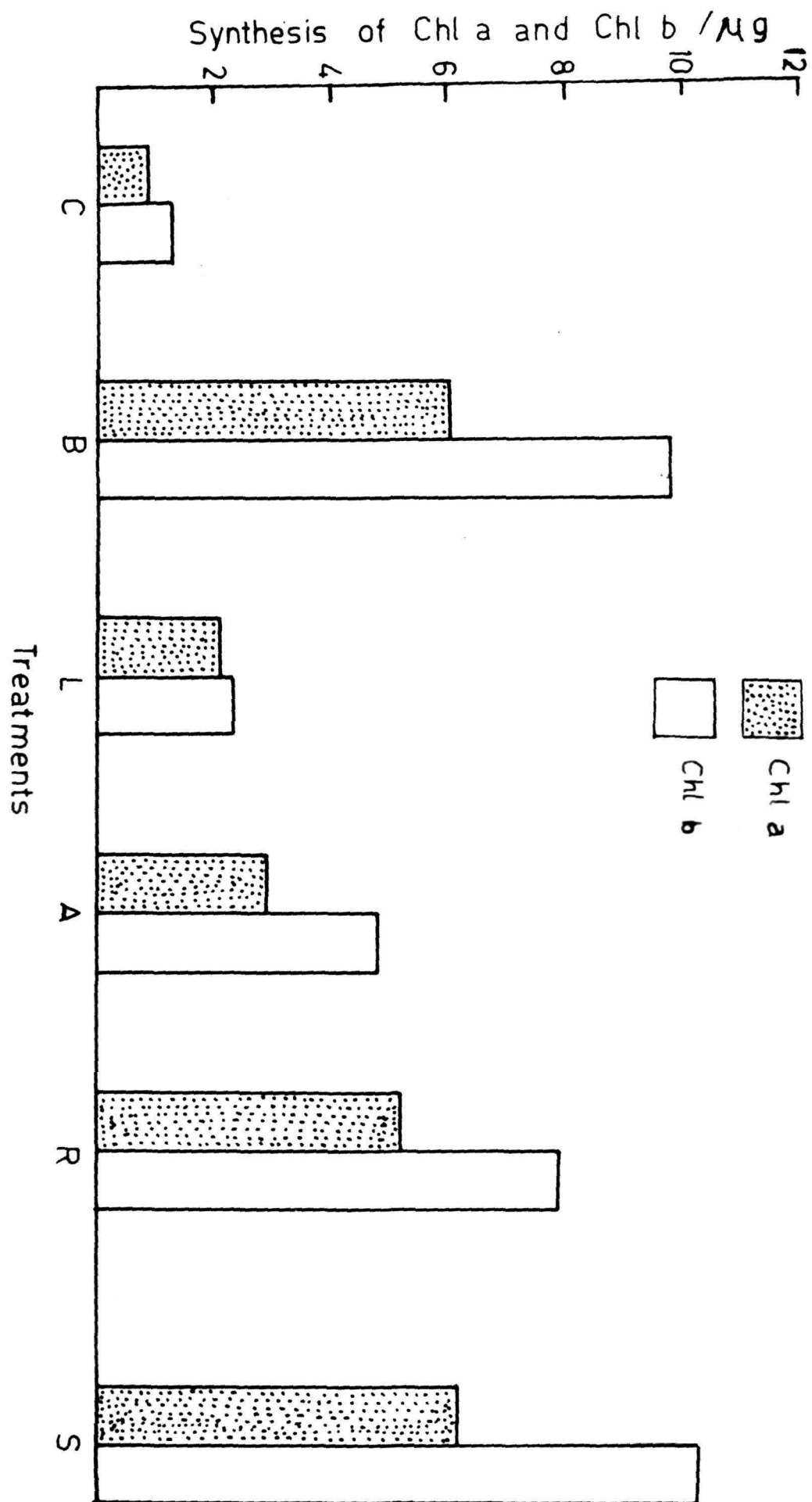
Explanation of Plate 27.

Showing the total chlorophyll a and chlorophyll b
in pine seedlings inoculated with different fungal
symbionts (Abbreviations are explained in Plate 12).



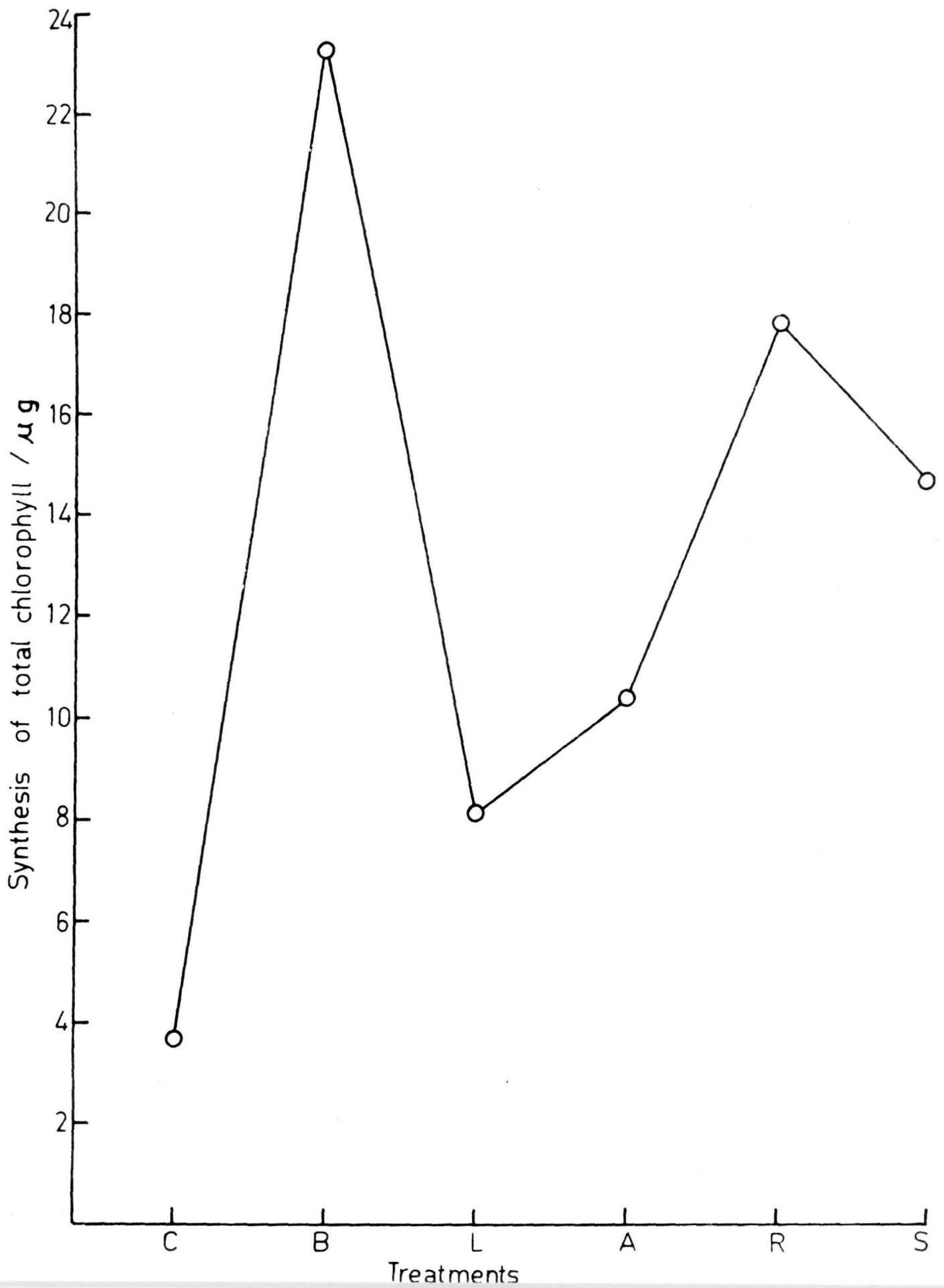
Explanation of Plate 28.

**Effect of mycorrhizal fungi inoculation in pine on
production of total chlorophyll in pot culture studies
(Abbreviations are explained in Plate 12).**



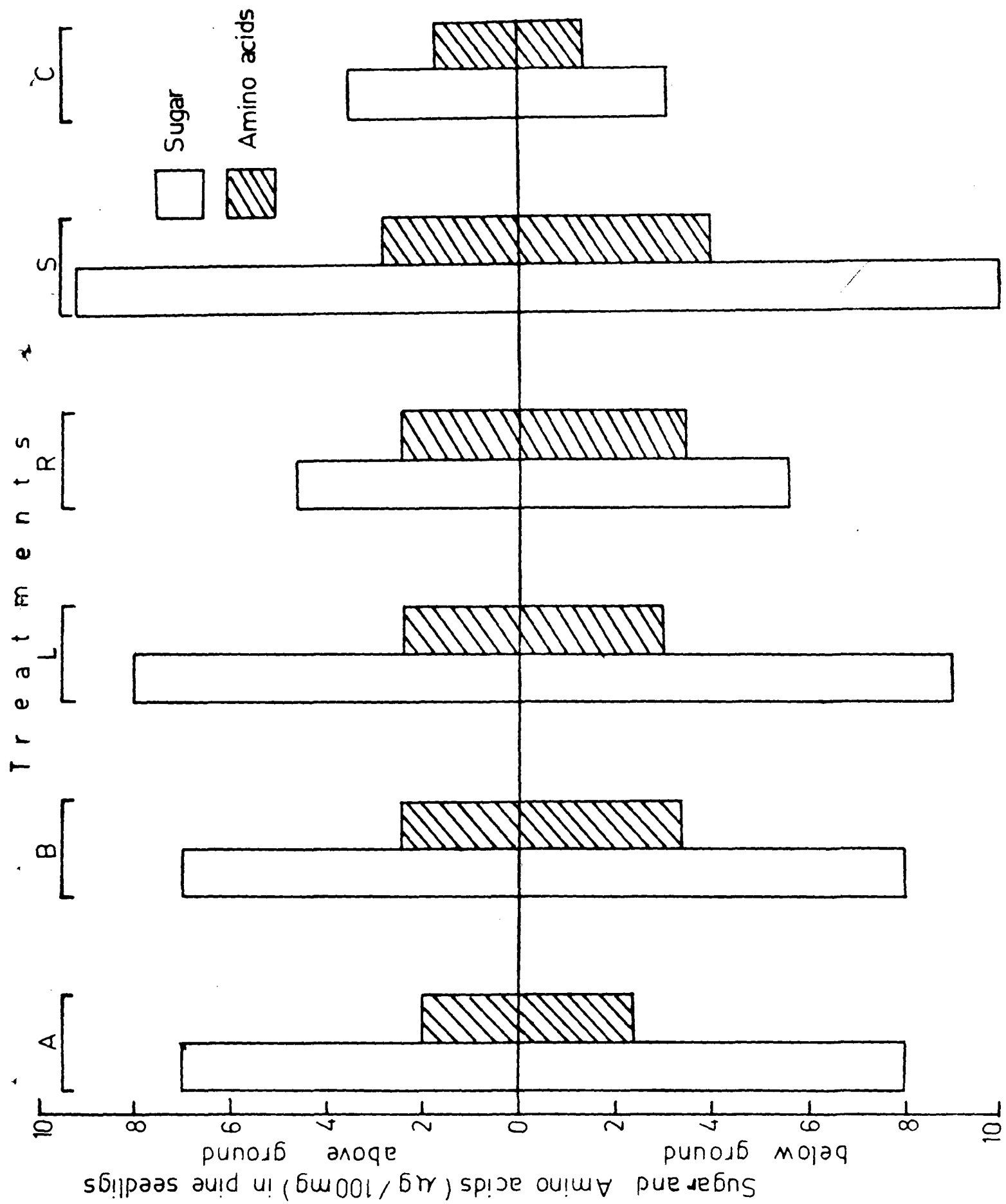
Explanation of Plate 29.

Effect of mycorrhizal fungi on production leaf
numbers and size in pine seedlings in pot culture
studies (Abbreviations are explained in Plate 12).



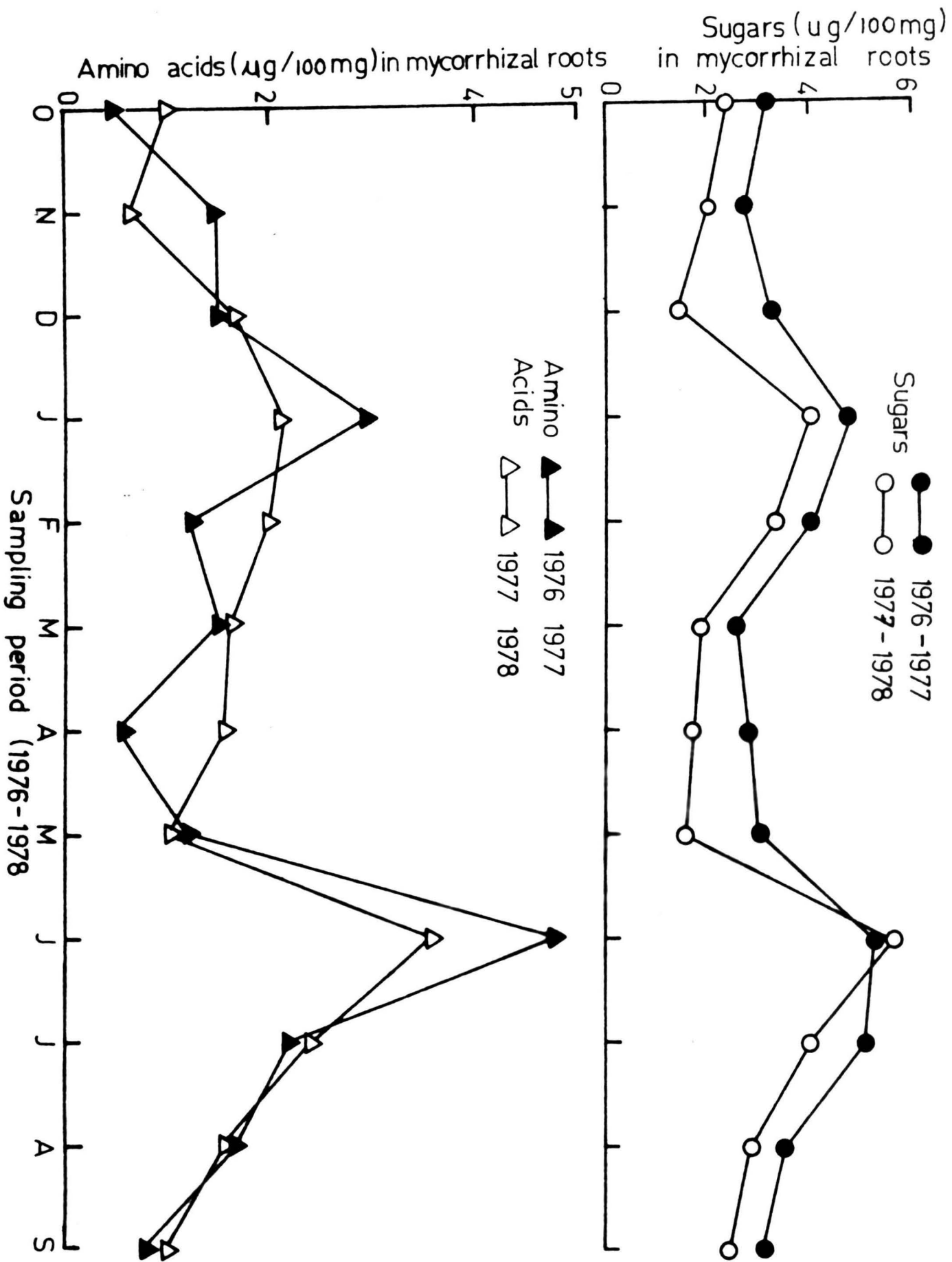
Explanation of Plate 30.

Total sugar and amino acid contents of above and below ground parts of pine seedlings inoculated with different fungal symbionts (Abbreviations are explained in Plate 12).



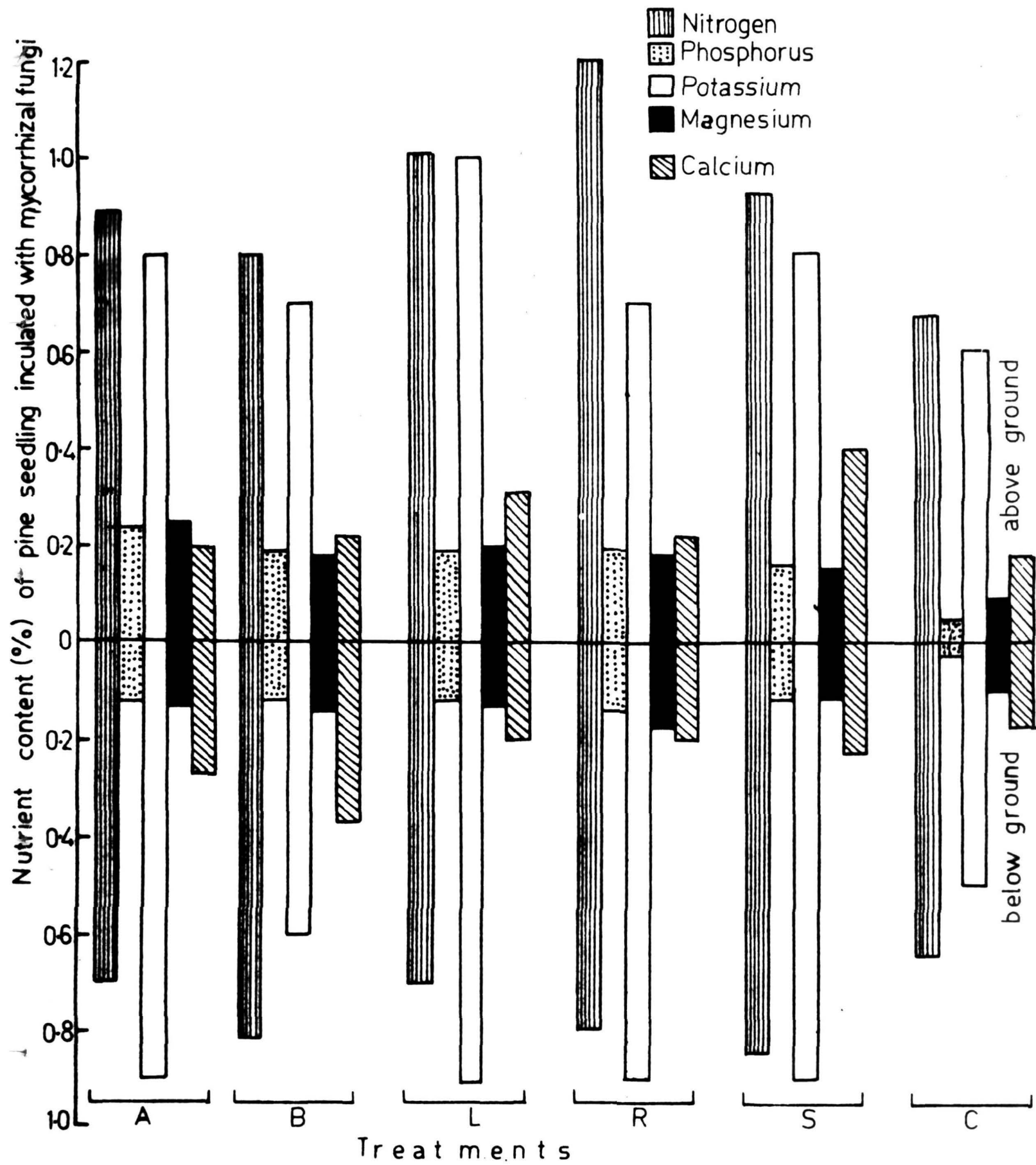
Explanation of Plate 31.

Monthly variation in total sugar and amino acid
production (1976-1978) in mycorrhizal roots.



Explanation of Plate 32.

Effect of mycorrhizal fungi in different nutrient status of pine seedlings. (Abbreviations are explained in Plate 12).



DISCUSSION

A large number of agaricales and puff ball are involved in development of mycorrhizal association with trees in their natural habitat (Ashton, 1976 Hacskeylo and Bruchet, 1972). Some of them may be short lived in natural conditions and may be influenced by environmental conditions (Marx and Bryan, 1969; Zak and Larsen, 1978).

The consistent occurrence of sporocarps with intimate association of roots of trees is indicative of mycorrhizal formation by a fungus. The sporophores of thirtytwo mycorrhizal fungi were observed during late spring and rainy season(Appendices 1 & 2). No sporophore was found during the winter months. The formation of sporophores may be attributed to climatic conditions of the place. The characteristic black jet like mycorrhiza formed by Genococcum graniforme was also observed. Fruiting bodies of Amanita, Boletus, Lactarius, Russula and Scleroderma were dominant and were commonly associated with Pinus kesiya Royle in natural stands. Among thirtythree mycorrhizal fungi, fifteen could successfully be isolated in culture and the rest could not be grown on synthetic media inspite of repeated attempts. Probably, the technique adopted and the media used were not suitable for their isolation.

The highest sporophore production of the mycorrhizal fungi was observed during mid rainy season

(Plate 4-9). In the beginning and the end of the ^{rainy} season_A the fruiting bodies of higher fungi were less. A positive significant correlation was noted between total sporophore production and temperature, rainfall, humidity and soil moisture. Hacskeylo (1967) and Marx (1970) have reported that temperature, light intensity, pH and other environmental factors affect the development of mycorrhizal fungi.

It was observed that sugar content of pine root exhibited a positive correlation with the development of mycorrhizal fungi (Table 33). This is in conformity with the views of earlier workers that the concentration of carbohydrate in root system is responsible for production of the sporophore (Bjorkmann, 1942; Harley, 1975).

During the present investigation, different types of mycorrhizae were observed (Table 5). The most dominant was the dichotomous type. However, other forms like digitate coralloid, simple, tuberculate and racemose were also observed (Table 5). The variation in colour of mantle, its surfaces, mycelium or rhizomorph and colour reactions was observed (Table 5). This variation may be correlated to the chemical composition of fungus forming the mycorrhizae (Zax, 1973; Alvarez and Cobb Jr, 1977). Such criteria are often used in classifying the ectotrophic mycorrhiza (Dominik, 1959; Zak, 1973). The problem of mycorrhizae identification

in natural conditions, however, still remains unsolved and it needs further investigation.

No mycorrhizal fungi specific to a pine stand was recorded. The age of plant significantly influenced the species composition (Appendices 1 & 2). The highest number of species was recorded in oldest plantation. Besides the nutrient availability in root, this could also be correlated to the edaphic conditions of the place.

All the fungi which could be cultured developed mycorrhizal association. The synthesis of symbiotic relations confirmed the findings of mycorrhizal association in natural habitat (Table 20 & 21, Plate 16-20) with pine tree. Due to lack of isolation of all fungi observed in natural conditions in culture it was not possible to test their ability for mycorrhizal development in aseptical conditions.

It was noticed (Table 20 & 21) that certain genera i.e. Boletus, Cantharellus, Suillus, Scleroderma, Tricholoma and a member of fungi imperfecti exhibited greater intensity of mycorrhizae development, while certain other genera formed relatively lesser number of ectomycorrhizae. Many fungi have been shown to have a broad spectrum of host plant and are able to form mycorrhizae from coniferous to deciduous trees (Trappe, 1962; Pachlewski and Pachlewska,

1974; Marx and Ross, 1978; Molina, 1979).

Time taken for the first appearance of mycorrhiza varied from species to species (Table 20 & 21). Earliest development of mycorrhizae was shown by few genera e.g. Boletus, Suillus, Cenococcum and the member of imperfecti, whereas maximum time was needed by Russula and Lactarius (Table 20 & 21). The appearance of first mycorrhizae which is reported to be a good indices of assessing mycorrhizal activity may differ from fungus to fungus and also with the range of host plants. Certain genera possess capacity to form mycorrhizae efficiently while others may lack this ability, (Melin, 1953; Trappe, 1962; Pachlewski and Pachlewska, 1974; Wilcox et al., 1974; Palmer and Hacskeylo, 1970).

Different pattern of branching in mycorrhizae was observed (Plate 16-20). Majority of the fungi developed dichotomous bifurcated mycorrhiza while others formed coral and simple straight structures (Plate 16-20). The intensity of bifurcation and length of mycorrhizae differed from species to species, which may be accounted to the type of growth hormones produced by ectomycorrhizal fungi (Miller, 1971; Slankis, 1973; Pachlewski and Pachlewska, 1974; Froidieux, 1973; Froidieux and Amiet, 1975a,b,c). The formation of ectomycorrhizae in and above the substrate

differed with the fungal associates. Mycorrhizae formed even inside the substrate had an aerial mycelium which indicated the need for good aeration.

The surface of mantle and structure were conditioned by the type of ectomycorrhizal fungi. Smooth mantle with pubescent appearance was observed with Amanita, Lactarius and Russula (Plate 16-20), while rough, creamy to brown coloured mantle was formed by others. However, colour variation in aseptic agar condition was not remarkable.

However, a black coloured characteristic mantle was observed. Such macromorphological characters may be of great help in identification of mycorrhizae in natural habitat (Trappe, 1962; Zak, 1976).

Large microbial population in an unstable equilibrium state has been recognised in root region (Brown, 1975). Rhizosphere stimulates the competition for space and nutrition at inter and intra-specific level among different organisms like bacteria, fungi, actinomycetes, free living nematodes and protozoans (Garrett, 1963).

Higher microbial population was observed in rhizosphere region (Plate 21-26) and this could be accounted to the amount of nutrient present in root (Brown, 1975;

Rovira, 1969; Campbell, 1977) and to the age of plants (Vancura and Hanzlicova, 1972; Mishra and Kanaujia, 1972). The number may differ from slight to many fold in rhizosphere of different plants (Rambelli, 1973). The increase in microbial number has also been correlated to the presence of senescent and lysed microbial cells on root surfaces by SEM and light microscopical studies (Martin, 1978; Bowen and Foster, 1979). The rhizospheric effect has been shown by various other methods like contact slide method (Hulpoi, 1936; Linford, 1939), SEM (Campbell and Rovira, 1973; Elliott, et al, 1979) and transmission electron microscopy (Foster and Rovira, 1978; Rovira et al 1980).

It was observed that nonrhizosphere region (Table 25) was dominated by Absidia, Mucor, Trichoderma, Penicillium, Cladosporium and Fusarium species which produce propagules capable of rapid growth and occupy the habitat away from root. This may probably be due their low competitive ability and thus tend to follow the r strategy in r and k systems (Southwood, 1976). In rhizoplane region of Pinus kesiya the whole rootlet is enclosed by slow growing fungal forms which are characterised by possessing high competitive ability (Bowen, 1980) and the organisms may follow the k strategy. Therefore, the manipulation of certain forms present in

the environments for their feasibility to go from r to k or vice versa will provide a new opening in the field of biological control.

Table 5 and appendix 1 and 2 revealed that certain fungal species i.e. Aspergillus nidulans, Ulocladium chartarum, Memnoniella echinata, Phialophora fastigiata, Metarrhizium anisophae, Curvularia lunata, Trichurus spiralis and Coniothyrium sp. in nonrhizosphere; Neurospora sitophila, A. flavus, Penicillium fumoserosens, Eupenicillium sp, Mycogone perniciosa, Cladosporium herbarum, Bauveria bassiana, Epicoccum purpurascens and Stachybotrys in rhizosphere; and Nectria haematococca, A. glauca, C. verticillatum, Pleochaeta^{sp} and an unidentified Basidiomycetes in rhizoplane, were specific to the environments. Zukovskaya (1941) and Berozova (1941) while studying the rhizosphere of various plants, found that the plants possessed a capacity to stimulate individual specific microflora and this difference even may occur between and along roots. Sivasithamparam and Parker (1979) observed different microbial population on nodal and seminal roots of wheat plant. Bowen (1980) and Bowen and Rovira (1973) by inoculating organisms on root surface found different colony development on different sites. It has also been reported that certain microorganisms in rhizosphere also stimulate the penetration of fungal symbionts (Rambelli, 1973;

Poster and Marks, 1967; Bagyaraj and Menge, 1978; Katznelson et al 1962).

During the course of investigation, various climatic and edaphic factors influenced the microbial activity differently (Table 33). Mishra and Sharma (1977) and Widden (1979), have emphasized the role of soil and other factors in regulating the microbial population. Fungal population in nonrhizosphere was significantly correlated with soil moisture and available potassium (1977-78). In rhizosphere and rhizoplane regions available Phosphorus, available Potassium and soil moisture significantly influenced the population of fungi. pH played a significant role on bacterial and actinomycetes population in nonrhizosphere and rhizosphere regions (Table 33). Since a majority of microbes live in water film, they may be governed by soil structure and water conditions (Campbell, 1977) and pH plays a complex role in determining the microbial population because of its control on nutrient availability, absorption and enzymatic activity.

Significant correlation was shown by bacteria and actinomycetes with climatic factors e.g. temperature, humidity and rainfall (Table 33). Campbell (1977) mentioned that a variety of factors govern the microbial population and inspite of that, it is an apparent paradox

that overall soil population could be seen stable, though having mosaic of local environmental and neighbouring interactions in this dynamic equilibrium.

The variability in nutrient contents of mycorrhizal roots (Plate 31 and Table 37) in different seasons also affected the microbial population. Nitrogen and total sugar exhibited a positive significant correlation with actinomycetes (1976-77) and bacteria (1977-78) respectively in rhizosphere region. Goring and Clark (1948) have showed that rhizosphere microorganisms are affected by nitrogen availability. The amino-acid and sugar spectrum in root differed according to the activity of roots (Plate 31) and it is reported that the relative amount of each compound may influence the rhizospheric population (Vancura and Hanzlikova, 1972). This effect varies from plant to plant (Rambelli, 1973). Bowen and Rovira (1976) while analysing the amino acids and sugars of seedlings grown in controlled conditions, have indicated that glucose and fructose are widely distributed simple sugars. Other high molecular weight polysaccharides and amino-acids like Alanine, Serine, Leucine, Valine, Glutamic and Aspartic acids, however, are also important in the nutrition requirements of microorganisms. Das (1980) found that soil microbial population is also effected by different degree of litter decomposition in pine forest,

which may add different types of organic acids, sugars and amino acids to the soil environment. In symbiotic system, it has been reported that from few percent to a quarter of tree's photosynthate could be used by ectomycorrhizas (Newman, 1978; Crush 1976) and in similar way microbes can sustain a concentration gradient and diffusion parallel to mycorrhizal fungi for glucose and fructose (Harley, 1975). Rovira et al (1979) have recognised that substances in rhizosphere region may be of various root origins like exudates, secretions, mucilages, mucigel and lysates. Further, Rovira et al (1980) have indicated that fungal gels are more stable than mucilage derived from roots and this was correlated with the capacity of vesicular-arbuscular mycorrhizae to increase the aggregation of soil (Sutton and Sheppard, 1976). The carbon budget of mycorrhizal root may influence the normal soil microflora (Harley, 1975; Bowen, 1980) and the use of amino-acids in rhizosphere of mycorrhizal roots of various trees by fungi has been widely reported (Rambelli, 1962, 1966, 1973).

It has been suggested that nutrition and energy are two limiting factors in rhizosphere microbial competition (Rambelli, 1973) and excretions of mycorrhizal roots may probably play an important role in selection of microorganisms. Inhibitory or stimulatory effect may also be due to quality

and quantity of excretions through the fungi of mycorrhizal roots and thus they may effect the development of certain microorganisms in the region. Different amino acids (Schinner, 1976; Krupa et al 1973) have been reported in pine roots, whose leakage may alter the microbial population.

A positive significant correlation between mycorrhizal fungi and population of fungi and actinomycetes of rhizoplane and rhizosphere revealed that mycorrhizae of pine have stimulatory effect on most probable number of these organisms (Table 33). Neal et al (1968) while studying the rhizosphere of mycorrhizae of red alder and douglas fir, found that microbial population (ammonifying and nitrate reducing microbes) differs qualitatively as well as quantitatively in both nonrhizosphere and rhizosphere regions. A stimulatory effect of mycorrhizal fungi on the population of streptomycetes has also been observed (Mikola, 1965). It was suggested that the increased actinomycetes population through the production of the antibiotics may inhibit the growth of certain micro-organism. An obscure effect of actinomycetes by mycorrhizosphere has also been reported (Rambelli, 1966). Filtrates of symbiotic fungi have also been found to have stimulatory effect on rhizospheric population in good garden soils (Rambelli, 1973). Khan and Saif (1974) found

a synergistic effect on bacteria and Endogone in rhizosphere of wheat plant. Ogawa (1977) found that Tricholoma matsutake a mycorrhizal fungus significantly influenced the composition of fungal species at different sites. He (1978) further pointed that this influence may differ in recovery of soil fungal flora, from one mycorrhizal species to another. In rhizosphere of certain mycorrhizal trees an irregular pattern of microbial population in different seasons has also been reported (Rambelli et al., 1972). It has been further noted that rhizosphere of yellow birch mycorrhiza may inhibit fungal population and also has stimulatory effect on the population of actinomycetes and bacteria. Thus it may be indicated that the microbial population may significantly be altered by the nature of mycorrhizae (fungal symbiont). Therefore, further investigation in rhizosphere microbiology in relation to different symbionts and their effect on excretions is necessary and it may provide enough information on interactional studies. Further, it may be stated that the study of the nature of exudates through fungal symbiont would also be informative in understanding of the selectivity of mycorrhiza (Slankis, 1973).

Ectomycorrhiza is characteristic feature of most woody plants. The association helps in survival and better

growth of the plant. The fungal symbiont also play a significant ecological and evolutionary role in existence of vascular plants (Malloch, et al, 1980).

The pine seedlings inoculated with mycorrhizal fungi induced better growth in the plants and reduced the mortality rate in early stages of the seedlings. On the other hand, uninoculated seedlings had high mortality rate and even seedlings which could survive were weak, pale and dwarf (Table 9 and Plate 12 & 13). Failure of high mortality has been reported in certain nonmycorrhizal trees (Wright, 1964; Bakshi, 1974) and survivalship (60 percent or more) has been assured in plants with mycorrhizal infection at the time of transplanting. Mycorrhizal fungi had to play a crucial role in establishment of host plant because they increase absorb~~ing~~ing surface area of the roots and also enhance the nutrient uptake. Mycorrhizae have been suggested as underground energy suppliers to host plants (Melin, 1925, Bjorkmann, 1949). The woody plants particularly pines have poor development of root hair and seedlings at early stage of development require an additional organ to cope up with the nutrient requirement in general and phosphorus in particular (Baylis, 1970). Mycorrhiza in absence of proper root hairs may act as an alternative for phosphorus uptake from soil which is

not readily available, like other elements. This nutrient build mechanism at early age of tree may probably be helpful in reducing the mortality in pine (Table 9). Higher survivalship has frequently been reported in seedlings inoculated with mycorrhizal fungi (Moser, 1958,1959; Shemakhanova, 1967; Theodorou and Bowen,1971; Hacskeylo and Vozzo, 1971; Lamb and Richard, 1974; Shenck and Kinlock, 1980). Another probable reason for reducing mortality rate in mycorrhizal seedlings may be attributed to their ability to check the root pathogen (Marx, 1969a, b; Marx and Davey, 1969c,d) and root rot causing nematodes (Kellam and Schenck, 1980). In pine, it has been observed that seedlings at early stage of growth are heavily infected by soil borne organism, Fusarium oxysporum which is responsible for wilting and high mortality in natural environment (Mishra et al 1981). In mycorrhizal roots, they may check the entry of this organism either by production of certain chemical substances (Marx, 1969b) or by bringing a change in physical and chemical characteristics of seedlings (Pope, 1980; Nagy et al, 1980). The control of F. oxysporum and other organisms by mycorrhizal fungi has been very well demonstrated (Marx, 1969b; Dehne, 1977; Shonbeck, 1978; Sikora and Schonbeck, 1975; Nemec, 1978).

It was observed that the relative growth of pine

seedlings was enhanced by mycorrhizal fungi within a period of six months since their inoculation (Table 10, 11 and 12). Further, increase in shoot and root dry weight in inoculated seedlings was also observed (Table 13). Similar pattern of increase has also been reported in other crop plants by the mycorrhizal fungi (Hayman and Mosse, 1971; Sanni, 1976). Inducement in growth and development of green colour in pine seedlings (Pinus caribaea) was observed by Hacskeylo and Vozzo, (1967). Uninoculated seedlings on the other hand exhibited the symptoms of chlorosis and stunting which could not be reverted back, even after fertilizing them, till suitable mycorrhizal fungi were inoculated. Hence, it may be assumed that mycorrhizal inoculation in pine is necessary for their establishment, survivalship and better growth in natural habitat. Significant increase in plant height and leaf number was also observed in woody plants propagated vegetatively (Kormanik et al, 1969). It was observed that few mycorrhizal fungi i.e. Russula lutea and Amanita sp. had better response to stem growth of the seedlings (Table-10). Other fungal symbionts on the other hand affected different growth parameters of the seedlings. The use of such specific highly beneficial fungal symbiont may be of great help in artificial regeneration programmes. Mexal and Reid (1973) stated that few selected ectomycorrhizal fungi (Cenococcum graniforme, Suillus luteus and Thelophora

terrestris) not only differ with respect to inducement of growth but also have different response to water stress in controlled conditions. The differences in mycorrhizal production and dry weight of the plants were statistically insignificant. However, significant differences were observed in shoot height and leaf production of seedlings which may be attributed to the level of efficiency of the mycorrhizal fungi (Table 15-19). Mejsirik, 1975; Mason, 1975; Majstrik and Krause, 1973; Daft and Elgiahmi, 1978; Hayman, 1980 have also observed better growth in seedlings of Eucalyptus on semi-sterile lateritic soil inoculated with ectomycorrhizal fungi.

Mycorrhizal fungi not only stimulated shoot height and dry weight of above and below ground parts of seedlings but also the total root length (Table 11). Thus they provided a relatively larger absorptive surface area in a given soil volume, which may be an explanation for enhancement in growth through increased uptake of nutrients. The increase in root length by a Pisolithus tinctorius a mycorrhizal fungi has been reported (Marx, 1969a,b; John, 1980).

The pattern of establishment and development of mycorrhizae in seedlings varied from symbiont to symbiont (Table 8). The colour of mantle and size of mycorrhizae

also differed from species to species (Table 14, Plate 14 & 15). These macromorphological characters may be of some help in identification of types of mycorrhizae in nature (Zak, 1973). Differences in development of mycorrhizae could also be attributed to their variation in growth behaviour and actual number of fungal propagules in contact with root of seedlings (Trappe, 1977 and Marx, 1976) and thus their clear understanding may again supplement previous verdict in selection of efficient mycorrhizal fungi.

Analysis of nutrients in pine seedlings highlighted that phosphorus uptake was fourfold greater in mycorrhizal seedlings than uninoculated ones (Plate 32). The enhanced growth is generally correlated with increased phosphorus uptake (Gerdemann, 1968; Mosse, 1973). Though the source of phosphorus for mycorrhizal and nonmycorrhizal plants may remain same, but substantial evidences favour that mycelium of mycorrhizae is responsible for increased phosphate uptake and translocation to host plants (Sander and Tinker, 1971; Hayman and Mosse, 1972a,b; Pearson and Tinker, 1975). Increased uptake of phosphorus by mycorrhizal fungi is reported (Daft and Nicolson, 1969a,b; Mosse and Hayman, 1971; Pearson and Read, 1975; Meyer, 1974; Mosse, et al., 1976; Johnson, 1977; Davis and Menge, 1980; Heap and Newman, 1980b) in many plants and a phosphate pool is

streaming to the host plant and thus fungal hyphae make link between root and soil (Heap and Newman, 1980a). It is assumed that the multiphase uptake mechanism in which each phase obeys Michaelis Menten Kinetics, is involved in mycorrhizal root system as has been reviewed in case of certain crop plants (Nissen, 1974). The recent studies on enzymatic mechanism (Gianinazzi and Gianinazzi, 1976) revealed that new bands of alkaline and acidic phosphates appeared in mycorrhizal roots, which were absent in nonmycorrhizal roots and these specific bands accounted for 22-32 percent of total alkaline phosphate activity (Gianinazzi and Gianinazzi, 1978). Based on their observation, they further (1979) speculated that this MSP (Mucorhizal specific phosphate) is of fungal origin. In our studies on enzymatic level, in mycorrhizal and non-mycorrhizal pine seedlings (Mishra and Sharma, unpublished), different patterns of phosphatases, (alkaline and acidic) peroxidases, dehydrogenases and esterases in different fungal inoculated mycorrhizae were observed. Variation was also seen in some fungal symbiont inoculated in different pine populations. This is of interest that these fungal symbionts enhance the enzymatic pool in host plant and not only phosphatases but also other enzymes may differ with different population of same host or with a variety of host. In ultrastructural studies, a greater acid phosphatase activity has been recorded on the tips of

arbuscules but not in either host cells or senescent fungal hyphae (Scannerini et al., 1975) and the great alkaline phosphatase activity was correlated with MSP and finally with the number of arbuscules (Gianinizzi and Gianinizzi, 1979). The occurrence of dehydrogenase and acid phosphatase has also been shown by EMS studies in vascular plants (Macdonald and Lewis, 1973). SEM and X-ray micro-analysis studies have also indicated a larger amount of phosphorus pool in cells with intimate association of arbuscules (Schoknecht and Hattingh, 1976). However, the actual mechanism involved in phosphate uptake needs further investigation.

Plate 32 indicates that uptake of other major nutrients i.e. Nitrogen, Potassium, Calcium and Magnesium is also enhanced by mycorrhizal fungi. This enhancement may probably be responsible for better growth of the seedlings inoculated with fungal symbionts (Plate 12 & 13). However, the difference in the nutrients was not significant. (Table 33). It has been reported (Mosse, 1957; Baylis, 1959; Gerdemann, 1964; Ross and Harper, 1970; Bowen et al. 1974 and Bartschi and Garrec, 1980) that mycorrhizal fungi increased the total amount of Calcium, Magnesium, iron, Sodium, Boron, Copper and Manganese. Further Zinc deficiency was also not observed in mycorrhizal peach seedlings (Gilmore, 1971).

Nevertheless, uptake of nutrients though low

(Plate 32) would be of significance especially to pine trees which have a slow growth rate. Enhanced absorbing capacity by mycorrhizal fungi has been reported to be more active in field conditions (Bowen, 1968,1973). Phosphate uptake by mycorrhizal fungi also improved bacterial nodulation and nitrogen fixation in leguminous (Mosse, et al, 1976), and actinomycetous nodules in nonleguminous (Mishra and Sharma, 1980) plants.

The amount and type of sugars and amino acids were different in symbiotic system of inoculated seedlings than noninoculated ones (Table 38 and 39). Nonmycorrhizal seedlings possessed only few simple sugars whereas a variety of sugars and aminoacids were detected in mycorrhizal roots (Table 39). It has been found (Harley, 1969,1975) that heterotrophs fungal symbionts are well adapted to receive the supply of carbon as photosynthate of autotrophs rather than from humus or soil, and they convert these sugars into unavailable form to host plant. Clement et al (1977) have reported that mycorrhizal fungi eliminate chlorosis, correct the amino acid and protein synthesis pathways in Pinus nigra. It has also been reported that upto ten percent of carbohydrates and aminoacids could be made available to the root fungi (Richard, 1964). The flow of carbohydrate from the site of photosynthesis to the accumulation has been suggested (Harley, 1975) and these

sinks are known to be regulated by hormones (Kumar and Wareing, 1974).

The radioactive studies (Lewis and Harley, 1965) have revealed that carbohydrate sink in the form of organic acids is located into the tips of mycorrhizae. This may probably be due to the entrance of substances into metabolic pathways of symbionts and host at the region. The nature of mechanism involved in allowing the carbohydrates across the fungal membrane to the host plant in operating metabolism is opened for further course of investigation. The reversal movement of carbohydrate and amino acids from the site of storage is not yet fully known and it deserves detailed study.

S U M M A R Y

Pinus kesiya Royle ex Gordon is an early colonizer among the tree species at higher altitude (1100 - 1600 M) of Meghalaya. Forests are rapidly vanishing due to shifting agricultural practice prevailed in north-eastern India. Long and continuous harshment of site, in slash and burnt agricultural practice may initiate the colonization of nonmycorrhizal grasses and other plant species, which may inhibit the germination and propagation of mycorrhizal fungi and tree species. Furthermore, it may thus slow down the process of succession and may alter the pattern of species composition (Reeves et al, 1979, Miller, 1979). North-eastern India receives maximum precipitation (Mawsmal-Cherrapunji) in world but it is provoked towards desertification due to less mycorrhizal symbionts population (Mishra et al unpublished). On the other hand, fast growing demand for energy and timber due to increasing human population pressure, provided an opportunity to work on this topic to serve two broad ideas. I. Analysis of fungal symbiont (Mycorrhizal fungi) with pine tree, which may be used in afforestation, reclamation and silvicultural programmes on waste land. II - To improve the growth of tree, wood quality and protect them from fungal pathogen by exploiting certain effective mycorrhizal fungi. Thirtythree mycorrhizal fungi were observed with pine (*Pinus kesiya* Royle) in nature. Among basidiomycetes agaricales were maximum in number to form mycorrhizal association. Age of pine tree influenced the

population of sporocarp of fungal symbionts. Middle rainy season i.e. June to September was most conducive for the development of sporophores of mycorrhizal fungi. No sporocarp was observed during winter months. Boletus, Clitocybe, Collybia, Cortinarius and Tricholoma were first to produce their fruiting bodies. Boletus edulis, B. luteus, Lactarius deliciosus, L. Sp., Russula delica and Scleroderma geaster were dominant mycorrhizal fungi. Different kind of mycorrhizae were observed in nature but bifurcate to coralloid forms occurred frequently. Mycorrhizal fungi were found to influence significantly the bacterial, actinomycetes and fungal population in rhizosphere of pine. Significant variation in higher fungal species in pines of different age groups was also obtained.

Pure culture studies on agar with pine and mycorrhizal fungi confirmed their symbiotic relationship. Different mycorrhizal fungi induced different kind of mycorrhiza, however, bifurcate type was most common. Variation in time for mycorrhizae establishment with different forms was also observed. Intensity of mycorrhizae varied from species to species. Variation in mantle, Hartig net and colour of mycelium was also recorded.

Studies on mycorrhizae in pot culture exhibited that mycosymbionts reduce mortality and induce robust growth

in pine seedlings. Efficiency of mycorrhizae development varied from symbiont to symbiont. Mycorrhizal inoculation also stimulated stem growth, root length, leaf production and leaf size. Uninoculated pine seedlings grew weak and exhibited the yellowish leaves and symptoms of stunting. Photosynthetic pigment (Chlorophyll) was greater in inoculated seedlings than uninoculated ones. Nutrient status analysis revealed that uptake of almost all the cations and anions is enhanced in mycorrhizal seedlings than non-mycorrhizal ones. Phosphorus uptake was found four times greater in inoculated seedlings than uninoculated ones. Bifurcate to coralloid types of mycorrhizae were found dominant. Chromatographical and Colorimetric studies on sugar and amino-acid flux of roots of mycorrhizal inoculated and uninoculated ^{seedlings} indicated that mycorrhizal roots have different kind of sugars and amino-acids and also possessed more sugars and amino-acids than nonmycorrhizal root system. Allocation of higher biomass in above ground and below ground parts was observed in seedlings inoculated with mycorrhizal fungi. Significant variation in dry matter production and mycorrhizae development was observed.

Nintynine fungal species were isolated from nonrhizosphere (NR), rhizosphere(RS) and rhizoplane(RP) regions of mycorrhizal roots of pine. Highest number of fungal species was recorded in rhizosphere region. Absidia,

Mucor, Trichoderma, Penicillium, Cladosporium and Fusarium dominated nonrhizosphere while in rhizosphere region Trichoderma, Penicillium and Cladosporium were dominant. Rhizoplane was surrounded by basidiomycetes throughout the study. Trichoderma and Penicillium were also dominantly associated in this region. Highest microbial population was observed during rainy season in all the three regions of mycorrhizal roots. Edaphic and climatic condition significantly influenced the microbial population. Nitrogen, Phosphorus and Potassium content varied seasonally in mycorrhizal roots. Sugars and aminoacids status was also different in different months of mycorrhizal roots. Nutrient status of mycorrhizal roots influenced significantly the microbial population.

Present study emphasised that a variety of mycorrhizal forms are associated with pine which increase absorptive surface area of roots and enhance nutrient uptake and survivality of pines. Mycorrhizae also influence the microbial complex in rhizosphere region.

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 Acc. by.....
 Class by.....
 Sub. Heading by.....
 Date by.....
 Transcribed by.....

Appendix 5

List of fungi.

Absidia corymbifera (Cohn) Sacc.

A. cylindrospora Hagem

A. heterospora Ling yong

Rhizopus nigricans Ehren.

Mucor cercinelloides Vantieghem

M. hiemalis Wehmer

M. racemosus Fresenius

Cunninghamella echinulata Thax

C. elegans Landner

Chaetomium globosum Kunze ex stand.

Neurospora sitophila Sheer & Dodge

Phoma sorghina

Pestalotiopsis dissiminata (Thum) Steyaert

Trichoderma hamatum (Bonn) Bain

T. harazinum Rifai aggr

T. koningii Oud aggr

T. longibrachiatum Rifai aggr

T. viride Pers ex SF Gray

Aspergillus aculeatus Iizuka.

A. fischeri Var spinosus Raper and Funnell.

A. flavus Link extr.

A. nidulans (Eidan) Winter

A. niger Vantiegham
A. sulphureus (Pres) Thom and Church
A. tamaris Kita
A. versicolor (Vuill) Tirab
Penicillium brefeldianum Dodge
P. brevicompactum (Vize) Brown & Smith
P. chrysogenum Thom
P. citrinum Thom
P. corvillophillum Dierckx and others
P. cyclopium Raper & Thom
P. daleae Zaliski
P. fellutinum Biourge
P. frequentans Westling
P. fumosoroseus (Vize) Brown & Smith
P. helicum Raper & Thom
P. implicatum Biourge
P. islandicum Sopp
P. lanthanellum Biourge
P. liliacinum Thom
P. nigricans Bainer ex Thom
P. oxalicum Currie & Thom
P. rubrum Stoll.
P. variotii Bainer
P. verrucosum Westling Samson, Stolk & Hodlok
Scopulariopsis brevicaulis (Sacc) Bainer

Gliocladium roseum (Link) Thom
Ulocladium chartarum (Preuss) Simmons
Trichothecium roseum Link
Mycocone periciosa (Magn) Delacroix
Popularia pullulans (deBary) Berkhout
Mamaoniella echinata (Riv) Galloway
Cladosporium cladosporioides (Fres) devries)
G. herbarum (Person) Link
G. oxysporum Berk & Curt
G. sphaerospermum Penz
G. tenuissimum Cooke
Phialophora fastigiata (Lagerb Lunderberg & Melin) Conant
Metarrhizium anisopliae (Metschn) Sorokin
Alternaria alternata (Fr) Keissler
Trichurus spiralis Hasselbr.
Fusarium acuminatum Ell & Ev
F. arthrosporioides Sherb
F. moniliforme Sheld.
F. oxysporum Schlecht
F. solani (Mart) Sacc.
Bauvaria bassiana (Bal) Vuillimin

Appendix 6.

List of mycorrhizal fungi.

A. verna (Bull. ex Fr.) Pers. ex Vitt.

Boletus edulis Bull. ex Fr.

B. luteus (L. ex Fr) S.F. Gray.

Cantharellus floccosus Schweinitz

Genococcum graniforme

Gollybia confluens (Pers. ex Fr) Kummer

G. maculata (A. et S. ex Fr.) Kummer

G. radicata (Fr.) Quelet.

Cortinarius mucosus (Bull. ex Fr.) Fr.

L. deliciosus (L. ex Fr.) Bk et Br.

Lepiota clypeolaria (Fr) Quelet.

Lycoperdon pyriforme Schff.

Marasmius rotula Fries.

Mycena atroalba (PK.) Saccarado

Rhizopogon luteolus Fr.

Russula delica Fries.

R. lutea (Huds. ex Fr.) Fr.

Scleroderma aurantium Vaill. ex Pers.

S. geaster

Suillus bovinus (L. ex Fr.) S. F. Gray.

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* LIST OF PUBLICATION *
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1. Mishra, R.R. and Sharma, G.D. 1976: Studies on Soil Microflora. Comparison of soil microflora of different localities. Proc. Nat. Sci. Acad. (45th) p.14
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