ECO-PHYSIOLOGICAL STUDIES ON ENDOMYCORRHIZAE OF CERTAIN IMPORTANT TIMBER TREE SPECIES OF NORTH-EASTERN INDIA

By

Satish Kumar Sharma

A THESIS
SUBMITTED
IN
FULFILMENT OF THE REQUIREMENT OF THE DEGREE OF

DOCTOR OF PHILOSOPHY IN BOTANY

To

DEPARTMENT OF BOTANY
SCHOOL OF LIFE SCIENCES
NORTH-EASTERN HILL UNIVERSITY
SHILLONG-793 014
INDIA

JULY, 1987

North-Eastern region of India is endowed with rich natural vegetation and forest resources. The survey of 26 important timber tree species from a natural mountain forest ecosystem of the region indicated their highly mycotrophic nature. Six tree species were ectomycorrhizal and twenty others were endomycorrhizal. In endomycorrhizal group two species had ericoid and remaining eighteen had vesicular-arbuscular type of endomycorrhizal association. The rhizospheric soil of all the tree species was acidic (pH:4.5-6.0), rich in soil moisture (17-40%) and organic matter (5-15%). However, the soil was poor in available phosphorus (1-10ppm).

Cryptomeria japonica, *Exbucklandia populnea*, *Acacia dealbata*, *Schima khasiana*, *Machilus kingii*, *Cinnamomum tamala*, *Manglietia insignis*, *Lindera latifolia* and *Casearia verica* exhibited seasonal variation in the level of VA mycorrhizal infection. The percent VA mycorrhizal infection increased during spring and rainy seasons and declined towards winter or late winter depending on the tree species in both the years viz. 1983-84 and 1984-85. In *Daphniphyllum himalayanse* and *Mallotus nepalensis*, VA mycorrhizal infection was high in all the seasons. Tree species exhibited higher population of endogonaceous spores in their rhizospheric soil and it varied among various tree species and in different seasons. *Cryptomeria japonica*, *Exbucklandia populnea*, *Cinnamomum tamala* and *Lindera latifolia* displayed maximum endogonaceous spore population in rhizospheric soil in rainy season, whereas, in *Daphniphyllum himalayanse* and *Machilus kingi* it was maximum in winter. *Acacia dealbata*, *Schima khasiana* *Mallotus nepalensis*, *Manglietia insignis* and *Casearia verica* did not show seasonal variation in endogonaceous spore population in the rhizospheric soil.

Seasonal variation in soil pH was low. Moisture content, organic matter, total nitrogen, available phosphorus, exchangeable potassium and exchangeable calcium of the soil exhibited greater seasonal variation.

Spores of *Glomus* spp, *Acaulospora* spp, *Gigaspora* spp and *Sclerocystis* spp were observed in the rhizospheric soil of the tree species. Spores of different types representing more than one gen~~era~~us of endogonaceous fungi were present in the rhizospheric soil of tree species. Spores of *Glomus* spp and *Acaulospora* spp were abundant. The ~~F~~former was found in all the seasons, whereas, latter was common during winter season. The ~~S~~spore populations of *Gigaspora* spp and *Sclerocystis* spp w~~as~~ere low and were observed during warmer months of April and June.

Ten species of endogonaceous fungi were identified from the soil samples, viz; two species of *Acaulospora*, namely *A. laevis* and *A. scrobiculata*· three species of *Sclerocystis*, namely *S. rubiformis*, *S. coremioides* and *S. microcarpus*; two species of *Glomus*, namely *G. mosseae* and *G. macrocarpus* var. *geosporus*; three species of *Gigaspora*, namely *G. calospora*, *G. gregaria* and *G. gigantea*.

Seedlings of *Exbucklandia populnea* developed typical vesicular-arbuscular mycorrhizal infection with *Glomus* spp in pot culture using nutrient deficient soil. Mycorrhizal seedlings grew better than the non-mycorrhizal ones. Growth response of plants to mycorrhiza and VA mycorrhizal development depended on fertility level of soil. Mycorrhizal inoculation supplemented with low fertilization (14 kg ha^{-1}NPK) of soil resulted in better growth of seedlings than all other mycorrhizal and fertilizer treatments both in combination or alone. At higher soil fertility (56 kg ha^{-1}NPK) both mycorrhizal and non-mycorrhizal plants exhibited similar growth responses. Increase in level of soil fertility suppressed VA mycorrhizal infection. Higher levels of NPK (56 kg ha^{-1}) added to the soil inhibited VA mycorrhizal infection of plants.

Growth characteristics of mycorrhizal plants of *Exbucklandia populnea* were improved when soluble phosphate (sodium hydrogen phosphate) at low levels (0.34g per pot) and bone meal at relatively higher levels were added to the phosphorus deficient soil. In the presence of soluble phosphate VA mycorrhizal infection was reduced and was inhibited at higher levels (3.47g per pot and above). VA mycorrhizal infection was high in presence of bone meal upto intermediate levels of 17.2g per pot but was reduced to 20% at higher levels of bone meal (34.4g per pot). The concentration of phosphorus in the root was negatively co-related to percent VA mycorrhizal infection. The results suggested that phosphorus status of the host may regulate mycorrhizal infection of root.

Root/shoot ratio of mycorrhizal plants was lower than that of non-mycorrhizal plants at lower nutrient status (NPK levels) and phosphorus status of soil.

Effect of VA mycorrhiza on the mineral nutrition of the seedlings of Exbucklandia populnea was studied at different fertility regimes and phosphorus amendments of soil. Mycorrhizal infection improved phosphorus nutrition of seedlings. Mycorrhizal plants had higher concentration of phosphorus in comparison to non-mycorrhizal plants at lower fertility levels of soil (0,14kg ha^{-1}NPK). Mycorrhizal plants had higher concentration of phosphorus than non-mycorrhizal plants grown in both sources of phosphorus but, the difference in concentration between mycorrhizal and non-mycorrhizal was observed with relatively higher levels of bone meal and only at lower levels of sodium hydrogen phosphate (0.34, 0.69g/pot). The concentration of nitrogen in the leaves did not differ between mycorrhizal and non-mycorrhizal plants of Exbucklandia populnea but, stem and roots of mycorrhizal plants had higher concentration of nitrogen than non-mycorrhizal plants at lower levels of soil fertility (O, I, II levels of NPK). Concentration of potassium in the leaves and stem of non-mycorrhizal plants was higher than that of mycorrhizal plants at lower levels (O,I) of NPK added to the soil. No significant differences were observed in the concentration of calcium between mycorrhizal and non-mycorrhizal plants except for leaves which differed at higher levels of NPK. The growth improvement of mycorrhizal plants could be due to phosphorus nutrition.

The activity of acid phosphatase did not differ between the mycorrhizal and non-mycorrhizal roots of seedlings of _Exbucklandia_ _populnea_.and there was no consistent effect of addition of single super-phosphate to the soil on the activity of this enzyme. Mycorrhizal infection did not affect soluble alkaline phosphatase activity at 'O' level of single superphosphate. Soluble alkaline phosphatase activity was decreased by addition of single superphosphate at 5.0g per pot level in both mycorrhizal and non-mycorrhizal plants. The results suggest that pathway of phosphorus metabolism in mycorrhizal plants may be different from that of non-mycorrhizal plants.

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CERTIFICATE

I certify that the thesis entitled, "Eco-Physiological Studies on Endomycorrhizae of certain important Timber Tree Species of North-Eastern India." submitted by Mr. Satish Kumar Sharma 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.

R.R. Mishra

Signature of Supervisor.

Place : Shillong
Date : 29th July, 1987.

Forwarded
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S. Sharma
(S.K. Sharma)

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CONTENTS

Page

Chapters :

GENERAL INTRODUCTION

Mycorrhizal association is symbiotic association between the roots of plants and fungi. The term mycorrhiza was first introduced by Frank in 1885. All plants in nature develop mycorrhiza to varying degrees, with only few exceptions. Wilhelm (1966) stated that under field conditions plants do not strictly speaking have roots but they have mycorrhizae. Three different types of mycorrhiza have been recognised namely, ectomycorrhiza, endomycorrhiza and ectendomycorrhiza (Harley, 1969).

Endomycorrhizae are mainly formed by two types of fungi i.e. those with septate hypha and non-septate hypha. The former type are common in orchidaceous and ericalean groups of plants. Whereas, fungi of the later group are commonly called vesicular-arbuscular type. VA mycorrhiza are common in bryophytes, pteridophytes, gymnosperms and angiosperms. VA endophytes develop arbuscules and vesicles. They also produce azygospores, chlamydospores and sporocarps. Infection of host root takes place by germinating spores or preinfected root segments. The VA endophytes are phycomycetes and belong to the family Endogonaceae (Gerdemann and Trappe, 1974). Nine genera of these fungi have been recognised (Trappe, 1982). These fungi are obligate symbionts and are cultured on host plant (Gilmore, 1968; Allen et.al, 1979; Macdonald, 1981). Attempts to grow them in-vitro have yielded poor results (Daniel and Trappe, 1980).

Endomycorrhiza of VA type helps in growth, development and yield of plants (Gerdemann, 1968; Mosse, 1973a).

Growth stimulation is more marked at low soil fertility (Csinos, 1981; Banister and Norton, 1974). Plants growing in nutrient deficient habitats frequently form VA endomycorrhiza. Growth improvement of plants is attributed to increased nutrient inflow into plants helped by VA endophyte (Rhodes and Gerdemann, 1980). VA endophyte increases the absorption area and the rhizospheric zone of the root (Bethlenfalway et.al., 1982). Growth stimulation may also be due to interaction of one or more of metabolites of the fungus which alters the physiological processes of the host which may directly or indirectly affects its growth (Plenchette et.al., 1983). The activity of some enzymes like phosphatases has been related to the development of mycorrhiza in roots (Gianninazzi-Pearson and Gianninazzi, 1978; Allen et.al., 1981). The process which process ? may enhance nutrient uptake by endomycorrhizal roots.

The development of VA mycorrhiza and growth of host are inter-related processes and the relationship depends on various ecological factors. The interaction between VA endophyte and the host at species level in relation to ecological factors needs to be investigated. The studies are important in natural forest ecosystems where nutrient requirement of the plants can not be managed through artificial means and dependence on mycorrhiza for sustained growth of plants is a vital force. Poor growth of the tree seedling was attributed to the lack of endomycorrhizal infection of roots and when seedlings were inoculated with endomycorrhizal fungi, growth was enormously increased (Bryan and Ruehle, 1976; Schultz et.al., 1981· Kormanik et.al., 1982; Furlan, et.al., 1983; Pope, 1980). Endomycorrhiza may be an obligate physiological requirement of the seedlings for their establishment and growth (Bryan and Kormanik, 1977). Development of mycorrhiza is important in production of high quality seedling in the nursery before they are planted into fields (Kormanik et.al., 1982). Techniques for increasing fungal inoculum in forest nursery soils have been evolved (Kormanik, et.al., 1980). An assessment of ecological factors and their impact on mycorrhiza in tree species is warranted for the better understanding of the indigenous natural forest ecosystem.

VA mycorrhiza help~~s~~ in uptake of nutrients A review (Rhodes and Gerdemann, 1980). The most accepted hypothesis is that hypha of VA endophyte absorb and translocate nutrients directly to the roots of the host. The hypothesis has been repeatedly tested demonstrated with several types of plants. The absorption and translocation of nutrients to the host root via hypha of VA endophyte is an active process which can not be explained on the basis of mass flow or diffusion of phosphorus in the soil (Sanders and Tinker, 1971). Rapid movement of phosphorus takes place through VA endophyte in mycorrhizal roots than non-mycorrhizal ones. The use of radioactive in split-plate culture technique had also supported the translocation of phosphorus from soil to host through fungal hypha which may either be due to protoplasmic streaming or as bulk flow of hyphal contents. However, polyphosphate granules in external hypha have also been observed (Cox et. al., 1975; Linglee et.al., 1975). Most of the studies have been devoted to phosphorus uptake, perhaps, because it is a limiting factor for growth of plants in soils. Endomycorrhiza also helps in uptake of other important nutrients like sulphur, nitrogen, potassium, zinc and copper (Rhodes and Gerdemann,1978; Bagyaraj,1980; Banister and Norton, 1974). It is still unanswerable whether VA mycorrhizal fungi are involved in solublization of insoluble phosphates. Studies indicated that mycorrhizal plants have better utilization of relatively insoluble form of phosphorus than non-mycorrhizal ones (Mosse,1977; Powell and Daniel, 1978; Pairunan et.al., 1980).

see also Cooper & Tinker

No. read the paper again!

Studies on enzymes at different stages of mycorrhizal infection, during absorption and translocation of nutrients are meagre (Gianinazzi-Pearson and Gianinazzi, 1983). Activities of some enzymes like phosphatase and nitrogenase have been reported to be stimulated by VA mycorrhizal infection of roots (Gianinazzi-Pearson and Gianinazzi, 1978; Assimi et.al.,1980; Allen et.al., 1980 understanding of phosphatase activity may provide a clue for mechanism of phosphorus uptake by plants. Most of basic VA mycorrhizal studies have been accomplished with herbaceous plants with emphasis on agronomic plants. Many species of forest trees form VA mycorrhiza and in natural vegetation like forest ecosystem it may be an important ecological requirement of the trees. However, little is known about the species of VA endophytes involved and the nature of interaction in forest ecosystem. Some workers have investigated the role of VA mycorrhiza in forest tree species (Kormanik *et.al.*, And others, 1982; Furlan *et.al.*,1983; Pope,1980). Studies are needed to investigate mycorrhiza at species level with respect to indigeneous ecological conditions.

In India, Bakshi (1974) emphasised the role of ectomycorrhiza in various tree species. North-Eastern region which possess luxuriant growth of broad leaved tree species remained untouched. Sharma (1981) studied the ectomycorrhiza of pine (*Pinus kesiya*). Other important tree species of commercial timber value needed to be examined.

Exbucklandia populnea is a successful competitor and grows luxuriantly in pine forest of North-Eastern India. It is commercially important timber tree. The tree form endomycorrhiza of VA type. For the natural regeneration and successful plantation and to meet the increasing demand for production has stimulated interest to take up mycorrhizal studies of this tree species.

The present investigation was aimed to study

- The distribution and association of mycorrhizae with certain important tree species in natural forest ecosystem of N.E.India.

- Isolation, identification and establishment of VA mycorrhizal fungi in culture.
- Effect of vesicular-arbuscular mycorrhiza formation on establishment, growth and yield of tree seedlings.
- Effect of VA mycorrhiza on uptake of nutrients with special reference to phosphorus.
- Effect of VA mycorrhiza formation on activity of enzymes of tree seedlings.

REVIEW OF LITERATURE

In the year 1849 Reissek observed the roots of an orchid *Neottia nidus* and described in it the presence of a septate fungus. The identity and the significance of presence of fungus was not recognised. Then in the year 1881 Kamienski observed the presence of fungal hyphae in the roots of ericalean plants. Frank, a German botanist, coined the term 'Mycorrhiza' in the year 1885 for the fungus root-organs possessed by certain tree species. In the same year an endophyte which was certainly of VA type was observed by Treub in the roots of sugarcane. Schlicht (1889) described similar fungal infections in the roots of 71 spp. of flowering plants. Extensive studies by Janse (1896) in Java revealed VA endophyte in several plant species. He observed that the fungus was distinct from those found in orchidaceous and ericalean plants.

Dangeard (1900) described VA infections in poplar roots. He considered the infection a disease and named the fungus to be *Rhizophagus populinus*. Peyronnel (1923) was the first to recognise that VA infection was caused by genus *Endogone* by demonstrating hyphal connections between *Endogone* sporocarps and the mycorrhizal roots of certain alpine plants. These findings were confirmed by Mosse (1953), when she observed similar inter-connections between sporocarps and strawberry roots. Hawker (1962) and Hawker *et.al.*(1957) identified VA endophyte to be *Pythium spp*. When inoculated they found it to form typical VAM infection.

Unconfirmed
Now largely
discredited

Mosse (1959) described the morphology of extramatrical mycelium and measured the number of hyphal connections with the VA endophyte within mycorrhizal roots. In the same year she successfully established a typical VAM infection in the roots of aseptically grown apple and strawberry seedlings inoculated with surface sterilised spores of an Endogone sp.

Mosse and Bowen (1968a) gave a key for the identification of different types of Endogone spores.The key was based on morphology of spore wall.They reported that seven of nine spore types formed VAM with inoculated host plants. They (1968b) concluded that Endogone spores were more numerous in cultivated soil than in natural vegetation.

Mosse and Phillips (1971) studied the effect of phosphate and other nutrients on establishment of mycorrhiza in Trifolium parviflorum inoculated with Endogone spores. They observed that Calcium phytate, Calcium monohydrogen phosphate, Sodium phytate, Irion phytate, phytin, lecithin and DNA were good sources of phosphorus for both host plant and the symbiont.

Mosse (1973) reviewed the work on mycorrhiza.

Mosse and Hepper (1975) established Vesicular-Arbuscular mycorrhiza in clover root organ cultures. They found that pH of the medium was critical factor for the initiation and spread of mycorrhizal infections.

Mosse et.al.(1976) studied the utilization of rock phosphate by three legumes and onion plants in phosphorus deficient soils at variable pH in relation to inoculation with VA endophytes and *Rhizobium*. They observed that inoculation of VA endophyte increased the phosphorus uptake in plants. Plant responded to dry weight increase when phosphorus concentration of plant was low (0.15%). VAM improved phosphate utilization in all acid soils whereas in neutral and alkaline soils, rock phosphate was unavailable to both non-mycorrhizal and mycorrhizal plants. VA inoculation and added phosphates greatly improved nodulation of legumes.

Mosse (1977) studied the growth of pot grown *Stylosanthes guyanensis* and maize in unsterile soil with and without added rock phosphate and inoculated with a VA endophyte. She observed that inoculated plants grew better than the control. She concluded that inoculation improved utilization of rock phosphate but availability of phosphorus was not increased. Inoculum density affected response more than phosphate status. *Rhizobium* nodulation was stimulated by VAM.

Nicolson (1959) recognised two phases of VAM fungal mycelium. One internal to root, the other external, the latter consisting of thin walled hyphae bearing vesicles with rudimentary hyphal attachments which in some cases formed aggregations like *Endogone* sporocarps.

Baylis (1959) observed that growth of *Grisalinia litoralis* was not stimulated in sterilised soil when mycorrhiza was not formed due to techniques of inoculation, P uptake was enhanced due to the effect of mycorrhiza. He failed to get pure culture of endophyte on agar media except its ephemeral growth.

Baylis (1967) reported phycomycetous mycorrhiza in several tree species including gymnosperms from a rainforest of New Zealand. Mycorrhiza failed to establish in soil heated above 50°C and small amount of phosphorus added to unheated soil resulted in plants with many mycorrhizas, these plants were healthier than plants with no infection and no phosphorus addition. From these experimental findings he justified the ecological significance of mycorrhiza in forest trees where phosphorus availability in the soil was low and that mycorrhizal association increased phosphate uptake in plants.

Baylis (1975) justified the evolutionary status of magnoliales on the basis of morphological observations of mycorrhizae of these plants.

Gerdemann and Nicolson (1963) gave a method of isolation of extramatrical spores of *Endogone* from the soil and described 6 different types of spores they isolated from soil and believed them to represent distinct spp.

Gerdemann (1968) gave an excellent review on vesicular-arbuscular mycorrhizae which is still considered as an important reference.

Gerdemann and Trappe (1974) monographed the family *Endogonaceae* with seg~~g~~regation of genus *Endogone* into seven genera and gave a detailed description of the species encountered in Pacific Northwest region.

Gerdemann and Bakshi (1976) described two mycorrhizal fungi namely, *Glomus-multicaulis* and *Sclerocystis sinuosa* collected from forest trees of India.

Wastie (1965) successfully established *Endogone* infections in pot culture of *Hevea braziliensis* but could not ascertain the role of infection in this species.

Daft and Nicolson (1966) examined the effects of three mycorrhizal *Endogone* endophytes on tobacco, tomato and maize and found that stimulus obtained depended on nutrient conditions and the subsequent level of infection developed in the root system. Marked stimulation occurred with low phosphate availability and high root infections.

Daft and Nicolson (1969a) demonstrated that amount of phosphate added to the soil and the endophyte produced within the root-system were related inversely. They also observed that repeated doses of phosphorus given for longer periods had more suppressing effect than phosphorus given for shorter periods. They (1969b) found that the effect of *Endogone* on growth stimulation of tomato depended on amount of inoculum, however, the level of infection at final harvest was independent of inoculum concentration used in the beginning.

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

Daft and El-Giahmi (1974) observed that VAM inoculation of *Phaseolus* improved *Rhizobium* nodulation and various characteristics over non-mycorrhizal plants.

Daft and Nicolson (1974) and Daft, Hackskaylo and Nicolson (1975) described *Endogone* mycorrhizal association in plants growing on coal strips. They found infection in these plants to be heavy and stressed the ecological significance of mycorrhizae in such nutrient deficient harsh habitats.

Daft (1983) found that multiple mycorrhizal infections are common in nature. In mixed inocula *Glomus clarum* was a good fungal competitor than *G. caledonium*. Phosphate application reduced sporulation in *G. clarum* much than in *G. caledonium*.

Daft and Hogarth (1983) observed that inocula containing more than one endophyte gave more consistent results to growth enhancement, although, spore yield was low than with inoculum of single species. They suggested mixed inocula for field inoculations.

Redhead (1968) investigated the incidence of mycorrhizal association in several indigeneous and exotic tree species from Nigeria and found endomycorrhizae to be the dominant type. He also observed a positive correlation between amount of external mycelium and the internal root infection.

Redhead (1975) studied the effect of many ecological factors on mycorrhizal association of *Khaya grandifoliola* and found environmental variables like moisture level, light and nutrient supply to exert greatest effect on infection and sporulation of VA fungus He (1977) found that spores (endogonaceous) were distributed from lowland rainforest to dry zones, frequent in areas with marked dry seasons in which herbaceous growth died back. Spores could only be recorded from top 15cms of soil.

Hayman (1970) demonstrated seasonal variability in *Endogone* spore population and VAM infection of wheat in the field and found a correlation with growth and development of plant. He also noticed that soil treatment with formalin and application of nitrogen or any other fertiliser suppressed the spore population in soil.

Hayman and Mosse (1971) found in onion a large increase in shoot dry weight upto eighteen fold and with *Coprosma robusta* nineteen folds by adding phosphate. Mycorrhiza had similar type of effect in the absence of phosphate fertilizer. They concluded that mycorrhizal roots could make available phosphorus to plants while non-mycorrhizal failed to do so.

Hayman (1978) reviewed work on Endomycorrhizae in plants. He (1982) reviewed the occurrence, survival, adaptability and symbiotic activity of VAM fungi. He (1983) reviewed the physiological aspects of vesicular-arbuscular mycorrhizae.

Hayman and Tavares (1985) tested nine VAM endophytes for their infectivity and ability to improve growth of alpine strawberry seedlings in low 'P' soils. They noted that different endophytes vary enormously in their symbiotic effectiveness at different soil pH.

Sanders and Tinker (1971) observed, that rate of phosphorus inflow into mycorrhizal plants was higher and can not be accounted for mass flow or diffusion of phosphorus through soil. They concluded that rapid movement of phosphorus takes place through VA endophyte hyphae.

Sanders (1975) observed that the phosphorus status of the host plant controls the spread of mycorrhizal infection.In an experiment on effect of foliar application of phosphorus he found that phosphorus application reduced the rate of spread and intensity of mycorrhizal infection and weight of external mycelium associated with the infected roots.

Sanders et.al.(1977) indicated the presence of some specificity in VA endophytes. They studied the [illegible]iciency of VA endophytes on growth effects of onion and found that three had similer growth increments, hyphal inflows and only one endophyte did not enhance growth.

Khan (1971) found more than two type of spores in most of the soil samples and concluded that there was a close relationship between spore types and their numbers with soil categories. He(1972) found that vesicular-arbuscular mycorrhiza increased growth, dry matter, grains number per ear and grain weight in maize. The association also helped in greater uptake of phosphorus.

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

Khan (1978) reported heavy VAM infection in plant species growing in coal strips and identified three VAM fungi from them.

Khan (1981) studied the effects of different introduced VA endophytes on growth of onion in unsterilised coal wastes and found that mycorrhizal plants showed better growth characteristics than non-mycorrhizal plants. Also there were differential growth effects with respect to different VA endophytes. Sclerocystis rubiformis gave poorest while E_3 strain of Endogone gave most consistent results. He observed the better performance of E_3 as it also formed more extrametrical hyphae.

Kleinschmidt and Gerdemann (1972) studied the effect of methyl bromide and chloropicrin on the development of mycorrhiza and growth of citrus seedlings and found their effect to be toxic to development of VA infection.

Gray and Gerdemann (1973) studied the uptake of radioactive sulphur by the mycorrhizae and found that mycorrhizae took up sulphur faster than uninfected roots of red clover and maize growing in sand and supplied with nutrient solution.

Hatting et.al.(1973) through radioactive and split-plate culture technique established that translocation of phosphorus takes place through external hyphae of mycorrhizal fungi.

Meyer (1973) estimated that most phanerogams have endomycorrhizae (VA type), however, about 3% plants have ectomycorrhiza.

Crush (1974) reported that in phosphorus deficient soils Vesicular-arbuscular mycorrhizae strongly stimulated the nodulation and growth of four herbage legumes. He found that tropical legumes are much more dependent on mycorrhiza than temperate legumes.

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

Banister and Norton (1974) noticed that mycorrhizal plants in drained regimes with low nutrient grew better than non-mycorrhizal plants, however, in presence of high nutrients growth was decreased. They observed higher concentrations of nitrogen, potassium and phosphorus in shoots of plants growing in well drained regimes with low nutrient additions. They concluded that mycorrhizal infection had beneficial effect in freely drained infertile soils, a characteristic of well developed heathland.

Koske (1975) studied the distribution of Endogone spore types in coastal sand dunes of N.S.Wales and noticed that density of spores was greater in older more stabilised sand dunes than in younger foredunes and mobile dunes.

Koske and Halvorson (1981) found that plants forming a vegetation on a barrier dune of south coast of Rhode Island were mycorrhizal. They identified 6 species of VAM fungi and noticed that Gigaspora gigantea and Acaulospora scrobiculata were abundant. Spore population could not be correlated with the physical properties of the soil.

Pearson and Tinker (1975) observed that concentration of phosphorus in the hyphae of VA endophyte was more than the concentration of P in the soil solution. They concluded that absorption of phosphorus by VA endophyte takes place by an active process.

Cox *et.al.*(1975) observed polyphosphate granules in the hyphae of VA endophyte and suggested that these granules move in hyphae by cytoplasmic streaming and are the means of phosphorus translocation from the fungus to the root cells.

Kruckelmann (1975) noticed that *Endogone* chlamydospores were more frequent in loamy soils than in sandy ones and that spore number is influenced by agricultural practices such as manuring and soil tillage. Long term soil fertilization reduced the number in silty clay loam. But in sandy soil manuring increased number of spores. He also observed that spore number appeared to be more closely related to soil pH.

Ramiraj *et.al.* (1975) compared the effects of three VAM fungi on the growth of Papaya.

Ling-lee *et.al.*(1975) reported the occurrence of polyphosphate granules in hartig net and sheath hyphae of *Pinus* ectomycorrhiza; in sheath, intercellular and intracellular hyphae of *Arbutus* ectendomycorrhiza and in the hypha and vesicles of *Liquidambar* endomycorrhiza.

Powell (1975) studied the growth of onion and red clover inoculated with various types of *Endogone* spores and grown in phosphorus deficient ^{32}P labelled soils. He observed higher concentration of P in mycorrhizal plant as compared to non-mycorrhizal plants. However, he noticed that the specific activity of a sorbed phosphorus was identical in all mycorrhizal and control plants.

Powell and Daniel (1978) studied the utilization of soluble phosphate and Nauru rock phosphate by mycorrhizal crops of clover and ryegrass in sterile soil with inoculation of *Glomus tenuis*. They found that Nauru phosphate was utilised by the mycorrhizal plants and not by non-mycorrhizal ones. Also they observed that mycorrhizal plants recovered more fertilizers from soil as compared to non-mycorrhizal plants.

Powell (1979) compared the efficiency of indigeneous VAM fungi and the introduced strains of *Glomus tenuis* and *Gigaspora margarita* on growth stimulation of white clover and ryegrass in unsterile soil under glass house and field conditions and found that growth of ryegrass was more with *Glomus tenuis* than with indigeneous VAM fungi. Clover showed more growth in glasshouse than in field and *Gigaspora margarita* increased growth more than any other VAM fungi in field.

Strzemska (1975) investigated several cultivated crop plants with respect to the occurrence of mycorrhizal associations and gave a quantitative information of VAM infection in these plants.

Sparling and Tinker (1975) observed that change in soil nitrogen and pH levels caused by higher lime dressing had no effect on mycorrhizal infection. They explained this in the light of the nutrient level of soil and suggested that pH tolerance of host accounted for such discrepancy. Phosphate dressing also decreased infection.

Hall (1975) studied the effect of mycorrhiza formation of *Metrosideros umbellata* and *Weinmannia racemosa*. With addition of soluble phosphorus both mycorrhizal and non-mycorrhizal plants grew well and there were no significant differences between the two. He (1976a) reported VA endophyte from the roots of an orchid *Corybas macranthus*.

Hall (1976b) noticed that rapid growth stimulation of *Coprosma robusta* seedlings took place when inoculated with infected root segments than with spores.

Hall (1977) described several endogonaceous VAM fungi from New Zealand. From the studies he concluded that except in the case of polymorphic vesicles associated with *Acaulospora spp.* infections and external vesicles of *Gigaspora spp.*, the other characters of mycorrhizal spp. for diagnosis of the species does not seem warranted. He also observed that spore number tended to be low in bush soils but high in disturbed areas and seral vegetation.

Hall and Fish (1979) gave a dichotomous key based on computer programme for the identification of various spp.

of VAM fungi known at that time.

El-Giahmi et.al.(1976) described VAM fungi collected in the rhizosphere of agronomic plants and tree species.

Godse et.al.(1976) described occurrence of Endogone mycorrhizal infections in eight important crop plants of Karnataka.

Sahni (1976a) studied the effect of inoculation of isolated indigeneous fungi on growth and nutrient uptake by plants of cowpea, maize and tomato. He noticed a positive correlation between VAM infection and amount of phosphorus and nitrogen in the tissues of these plants. With mycorrhizal rice he(1976b) further observed a positive correlations between numbers of spores in individual root system and growth of rice plant and percent VAM infection.

Johnson (1977) observed variable spore density of nine VAM fungi and concluded that nonsporing races were pre-dominant. He did not observe any host specificity, however, they were specific to soil types. In rock areas or logs he observed Rhizophagus tenuis to be the pioneer endophyte.

Johnson et.al.(1982) observed that VAM formation was reduced during early stages of flower bud development and not during later bud expansion. They suggested that reduction of VAM formation resulted from a decrease in availability and net likage of metabolites necessary to sustain mycorrhizal fungus during pre and post infection.

Becker and Gerdemann (1977) described a colorimetric method for VAM quantification depending on yellow pigments in VAM infected roots in onion.

Barrow et.al.(1977) compared the responses of VAM to freshly added phosphate and firmly bound phosphates and found that phosphate utilization increased more than double by VAM in Trifolium subterraneum and Allium cepa for freshly added phosphate, however, difference was not significant for firmly held phosphate.

Furlan and Fortin (1977) found that rate of VA infection and percentage infection at low light intensity was higher than at higher light intensities. Spore production increased with light intensity.

Furlan and Fortin (1982) noticed that different fungi produced varying effects according to plant root. There was no correlation between the percentage of root infection and stimulation of growth in case of Strawberry but in other three hosts significant correlation was observed. No fungal specificity of the endophyte for the host plant was suggested.

Subsequently, Furlan et.al.(1983) studied the response of white ash seedlings to inoculation with five different VAM fungi and observed variation on growth with respect to different fungal species, however, difference decreased after 82 days of culture. They noticed a significant growth enhancement with low level of root colonization.

Saif (1977) observed that a relationship of different phases of mycorrhizal development and spore number in the soil existed with the physiological changes in the host.

Saif (1981) observed that O_2 level in the soil affected mycorrhizal development in plants of Eupatorium odoratum.

Saif (1987) compared growth and mineral uptake of mycorrhizal and non-mycorrhizal plants of 24 tropical forage legumes and found that root and shoot dry weights and total uptakes of P, N, K, Ca and Mg significantly increased by mycorrhizal inoculation. Also mineral content was high for mycorrhizal than non-mycorrhizal plants whereas R/S ratio decreased.

Abbott and Robson (1977a) compared the growth response of subterranean clover inoculated with two type of VA endophytes grown in sterilised and unsterile soil with five levels of applied superphosphates in high phosphate fixing soil. They observed marked increase in the growth and phosphorus content of plants inoculated with VAM fungi. Non-mycorrhizal plants produced more dry weight at a given phosphorus concentration in tops than did mycorrhizal plants.

Abbott and Robson (1977b) studied the distribution and abundance of different VA endophytes. They found five spore types. Honey colored sessile spores were most common in distribution. Yellow vacuolate type was mostly confined to cultivated and fertilised soils. The other three endophyte types occurred on both virgin and agricultural soils. They concluded that distribution of honey colored sessile and yellow vacuolate spores in cultivated soil appear to be associated with variation in soil pH.

Abbott and Robson (1978) investigated the effect of three introduced VAM fungi in the field soil infested with four indigenous endophytes and found that introduced endophytes differed in their ability to increase growth and phosphorus content of subterranean clover and these differences in growth were related to the rate of mycorrhizal development when limited by phosphorus supply. They (1982) reviewed the role of VAM in agriculture and discussed the methods of field inoculation of VAM fungi in agricultural soils.

Cooper and Tinker (1978) through radioactive and split-plate culture techniques observed that the molar amounts of phosphorus, sulphur and zinc translocated were in the ratios of 35:5:1 whereas the mean influx in the hyphae was in the ratio of 50:8:1. They (1981) studied the effect of some environmental variables viz. transpiration rate of host, temperature, cytoplasmic streaming inhibitor (Cytochalasin-B) and osmotic pressure on the translocation of phosphate by external hyphae of _Glomus_ _sp_ infecting

Trifolium repens across a barrier in soil-agar split-plate culture. They found that phosphorus translocation was increased by increasing the rate of transpiration. They observed maximum phosphorus transport at 20-25°C and low transport at 5°C. Cytoplasmic inhibitor inhibited either uptake or translocation of P by the fungus. Osmotic pressure at 1-2 bars did not affect transport of P. They concluded that translocation of P in the hyphae takes place by protoplasmic streaming or by bulk flow of hyphal contents under water potential gradient.

Hirrel et.al. (1978) demonstrated the occurrence of VAM in 9 spp of chenopodiaceae and 2 spp of Cruciferae. They did not find arbuscules in them.

Molina et.al. (1978) investigated the VAM infection in five species of Festuca growing at five different sites and found that percentage infection did not differ much between different species or at different sites.

Herkowitz and Easty (1978) found a seasonal variability in spore population of VAM fungi in Quebec soils.

Boatman et.al. (1978) observed that formation of mycorrhizae was prevented and also its spread in established infections halted in clover roots by soil drenches of two fungicides they used. Immersion of fungal inoculum in suspension of fungicide reduced its infectivity.

Ratnayake et.al.(1978) observed that high phosphate inhibited mycorrhizal association in Sorghum vulgare. They proposed a hypothesis that 'P' inhibition of mycorrhizal symbiosis is associated with a membrane mediated decrease in root exudation.

Menge *et.al.*(1978) using split root and vial embedding techniques concluded that concentration of 'P' within the plant rather than soil phosphorus led to reduction in colonization, infection and spore production of *Glomus fasciculatus*.

Menge *et.al.*(1979) studied the effect of several heat treatments, a nematicide, and two fungicides on survival, infection and sporulation by *Glomus fasciculatus*. They found that heating inoculum at 52.5°C for 10 mts killed it. They concluded that pesticide and fungicide could be used to reduce contamination and to increase the quality of inoculum of *Glomus fasciculatus.*

Menge *et.al.*(1983) reviewed various methods for utilization of VAM fungi in agricultural fields.

Mac donald and Lewis (1978) demonstrated occurrence of acid phosphatase, glutamate dehydrogenase, glyceraldehyde -3-phosphate, NAD, NADH diaphorases in *Glomus mosseae*. Acid phosphatase activity was found in lysing and growing fungal structures.

Mac donald (1981) devised a compact autoclavable hydroponic system for production of axenic VAM between *Trifolium parviflorum* and *Glomus caledonium.*

Gianninazzi-Pearson and Gianinazzi (1978) found that the activity of soluble alkaline phosphatase enzymes in the root tissue and development of mycorrhiza were closely related.

Gianninazzi _et.al._(1979) observed distribution and localization of acid and alkaline phosphatase and found active acid phosphatases in arbuscules.

Gianninazzi-Pearson and Gianninazzi (1983) reviewed the physiological aspects of vesicular-arbuscular mycorrhizae.

Gianinazzi-Pearson _et.al._(1985) concluded that propagule number, rate of root colonization and plant growth responses to VAM were not necessarily related to each other or to the physico-chemical properties of soil.

Black and Tinker (1979) observed a slight correlation of %VAM infection of barley with clay content and soil pH.

Nicolson and Johnston (1979) investigated VAM infections of pioneer grasses in sand dune system and found _Glomus fasciculatum_ to be the only endophyte colonizing the roots.

Nicolson and Schenck (1979) described 21 species of Endogonaceae.

Ho and Zak (1979) studied the acid phosphatase activity of six ectomycorrhizal fungi and discussed their role in utilization of complex phosphates by tree roots.

Haselwandter (1979) found a close relationship between %VAM infection and host vigour. He observed that infection level increased with decrease in altitude of sampling site.

Vaidyanatha et.al.(1979) observed that growth and phosphorus uptake was increased by addition of rock phosphate to VAM inoculated plants. The source of phosphate gave different growth and P uptake stimulii in Pueraria.

Owusu-Bennoah and Mosse (1979) observed that % infection of barley, lucerne and onion was less with indigenous fungi than with introduced fungi. Indigenous fungi failed to sporulate.Spore production was affected little by host or soil phosphorus and was not related to % root infection.

Miller (1979) observed that plants were mycorrhizal on undisturbed sites but he found non-mycorrhizal plants on disturbed sites.

Bagyaraj et.al.(1979) reported the occurrence of VAM endophyte in 12 tropical aquatic plants. It was earlier believed that VA infection is absent from very wet habitats especially where root system is submerged.

Bagyaraj et.al.(1979) observed that dual inoculation with VAM and Rhizobia increased significantly the dry weight of shoot and its nitrogen content over their single inoculation with either Glomus or Rhizobia. They suggested that VAM inoculation in fields may help in nodulation and nitrogen fixation of field grown soyabean inoculated with Rhizobia.

Bagyaraj (1980) found that VAM inoculation in three crop plants increased root, shoot weights, P and Zn content. Mn content was unaffected.

St.John (1980) examined mycorrhizal infection of 89 tree species from Amazonian rain forest and a strong association between root depth and mycorrhizal condition. Surface roots were much more mycorrhizal than deeper ones. He interpreted deep root mycorrhizal condition as an ecological strategy for avoiding competition with more efficient mycorrhizal spp. He (1980b) correlated the VAM status of 89 Brazilian forest spp. with various root characteristics and found a significant association between magnolioid root characteristics and VAM infection.

Herbaceous plants generally form endomycorrhiza, however, Warcup (1980) found that some indigenous herbaceous plants of Australia possess ectomycorrhizae ranging from beech type to loose sheath type. Besides ecto these spp. also form endo-mycorrhizae(VA type)also.

Reports on mycorrhizal association in mosses are scanty and inconclusive. Parke and Linderman (1980) described endomycorrhizae in Funaria hygromatrica and discussed its ecological significance in production of VA inoculum.

Nemec (1980) investigated the effect of eleven different fungicides on development of endomycorrhizae (VA type) in sour orange. Nemec (1981) demonstrated histochemically the distribution of dehydrogenases in young arbuscules, hyphae and vesicles. He detected peroxidase and catalase in sensing arbuscules.

Rose (1979) reported the occurrence of VAM in nonleguminous actinomycetous nitrogen fixing plants. Rose (1981) described VA endophyte in endemic plants of Sonoran desert and discussed its significance in harsh habitat.

Rose and Youngbeg (1981) observed that dual infection of *Ceanothus velutinus* with actinomycete and VAM fungi gave better growth, nutrient level, yield, nodulation and nitrogenase activity in this plant.

Pope (1980) observed better growth and nutrient uptake of mycorrhizal seedlings than non-mycorhizal ones. Intermediate level of nutrient regimes in the Hoagland solution gave better response to growth and nutrient uptake. He noticed that seedling growth attributed to VA inoculation is directly related to degree of mycorrhizal colonization and inversely related to amount of extractable soil phosphorus.

Pairunan, Robson and Abbott (1980) studied the effect of VAM on growth and 'P' uptake of subterranean clover supplied with phosphorus from three sources differing in their solubilities. They found that at intermediate level of P, VA inoculation increased growth and 'P' content of tops of plants. Effectiveness of phosphorus was independent of solubilities of phosphorus source. At given conc. of phosphorus in tops non-mycorrhizal plants had more dry matter than mycorrhizal clover supplied with SSP. Non-mycorrhizal and mycorrhizal plants supplied with c-grade rock phosphate had same dry weight at given 'P' conc. in their tops.

Lambert et.al.(1980) noticed that indigenous strains of VAM fungi had better adaptation to edaphic factors than introduced strains which, otherwise are more efficient.

Heap and Newman (1980) demonstrated that mycorrhizal association can increase phosphorus transfer between plants but it was not clear whether transfer was via interconnecting hyphae or P must leave donor root before being taken up by mycorrhizal hyphae.

Giovanetti and Mosse (1980) reviewed the various methods of assessment of VAM infection in plants and calculated the standard error of four method used extensively by most workers.

Ocampo et.al.(1980) demonstrated that barrier of mycorrhizal infection in non-host crucifers and chenopods are intrinsic and more probably related to characters of root cortex or epidermis than to any infection inhibiting factors that might be released in root exudates.

Rhodes and Gerdemann (1980) reviewed nutrient translocation by mycorrhizae in plants.

Assami *et.al.*(1980) found that activity of nitrogenase enzyme of soybean roots were stimulated by mycorrhiza formation and preceded plant growth responses. Not the only are

Smith (1980) reviewed work on mycorrhiza of higher plant.

Smith (1981) found that small amount of spore inoculum of VA endophyte was insufficient to induce mycorrhizal infection of soyabean unless pre-infected roots were also inoculated. He concluded that inoculum level was not of major importance provided it was sufficient to assure 50% infection.

Smith and Smith (1981) studied the effect of soil sterilization which alters the properties of soil on the growth and VAM formation in *Trifolium subterraneum* and concluded that sterile soil can be used as a control treatment to VAM inoculation.

Smith (1982) demonstrated that inflow of phosphate into the roots of mycorrhizal plants of *Trifolium subterraneum* was more than that of non-mycorrhizal plants during early growth of plant.

Smith et.al.(1985) studied the activities of Glutamine dehydrogenase and Glutamate synthetase in relation to phosphorus nutrition and mycorrhiza development. They found that activity of Glutamine dehydrogenase was unaffected while that of Glutamate synthetase was affected by phosphorus application and mycorrhiza formation.

Smith et.al.(1986) observed that the growth responses to VAM were larger at low phosphorus in soil and smaller at high phosphorus level. They concluded that mycorrhizal infection increased the rate of absorption of phosphorus from the soil.

Manjunath and Bagyaraj (1981) assessed the effects of different components of VAM inoculum on growth of onion and found that plants inoculated with chlamydospores produced more dry weight and contained more phosphorus.They concluded that chlamydospores were the important components of inoculum.

John et.al.(1981) described a two membered system for obtaining pure inoculum of VAM infection without specialised media.

Hepper (1981) developed a method for growing axenic seedlings infected by VAM using agar, paper or glass as a support. The technique is suitable for studying the interactions between fungus and host plant.

Bierman and Linderman (1981) gave a novel method for VAM assessment using less number of root segments. The results can be obtained within confidence limit of 10% of the mean.

Chilvers and Daft (1981) observed morphological diversities of VAM infections in different genera of host plants. They did not get any relationship between spore population and %VAM infection. They also observed that mycorrhizas were significantly less likely to bear root hairs.

Azcon and Ocampo (1981) observed that conc. of nutrients in plant tissue did not affect level of infection in wheat cultivars inoculated with Glomus mosseae. They demonstrated that lack of sugar exudation from roots in some wheat cultivars is responsible for absence of mycorrhizal infection in some wheat cultivars.

Csinos (1981) demonstrated that VA inoculation increased the growth response of tobacco at low levels of soil fertility.

Clarke and Mosse (1981) observed that yield of barley was increased by VAM inoculation supplemented with phosphate addition to soil.

Hardie and Leyton (1981) found that VAM inoculation enhanced growth of root, shoot and concentration of phosphorus in clover plant and reduced its R/S ratio when grown in P deficient soils, addition of phosphorus below optimum level stimulated the growth of both mycorrhizal and non-mycorrhizal plants.

Singh and Varma (1981) described various endogonaceous spores associated with some xerophytic plants of northern India.

Allen et.al.(1981) compared the effect of different phosphorus sources on percent VAM infection of Boutelova gracilis in a defined media and found that mycorrhizal infection was highest in roots of plants grown in the presence of phytate (75%). Infection was low with sodium phosphate (19%) and mixed phosphate (22%). No penetration in plants occurred with sodium phosphate + inositol or inositol and calcium media.

Plenchette et.al.(1981) concluded that inoculation of seedlings prior to field planting in unsterilised, phosphorus poor soil can lead to a significant growth stimulation.

Plenchette et.al.(1982) demonstrated that turface is a good growing media for mycorrhizal studies as it permit good plant growth and abundant mycorrhizal formation. The fungi produced different growth effects according to plant host. They did not observe fungal specificity in VA endophyte for the host plant.

Plenchette et.al.(1983a). suggested that a thorough evaluation of the infectivity and the efficiency of a given endomycorrhizal fungus on host with different levels of phosphorus should be conducted.

Plenchette et.al.(1983b) observed that neither total 'P' uptake nor nutrient conc. of host can explain growth stimulation of mycorrhizal plants when phosphorus fertilization was higher, they suggested that stimulation might be attributed to the interaction of one or more fungal metabolites with the growth processes of host plant.

Schultz et.al.(1981) studied the mycorrhizal growth stimulation of seedlings of eight hardwood tree spp.

Iqball et.al.(1981) reported heavy mixed VAM infections in ferns of Pakistan.

Trappe (1982) proposed a synoptic key for the identification of genera and species of zygomycetous mycorrhizal fungi.

Trappe and Schenk (1982) discussed the methods of observations of VAM fungi for the purpose of their taxonomy.

Walker et.al.(1982) noticed that change in spore population was correlated with soil moisture content. Ploughing and herbicide treatments depressed spore production.

Hogberg (1982) studied the mycorrhizal infections in various forest ecosystems of Tanzania and reported a tropical dominance of endomycorrhizae.

Toth and Toth (1982) gave a morphometric method of VAM quantification using ocular grid in the microscope.

Toth et.al.(1984) found a positive correlation between ear leaf phosphorus content of inbreds of maize and their %mycorrhizal colonization. They suggested that mycorrhizal colonization may be a significant component of genetic differences expressed in the field.

Stahl and Christensen (1982) found that relative prominence of different species of VAM fungi was related to the geographical locations, abiotic factors and identity of plant symbiont.

Warner and Mosse (1982) found that VAM colonization and linear spread of infection were favoured by greater root density.

Schenck and Smith (1982) identified many new species of endogonaceae from Florida.

Howler et.al.(1982) studied the effect of phosphorus concentration on endomycorrhizal association on Cassava in hydroponic system and found that the Cassava had heavy VAM infection at low phosphate.

Bethlenfalvay et.al.(1982) determined fungal biomass both extra and intra by chitin assay method and found that intra radical mycelium increased throughout life span of host whereas extraradical was maximum till onset of soyabean pods and decreased afterwards, the ratio of extra and intra also decreased through association.They considered this ratio to be an index of usefulness to host growth and its high value coincided with the significant growth enhancement of the host plant.

Jensen (1982) observed that different VAM fungi had different effects on growth and nutrient uptake of barley plants.

Fairweather and Parbery (1982) also reported differential effectiveness of four VAM fungi on growth improvement of tomato.

Whittingham and Read (1982) demonstrated that direct transfer of nutrients between living 'source' and 'sink' plants occur by way of connecting mycorrhizal hyphae.

Zak (1982) studied the effect of amendment of mine spoil with fertilizer or liquid sewage sludge and concluded that sewage sludge application has adverse effect on reclamation programme.

Marx et.al.(1982) studied the distribution and localization of ATPase activity and supported the hypothesis that nutrient exchange in VA mycorrhizae occurs across the host fungus interface and active transport mechanism is involved.

Kormanik et.al.(1982) observed differential growth response of mycorrhizal seedlings of eight hardwood spp. to inoculation with different VAM fungi.

Kormanik (1985) observed that VAM inoculation improved leaf retention of seedlings the growth characteristics of VAM plants did not differ with different phosphorus treatments.

John et.al.(1983) noticed suppression of VAM infection in the presence of potassium permanganate. They concluded that oxidiazable compounds have a largely stimulatory effect on VAM infection.

Anderson et.al.(1983) demonstrated that spore population in soil was positively correlated with plant cover and negatively with soil moisture. They (1984) observed that plant cover, abundance of VAM spores, plant species richness and number of VAM fungi representing as spores had significant correlation with each other and with percent organic matter in soil. Spore population had significant negative correlation with soil pH and available nutrients and moisture content. They noticed that calcium was found to be the best predictor of spore abundance.

Tommerup (1984) suggested that chemical rather than physical factors associated with Brassica napus appeared to be involved in reducing rate of development of VAM.

Ames et.al.(1984) suggested that at intermediate fertilizer level where plants produced most growth, mycorrhizal sorghum plants derived nitrogen from a source that was less available to non-mycorrhizal plants.

Wilson (1984) compared effect of inoculum density of three VAM fungi on development of infection and found that fungi showed different strategies for spread of infections. Inoculum density rather than age of infection affects intensity of infection.

Graham and Timmer (1985) found that addition of superphosphate reduced root colonization and growth response of root stock of Carizo citrange and sour orange. They concluded that rock phosphate has a long term available 'P' compared with superphosphate which leached fast. No further phosphorus fertilization is necessary if rock phosphate is used and VAM is inoculated into soilless media.

Frankland and Harrison (1985) reported that VAM infection in Acer was highly correlated with soil factors viz. pH, organic matter, phosphorus and Iron, growth of Acer has greater influence on VAM infection than soil factors.

Kurakov (1985) reviewed the effects of environmental variables on endomycorrhiza formation in plants and distribution of VAM fungi in nature.

Schubert and Hayman (1986) studied the effectiveness of different endophytes at different levels of soil phosphate.They found that mycorrhizal infection was decreased by addition of phosphates to soil. Different endophytes had different rates of colonization and infection. Growth response was large at low and medium phosphorus. They stressed that selection of field inoculation should take special account of endophyte behaviour in soil given extra phosphate.

Thomson et.al.(1986) showed that increase in supply of phosphorus decreased % root infection.At low 'P' infection decreased inside the root and at high P infection outside the root was also decreased. They observed that growth response to inoculation depended on species of VAM and phosphorus level of soil.

CLIMATE, VEGETATION & SOIL

The present study was carried out at Shillong, the capital of Meghalaya which is located at 25°34'N and 91°56'E. The entire area is hilly and altitude varies from 1250-1960 meters (MSL).The vegetation consists of mixed evergreen broad leaved forests and pine forests.The former chiefly consisting of oaks, laurels, magnolias, *Schima* spp. at lower altitudes and dwarf ericaceous association of *Rhododendron* spp. alongwith other dwarf trees like *Symplocos spicata*, *Elaegnus pyriformis* etc. at higher level~~s~~. Pine forests consist~~s~~ of species of *Pinus kesiya* mixed with species of *Exbucklandia* and *Alnus nepalensis*.

The soil is red laterite under red loam or brown loam types. At some places the sand content of the soil is upto 90%.The soil is acidic in reaction with pH ranging from 4.9 to 6.7.The soil is rich in nitrogen content (0.338-0.543%) in the form of organic matter (9.9 to 13.9%). However, the amount of phosphorus is very low ranging between 20kg/acre to 50kg/acre.

The climate of Shillong is cool with winter temperature dropping down to 4-5°C in the month of January. The maximum temperature goes upto 25°C in the month of April. The average maximum temperature is 20.72°C and minimum temperature is 12.77°C. The rainfall is spread over all the months except November, December and January when it is nil or very low. The average annual rainfall is 173.53~~m~~cm. Similarly, average humidity is very high ranging between

71.38 to 84.21 in the diurnal cycle.

The typical summer season is not found at Shillong. However, based on meterological conditions the year can be divided into following seasons.

1. Winter season : Winter season starts at the end of October and continues upto the middle of February. The average lower temperature during winter is 7.9°C and maximum temperature is 16.61°C. During winter season rainfall is very low.

2. Spring season : The period from middle of February upto the middle of April covers the spring season which experiences high wind velocity and less humidity and moderate temperature.

3. Rainy season : The rainy season extend from the middle of April to the middle of October. The early period of rainy season is a bit warm representing summer while the later period of the season is comparatively cool.During rainy season humidity is very high due to heavy rainfall.

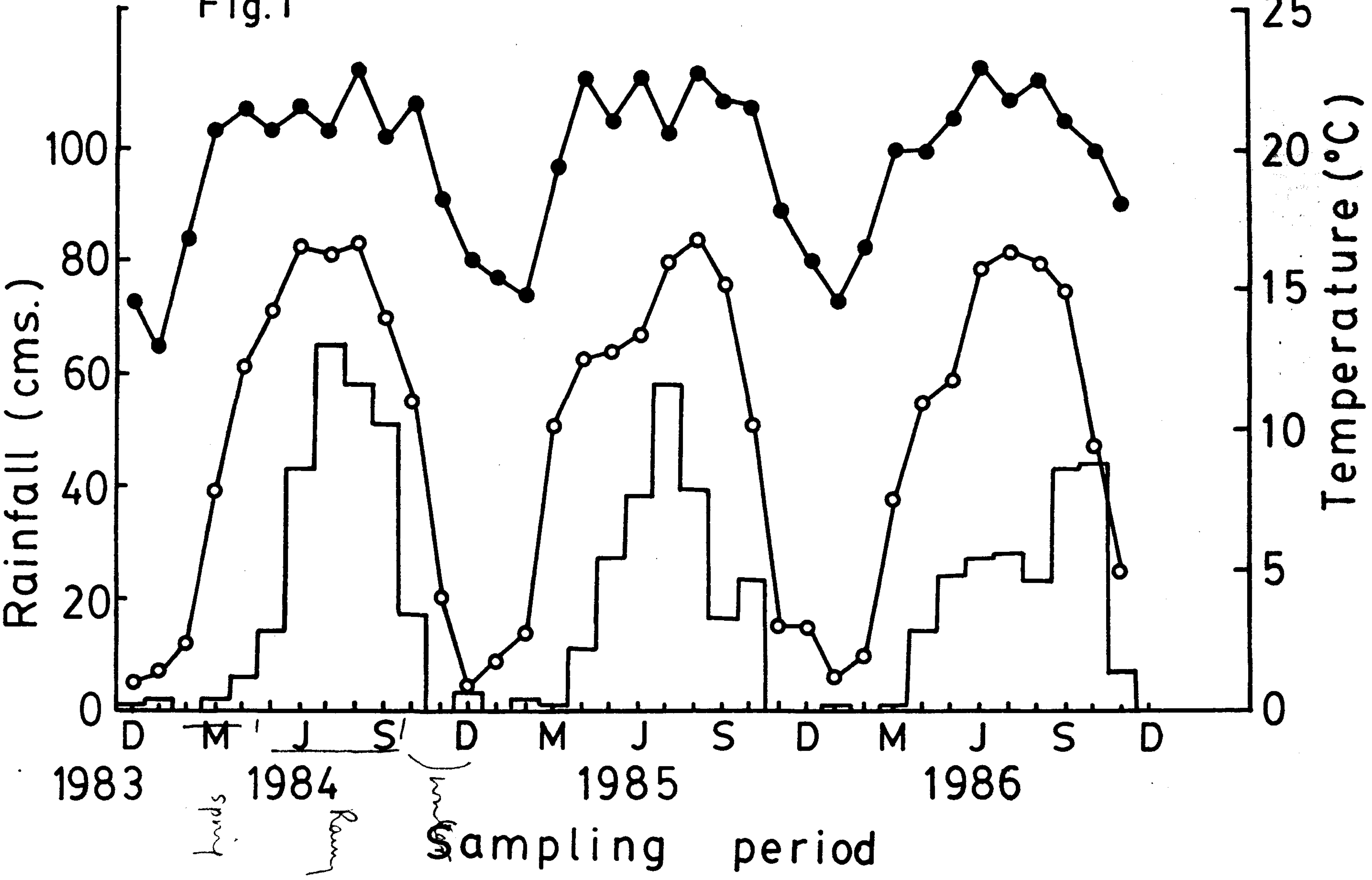
The data of average monthly temperature and rainfall during the study period (1983 to 1986) is presented in fig. 1.

Fig. 1 Data of temperature and rainfall during the study period of 1983 to 1986.

Histograms : rainfall; ●——● : maximum temperature;

○——○ : minimum temperature.

Fig.1


Rainfall (cms.)
0
20
40
60
80
100
Temperature (°C)
0
5
10
15
20
25
D
M
J
S
D
M
J
S
D
M
J
S
D
1983
1984
1985
1986
Sampling period
buds
Rain

CHAPTER - I

DISTRIBUTION, SURVEY AND SEASONAL VARIATION IN VESICULAR-ARBUSCULAR MYCORRHIZA

INTRODUCTION

About 95% of the world plant species are mycorrhizal, of which the great majority form vesicular-arbuscular mycorrhiza (Malloch et.al.,1980, Trappe,1977). However, unlike ectomycorrhizae which predominate~~s~~ in the temperate climate, VA mycorrhiza is a dominant feature~~s~~ of tropical plants (Readhead, 1968). Most of cultivated crop plants form VA mycorrhizae (Godse et.al.,1976; Bloss,1979; Kianmehr,1981; Strzemska,1975 and Trappe,1982). In natural vegetation also many plant species possess VA type of mycorrhizae (Read and Haselwandter, 1981; Miller,1979). In forest ecosystem most of the tree species develop vesicular-arbuscular mycorrhizae (Redhead,1968; Baylis,1967; St.John,1980; Johnson,1977; Malloch & Malloch, 1981,82).

VA mycorrhizae is a common feature of plants growing in harsh habitats marked by deficiency of nutrients. Such places are coal/mine/industrial wastes, deserts and other disturbed sites (Daft and Nicolson, 1974; Daft et.al.,1975; Khan, 1978; Miller, 1979; Koske and Halworson,1981; Mejstrik and Cudlin, 1983).

The incidence and extent of mycorrhiza development in plants may vary with season (Staffeldt and Vogt, 1975) and nutrients status of soil (Daft and Nicolson, 1969; Allen et.al.,1981). Plants growing in nutrient deficient habitats frequently develop VA mycorrhizae (Baylis, 1967). Several other physical and chemical factors of soil affect

VA mycorrhizal infection (Furlan and Fortin, 1977). Incidence of mycorrhizal colonization may also depend on composition of plant community (Hirrel et.al.,1978).

VA mycorrhizal fungi are ubiquitous in nature. They multiply and survive through the formation of spores, mostly in soil (Gerdemann & Nicolson, 1963). Distribution and number of spores are affected by soil types (Khan,1971), cropping history and agricultural practices (Kruckelmann.,1975), nutrient status and various other edaphic factors (Walker et.al.,1982; Anderson et.al.,1984) and season (Herkowitz and Easty, 1978; Redhead, 1977). VA endophytes are not host specific (Johnson, 1977). However, the relative prominence of diferent species depends on geographical locations, biotic and abiotic factors (Stahl and Christensen, 1982).Agricultural soils possess more spores than natural vegetation (Mosse and Bowen, 1968b). VA endophytes with honey colored sessile and yellow vacuolate spores are more common in cultivated and fertilised soil (Abbott and Robson, 1977a).

Timber is the main source of economy of North-East region. The climatic conditions favours luxuriant growth of forest trees. VA mycorrhiza may be an essential ecological requirement of tree spp. Therefore, the present study is devoted to the distribution and extent of vesicular-arbuscular mycorrhizal development in a forest ecosystem of Meghalaya. The study is extended to the ecology of seasonal variability to evaluate the requirement and significance of the association in forest trees.

MATERIAL AND METHODS

The outdoor field study comprised of collection of mycorrhizae alongwith its rhizospheric soil. It was conducted in two parts. In the first part a general survey of about 26 important tree species in a natural mountain, mixed evergreen forest ecosystem was carried out in the month of September, 1983 to evaluate the occurrence, type and status of mycorrhizae and its ecological significance. The second part comprised of a seasonal study of endomycorrhizae of 11 tree spp. from the same habitat. The seasonal study was conducted in five surveys each carried out in the months of December, February, April, June and August covering three successive seasons namely, winter, spring and rainy in each of two successive years viz.,1983-84 and 1984-85.

Collection of samples : For each survey five individuals of each tree species were sampled randomnly with three collections made from each individual plant. All the 15 sub-samples of each tree species for each survey were thoroughly mixed. The composite samples of mycorrhizae and soil were brought to the laboratory. The roots were separated from the soil, washed with tap water and preserved in FAA (Kormanik et.al.,1980). The rhizospheric soil was ground~~ed~~ and mixed thoroughly. pH and moisture content of soil were determined with fresh soil samples and the rest of the soil was air dried for analysis of chemical properties and assessment of endogonaceous spore population.

Assessment of Mycorrhizal Infection : The preserved root specimens were washed repeatedly with tap water and cut into 1 cm root segments. 100 segments were randomnly selected and transferred to 100ml conical flask containing about 30ml of 10% KOH solution. The mycorrhizae segments were cleared and stained by the autoclaved method of Kormanik et.al.(1980). After KOH treatment the colored roots were decolorised with alkaline H_2O_2 and stained in 0.05% Trypan blue. The root segments were mounted in groups of 10 segments on the microscopic slides and pressed for observation under microscope. A root segment was considered infected when one or more of the structures characteristic of VA mycorrhizal infection were observed and percent root infection was determined by foot slide technique of Read et.al.(1976) and calculated as follows.

Be more Specific

$$\%VAM\ infection = \frac{No.\ of\ infected\ root\ segments}{Total\ number\ of\ segments\ examined} \times 100$$

Assessment of Endogonaceous spore population : The endogonaceae spores were isolated by wet sieving and decanting technique of Gerdemann and Nicolson (1963). 10g air dried soil from each sample was suspended in 200ml water in a 500ml bottle. The suspension was shaken vigorously for 5 minutes and washed into a 1 litre measuring cylinder and allowed to stand for a minute. The suspension was passed through a series of sieves of different pore size ranging from 500-$\overset{\mu m}{63U}$. The sievings were transferred into glass dishes and filtered through Whatman filter paper No.1. The

m

debris on filter papers were scanned under stereomicroscope for the endogonaceous spores. The spores were hand picked by a needle and mounted on glass slides. A count of total spore number was recorded. The spores were separated into genera based on generic description of Gerdemann and Trappe (1974) and the generic population was also recorded.

Analysis of Soil : Each composite soil sample was ground~~ed~~ and thoroughly mixed and passed through 2mm sieve. The fraction greater than 2mm was discarded. The pH and percent moisture content were determined with fresh soil and the chemical analysis was performed with air dried soil. For determination of available phosphorus, calcium and potassium 2mm sample was used and for the percent organic matter and percent nitrogen the 2 mm sample was ground in pestle and mortar and passed through 0.2mm sieve. The fraction which passed through 0.2mm sieve was used. The methods suggested by Allen (1974) and Jackson (1972) were followed and adapted as follows.

Determination of Soil pH : For the determination of pH, 10g fresh soil (2mm) was suspended in 25ml of distilled water in a 100ml beaker. The suspension was stirred for 15 minutes and pH read with electric digital pH meter.

Determination of percent moisture content : 10g fresh soil (2mm) from each composite samples were taken in petri dishes and oven dried at 105°C for a period of 24 hours. The dry sample was reweighed and percent moisture content calculated as follows.

$$\%\text{moisture content} \quad \frac{\text{Loss in weight on drying}}{\text{Initial weight of sample}} \times 100$$

Determination of organic matter : Organic matter was determined by the method of Walkley and Black (1934). For the analysis 100mg of air dried soil was taken in a 500ml conical flask alongwith 10ml of 1N.$K_2Cr_2O_7$ solution and flask was swirled to mix soil with $K_2Cr_2O_7$solution. About 20ml of conc. $H_2SO_4$was added rapidly and left for 30 minutes. Thereafter about 200ml of distilled water and 10 ml of 85% phosphoric acid were added and allowed to stand for another 10 minutes. The mixture was then titrated against IN $FeSO_4$ using diphenylamine as the indicator. For the blank titration the procedure was repeated without soil. Percent organic matter was calculated according to following formula :-

$$(1)\ \%\ \text{Organic matter} = \frac{B-S}{W} \times 0.3 \times M \times 1.72$$

where

B = ml. volume of $FeSO_4$ used for blank titration

S = ml. volume of $FeSO_4$ used for sample titration

W = weight (g) of soil taken

M = Molarity of $FeSO_4$ solution used

Determination of Percent Nitrogen : The semimicro Kjeldahl method suggested by Allen (1974) was adapted as follows.

(a) Digestion : 250 mg air dried soil (0.2mm) was taken in a 100ml semimicrokjeldahl flask alongwith 2g K_2SO_4-HgO mixture and 3ml of conc. H_2SO_4. The mixture was digested for about 40 minutes till the digest became colorless. The digest was cooled, diluted to 100 ml with distilled water and filtered on Whatman no.1 filter paper. The filtrate was used for the estimation.

(b) Estimation : Percent nitrogen was estimated by Indophenol blue method, suggested by Allen (1974) and percent nitrogen calculated as follows.

$$\%N = \frac{C(mg) \times \text{Solution volume}}{10 \times \text{aliquot} \times \text{sample wt(g)}}$$

In present case solution volume = 100 ml; aliquot = 2 ml; sample weight = .250g and C = concentration reading (mg) in the aliquot.

Determination of Available phosphorus : The available phosphorus was estimated by the method suggested by Jackson (1972) and adapted as follows.

(a) Extraction of phosphorus : Brays and Kurtz I reagent was used for the extraction and was prepared by mixing 1.11g of solid Ammonium fluoride and 4.16ml of 6N HCl in 1 litre of HCl distilled water. For the extraction of phosphorus 2.85g air dried (2 mm) soil was shaken for 1 minute with 20ml of extractant in a 100ml volumetric flask and filtered through Whatman no.44 filter paper.

(b) Estimation : The Molybdenum blue method suggested by

Allen (1974) was used for the estimation of phosphorus in the extract (filtrate) and calculated as follows.

$$\underset{(\text{mg } 100\text{g}^{-1})}{\text{Extractable phosphate}} = \frac{\text{C(mg)} \times \text{solution volume} \times 10^{3}}{10 \times \text{aliquot} \times \text{sample weight}}$$

where solution vol. = 20ml; aliquot = 5 ml; sample weight = 2.85g and 'C' value is concentration reading (mg) in the aliquot.

Determination of Exchangeable Potassium and Calcium :

Potassium and calcium were extracted together and estimated separately from the same extract. The methods suggested by Allen (1974) were followed.

(i) Extraction : Ammonium acetate solution (pH=7.0) was used for the extraction. The extractant was prepared by mixing 575ml of glacial acetic acid with 600ml. of ammonia solution and diluted to 10 litre with distilled water. 10g air dried (2mm) soil was suspended in 150ml of Ammonium acetate extractant solution and stirred mechanically for 30 minutes. The suspension was filtered over Whatman no.44 filter paper.

(a) Estimation of Exchangeable potassium : The exchangeable potassium in the filtrate was estimated by flame photometer and calculated as follows.

$$\underset{(\text{mg } 100\text{g}^{-1})}{\text{Exchangeable Potassium}} = \frac{\text{C(ppm)} \times \text{Solution volume} \times 10^{3}}{10^{4} \times \text{sample weight}}$$

where C = ppm potassium given by flame photometer

Solution volume = 150ml
Sample weight = 10g.

(b) Estimation of calcium : Calcium was estimated in the filtrate by EDTA titration method as suggested by Allen (1974) and adapted as follows.

5ml of the extract was taken in a 100ml beaker and mixed with 5 ml of 1M NaOH solution. About 100mg of murexide (an Indicator) was added to the above mixture and titrated against IN EDTA solution. Extractable calcium was calculated as follows.

$$\text{Extractable Calcium } (\text{mg } 100\text{g}^{-1}) = \frac{\text{T(ml)} \times \text{solution volume} \times 10^3}{10^2 \times \text{aliquot} \times \text{sample weight(g)}}$$

where T=ml of EDTA solution used against sample titration, solution volume = 150ml; aliquot = 5 ml and sample weight = 10g.

RESULTS

I. Occurrence, type, status of mycorrhizae and its rhizospheric soil properties :

All the twenty six tree species were found to be mycorrhizal (Table 1.1). Six tree species namely, *Quercus dealbata*, *Q. griffithi*, *Castanopsis hystrix*, *Myrica esculenta*, *Pinus kesiya* and *Alnus nepalensis* had ectomycorrhizae and others possed endomycorrhizae. Two endomycorrhizal

Table 1.1 Mycorrhizal status of tree species.

Plant species	Infection type and Intensity		
	VA mycorrhiza*	Ectomycorrhiza	Ericalean mycorrhiza
Hammamelidaceae :			
1. *Exbucklandia populnea*	91	-	-
Euphorbiaceae :			
2. *Daphniphyllum himalayanse*	89	-	-
Lauraceae :			
3. *Machilus kingi*	88	-	-
4. *Cinnamomum tamala*	97	-	-
5. *Lindera latifolia*	56	-	-
Magnoliaceae :			
6. *Manglietia insignis*	100	-	-
Ternstromiaceae :			
7. *Schima khasiana*	38	-	-
8. *Eurya acuminata*	90	-	-
Elaeocarpaceae :			
9. *Elaecarpus braceanus*	85	-	-
Leguminosae :			
10. *Acacia dealbata*	48	-	-
Bixaceae :			
11. *Casearia verica*	100	-	-
Symplocaceae :			
12. *Symplocus spicata*	87	-	-
Rosaceae :			
13. *Prunus cerasoides*	78	-	-
14. *Photinia notothiana*	98	-	-
Taxodiaceae :			
15. *Cryptomeria japonica*	59	-	-
Cupressaceae :			
16. *Cupressus* sp.	92	-	-
Juglandaceae :			
17. *Englehardtia spicata*	59	-	-
Oleaceae :			
18. *Ligustrum nepalense*	86	-	-
Fagaceae :			
19. *Quercus dealbata*	-	+	-
20. *Q. griffithii*	-	+	-
21. *Castanopsis hystrix*	-	+	-
Myricaceae :			
22. *Myrica esculenta*	-	+	-
Pinaceae :			
23. *Pinus kesiya*	-	+	-
Ericaceae :			
24. *Pieris ovalifolia*	-	-	+
25. *Rhododendron formosum*	-	-	+
Betulaceae :			
26. *Alnus nepalensis*	-	+	-

(+) sign denotes presence and (-) the absence of mycorrhizal infection.

* Figures represent percent number of infected root segments.

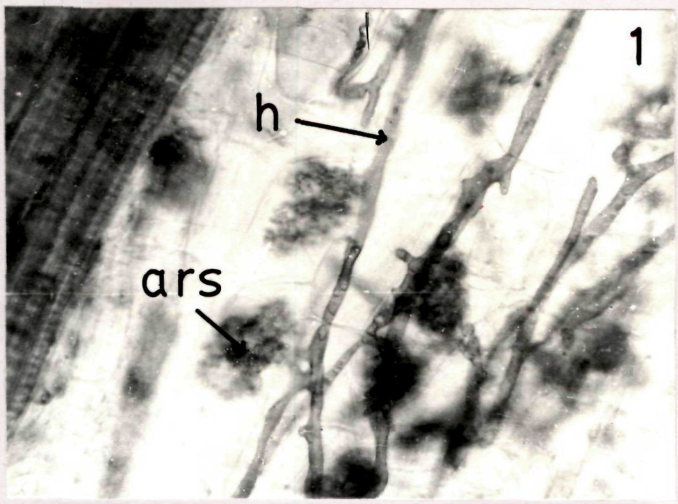
Table 1.2. Physico-chemical characteristics of rhizospheric soil of trees.

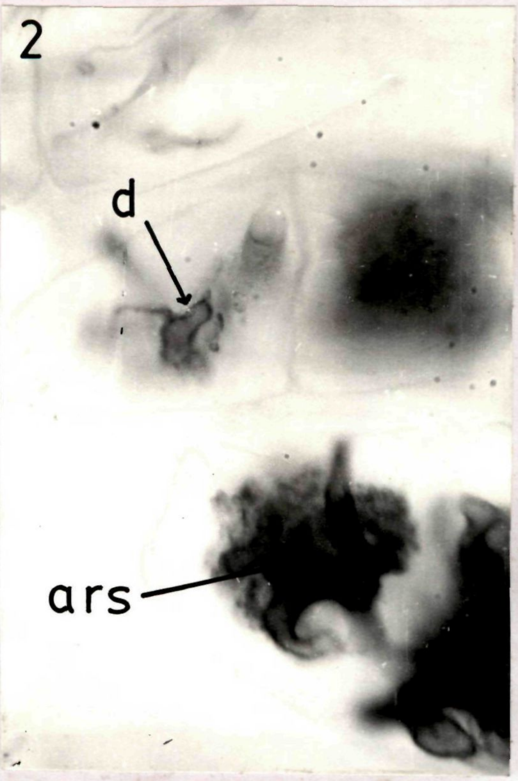
Plant species	pH	Moisture content (%)	Organic matter (%)	Available phosphorus (ppm)	Total Nitrogen (%)
1. *Exbucklandia populnea*	5.9	20	9.6	6.4	0.44
2. *Daphniphyllum himalayanse*	5.4	18	9.2	1.1	0.52
3. *Machilus kingi*	4.9	37	9.5	4.5	0.47
4. *Cinnamomum tamala*	5.6	49	13.5	2.3	0.50
5. *Lindera latifolia*	5.3	40	9.4	7.2	0.41
6. *Manglietia insignis*	5.2	26	15.5	4.8	0.52
7. *Schima khasiana*	5.5	17	5.6	2.8	0.22
8. *Eurya acuminata*	5.1	32	16.7	7.3	0.70
9. *Elaeocarpus braceanus*	5.7	37	16.5	1.5	1.00
10. *Acacia dealbata*	4.8	22	6.2	1.8	0.43
11. *Casearia verica*	5.5	36	12.5	2.5	0.43
12. *Symplocos spicata*	4.8	30	17.4	3.5	0.58
13. *Prunus cerasoides*	4.9	30	10.1	3.4	0.49
14. *Photinia notothiana*	5.1	53	9.2	1.3	0.53
15. *Cryptomeria japonica*	5.8	31	17.2	8.4	0.50
16. *Cupressus* sp.	5.0	28	5.3	1.6	0.50
17. *Englehardtia spicata*	4.8	36	5.6	2.6	0.37
18. *Ligustrum nepalense*	5.7	21	12.8	1.1	0.52
19. *Quercus dealbata*	4.7	38	15.5	2.3	0.55
20. *Q. griffithii*	5.1	29	14.3	3.2	0.61
21. *Castanopsis hystrix*	4.8	29	8.6	2.7	0.53
22. *Myrica esculenta*	5.3	40	10.3	3.4	0.39
23. *Pinus kesiya*	4.9	32	8.7	2.9	0.33
24. *Pieris ovalifolia*	5.0	37	9.8	6.5	0.49
25. *Rhododendron formosum*	4.8	39	15.2	2.9	0.42
26. *Alnus nepalensis*	5.5	30	7.1	1.4	0.38

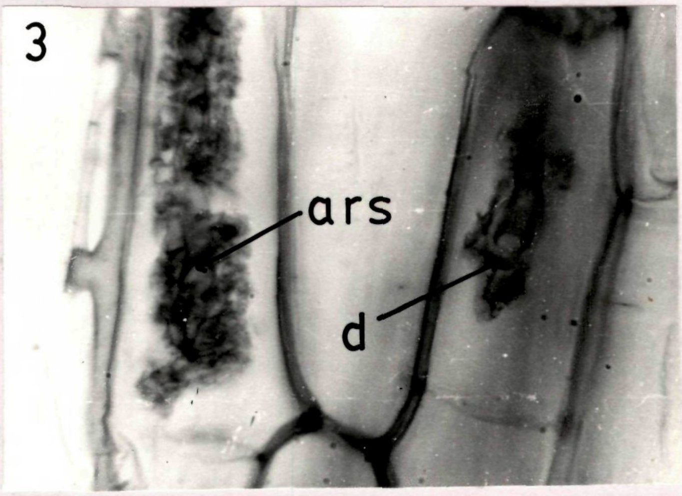
Plate 1.1

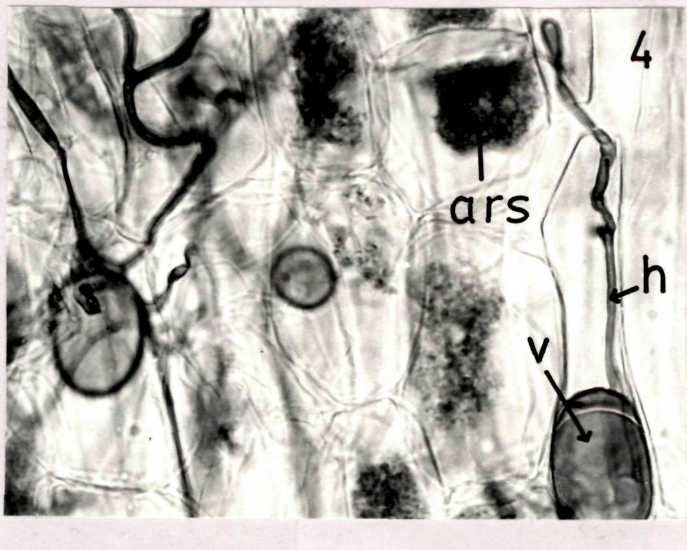
1. Young arbuscle (ars) and hypha (h) in the roots of *Daphniphyllum himalayanse* (X 290); 2. Young (ars) and degenerated (d) arbuscules in the roots of *Manglietia insignis* (X 290); 3. Young apical arbuscle in the roots of *Exbucklandia populnea* (x 727); 4. Young arbuscule (ars) and vesicles (v) in the roots of *Cryptomeria japonica* (X 290); 5. Young arbuscules (ars) and hypha (h) in the roots of *Cinnamomum tamala* (X 290).

PLATE - 1.1









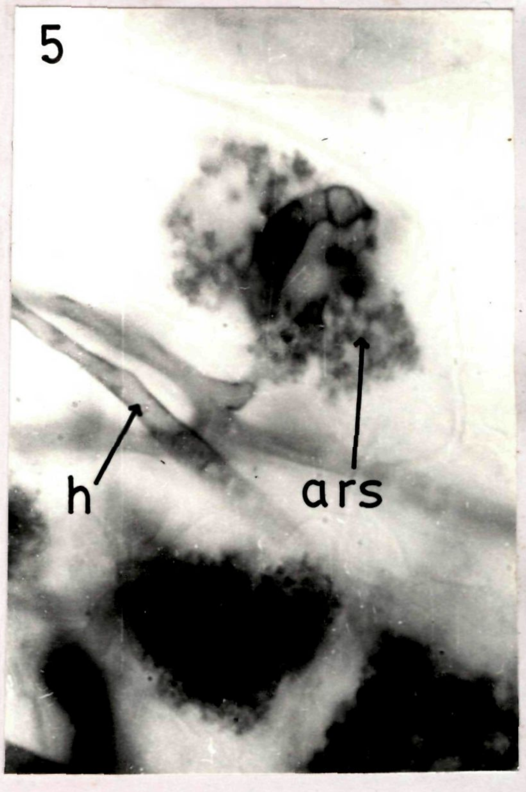
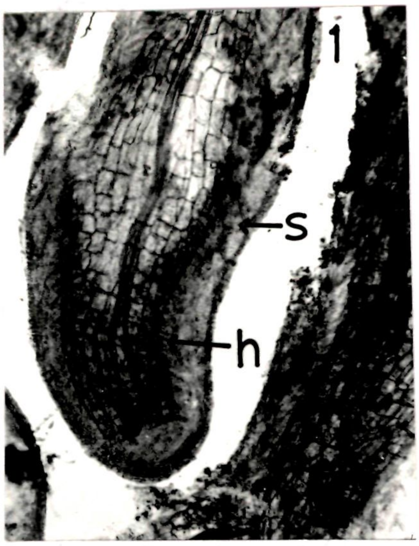
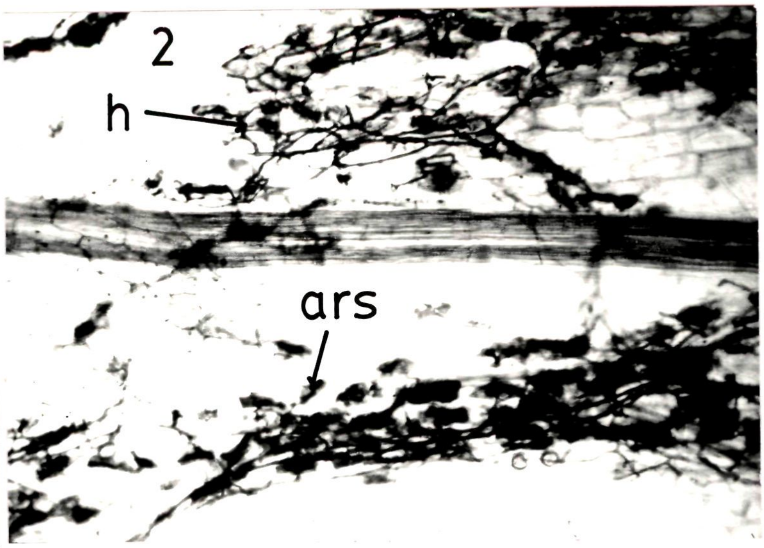


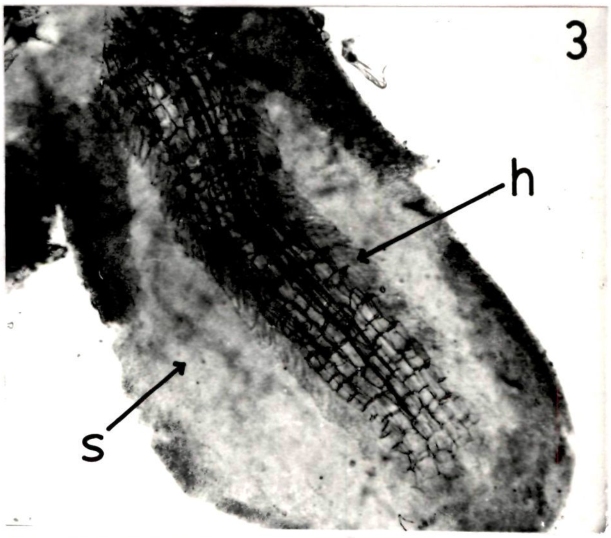
Plate 1.2

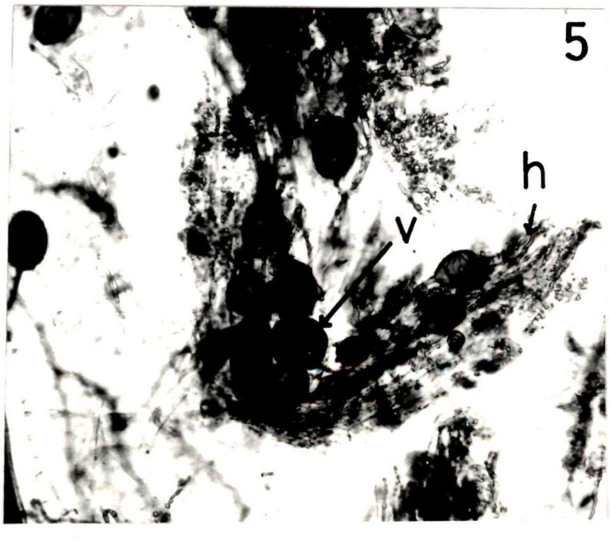
1,3. Ectomycorrhiza showing sheath (s) and hartig net(h) in Quercus griffithi and Q. dealbata (X 72); 2. VA mycorrhiza showing arbuscules (ars) and hyphae (h) in *Exbucklandia populnea* (X 150); 4. Ericalean mycorrhiza of *Pieris ovalifolia* (X 72); 5. Obovate vesicles (v), hypha (H) in the roots of *Manglietia* insignis (X 290); 6. VA mycorrhiza showing arbuscules (ars) and vesicles (v) in *Lindera latifolia* (X 150).

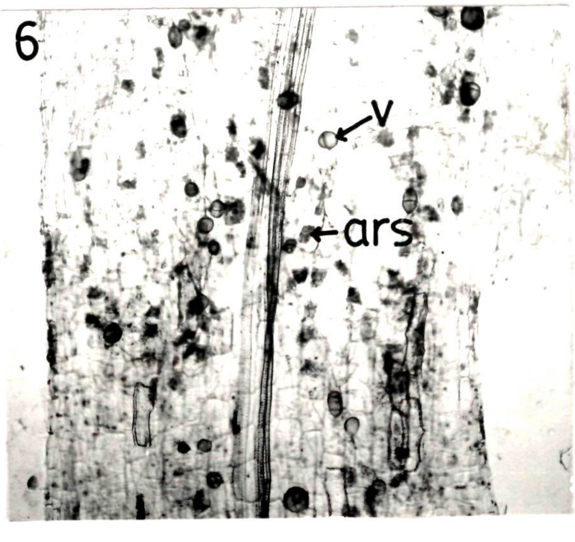
PLATE - 1.2











species namely, *Pyris ovalifolia* and *Rhododendron formosum* had ericoid type of endomycorrhizae and 18 other tree spp. namely, *Exbucklandia populnea*, *Daphniphyllum himalayanse*, *Machilus kingi*, *Cinnamomum sp.*, *Lindera latifolia*, *Manglietia insignis*, *Schima khasiana*, *Eurya acuminata*, *Elaeocarpus braceanus*, *Acacia dealbata*, *Casearia verica*, *Symplocos spicata*, *Prunus cerasoides*, *Photinia notothiana*, *Cryptomeria japonica* *Cupressus sp*. *Englehardtia spicata* and *Ligustrum nepalense* had VA type of endomycorrhizal association. Level of VA mycorrhizal infection varied from 50-100% depending on tree species. Thirteen spp. had more than 75% infection and other five had 50-75% infection (Table 1.1).

The rhizospheric soil of all the tree species was acidic(pH=4.8-6.0),rich in soil moisture (17-40%), organic matter (5-15%) but poor in available phosphorus (1-8ppm). The various soil properties varied among different tree species (Table 1.2).

II. Seasonal variations in status of Endomycorrhizae :

Seasonal variations in the level of VA endomycorrhizae, endogonaceous spore population, generic distribution of endophytes and various physico-chemical properties of rhizospheric soil was observed in eleven VA endomycorrhizal tree species.

(a) Level of VA mycorrhizal infection :

VA mycorrhizal infection was high (80-100%) in all

Fig. 1.1a Seasonal variation in percent VA-mycorrhizal infection of tree species during the study periods of 1983-84 and 1984-85.

Sampling period, D:December; F:February; A:April; J:June and A:August.

Tree species, 1:*Cryptomeria japonica*; 2:*Exbucklandia populnea*; 3:*Acacia dealbata*; 4:*Mallotus nepalensis*; 5.*Schima khasiana*; 6:*Daphniphyllum himalayanse*.

Fig. 1·1a

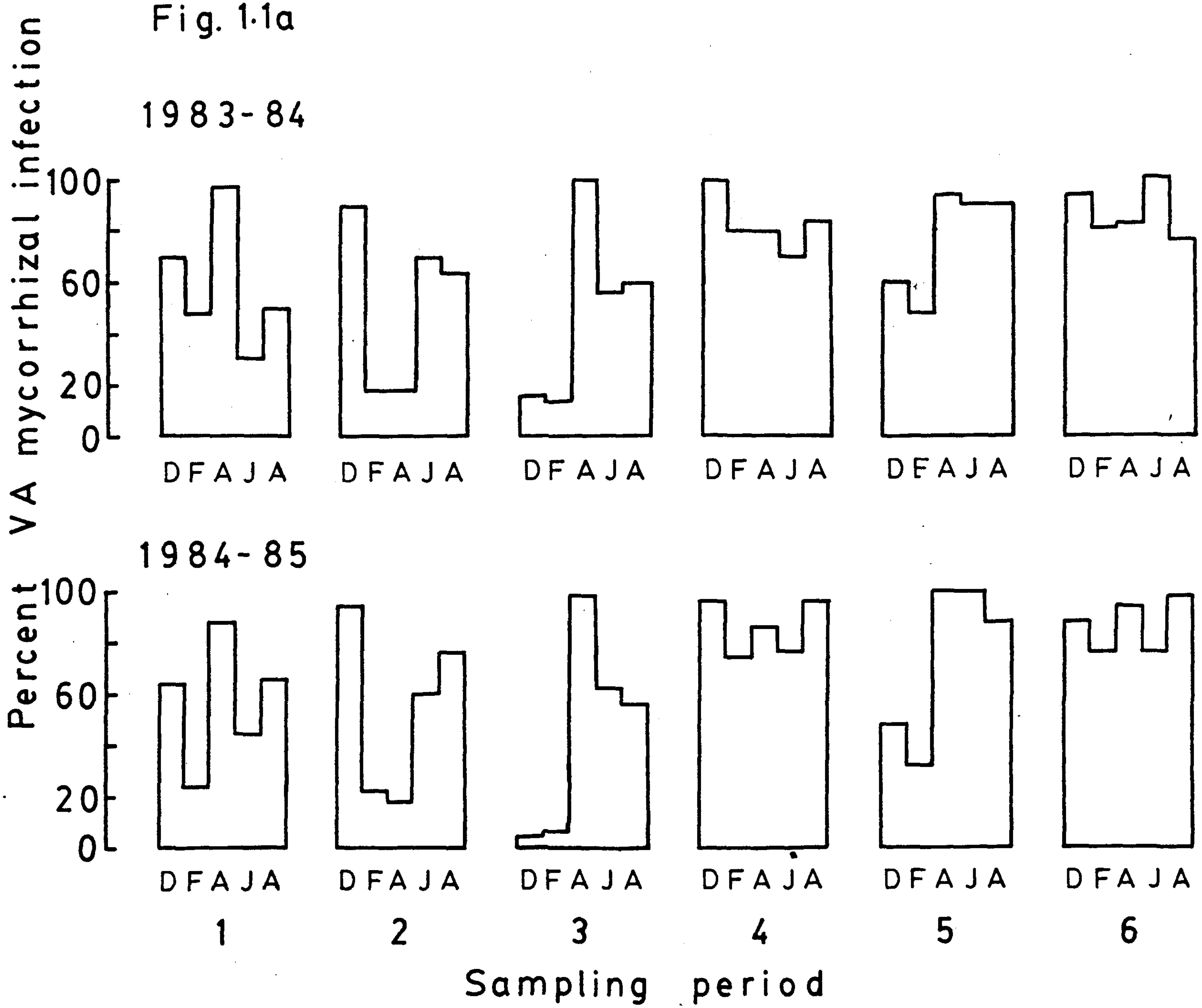
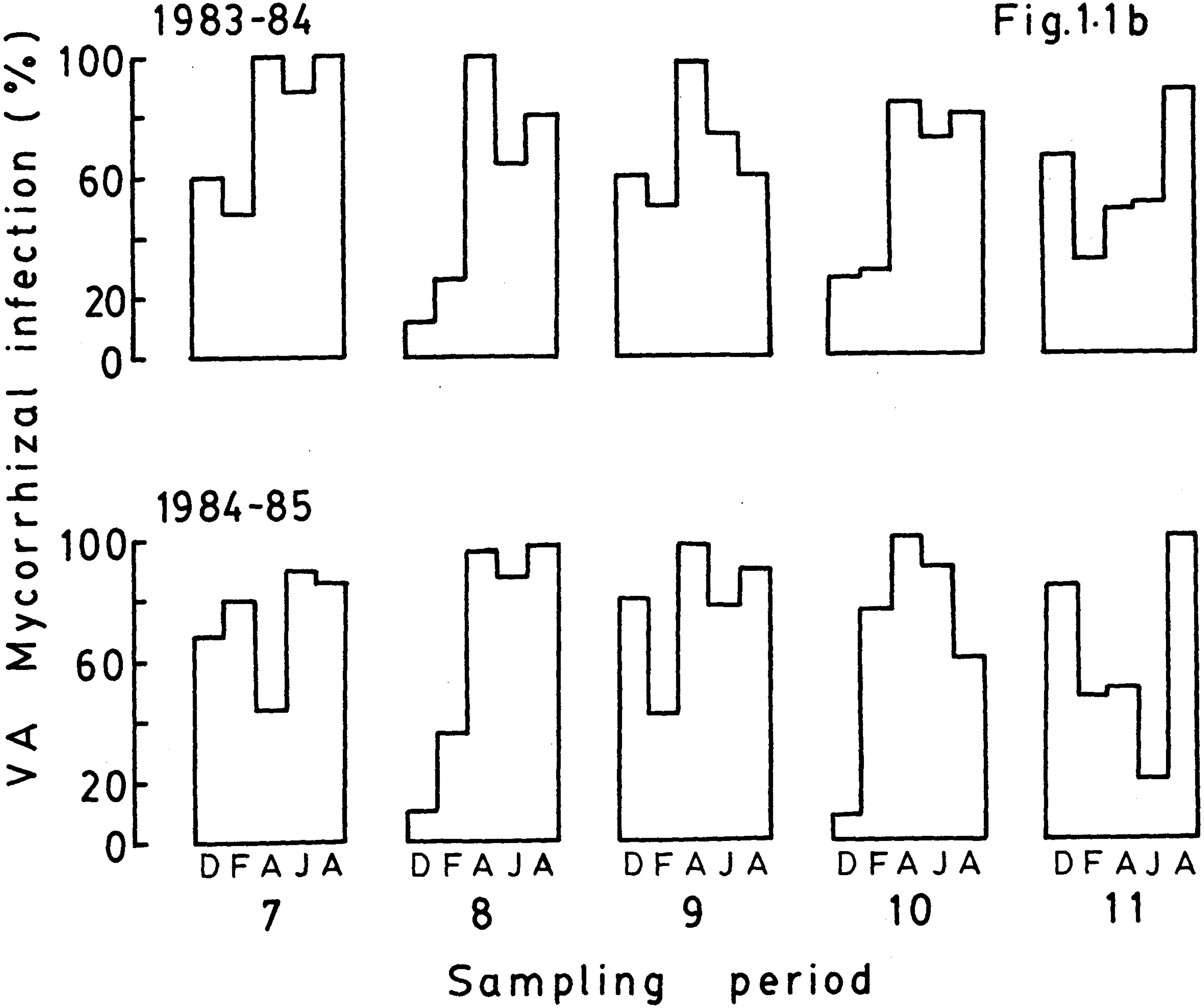

1983-84
1984-85
VA mycorrhizal infection
Percent
100
60
20
0
D F A J A
1
2
3
4
5
6
Sampling period

Fig.1.1b Seasonal variation in percent VA-mycorrhizal infection of tree species during the study periods of 1983-84 and 1984-85.

Sampling period, D:December; F:February; A:April; J:June; A:August.

Tree species, 7:*Machilus kingii*; 8:*Cinnamomum tamala*; 9: *Manglietia insignis*; 10: *Lindera latifolia*; 11:*Casearia verica*.

Fig.1·1b



1983-84
1984-85
VA Mycorrhizal infection (%)
100
60
20
0
100
60
20
0
D F A J A
D F A J A
D F A J A
D F A J A
D F A J A
7
8
9
10
11
Sampling period

the tree species in one or more seasons of surveys of both the years, viz: 1983-84 and 1984-85. The percent VAM infection varied among different tree species and there was the seasonal variability in a given tree species. Only two tree species namely, *Daphniphyllum himalayanse* and *Mallotus nepalensis* had more or less uniform and high infection in all the months of both the years (Fig.1.1 a,b). The pattern of seasonal variability was different for different tree species.

The VA mycorrhizal infection increased in spring in the case of *Acacia dealbata*, *Schima khasiana*, *Cryptomeria japonica*, *Cinnamomum tamala*, *Manglietia insignis* and *Lindera latifolia*. Subsequently, infection decreased during winter in *Acacia dealbata*. Whereas, in the case of *Schima khasiana*, *Cinnamomum tamala* and *Lindera latifolia*, VA infection remained higher during rainy season and decreased towards winter. In *Cryptomeria*, infection decreased during rainy season remained high during early winter and decreased during late winter.

In the case of *Exbucklandia populnea* infection increased during rainy season and reached to the maximum in December and remained low during late winter and spring seasons.

In *Machilus kingi* infection was found low during spring and high in all other seasons. Whereas in *Casearia verica* VA infection increased in late rainy season and remained high till December, however, infection was low from late winter to early rainy season.

(b) *Endogonaceous spore population* :

The number of endogonaceous spores per g soil varied among different tree species and also from season to season (Fig.1.2 a,b). Irrespective of season about 60-100 spores per g soil were observed in the rhizosphere of tree species namely, *Cryptomeria japonica*, *Exbucklandia populnea*, *Mallotus nepalensis* and *Schima khasiana*. Less often number was 100-150 in the rhizosphere soil of *Acacia dealbata*, *Daphniphyllum himalayanse*, *Machilus kingi* and *Cinnamomum tamala*. Rarely less than 50 spores/g rhizospheric soil were seen in the case of *Cryptomeria japonica*, *Exbucklandia populnea*, *Mallotus nepalensis*, *Daphniphyllum himalayanse* and *Lindera latifolia*. Occassionally more than 250 spores/g rhizospheric soil were observed in the case of *Casearia verica*. The magnitude and the pattern of seasonal variability varied among different tree species.

Variability

Endogonaceous spore population tended to be high in rainy season in the case of *Cryptomeria japonica*, *Exbucklandia populnea*, *Cinnamomum tamala* and *Lindera latifolia* and in winter in the case of *Daphniphyllum himalayanse*, *Machilus kingi*. While in other cases there was no regular increase or decrease in spore population in different seasons.

Fig.1.2a Seasonal variation in endogonaceous spore population in the rhizospheric soil of tree species during the study periods of 1983-84 and 1984-85.

Sampling period, D:December; F:February; A:April; J:June; A:August.

Tree species, 1:*Cryptomeria japonica*; 2:*Exbucklandia populnea*; 3:*Acacia dealbata*; 4:*Mallotus nepalensis*; 5:*Schima khasiana*; 6:*Daphniphyllum himalayanse.*

Fig. 1·2a

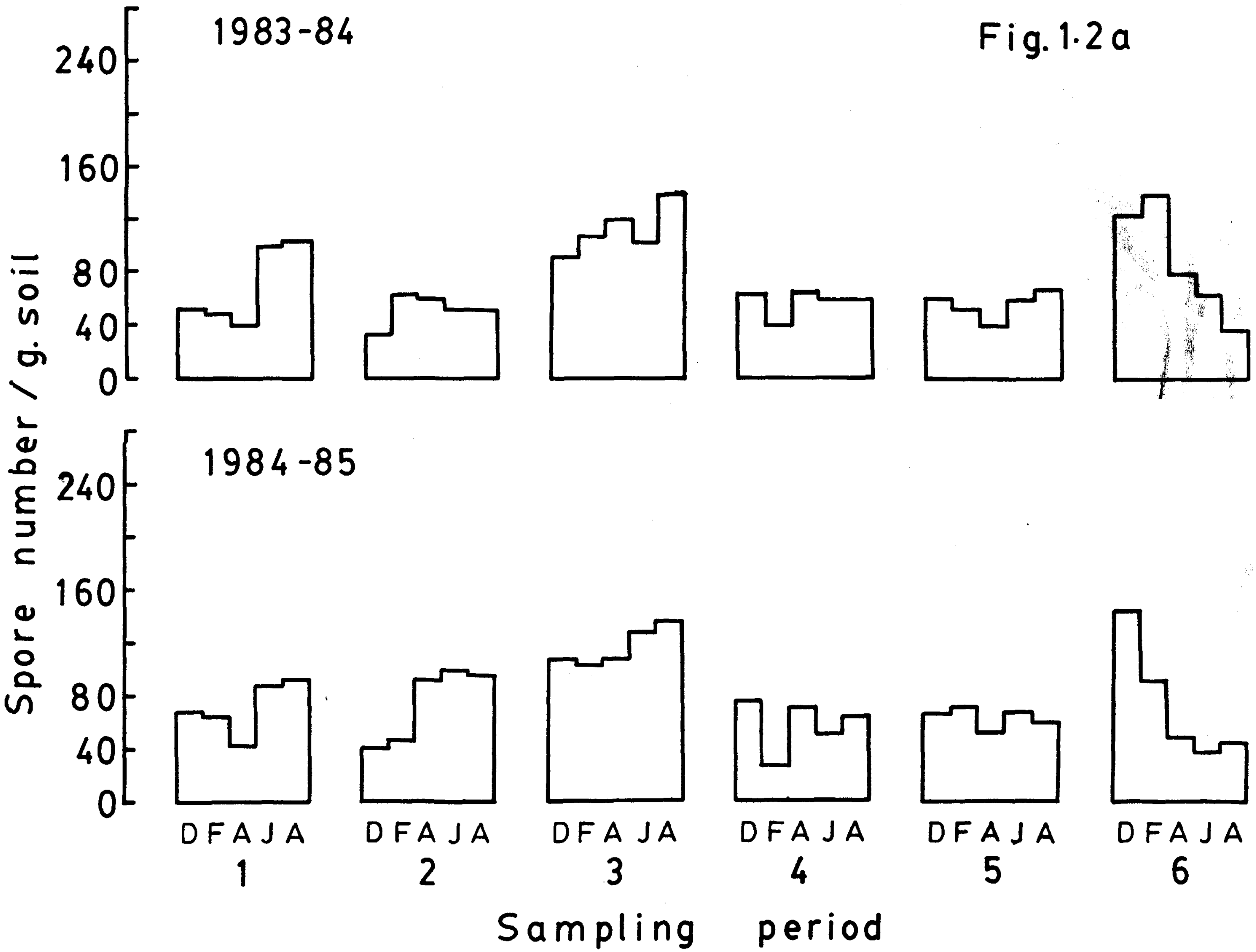

1983-84
1984-85
Spore number / g. soil
240
160
80
40
0
D F A J A
1
2
3
4
5
6
Sampling period

Fig.1.2b Seasonal variation in endogonaceous spore population in the rhizospheric soil of tree species during the study periods of 1983-84 and 1984-85.

Sampling period, D:December; F:February; A:April; J:June· A:August.

Tree species, 7:*Machilus kingi*; 8:*Cinnamomum tamala* 9. *Manglietia insignis*; 10: *Lindera latifolia*· 11:*Casearia verica.*

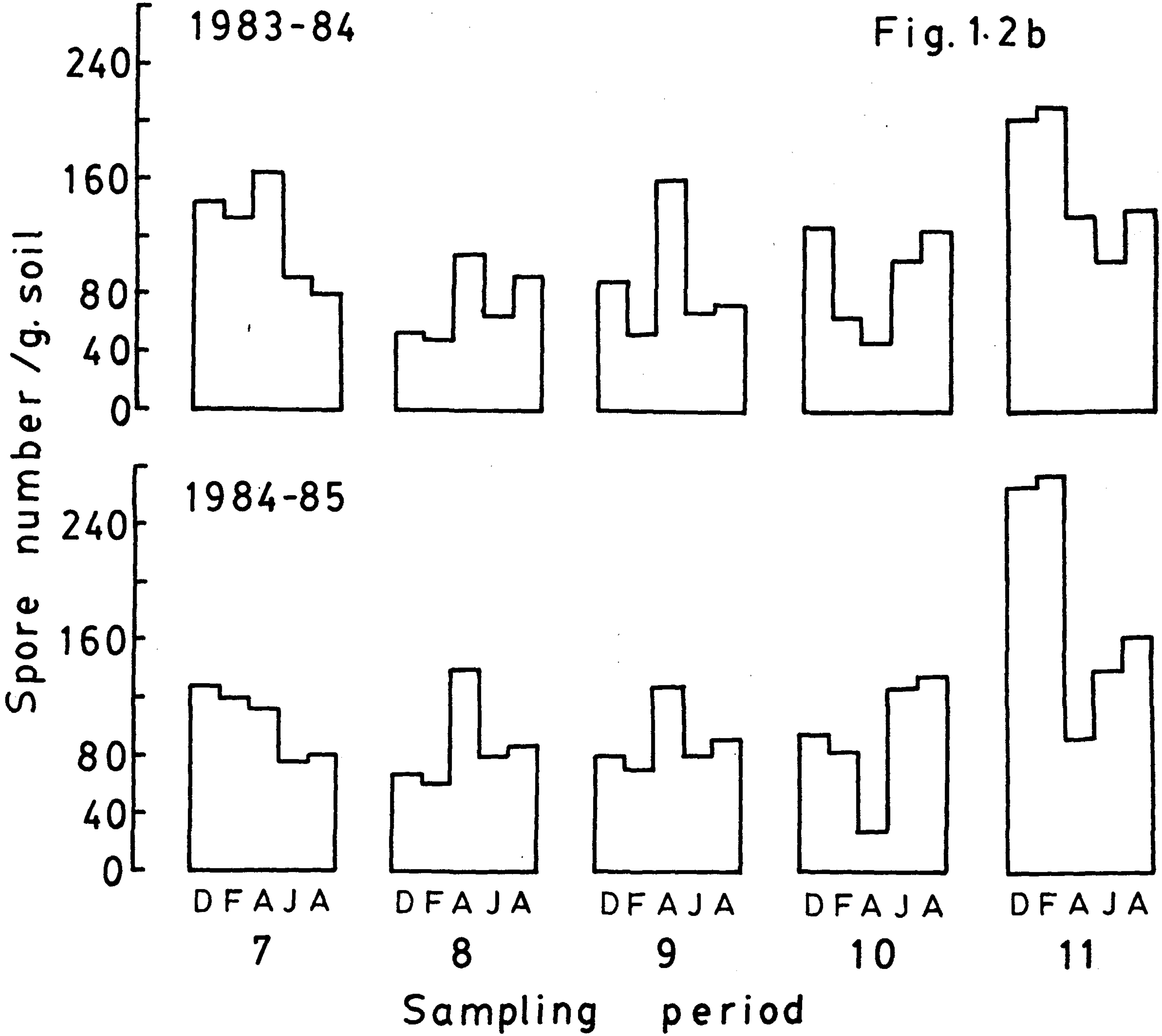


Fig. 1.2b

1983-84
1984-85
Spore number /g. soil
240
160
80
40
0
D F A J A
7
8
9
10
11
Sampling period

Table 1.3 Seasonal variability in generic population of Endogonaceous spores*.

Tree species	*Glomus spp*			*Gigaspora spp*			*Acaulospora spp*			*Sclerocystis spp*		
	S	R	W	S	R	W	S	R	W	S	R	W
1. *Cryptomeria japonica*	90.7	80.9	45.0	4.7	1.1	-	4.6	18.0	35.0	-	-	20.0
2. *Exbucklandia populnea*	98.9	67.7	66.5	11.1	18.2	0.5	2 -	14.1	12.0	-	-	21.0
3. *Acacia dealbata*	55.7	99.2	81.0	11.5	0.8	0.2	23.9	-	13.0	8.8	-	6.0
4. *Mallotus nepalensis*	86.1	94.2	67.0	-	-	-	11.1	5.9	33.0	2.8	-	-
5. *Schima khasiana*	81.5	79.4	38.0	1.8	1.5	0.3	11.1	19.1	40.0	5.6	-	21.0
6. *Daphniphyllum himalayanse*	76.3	74.3	50.0	5.5	5.7	-	7.3	20.8	47.0	10.9	-	3.0
7. *Machilus kingi*	77.7	100.0	17.0	5.3	-	-	23.0	-	81.0	-	--	2.0
8. *Cinnamomum tamala*	46.1	90.0	68.0	28.4	-	-	14.2	10.0	29.0	11.3	-	3.0
9. *Manglietia insignis*	84.5	75.6	85.0	-	-	-	11.6	24.4	15.0	3.9	-	-
10. *Lindera latifolia*	96.6	83.6	15.0	3.4	8.6	-	-	7.8	74.0	-	-	11.0
11. *Casearia verica*	61.6	35.0	27.0	28.3	0.3	0.2	6.1	5.9	67.0	4.0	58.8	6.0

S = Spring season; R= rainy season; W = winter season.

*The figures represent percent of total population of endogonaceous spores.

(c) Distribution and abundance of Endogonaceaus genera :

Four genera of endogonaceae were identified from the rhizospheric soil samples. These are *Glomus*, *Gigaspora*, *Acaulospora* and *Sclerocystis*. All the tree species harboured spores of more than one genus of Endogonaceae in their rhizosphere. The percent spore population of various genera and from season to season of endogonaceae varied among different tree species (Table 1.3). Spores of *Glomus* spp were dominant. ~~It~~ They was reported from all the eleven tree species and in all the seasons. ~~Its~~ the proportion of *Glomus* spp in the spore population varied from 15-100%. Generally spore population was found to increase during rainy season. Less rare common ? were the spores of *Acaulospora* spp. isolated in rhizosphere of all the tree species except *Exbucklandia populnea* in April, *Acacia*, *Machilus* in August and *Lindera* in June. The proportional representation in the spore population varied from 4-81%. Spore population was comparatively higher during winter. Spores of *Gigaspora* were not seen in the rhizosphere of *Mallotus nepalensis* and Manglietia insignis in any of the three seasons. Spore population was higher in spring. Spores of *Glomus* were found in all the seasons and likewise of *Acaulospora*. However, spores of *Sclerocystis* and *Gigaspora* were occassionally seen in rainy and winter seasons respectively.

(d) Edaphic factors :

All the edaphic factors namely, soil pH, soil moisture, organic matter, total nitrogen, available phosphorus, available potassium, available calcium showed variability among different tree species and in different seasons. Some of the factors like pH exhibited less seasonal variability whereas others had greater variations.

(i) Soil pH : Rhizospheric soil of all the tree species was acidic (pH:4.5-6.0). Soil pH was slightly higher in winter which decreased in summer and again increased in rainy season (Fig.1.3). Soil pH was correlated to %VAM infection of tree species, however, significant correlations were observed in *Cinnamomum tamala* in both years and in *Acacia dealbata* in 1984-85 (Table 1.4). No significant correlations were found between soil pH and endogonaceous spores population (Table 1.5).

(i) Moisture content :

Irrespective of season 20-45% soil moisture content prevailed. All the tree species exhibited seasonal variations in rhizospheric soil moisture content (Fig.1.4). Moisture content was correlated to the percent VA infection of tree spp (Table 1.4). Significant correlations were observed for *Machilus kingi* in 1983-84 and in *Cinnamomum tamala* and *Acacia dealbata* in 1984-85. Significant correlations were also observed between moisture content and endogonaceous spore population in *Exbucklandia populnea* and *Casearia verica* during 1983-84 and in *Daphniphyllum himalayanse* during 1984-85 (Table 1.5).

Fig.1.3 Seasonal variation in pH of rhizospheric soil of tree species during the study period of 1983-84 and 1984-85.

Sampling period, D:December; F:February; A:April; J:June; A:August.

Tree species, 1:*Cryptomeria japonica*; 2:*Exbucklandia populnea*; 3:*Acacia dealbata*; 4:*Mallotus nepalensis*; 5:*Schima khasiana*; 6:*Daphniphyllum himalayanse*; 7:*Machilus kingi*; 8:*Cinnamomum tamala*; 9:*Manglietia insignis*; 10:*Lindera latifolia*;11:*Casearia verica.*

Fig.1.3

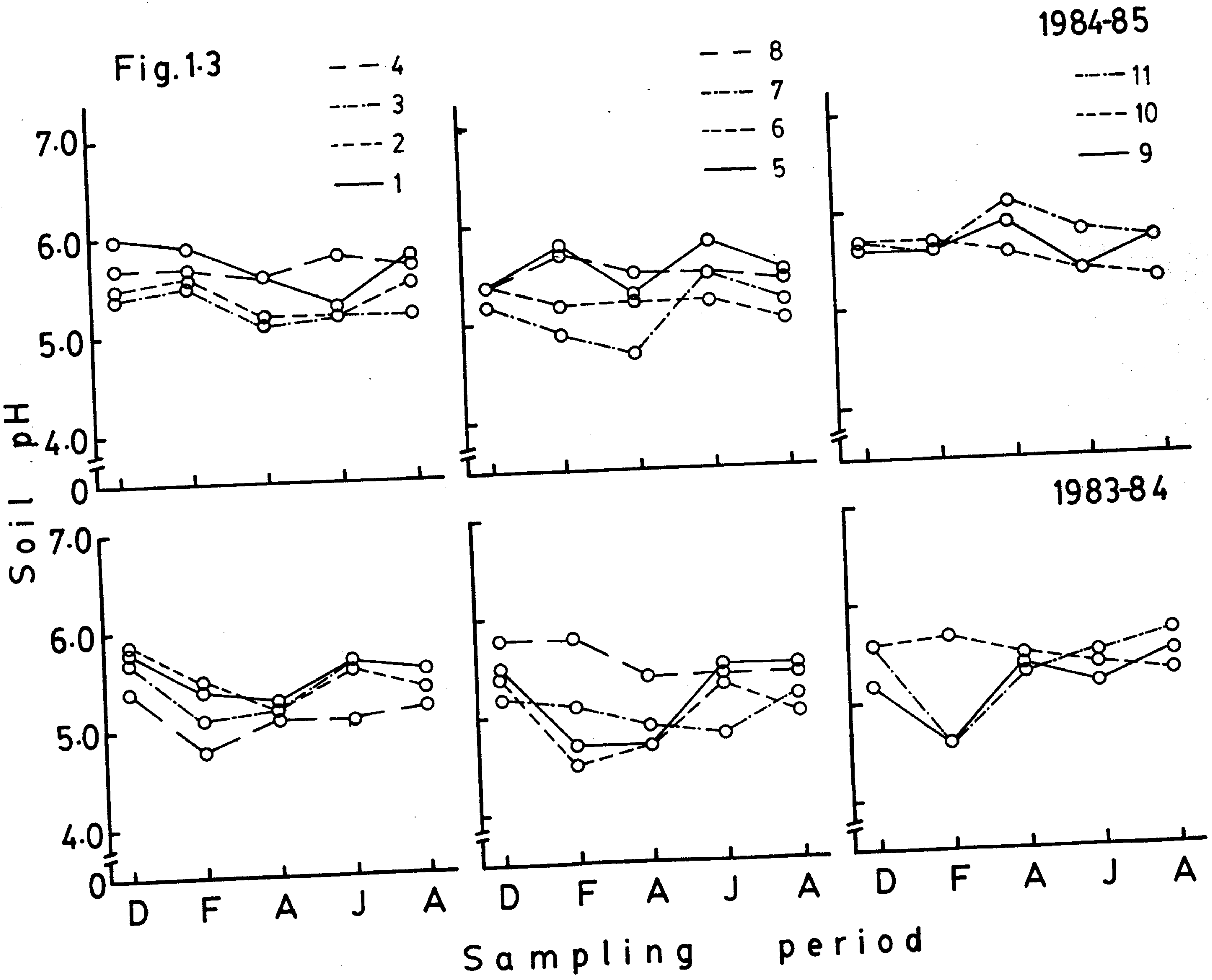
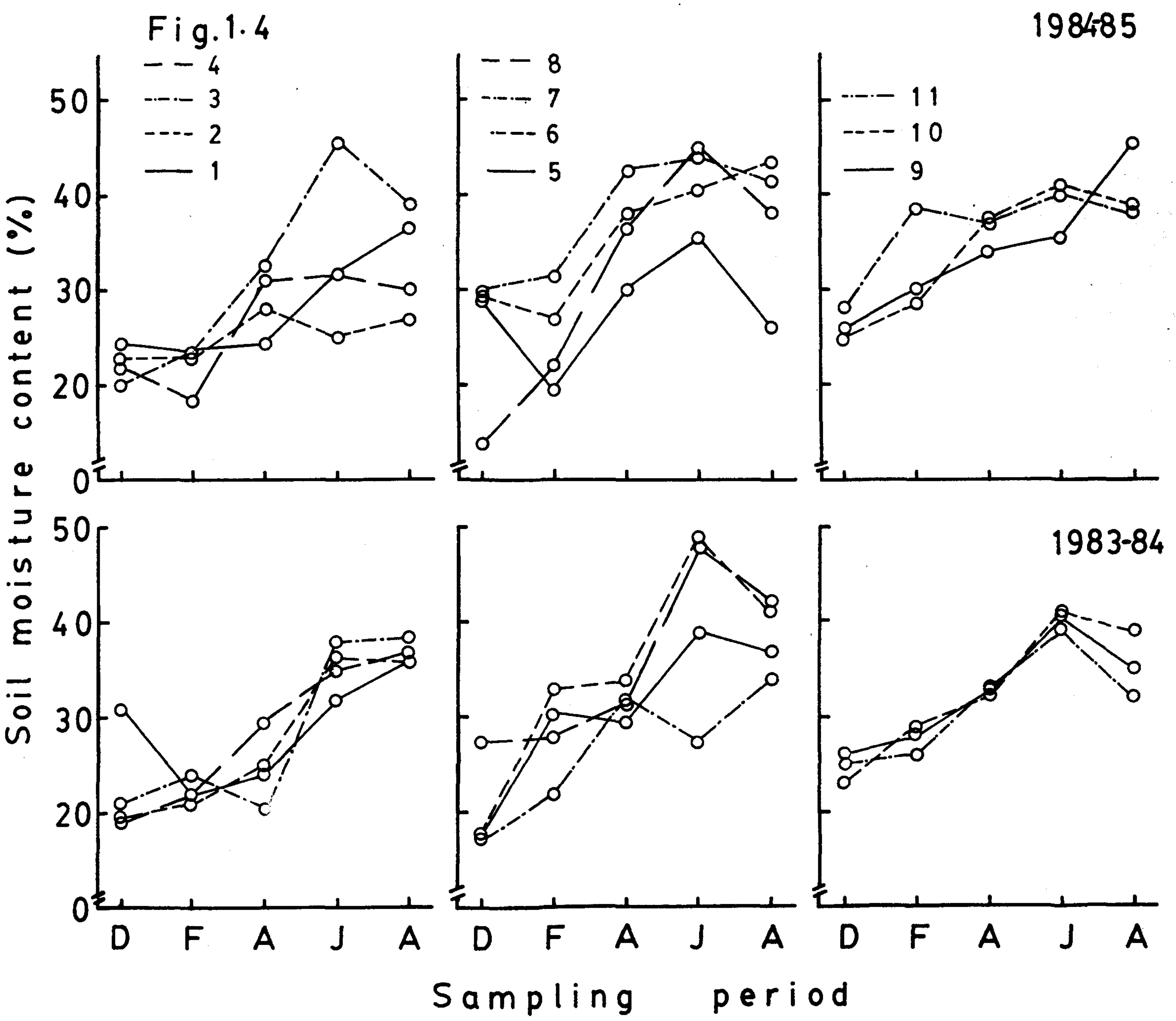

1984-85
1983-84
4
3
2
1
8
7
6
5
11
10
9
Soil pH
7.0
6.0
5.0
4.0
0
D
F
A
J
A
Sampling period

Fig. 1.4 Seasonal variation in moisture content of rhizospheric soil of tree species during the study periods of 1983-84 and 1984-85.

Sampling period, D:December; F:February; A:April; J:June; A:August.

Tree species, 1:*Cryptomeria japonica*; 2:*Exbucklandia populnea*; 3:*Acacia dealbata*; 4:*Mallotus nepalensis*; 5:*Schima khasiana*; 6:*Daphniphyllum himalayanse*; 7:*Machilus kingi*; 8:*Cinnamomum tamala*; 9:*Manglietia insignis*; 10:*Lindera latifolia*; 11:*Casearia verica.*

Fig.1·4



4
3
2
1
8
7
6
5
11
10
9
1984-85
1983-84
Soil moisture content (%)
0
20
30
40
50
D
F
A
J
A
Sampling period

(iii) <u>Organic matter</u> : Likewise soil organic matter was different in the rhizosphere of tree species and varied in different seasons (Fig.1.5). It varied from 9-20%. [redacted] Significant correlations were observed between %VA infection and organic matter for <u>Mallotus nepalensis</u> and <u>Casearia verica</u> during 1983-84 (Table 1.4). Significant correlation between spore population and organic matter in <u>Exbucklandia populnea</u> during 1983-84 and <u>Acacia dealbata</u> in 1984-85 were observed (Table 1.5).

(iv) <u>Percent total nitrogen</u> : About 0.27-0.71% nitrogen was observed. All the tree species exhibited seasonal variations (Fig.1.6). Soil nitrogen had significant correlation with %VAM infection of <u>Machilus kingi</u> in 1983-84 (Table 1.4), however, no significant correlation was observed with endogonaceous spore population in any of tree spp (Table 1.5).

(v) <u>Available phosphorus</u> : The rhizospheric soil of all the tree species was poor in available phosphorus (1.6-9.2 ppm) (Fig.1.7). Seasonal variations were correlated with % VA infections which was significant in <u>Cryptomeria japonica</u> during 1984-85 (Table 1.4). Available phosphorus was also correlated to the endogonaceous spore population. However, significant correlation were observed in <u>Machilus kingi</u> and <u>Casearia verica</u> during 1983-84 (Table 1.5).

cf earlier
kg/ha
constancy?

(vi) *Exchangeable potassium* : Soil was poor in exchangeable potassium (6-31 mg 100^{-1} g) (Fig.1.8). The seasonal variability was significantly correlated to %VA infection in *Machilus kingi* during 1983-84 (Table 1.4). With endogonaceous spore population was significantly correlated in *Exbucklandia populnea* during 1983-84 and *Acacia dealbata* during 1984-85 (Table 1.5).

(vii) *Exchangeable calcium* : (1.2-3.12) mg of calcium per g of rhizospheric soil was observed (Fig.1.9). The seasonal variation was significantly correlated with the %VA infection of *Schima khasiana* in 1983-84 (Table 1.4) and with endogonaceous spore population in *Exbucklandia populnea* in the same year (Table 1.5).

DISCUSSION

Vesicular-arbuscular mycorrhiza was prevalent and roots of the tree species were heavily colonised by VA endophytes. Various weed species of the region have also been reported to possess heavy VAM infections (Verma, 1982). Read *et.al.*(1976) discussed relationship between nutrient status of the habitat and occurrence of mycorrhizal infections. The conditions of nutrient stress and intense competition between plants seem to be the reason for the occurrence of heavy mycorrhizal infections in the plants. Root samples were collected from the top 5cms layer of soil where main source of nutrients was decomposing plant litter. St.John

(1980) observed strong association between root depth and mycorrhizal conditions. He also found higher infection level in surface roots of trees. The results showed that VA mycorrhizal infections were high during spring and rainy seasons and declined towards winter. Root growth is renewed in spring. Therefore, rapid development of VA infections takes place in spring. In older roots the endophyte is digested by host root cells and perhaps this may be the reason for the occurrence of low infection level during winter.

Endogonaceous spore population was high. However, the range of spore number per g soil was in consistent with the observations of Verma (1982). He also found higher *Endogone* spore population in the rhizosphere of the weeds of the region. The result of the present investigation are in contrast with observations of other researchers who have shown that virgin soils or seminatural grassland possess low spore number (Crush, 1975; Mosse and Bowen, 1968). In grasses root infection may occur by root contact and therefore, less need for production of endophyte propagules in the form of spores. But in the present case endogonaceous spores may be an important constituent of natural inoculum for future infections of roots. The difference could also be related to the heavy annual rainfall in the area which may cause contamination of sites. A higher population of *Endogone* spores have also been reported from the agricultural fields of the region (Bhattarai, 1983). In most of the tree species spore population was higher during rainy season and declined during winter and spring. Many researchers have reported similar seasonal variations. However, in other tree species (Fig.1.2a,b) the higher spore population during winter and spring may be due to renewed root growth which for some reasons may stimulate sporulation of these species.

Endogonaceous spores belonging to more than one gener~~a~~us were reported from the rhizospheric soil of these tree species. This accounts for the occurrence of mixed VAM infections which ~~is~~ are common in nature (Daft, 1983). Crush (1975) and Mosse and Bowen (1968) also observed diversity of spore types in New Zealand and Eastern Australian soils. Spores of Glomus spp were found from the rhizosphere of all the tree species (Table 1.3). Hetrick and Bloom (1983) also described the dominance of Glomus spp. in Kansas tall grass praire. Bakshi (1974) also reported various sporocarpic species of Glomus and Sclerocystis in forest soils. However, Mosse (1973) reported that sporocarpic species are not found in logged areas she examined.

Most of the correlations between percent VAM infections of roots and endogonaceous spore populations were not found to be significant with respect to soil properties. The topographic conditions of the north-eastern region could be considered. Heavy soil erosion occurs due to high annual rainfall. The water flow carry the soil particles and nutrients. Probably this may be the reason for lack of significant correlations.

Fig.1.5 Seasonal variation in organic matter of rhizospheric soil of tree species during the study periods of 1983-84 and 1984-85.

Sampling period, D:December; F:February; A:April; J:June; A:August.

Tree species, 1:*Cryptomeria japonica*; 2:*Exbucklandia populnea*; 3:*Acacia dealbata*; 4:*Mallotus nepalensis*; 5:*Schima khasiana*; 6:*Daphniphyllum himalayanse*; 7:*Machilus kingi*; 8:*Cinnamomum tamala*; 9:*Manglietia insignis*; 10:*Lindera latifolia* 11:*Casearia verica.*

Fig. 1.5

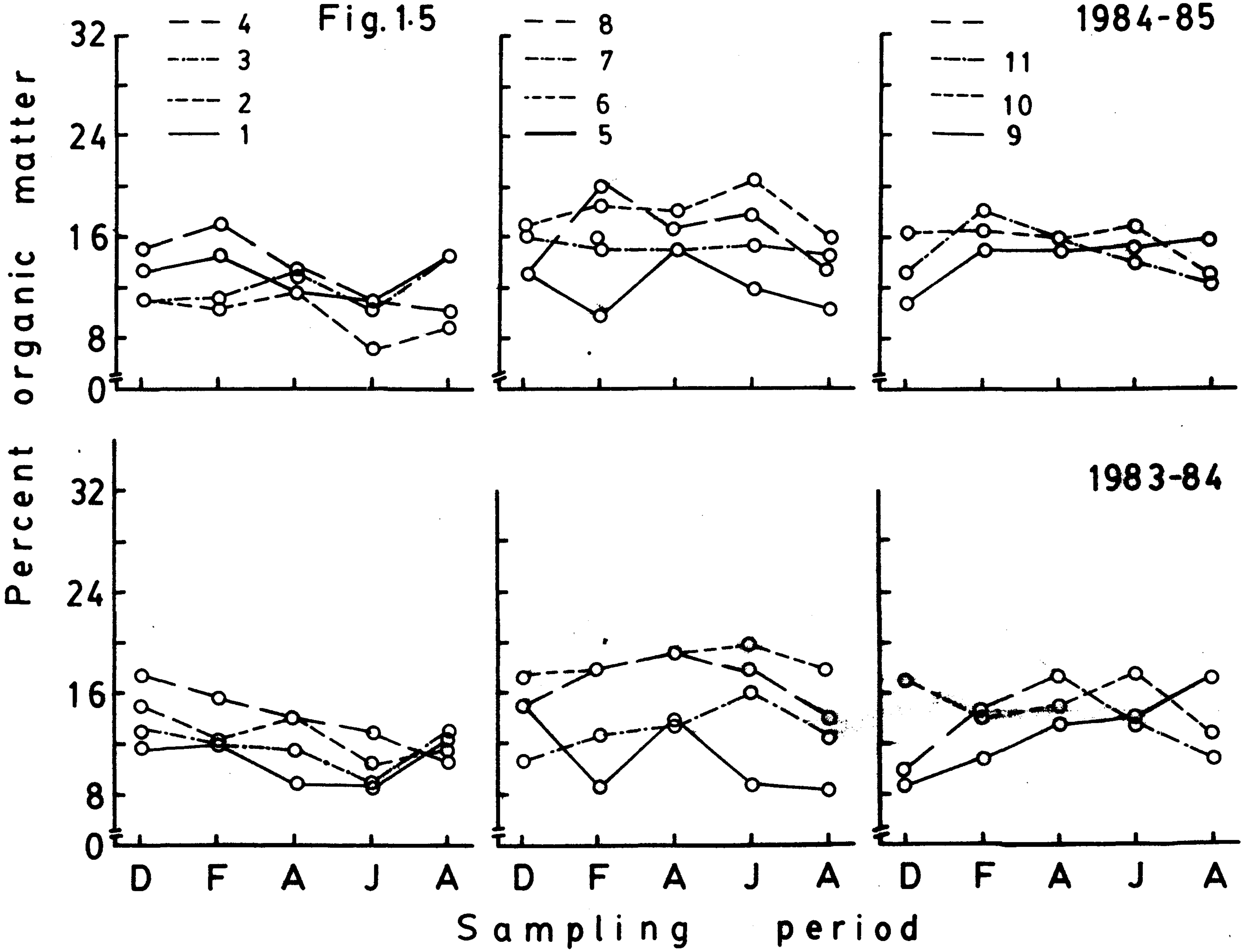
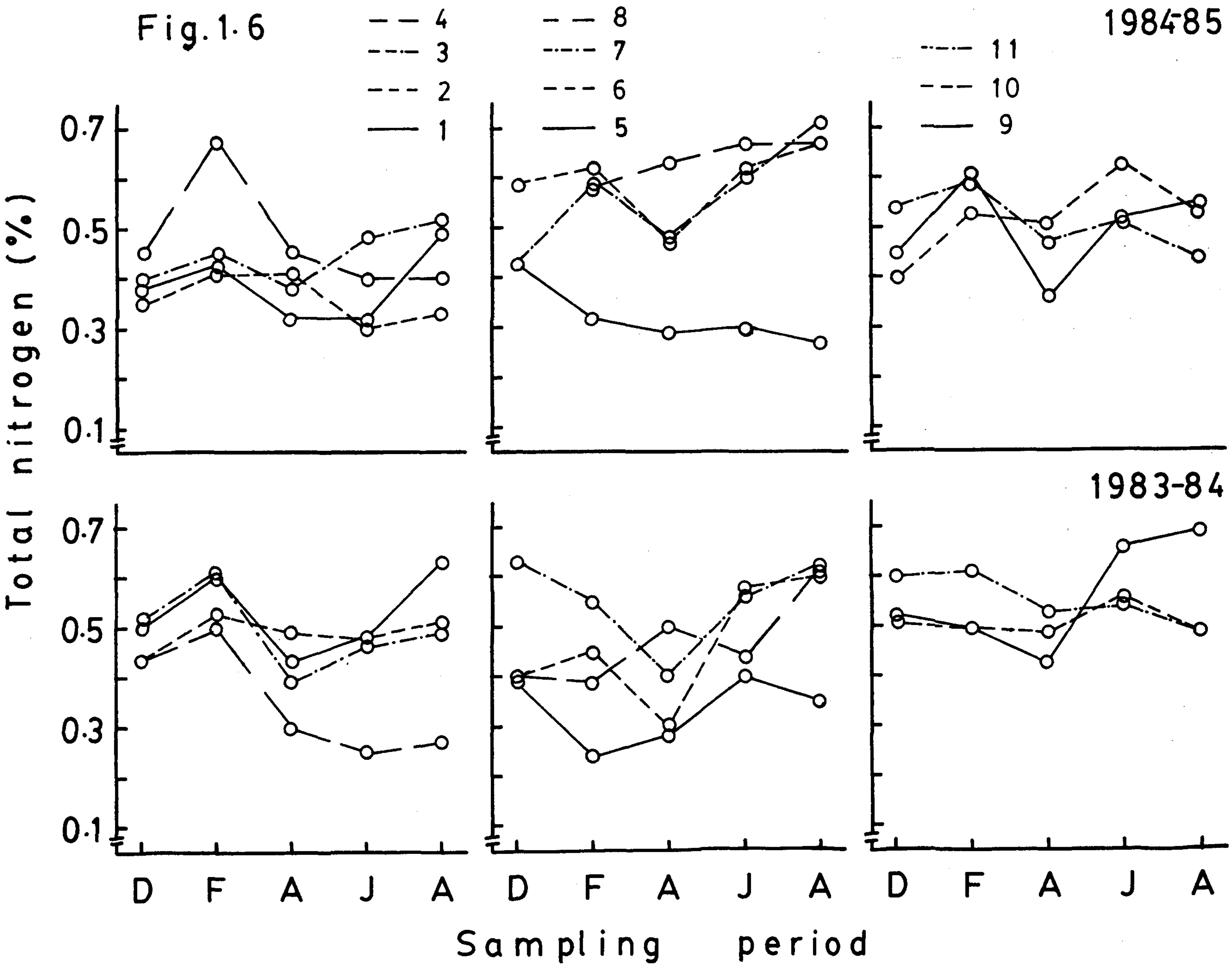

4
3
2
1
8
7
6
5
11
10
9
1984-85
1983-84
Percent organic matter
32
24
16
8
0
D
F
A
J
A
Sampling period

Fig.1.6 Seasonal variation in total nitrogen of rhizospheric soil of the tree species during the study periods of 1983-84 and 1984-85.

Sampling period, D:December; F:February; A:April· J:June; A:August.

Tree species, 1:*Cryptomeria japonica*; 2:*Exbucklandia populnea*; 3:*Acacia dealbata*; 4:*Mallotus nepalensis*; 5:*Schima khasiana*; 6:*Daphniphyllum himalayanse*; 7:*Machilus kingi*; 8:*Cinnamomum tamala*; 9:*Manglietia insignis*; 10:*Lindera latifolia*; 11:*Casearia verica*.

Fig.1.6



4
3
2
1
8
7
6
5
11
10
9
1984-85
1983-84
Total nitrogen (%)
0.7
0.5
0.3
0.1
D
F
A
J
A
Sampling period

Fig. 1.7 Seasonal variation in available phosphorus of rhizospheric soil of the tree species during the study periods of 1983-84 and 1984-85.

Sampling period, D:December; F:February; A:April; J:June; A:August.

Tree species, 1:Cryptomeria japonica· 2:Exbucklandia populnea; 3:Acacia dealbata· 4:Mallotus nepalensis; 5:Schima khasiana· 6:Daphniphyllum himalayanse; 7:Machilus kingi; 8:Cinnamomum tamala; 9:Manglietia insignis; 10:Lindera latifolia; 11:Casearia verica.

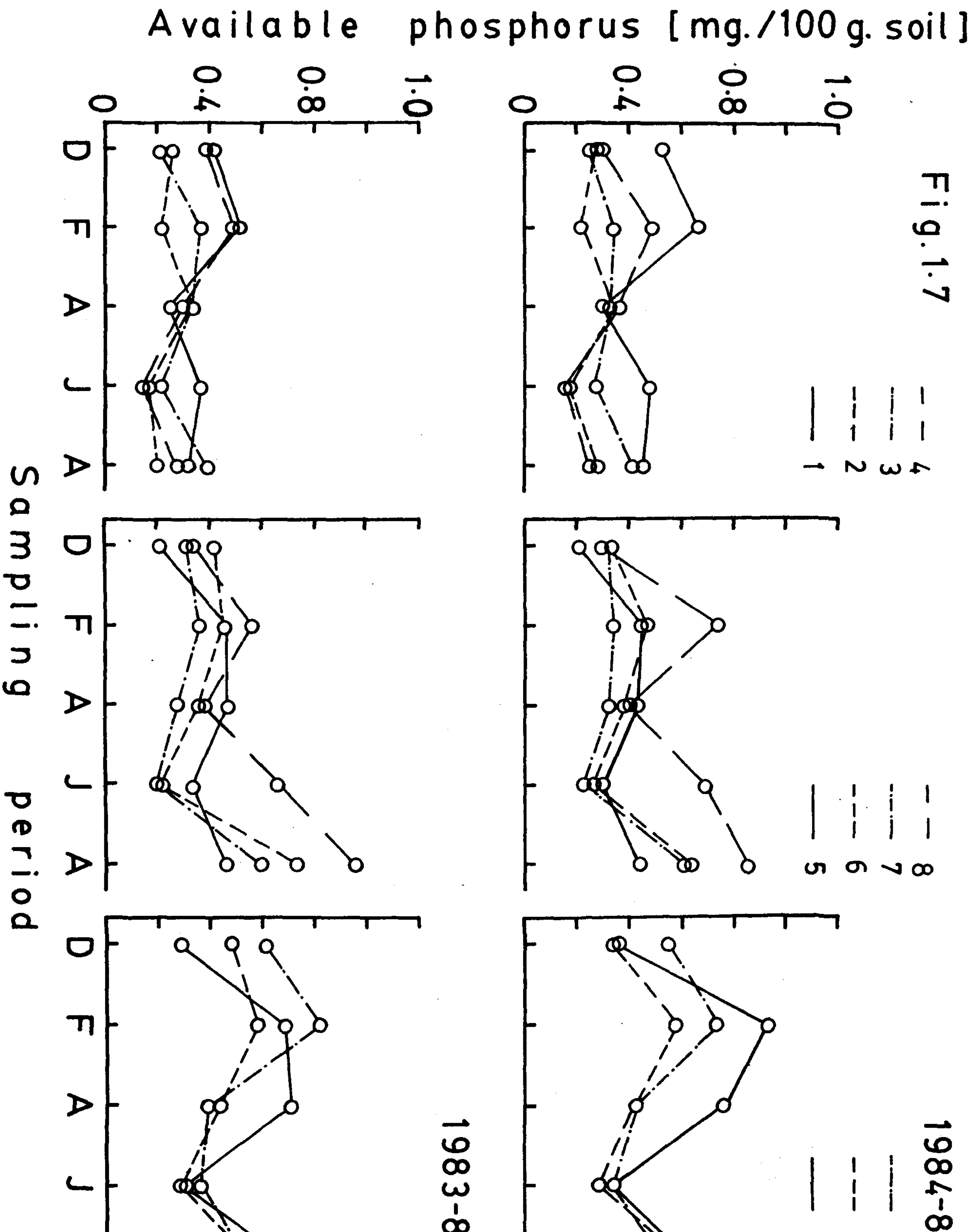


Fig.1.7

Available phosphorus [mg./100 g. soil]
0
0.4
0.8
1.0
D
F
A
J
A
Sampling period
1984-85
1983-84
4
3
2
1
8
7
6
5
11
10
9

Fig. 1.8 Seasonal variation in exchangeable potassium of rhizospheric soil of the tree species during the study periods of 1983-84 and 1984-85.

Sampling period, D:December; F:February; A:April; J:June; A:August.

Tree species, 1:*Cryptomeria japonica*; 2:*Exbucklandia populnea*; 3:*Acacia dealbata*; 4:*Mallotus nepalensis*; 5:*Schima khasiana*; 6:*Daphniphyllum himalayanse*; 7:*Machilus kingi*; 8:*Cinnamomum tamala*; 9:*Manglietia insignis*; 10:*Lindera latifolia*; 11:*Casearia verica.*

Fig. 1·8

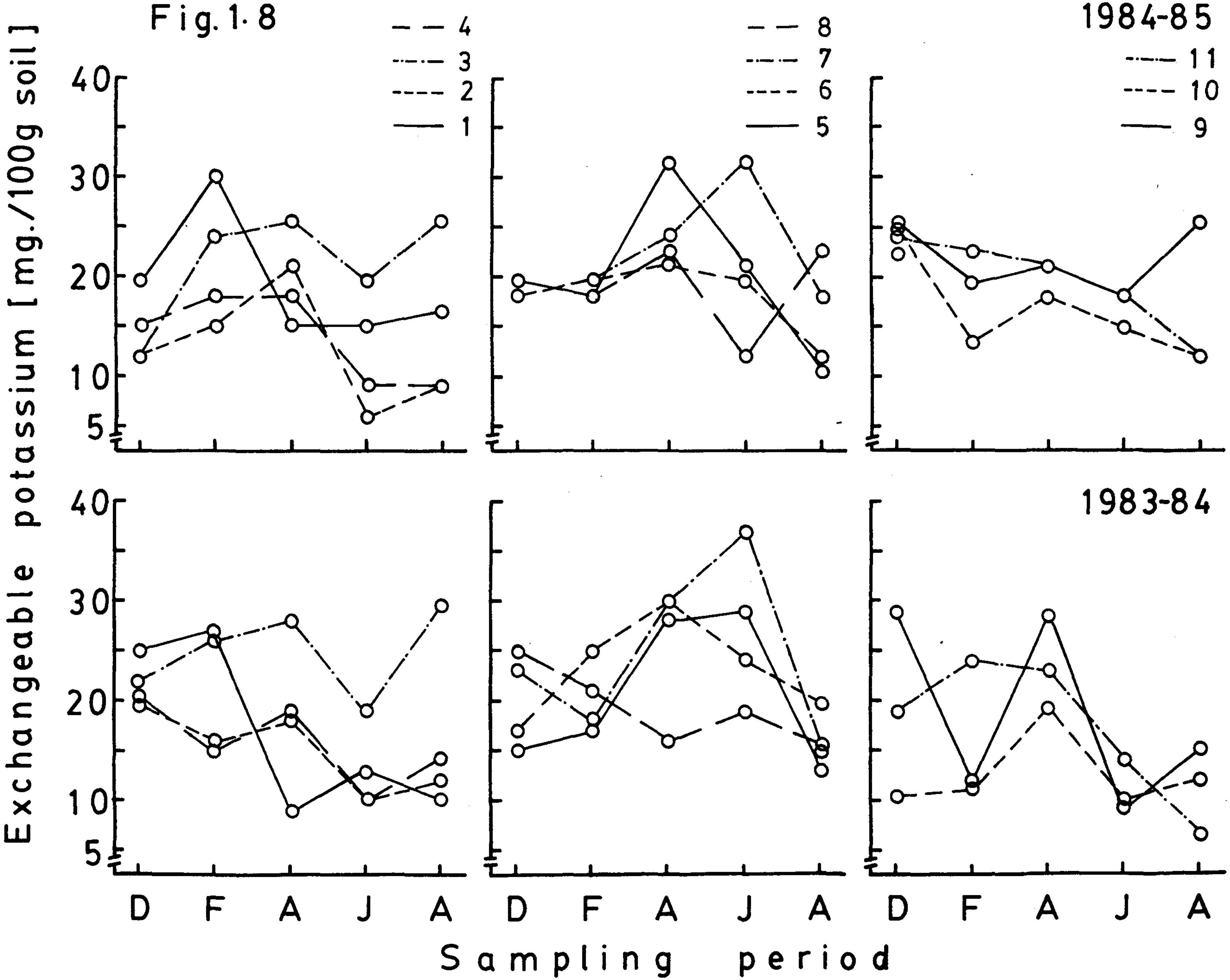

Exchangeable potassium [mg./100g soil]
40
30
20
10
5
4
3
2
1
8
7
6
5
1984-85
11
10
9
1983-84
D
F
A
J
A
Sampling period

Fig. 1.9 Seasonal variation in exchangeable calcium of rhizospheric soil of the tree species during the study periods of 1983-84 and 1984-85.

Sampling period, D:December; F:February; A:April; J:June; A:August.

Tree species, 1:*Cryptomeria japonica*; 2:*Exbucklandia populnea*; 3:*Acacia dealbata*; 4:*Mallotus nepalensis*; 5:*Schima khasiana*; 6:*Daphniphyllum himalayanse*; 7:*Machilus kingi*· 8:*Cinnamomum tamala*; 9:*Manglietia insignis*; 10:*Lindera latifolia*; 11:*Casearia verica*.

Fig. 1·9

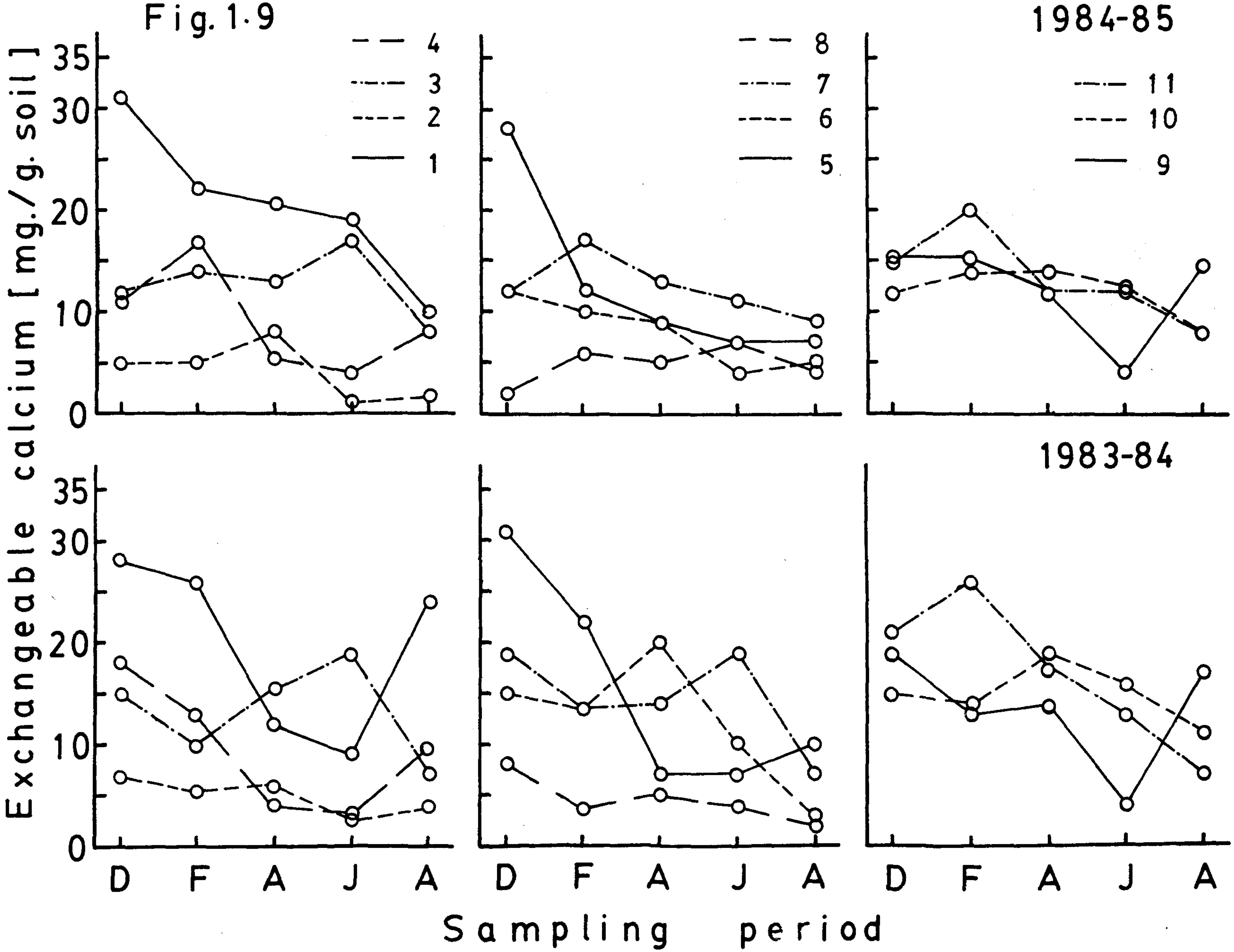

1984-85
1983-84
4
3
2
1
8
7
6
5
11
10
9
Exchangeable calcium [mg./g. soil]
0
10
20
30
35
D
F
A
J
A
Sampling period

Table 1.4 Relationship (r) of the percent VAM infection with the rhizospheric soil properties of the tree species.

Tree species	pH		MC		OM		N		P		K		Ca	
	I	II	I	II	I	II	I	II	I	II	I	II	I	II
Infection percentage														
1. *Cryptomeria japonica*	-.472	-.060	-.404	.038	-.124	-.346	-.510	-.260	-.510	-.905*	-.174	-.739	-.001	.184
2. *Exbucklandia populnea*	.772	.242	.241	-.194	-.017	-.415	-.715	-.731	-.456	-.202	-.154	-.673	-.147	-.574
3. *Acacia dealbata*	.052	.960**	.641	.893*	-.599	-.689	-.779	-.558	-.604	-.337	-.038	-.155	-.808	-.828
4. *Mallotus nepalensis*	.239	.337	-.474	-.441	.840*	.499	.267	-.041	-.117	-.165	.136	-.263	-.256	-.791
5. *Sghima khasiana*	.227	.264	.570	.791	-.033	.405	.319	-.590	.255	.084	.533	.306	-.867*	-.604
6. *Daphniphyllum himalayanse*	.714	-.336	.074	.467	.459	-.827	.081	-.190	-.806	.548	-.142	-.553	-.215	-.009
7. *Machilus kingi*	-.256	.765	.914*	.213	.484	.459	.877*	.289	.386	.756	-.864*	-.541	-.329	-.303
8. *Cinnamomum tamala*	-.926*	-.942*	.476	.964**	.526	-.680	-.614	.551	.138	.279	.279	.477	-.444	-.522
9. *Manglietia insignis*	.219	.466	.657	-.325	-.145	.017	-.512	.757	-.174	.393	.391	.400	-.406	-.215
10. *Lindera latifolia*	.843	-.286	.741	.719	-.168	.132	.038	.788	-.390	.020	.503	-.633	-.219	-.389
11. *Casearia verica*	.843	-.260	.057	-.501	-.861*	.484	-.618	-.518	-.222	.402	-.337	-.300	-.771	-.316

Mc = Moisture content; OM = Organic matter; N = Total nitrogen; P = Available phosphorus;
K = Exchangeable potassium; Ca = Exchangeable calcium; I = (1983-84); II = (1984-85).

*Significant at 5% level; ** Significant at 1% level.

Table 1.5 Relationship (r) of endogonaceous spore population with rhizospheric soil properties.

Tree species	pH		MC		OM		N		P		K		Ca	
	I	II	I	II	I	II	I	II	I	II	I	II	I	II
Spore population														
1.*Cryptomeria japonica*	.468	-.214	.821	.868	-.004	.180	.381	.427	-.174	.271	-.462	-.227	-.235	-.281
2.*Exbucklandia populnea*	-.489	-.711	.917*	.808	-.919*	-.344	.568	-.416	-.571	.227	-.941*	-.219	-.886	-.368
3.*Acacia dealbata*	.647	-.556	.096	.713	-.199	-.911*	-.118	-.738	.061	.817	.471	-.947*	.415	-.617
4.*Mallotus nepalensis*	.578	-.408	.015	-.079	-.844	.353	.677	-.868	-.060	-.145	.397	-.299	.102	-.337
5.*Sehima khasiana*	.825	.716	.226	-.446	-.333	-.626	.589	.481	-.250	-.360	-.631	-.533	.339	.370
6.*Daphniphyllum himalayanse*	-.253	.672	-.687	-.949*	-.497	-.213	-.531	.062	-.275	-.083	-.008	.188	.575	.858
7.*Machilus kingi*	-.024	-.810	-.376	-.607	-.288	-.718	-.481	-.582	-.905*	-.741	.316	.416	.655	-.275
8.*Cinnamomum tamala*	-.793	-.562	.223	.644	.207	-.328	-.576	-.192	-.272	.010	.087	.237	-.511	-.264
9.*Manglietia insignis*	.529	.810	.088	-.188	.046	.192	-.605	-.831	.247	.193	.760	.029	.214	-.086
10.*Lindera latifolia*	-.427	-.639	.006	.207	.148	-.310	.246	.332	-.018	.096	-.670	-.352	-.650	-.744
11.*Casearia verica*	-.498	-.828	-.978**	-.494	.273	.259	.812	.688	.888*	.790	.509	.481	.765	.734

Mc = moisture content; OM = Organic matter; N = Total nitrogen; P = Available phosphorus
K = Exchangeable potassium; Ca = Exchangeable calcium; I = (1983-84); II = (1984-85).

*Significant at 5% level; ** Significant at 1% level.

CHAPTER - II

TAXONOMY OF VA-MYCORRHIZAL FUNGI

INTRODUCTION

The fungi involved in mycorrhizal association generally produce spores outside the roots. The morphology of these spores mainly forms the basis of taxonomy of these fungi. The microscopic characters which include finer details complicates the identification of different species. As a result the taxonomical studies on endogonaceous members have been fragmentary, though, these fungi are frequently encountered in nature. Peyronel (1923) was the first to recognise that vesicular-arbuscular mycorrhizal fungi were members of Endogonales. Gerdemann and Nicolson (1963) developed procedure for collecting spores of these fungi from soil and described new species of genus Endogone. The family endogonaceae was monographed in (1974) by Gerdemann and Trappe and the genus Endogone was segregated into seven genera. Trappe (1982) recognised nine genera of VA mycorrhizal fungi. What about more recent work.

Little is known about geographical distribution of various species of VA mycorrhizal fungi. Hall (1977) from New Zealand, Nicolson and Schenck (1979), Schenck and Smith (1982) from Florida, Tandy (1975) from Australis, Iqbal and Parveen (1981) from Pakistan feported the mycorrhizal fungi.

Studies on taxonomy of endogonaceae from Indian soils are meagre (Bakshi, 1974; Bhattacharjee et.al.,1980 (a,b), 1982; Gerdemann and Bakshi, 1976; Singh and Varma, 1981; Thapper and Khan, 1985). Moreover north-eastern region of India remained uninvestigated untouched.During the present investigation on mycorrhizal association of tree species in a forest ecosystem of Meghalaya, ten different VAM fungi were identified and are described in this chapter.

(A) Methods of Isolation and identification of Endogonaceous fungi :

The spores of endogonaceous fungi were isolated from the soil by the method of Gerdemann and Nicolson (1963). On the filter paper the spores were separated from soil debris and transferred to watch glasses containing water. The identical spores were separated and stored in glass vials in Ringer's solution. The different types of spores were mounted in Polyvinyl lactophenol medium on glass slides for microscopic observations. Ringer's solution and PVL medium were prepared as follows.

(i) Ringer's solution

Sodium Chloride = 7.5 g.

Potassium Chloride = 0.075 g.

Calcium Chloride = 0.1 g.
(anhydrous)

Sodium bicarbonate = 0.1 g.
(anhydrous)

All the above constituents were dissolved in a litre of water and pH of solution was adjusted to 7.4 with

IN NaOH/IN Hcl.

(ii) PVL medium : Polyvinyl lactophenol medium was prepared by dissolving 15ml of Polyvinyl alcohol granules in 100ml of distilled water on a water bath at 80°C for 2 days. The resulting solution was stored in brown bottle. 50 parts of this solution were mixed with 22 parts each of lactic acid and phenol. The resulting mixture is PVL medium and was used for permanent mount of endogonaceous spores.

(iii) Histochemical tests : Histochemical tests of spores were performed with Meltzer's reagent which was prepared as follows.

Iodine = 0.5 g.
Potassium Iodide= 1.5 g.
Chloral hydrate = 20 g.
Distilled water = 20 ml.

The constituents were mixed and the reagent stored in dark bottle. The spores were mounted directly in the reagent.

(B) Establishment of culture of VA mycorrhizal fungi :

Isolated spores of Glomus spp. were inoculated in autoclaved garden soil on maize plants and grown till the host matured and dried. The method suggested by Gilmore (1968) was used. The suspension of isolated spores of VA endophyte Glomus spp. was poured in a hole in autoclaved garden soil taken in a plastic pot. A germinated seeds of maize were placed over the inoculum in the hole. The host plant was grown for a period of 2 months and allowed to dry. The roots of maize host were chopped and mixed with soil. This soil-root mixture was used as an inoculum.

(C) Description of endogonaceous fungi :

Ten species of endogonaceous fungi were isolated and identified from the rhizospheric soil samples of various tree species. These species are described below.

Genus Acaulospora is represented by 2 spp.

1. Acaulospora laevis Gerdemann and Trappe.

Sporocarps unknown, spores forming singly, sessile, borne laterally on a wide thin walled hyphae 20 µm in diameter that terminate nearby in a globose vesicle of the size of spore, shrunken at spore maturity, spores smooth, 300-400 µm, globose or sub-globose, dull yellow, spore wall continuous except for the occluded opening 5µm thick. Hypha attached below spores branched.

This form was collected from the rhizospheric soil of Machilus kingi. The characters of the present isolate resemble type description of Gerdemann and Trappe (1974) (Plate 2.1).

2. Acaulospora scrobiculata Trappe.

Sporocarp unknown, spores forming singly in soil, sessile, borne laterally on thin walled hyaline hyphae that terminate in a thin walled vesicle, collapsed vesicle attached to spore, spore shape varies from globose to ellipsoid, 100-200 µm, olive to light brown, spore sufface evenly pitted. This form was isolated from the rhizospheric soil of *Alnus nepalensis* and resemble the type description of Trappe (1977). (Plate 2.1).

Genus sclerocystis is represented by three species.

3. *Sclerocystis coremioides* Berk and Broome.

Sporocarp sub-globose, 200-250µm, in gregarious, ? dark-brown, peridium of interwoven hyphae (5 µm diam.) present. Chlamydospores arranged in a single laver around a central plexus of hyphae, obovoid to ellipsoid to broadly clavate, 50-75 × 40-53 µm, brown, spore wall 5 µm thick (Place 2.2).

4. *S. rubiformis* - Gerdemann & Trappe, sp. nov.

Sporocarp brown, subglobose, 400x450µm consisting of a single layer of chlamydospores surrounding a central plexus of hyphae, peridium absent or poorly developed of loose interwoven hyphae. Chlamydospores brown, obovoid to ellipsoid or sub-globose, 33-45x25-35 µm, spore wall laminate 3 µm thick with perforated projections on the inner surface.

This form was isolated from the rhizosphere of *Machilus kingi* and is also reported by Bhattacherjee *et.al*.

(1980) from agricultural field at Bangalore.

5. *S. microcarpus* Iqball & Bushra

Sporocarp brown, 112 µm in diam., globose, Chlamydospores formed radially in a single tightly packed layer around a central plexus of hyphae. Peridium absent. Chlamydospores 58-75x18-20 µm, narrowly clavate tapering towards basal hyphal attachment 5-8 µm in diam., spore wall 8-13µm thick at apex and 3-8 µm thick at the base.

The description of the present isolate resembles the type description given by Iqball and Parveen (1981). This species has not been reported from India earlier and constitute a new record. (Plate 2.2).

Genus *Glomus* : Two species of Glomus were identified namely, *G. macrocarpus* (Nicol & Gerd.) and *G. mosseae* (Nicol & Gerd.).

6. *Glomus macrocarpus* var. geosporus (Nicol. & Gerd.)

Chlamydospores formed singly in soil, globose or subglobose, 110-150x110-143µm, smooth, dark brown to black, spore wall 10-15µm thick, perforated, hyphal attachment 12-15µm in diam.

This form was isolated from the rhizosphere of *Manglietia insignis* and has also been described in agricultural fields by Bhattacharjee *et.al* (1980) (Plate 2,3).

7. *G. mosseae* (Nicol. & Gerd.)

Sporocarp not found. Chlam[illegible]res yel.

(1980) from agricultural field at Bangalore.

5. S. microcarpus Iqball & Bushra

Sporocarp brown, 112 µm in diam., globose, Chlamydospores formed radially in a single tightly packed layer around a central plexus of hyphae. Peridium absent. Chlamydospores 58-75x18-20 µm, narrowly clavate tapering towards basal hyphal attachment 5-8 µm in diam., spore wall 8-13µm thick at apex and 3-8 µm thick at the base.

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This form was isolated from the rhizosphere of Manglietia insignis and has also been described in agricultural fields by Bhattacharjee et.al.(1980) (Plate 2.3).

7. G. mosseae (Nicol. & Gerd.)

Sporocarp not found. Chlamydospores yellow to brown, globose to obovoid 90-110μm in diam. Spore wall 7μm thick.

This species was isolated from the rhizosphere of *Cinnamomum tamala* and has also been reported from agricultural fields by Bhattacharjee *et.al.*(1980) (Plate 2.3)

Genus *Gigaspora* : Three species of Gigaspora were identified from the collection. These are *G. gregaria* Nicol. & Schenck; *G. calospora* and *G. gigantea*.

8. *Gigaspora gregaria* Nicol & Schenck

Azygospores formed singly in soil, 200-300μm **india-**diam., globose, dark brown, spore wall 12-25μm thick, irregular, polygonal projections on the surface present. The suspensor like cell 50μm in diam. Concolorous with spore and giving rise to a slender hyphae that projects to the spore surface.

This species was isolated from the rhizosphere of *Alnus nepalensis* and differ from *G. coralloidea* in having smaller spores with surface projections. This species is being reported from India for the first time (Plate 2.4)

9. *G. calospora* (Nicol & Gerd.)

Azygospores formed singly in soil, globose or sub-globose, 150-160μm in diam., pale yellow, spore wall 2-5 μm thick, continuous, suspensor like cell 50 μm in diam. bulbous and concolorous with spore wall. This form was isolated from the rhizosphere of *Schima khasiana* and resembles the type description of Gerdemann & Trappe (1974).However, azygospores were more or less globose. This form has been collected from the forest and agricultural land in Uttar Pradesh by Bakshi (1974) (Plate 2.4).

10. *G. gigantea* (Nicol. & Gerd.)

Azygospores formed singly in soil, 260-370x260-350 µm, globose, greenish yellow, spore wall 12-25 µm thick, suspensor like cell bulbous, 42-48 µm in diam. giving rise to a slender hypha which projects to the spore, vesicles not observed in the collection.

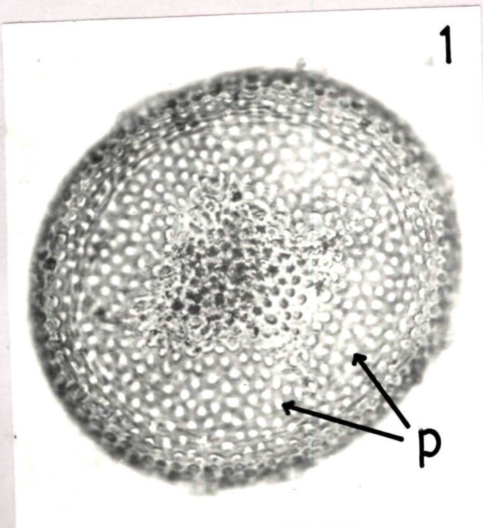
This form was isolated from the rhizosphere of *Manglietia insignis* and has also been reported in woodland by Bakshi (1974) (Plate 2.4).

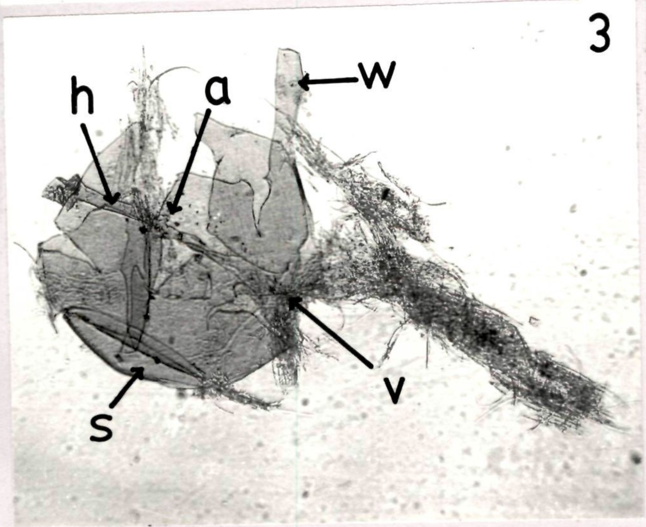
Plate 2.1

1. Spores of _Acaulospora_ _scrobiculata_ showing surface pits (X 100); 2. Spore with two distinct walls (X 400); 3. Sessile spore of _A_. _laevis_ and shrunken vesicle (X 100); 4. Spore with hyphal attachment (X 400); (h=hypha; i=innerwall; o=outer wall; p=surface pits; s=spore; v=vesicle; w=spore wall).

a = ?

PLATE-2.1





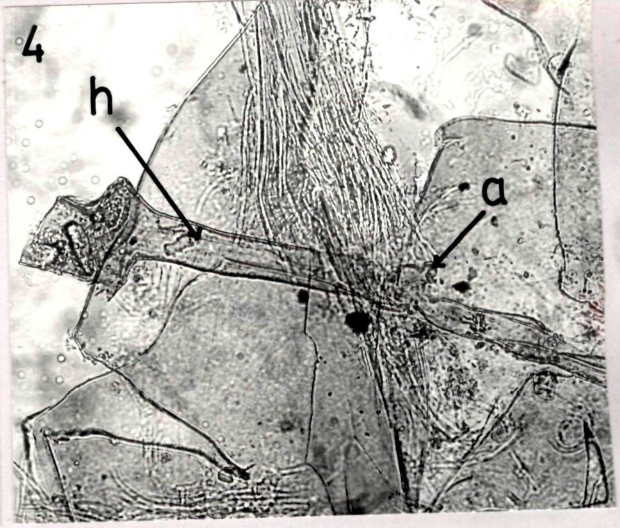
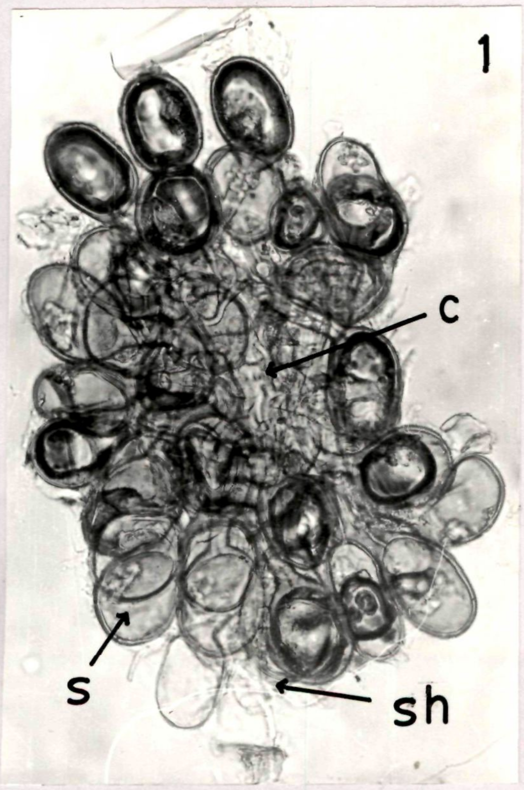
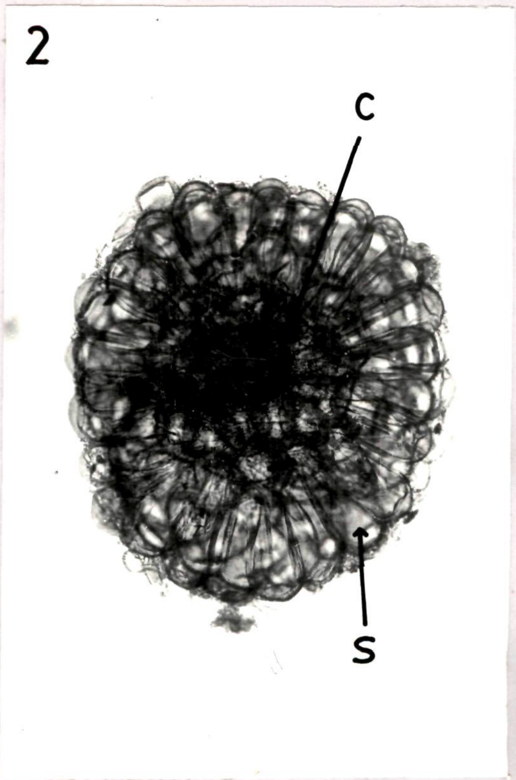


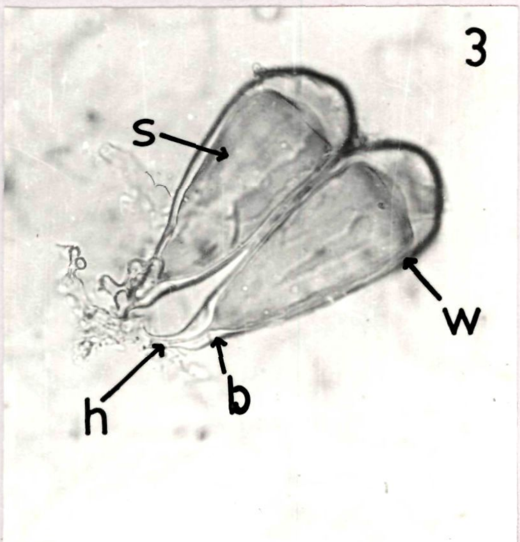
Plate 2.2

1. Sporocarp of *Sclerocystis coremioides* (X 100); 2. V.S. sporocarp of *S. microcarpus* (X 100); 3. Spores of *S. microcarpus* (X 400); 4. Sporocarp of *S. rubiformis* (X 100). (b=basal end; c=central plexus; h=hyphal attachment; S=spore; Sh=peridium; w=spore wall).

PLATE-2.2







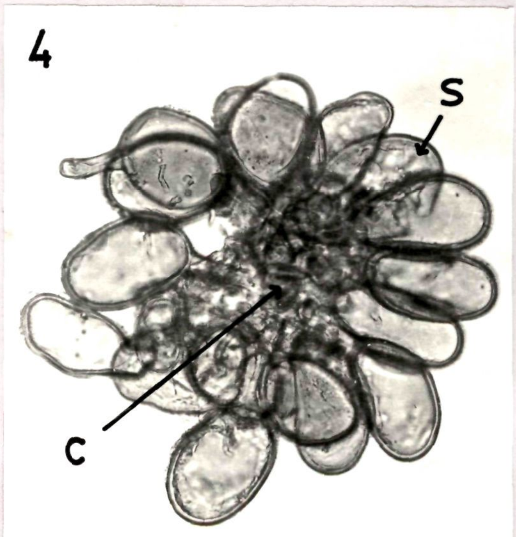
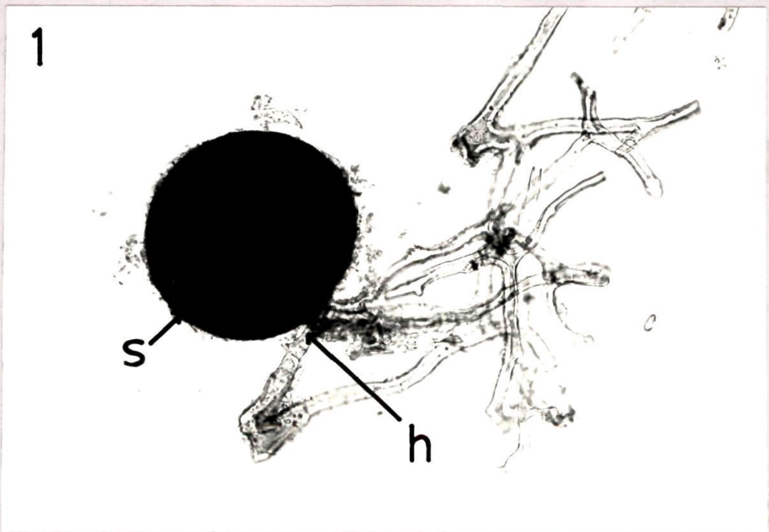


Plate 2.3

1. Spore of Glomus macrocarpus var. geosporus (X 100); 2. Spore of G. mosseae (X 100). (h=hyphal attachment; s=spore).

PLATE - 2.3



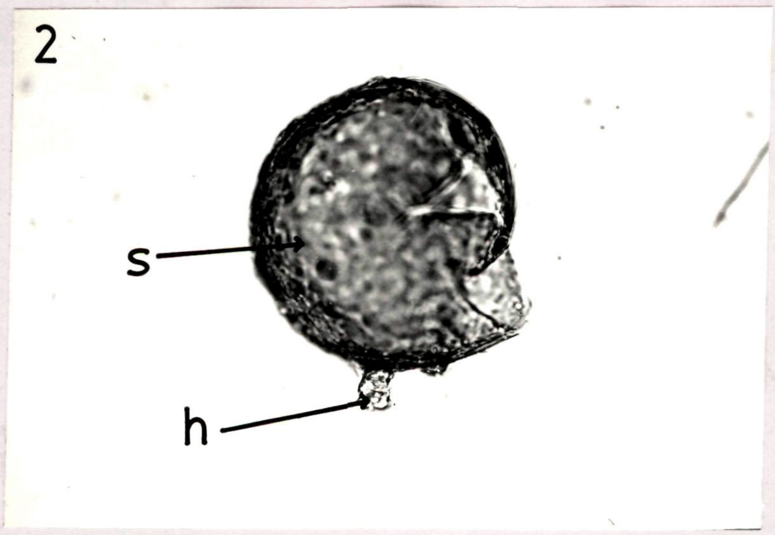
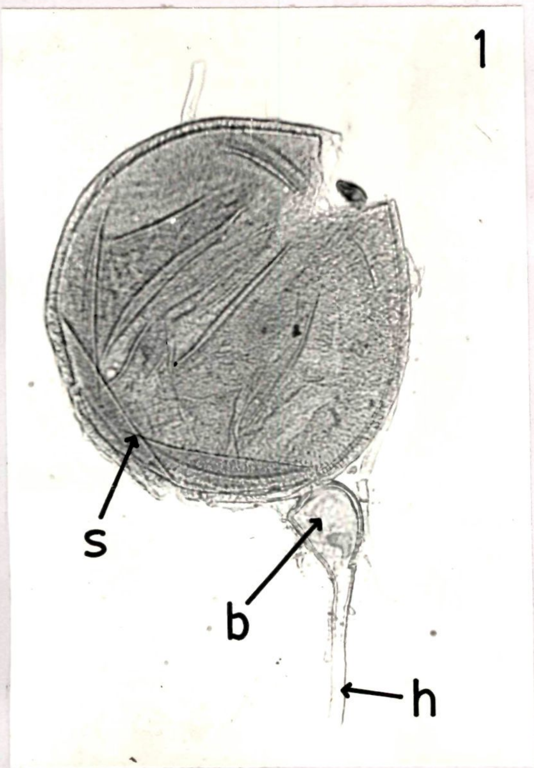
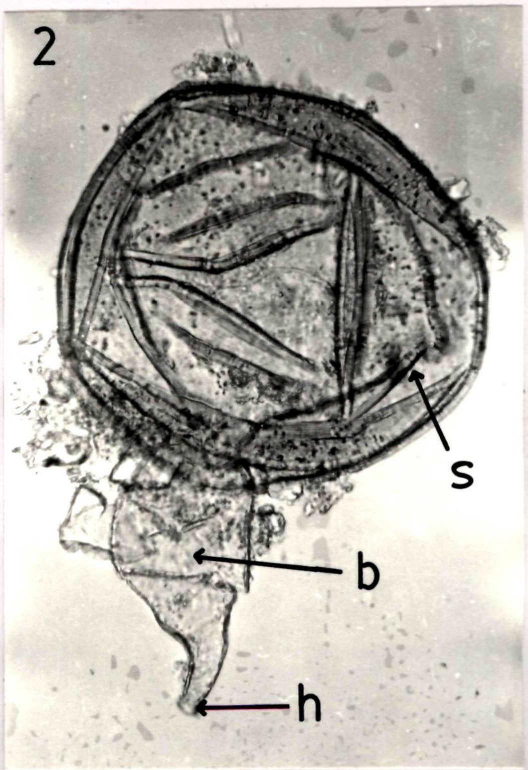


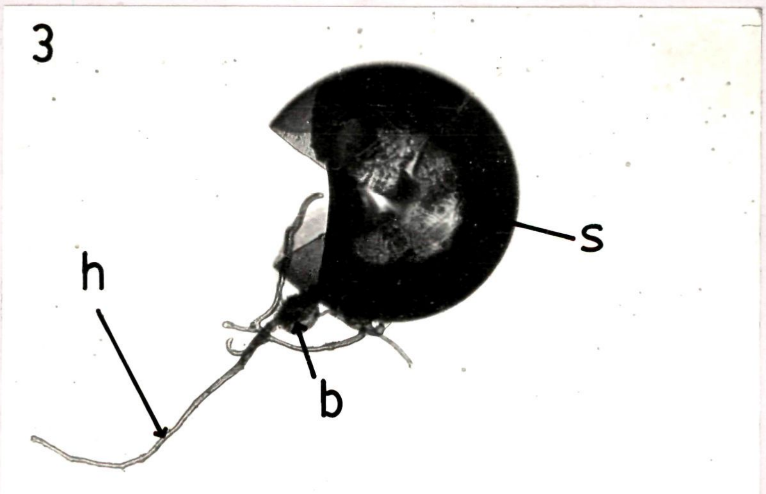
Plate 2.4

1. Spore of *Gigaspora gigantea* (X 100); 2. Spore of *G. calospora* (X 100); 3. Spore of *G. gregaria* (X 100) (b=bulbous base; h=hyphal attachment; s=spore).

PLATE - 2.4







CHAPTER - III

EFFECT OF VA-MYCORRHIZA ON GROWTH AND YIELD OF *Exbucklandia populnea*

INTRODUCTION

Endomycorrhiza of VA type helps in growth of plants (Gerdemann, 1968; Mosse, 1973a). Plants growing in nutrient deficient habitats frequently form endomycorrhiza and grow better than non-mycorrhizal plants (Banister and Norton, 1974; Csinos, 1981). However, when such plants are supplied with the nutrients below the optimum level better response to growth is observed (Pope,1980).

Phosphorus is a limiting factor in soil and plants growing in phosphorus deficient soil respond quickly to mycorrhiza formation and grow better than non-mycorrhizal plants. The mycorrhizal plants supplied with phosphorus enhance growth stimulation (Baylis, 1967; Hardie and Leyton, 1981 and Howler et.al.,1982). In presence of rock phosphate mycorrhizal plants grow better and utilize phosphate efficiently from such insoluble sources (Mosse,1977; Powell & Daniel, 1978; Vaidyanath et.al.,1979). In presence of additional phosphorus, endomycorrhiza can improve growth and yield of plants (Clarke and Mosse, 1981). Shoot and root growth is enhanced and R/S ratio is decreased when endomycorrhizal plants are supplied with additional phosphorus (Hardie and Leyton,1981). Plants given additional single super-phosphate at low levels and inoculated with VA endophytes grow better than non-mycorrhizal plants (Abbott & Robson, 1977b). Plenchette et.al.,(1983) suggested that efficiency of a VA endophyte with a host should be tested at different levels of phosphorus.

Many commercially important hardwood trees act as a host for fungi that form VAM. A few studies report the influence of VA mycorrhiza on hardwood species (Bryan & Kormanik, 1977; Kormanik et.al.,1977; Pope,1980; Riffle, 1980). Exbucklandia populnea is an important timber tree growing successfully in pine forest (Pinus kesiya) of N.E. India. Under natural conditions the plants form VA endomycorrhiza.

The present study was undertaken to assess the nature of interaction between endomycorrhizae and growth and development of seedlings in relation to various levels of soil fertility and phosphorus status of the soil.

MATERIALS AND METHODS

Seedlings of Exbucklandia populnea were raised from seeds and grown in plastic pots. The formation of VAM and its effect on survival, growth and yield of seedlings was observed. The study was planned in two separate experiments, each with a set of mycorrhizal and non-mycorrhizal plants grown separately.

In the first experiment the effect of vesicular-arbuscular mycorrhiza formation on the survival and growth of seedlings of Exbucklandia populnea was studied at five different levels of soil fertility. The fertility levels represented five different concentrations of each of three commercial fertilizers namely, single superphosphate, ammonium sulfate and muriate of potash added in combined form and expressed as 0,14,28,56 and 112 kg ha^{-1} of NPK. In the five treatments pots received an amount of 0,0.275, 0.550, 1.100 and 2.200g of single superphosphate; 0, 0.214, 0.428, 0.856, 1.712g of Ammonium sulfate and 0, 0.073, 0.146, 0.292 and 0.584 g of muriate of potash. For each level of soil fertility a set each of non-mycorrhizal and mycorrhizal seedlings was grown separately.

In the 2nd experiment the effect of addition of phosphorus on the formation of vesicular-arbuscular mycorrhiza and their effects on growth of seedlings of Exbucklandia populnea were studied. Two sources of phosphorus were used viz. bone meal and sodium dihydrogen phosphate.The two sources differ in the availability of phosphorus. Whereas, [illegible] bone meal represents non-available form. Bone meal was added at eight different levels i.e. 0, 0.34, 0.69, 1.72, 3.44, 17.2, 25.8, 34.4 and 51.6g per pot. Sodium dihydrogen phosphate was added at 0.34, 0.69, 1.72, 3.47 and 17.33 g per pot. In all there were fourteen phosphorus treatments, each with a set of mycorrhizal and non-mycorrhizal plants grown separately.

Methods of preparation of pots, application of nutrients, inoculation of VA endophyte, preparation and plantation of seedlings, growth conditions, harvesting and types of observations recorded are given below.

1. Preparation of pots : Plastic pots of 5L capacity were used for growing seedlings. Local garden soil and sand were filled in small gunny bags and autoclaved at 15Lb square inch pressure for 1 hour. Autoclaving was repeated twice with a gap of 24 hours. The soil was allowed to dry. The autoclaved soil and sand were mixed in the ratio of 1:1 and the mixture was filled in plastic pots which had hole at its bottom to allow free drainage of water and air.

2. Application of Nutrients : The required quantities of various fertilizers (stated above) were properly mixed with soil sand mixture in pots.

3. Inoculation of VA endophyte : The method of inoculation of VA endophyte was similar for both the experiments. The inoculum was pot soil infested with spores of *Glomus spp.* isolated from local field soil and pot cultured on maize plant as in Chap 2? Two sets of mycorrhizal treatments were given. The one designated as mycorrhizal and the other non-mycorrhizal. Mycorrhizal set received the inoculum of VA endophyte whereas, autoclaved inoculum and filtrate of inoculum washings were added to the non-mycorrhizal set. About 50g of soil inoculum was properly mixed with surface soil of pots.

4. Seedling preparation and plantation : The seeds of *Exbucklandia populnea* were procured from ripe fruits excised from trees growing at Upper Shillong and stored in dry polythene bags in refrigerator at 4°C till used.

Prior to planting in pots, the seeds were soaked in water for 12 hours and placed on moist filter paper in petri dishes. After about a week the germinated seeds were transferred to pots and burried 1/2 cm deep in holes in pot soil which has already received nutrient and inoculum treatments.

5. Growth conditions and harvesting : All the experiments were set up in the month of May, 1986 and were carried out in net house at University Campus, Shillong. Natural climatic conditions were provided for the growth of seedlings. Tap water was used.

The seedlings were grown for a period of about 6 months after which they were harvested for observations. The complete seedlings with intact root system were carefully excavated, washed with tap water and oven dried.

Measurements : Total number of surviving seedlings per treatment, length of seedling, number of leaves, stem collar diameter and oven dry weight of stem, root and leaves were recorded. About 25 fresh root segments (1cm) were randomly sampled from seedlings and fixed in FAA. The root segments were cleared and stained by the method of Phillips and Hayman (1970) and %VAM infection was assessed by the microscopic method of Allen *et.al*. (1982).

RESULTS

EFFECT OF INFECTION AS INFLUENCED BY FERTILITY LEVEL OF SOIL

(A) **Growth** Responses : The seedling of *Exbucklandia populnea* responded to VAM inoculation at O, I, II and III levels of added NPK. Both VAM inoculated and noninoculated plants died at IVth level perhaps because of supra optimal conc. of nutrients. VAM inoculation improved growth characteristics over its uninoculated counterparts. VAM inoculation combined with low levels of NPK gave better response than either of VAM inoculation or NPK additions. The details of various growth responses observed are as follows.

(i) Seedling Survivability : When the percent survivability of VAM inoculated and non-inoculated plants was compared, it was found that VAM inoculated seedlings had more survivability than their noninoculated counterparts ($p < .05$) at 'O' and I levels of NPK (Table 3.1). However, at other two levels i.e. II and III there was no significant difference between the two. On the other hand when the survivability of inoculated plants at different levels of NPK was compared, it was found that seedling survivability increased significantly at Ist level, however, it decreased subsequently at higher levels of NPK (Table 3.1).

(ii) Seedling length : The average seedling length of VAM inoculated plants was significantly higher ($p < .05$) than their non-mycorrhizal counterparts at 'O' and I levels of NPK. Seedling length of mycorrhizal plants increased at I level of NPK and decreased subsequently at higher levels of NPK (Fig.3.1).

(iii) Stem collar diameter : Stem collar diameter of VAM

Fig.3.1 Length of mycorrhizal and non-mycorrhizal plants grown at different fertility levels of soil.

bars are ?

Fig. 3·1

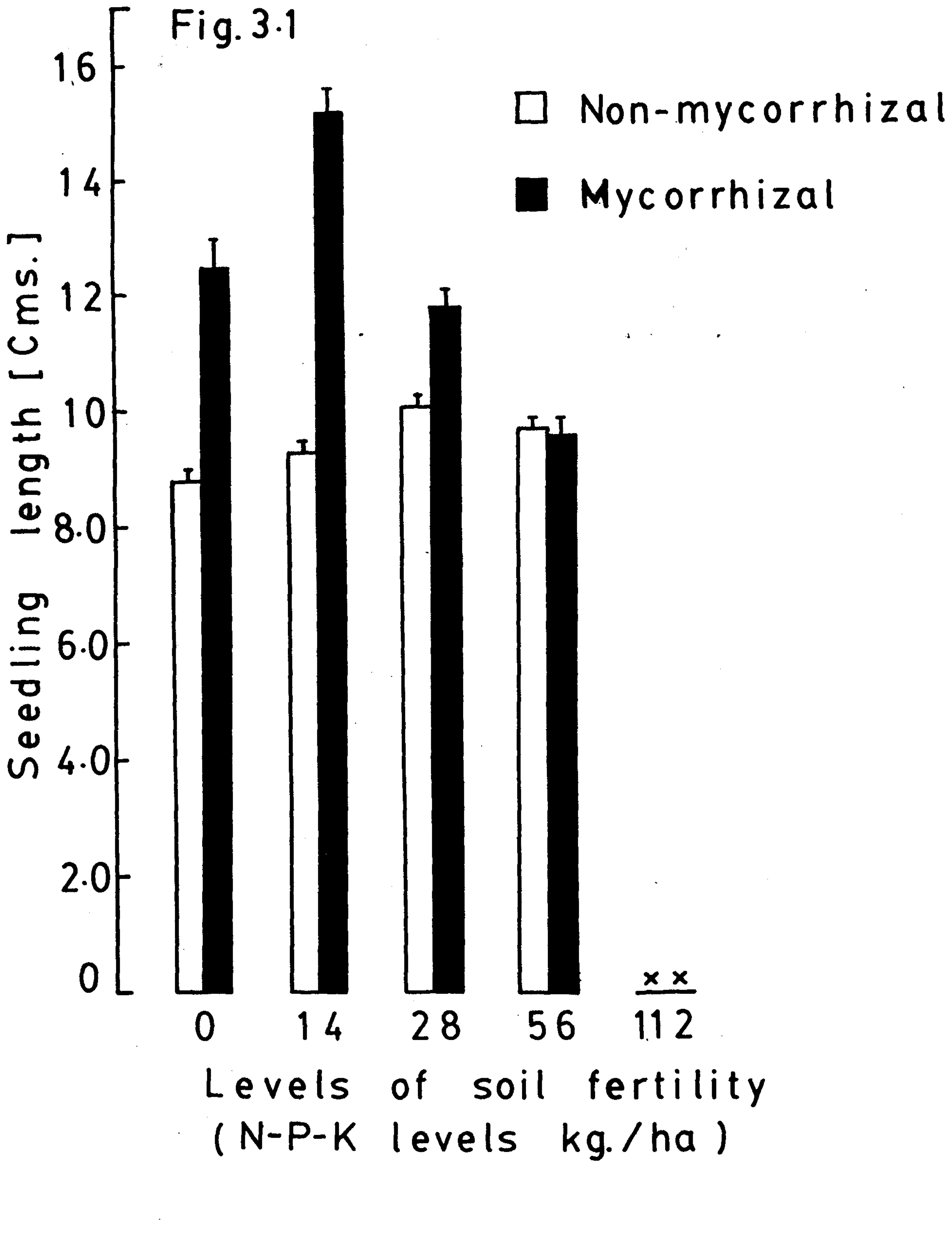
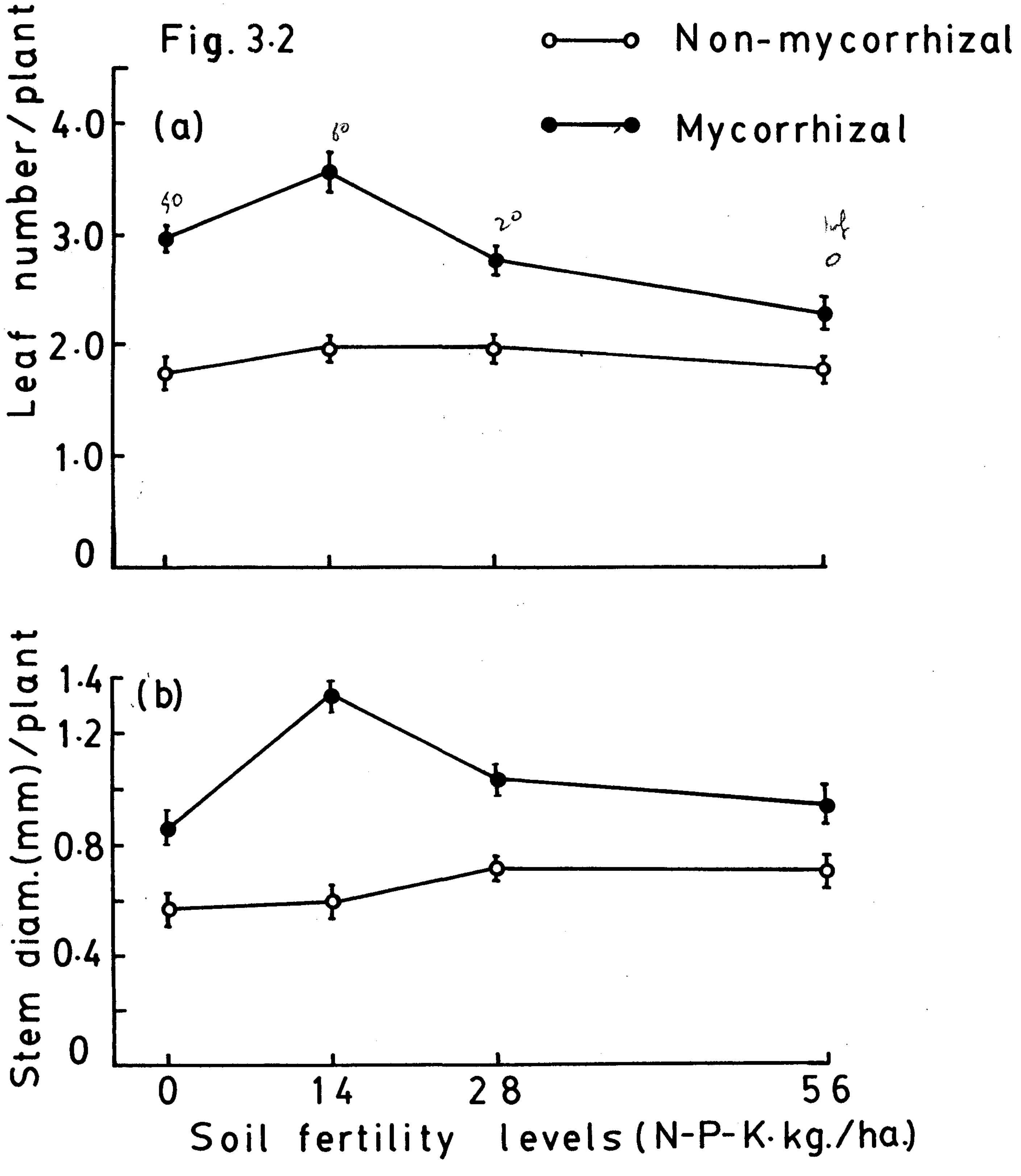

1.6
14
12
10
8·0
6·0
4·0
2·0
0
Seedling length [Cms.]
□ Non-mycorrhizal
■ Mycorrhizal
x x
0
14
28
56
112
Levels of soil fertility
(N-P-K levels kg./ha)

Fig.3.2 The differences in the leaf number (a) and stem collar diameter (b) of the mycorrhizal and non-mycorrhizal plants grown at different fertility levels of soil.

Fig. 3.2


o—o Non-mycorrhizal
●—● Mycorrhizal
(a)
Leaf number/plant
4.0
3.0
2.0
1.0
0
40
60
20
hf
0
(b)
Stem diam.(mm)/plant
1.4
1.2
0.8
0.4
0
0
14
28
56
Soil fertility levels (N-P-K.kg./ha.)

inoculated plants was significantly greater ($p < .05$) than that of noninoculated plants at O, I and II levels. However, at the III level the differences were insignificant. Stem collar diameter also increased with increased in concentration of NPK upto II level (Fig.3.2b).

(iv) Number of leaves per plant : The average number of leaves per plant was significantly more ($p < .05$) in mycorrhizal plants than their non-inoculated counterparts at O and I levels of NPK (Fig.3.2a).

From the above results it was noted that VAM inoculation of plants improved the growth characteristics of plants over their uninoculated counterparts. The improvement resulting from VAM inoculation depended on fertility levels of soil. At O level of NPK performance of mycorrhizal plants was better than non-mycorrhizal plants, but, at I level of NPK it was still better than that of 'O' level. From the results it can be inferred that growth stimulation of seedlings due to VAM can be improved by addition of NPK at suitably low levels. However, at higher levels of NPK both inoculated and uninoculated plants behaved alike and no significant differences in various growth characteristics were observed between them at these levels.

(B) Plant yield : Average dry weight of VAM inoculated plants was higher than their noninoculated counterparts at all levels of soil fertility (Table 3.2). However, differences were significant ($p < .05$) at O, I and II levels of NPK.

Table 3.1 Seedling survivability and percent VA mycorrhizal infection of seedlings of *Exbucklandia populnea* grown at different fertility levels of soil.

Reps.

Soil fertility level (N.P.K. kg ha^{-1})		Surviving seedling (%)	VAM infection (%)
0	-M	66.3±0.40*	50±12
	+M	79.4±2.41	
14	-M	82.4±2.88*	60±15
	+M	90.2±3.20	
28	-M	72.5±7.00	20±07
	+M	74.5±1.96	
56	-M	54.9±7.00	0
	+M	54.9±1.96	
112	-M	Seedlings died	n.d.
	+M	Seedlings died	

-M = Non-mycorrhizal; +M = Mycorrhizal

n.d.= not done due to mortality of seedlings

* = Significant at 5% level.

Table 3.2 Root dry weight, Shoot dry weight, total biomass and root/shoot ratio of the mycorrhizal and non-mycorrhizal plants grown at different levels of soil fertility.

Level of soil fertility (N-P-K.Kg ha^{-1})		Shoot dry weight (g)	Root dry weight (g)	Total Biomass (g)	R/S ratio
0	-M	.043±.015*	.012±.002	.055±.02*	.28±.02*
	+M	.084±.021	.017±.003	.101±.026	.20±.02
14	-M	.054±.020*	.017±.003*	.072±.030*	.31±.03*
	+M	.189±.035	.034±.008	.223±.050	.18±.02
28	-M	.055±.030*	.015±.002*	.071±.027	.27±.04
	+M	.093±.042	.028±.004	.121±.030	.30±.03
56	-M	.048±.020	.018±.003	.066±.015	.38±.05
	+M	.064±.027	.023±.006	.087±.024	.36±.03

-M = Non-mycorrhizal; +M = Mycorrhizal

*Significant at 5% level.

Average stem dry weight was increased by 100%, 272% and 101% by VAM inoculation and NPK addition at O, I and II levels. When the shoot dry weight of non-inoculated and inoculated plants was compared it was observed that average shoot dry weight of inoculated plants was significantly ($p < .05$) more than stem dry weight of uninoculated plants at 'O', I and II levels of NPK, However, at the III level both had comparable dry weights (Table 3.2).

VAM inoculated plants had more root dry weight than non-inoculated plants. However, significant ($p < .05$) differences were observed at I and II levels of added NPK (Table 3.2).

Root/Shoot ratio : R/S ratio of mycorrhizal plants was significantly lower ($p < .05$) than that of non-mycorrhizal plants at 'O' and I levels of NPK (Table 3.2).

(C) Percent Mycorrhizal Infection : Percent VA mycorrhizal infection of root was low (50-60%) being highest 60% at Ist level of NPK and decreased subsequently at higher levels. At the IVth level VA infection was not observed in the roots of VAM inoculated seedlings (Table 3.1).

II. EFFECT OF INFECTION AS INFLUENCED BY PHOSPHATE FROM DIFFERENT SOURCES :

(A) Growth responses : It was observed that when no phosphates were added to the soil, VA mycorrhizal seedling exhibited higher growth characteristics than non-inoculated ones and when bone meal or sodium hydrogen phosphate were mixed with the potted soil the growth of plants improved invariably for the inoculated plants. The results of various growth responses are as follows.

(i) Length of seedling : VAM inoculated seedlings were significantly ($p < .05$) higher than non-inoculated ones at 0, 0.34, .69 and 1.72g of applied bone meal per pot (Fig. 3.3). However, the relative difference between the two was maximum at 0.34g of bone meal. Whereas, at higher concentration of bone meal the difference between the VAM inoculated and non-inoculated seedlings showed a gradual increase with increase in rate of bone meal application, whereas, no such trend was observed with VAM inoculated plants. The length of mycorrhizal seedlings at low and higher levels of bone meal was Similar ~~comparable:~~ Also when sodium hydrogen phosphate was added to the soil, VAM inoculated seedlings were significantly ($p < .05$) higher than non-inoculated ones at 0.34 and 0.69g per pot of added phosphate (Fig.3.3). The length of seedling was maximum at 0.69g addition of NaH_2Po_4 , whereas, it declined at 3.47g of added sodium hydrogen phosphate. All seedlings both inoculated and uninoculated died at 17.33g per pot addition of sodium hydrogen phosphate. This was perhaps due to supra optimal conc. of the salt.

(ii) Stem collar diameter : Stem collar diameter of VAM inoculated plants was significantly ($p < .05$) more than those of non-inoculated ones at low levels viz. 0.34, 0.69

Fig.3.3 Length of mycorrhizal and non-mycorrhizal plants grown in a range of additions of bone meal and sodium hydrogen phosphate in the soil.

Fig. 3·3

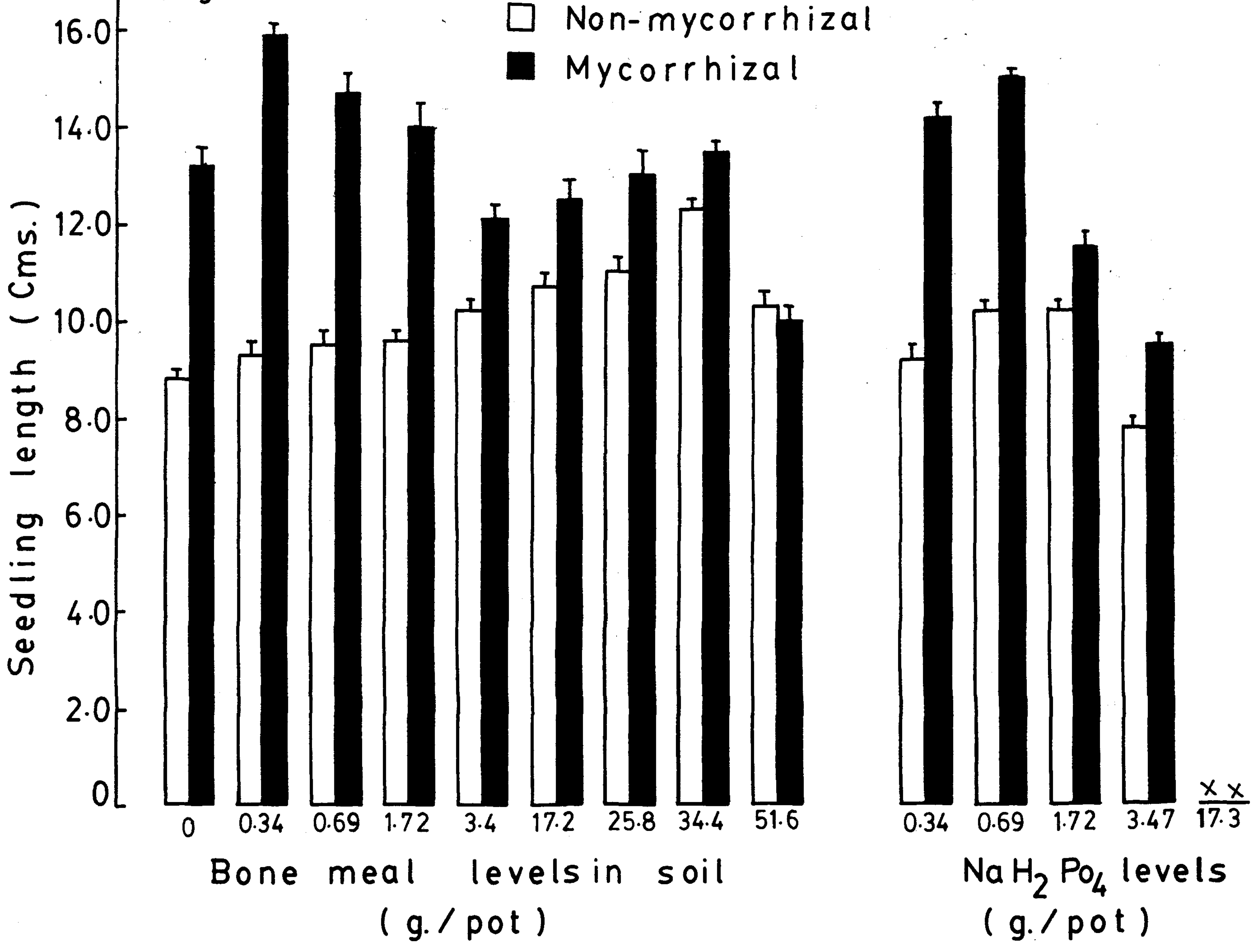
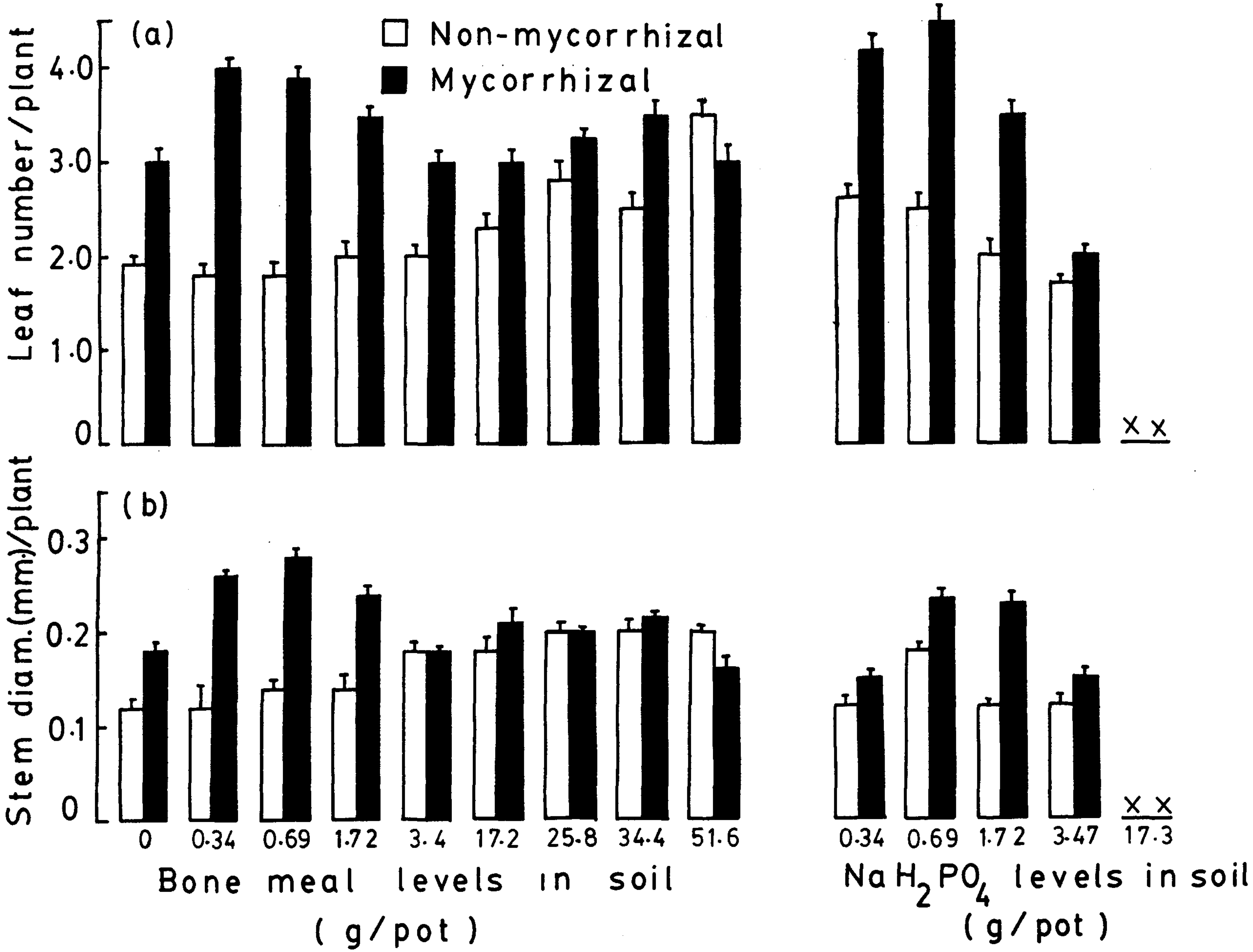

☐ Non-mycorrhizal
■ Mycorrhizal
Seedling length (Cms.)
16·0
14·0
12·0
10·0
8·0
6·0
4·0
2·0
0
0
0·34
0·69
1·72
3·4
17·2
25·8
34·4
51·6
Bone meal levels in soil
(g. / pot)
0·34
0·69
1·72
3·47
x x
17·3
Na H_2 Po_4 levels
(g. / pot)

Fig.3.4 The differences in the leaf number (a) and stem collar diameter of mycorrhizal and non-mycorrhizal plants grown in a range of additions of bone meal and sodium hydrogen phosphate in the soil.

Fig. 3.4


(a)
Non-mycorrhizal
Mycorrhizal
Leaf number/plant
4.0
3.0
2.0
1.0
0
x x
(b)
Stem diam.(mm)/plant
0.3
0.2
0.1
0
0
0.34
0.69
1.72
3.4
17.2
25.8
34.4
51.6
Bone meal levels in soil (g/pot)
0.34
0.69
1.72
3.47
x x
17.3
NaH2PO4 levels in soil (g/pot)

and 1.72g of bone meal. At these concentrations VAM inoculated plants were twice the thick~~er~~ness ~~than~~ of (or twice the diameter) the non-inoculated ones. When sodium hydrogen phosphate was used the significant difference ($p < .05$) was seen between mycorrhizal and non-mycorrhizal plants at 1.72g phosphate level (Fig.3.4).

(iii) Leaf number : VAM inoculated plants had more leaves than non-inoculated ones. However, the relative differences between the two were significant upto 3.4g addition of bone meal. With addition of sodium hydrogen phosphate the differences in the leaf number between VAM inoculated and non-inoculated seedlings were significant ($p < .05$) upto 1.72g phosphate addition (Fig.3.4).

(B) Plant Biomass : It was observed that average dry weight of VAM inoculated plants was significantly ($p < .05$) more than that of uninoculated plants at 0.34, 0.69, 1.72, 3.44g bone meal per pot (Table 3.3). The mycorrhizal plants showed the dry weight increase upto 0.69g bone meal addition. When sodium phosphate was added the dry weight of VAM inoculated plants was significantly ($p < .05$), more than non-VAM plants at 0.34 and 0.69g application of NaH_2PO_4 and at other levels there were no significant differences between them.

Shoot dry weight : There was significant difference ($p < .05$) in shoot dry weight between inoculated and uninoculated plants at 0.34, 0.69, 1.72 and 3.44g of addition of bone meal. With sodium hydrogen phosphate the differences between the two were significant only at 0.34 and 0.69g applications

Table 3.3 **Root dry weight, Shoot dry weight, total biomass and root/shoot ratio of the mycorrhizal and non-mycorrhizal plants grown with two phosphate sources at different levels.**

Source and level of phosphate (g/pot)		Shoot dry weight (g)	Root dry weight (g)	Total Biomass (g)	R/S ratio
0	-M	.043±.009	.011±.004	.054±.013	.26±.024
	+M	.084±.010	.017±.006	.110±.016	.20±.019
BONE MEAL					
0.34	-M	.041±.007*	.014±.003*	.055±.010*	.34±.020*
	+M	.184±.015	.042±.009	.226±.024	.22±.019
0.69	-M	.047±.005*	.013±.003*	.060±.083*	.28±.050*
	+M	.218±.010	.040±.011	.258±.020	.18±.030
1.72	-M	.042±.007*	.015±.007*	.057±.014*	.36±.020*
	+M	.157±.012	.047±.009	.203±.020	.29±.030
3.44	-M	.049±.009*	.020±.003	.069±.012*	.41±.060*
	+M	.175±.013	.035±.009	.210±.022	.20±.030
17.2	-M	.098±.009	.020±.002	.118±.010	.20±.060
	+M	.161±.012	.029±.008	.190±.020	.18±.050
25.8	-M	.130±.012	.026±.007	.156±.019	.20±.050
	+M	.184±.020	.032±.010	.216±.030	.17±.070
34.4	-M	.129±.030	.026±.007	.155±.025	.20±.020
	+M	.175±.021	.035±.006	.210±.027	.20±.040
51.6	-M	.121±0015	.031±.007	.152±.020	.25±.030
	+M	.107±.013	.028±.004	.135±.015	.26±.050
SODIUM HYDROGEN PHOSPHATE					
0.34	-M	.043±.007	.014±.003*	.057±.010*	.33±.020*
	+M	.148±.012	.036±.010	.184±.020	.24±.030
0.69	-M	.070±.009*	.027±.005	.097±.014*	.39±.040*
	+M	.172±.013	.038±.008	.200±.020	.22±.050
1.72	-M	.051±.011	.019±.007	.070±.020	.37±.017
	+M	.095±.008	.030±.012	.125±.020	.32±.021
3.44	-M	.053±.007	.017±.007	.060±.014	.32±.013
	+M	.065±.008	.025±.009	.090±.017	.38±.020
17.33	-M	n.d.	n.d.	n.d.	n.d.
	+M	n.d.	n.d.	n.d.	n.d.

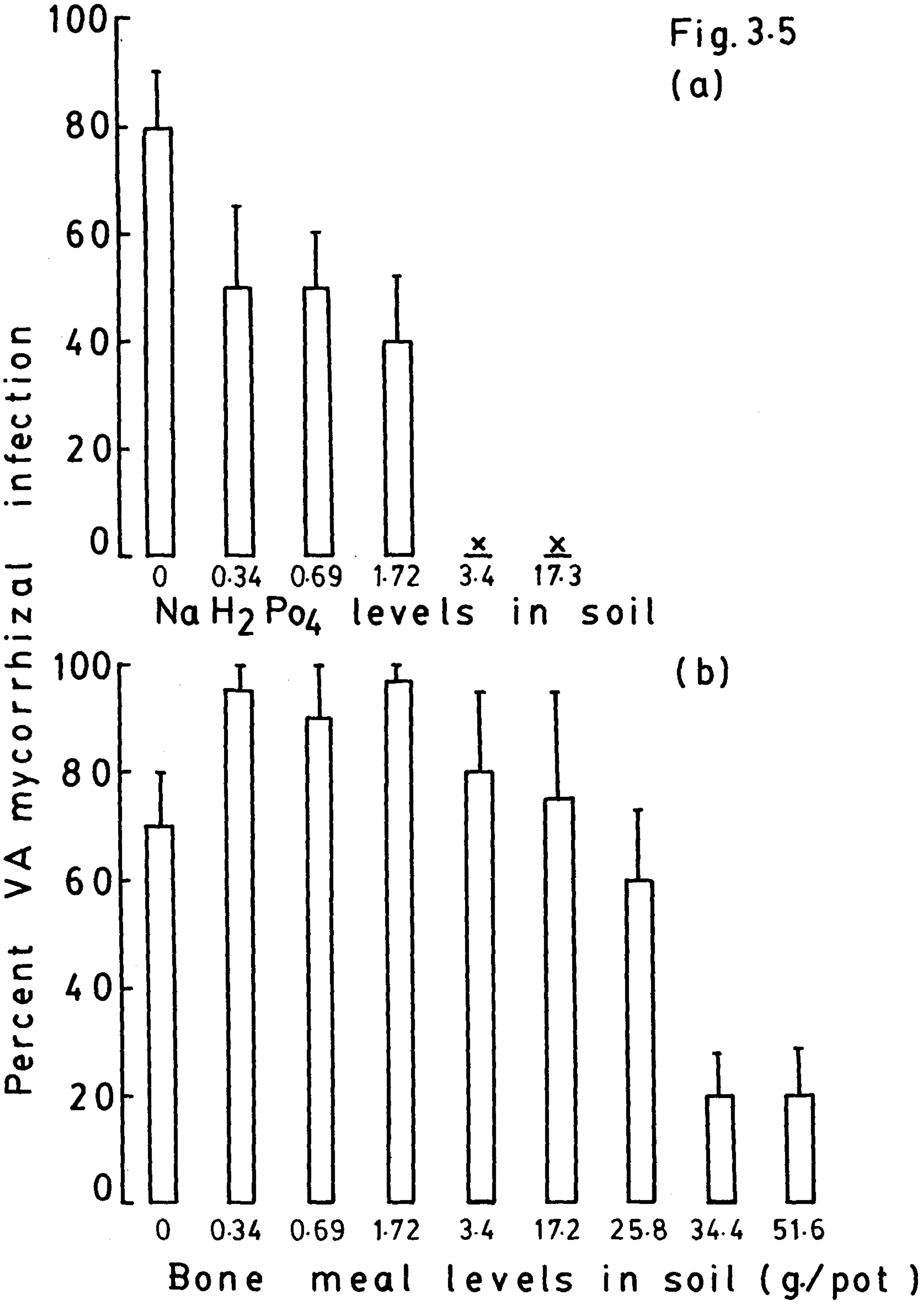
-M = Non-mycorrhizal; +M = mycorrhizal

n.d. = not done due to mortality of seedlings

* = Significant at 5% level.

Fig.3.5 Percent infection of VA-mycorrhizal plants grown in a range of additions of bone meal and sodium hydrogen phosphate in the soil.

Fig. 3·5


(a)
Percent VA mycorrhizal infection
100
80
60
40
20
0
0
0·34
0·69
1·72
x
3·4
x
17·3
NaH₂Po₄ levels in soil
(b)
100
80
60
40
20
0
0
0·34
0·69
1·72
3·4
17·2
25·8
34·4
51·6
Bone meal levels in soil (g./pot)

(Table 3.3).

(iii) Root dry weight : Root dry weight of VAM plants was greater than that of non-VAM plants. Dry weight increase was significant ($p < .05$) at 0.34, 0.69, 1.72g of bone meal applications, whereas, with sodium hydrogen phosphate the dry weight increase was significant ($p < .05$) at 0.35g only (Table 3.3).

(iv) Root/shoot ratio : Root/shoot ratio of inoculated plants was less than non-inoculated plants. Significant difference ($p < .05$) was observed at 0.34, 0.69, 1.72 and 3.44g of bone meal applications whereas, at higher levels the R/S ratio of two sets of plants was comparable. The ratio was also significantly ($p < .05$) low for VAM plants at 0.35g and 0.69g application of sodium hydrogen phosphate.

(C) Mycorrhizal Infection : 70% VAM infection was observed at 'O' level of added phosphate. Whereas, infection increased to 70-100% at bone meal application at the levels of 0.34, 0.69, 1.72, 3.4 and 17.2g per pot and at higher rates of bone meal application percent VAM infection was reduced to 20% at 34.4 and 51.6g bone meal per pot. Whereas, with sodium hydrogen phosphate VAM infection was 40-50% and was almost absent at 3.47g of sodium hydrogen phosphate application (Fig.3.5).

DISCUSSION

Seedlings of Exbucklandia populnea responded to inoculation with VA endophytes. At 0 and I levels of NPK marked differences in various growth characteristics were observed, whereas, at higher fertility levels, viz. II and III levels of NPK, there were no marked differences between inoculated and uninoculated seedlings. *Exbucklandia populnea* responded to growth enhancement by mycorrhiza like other hardwood tree species (Clark, 1963; Bryan and Ruehle, 1976; Riffle, 1980; Schultz et.al., 1981; Furlan et.al., 1983). Both fertility levels, viz., 0 and I represented comparatively poor nutrient conditions of soil. Banister and Norton (1974) also found that with low nutrients mycorrhizal plants grew better than non-mycorrhizal plants. However, with higher nutrients growth was decreased. Csinos (1981) found that VAM inoculation of tobacco increased growth response at low level of soil fertility. Mycorrhizal seedlings of *Platanus occidentalis* grew better in comparison to non-mycorrhizal ones when fertilised with Hoagland solution at intermediate levels of nutrient regimes in the solution (Pope, 1980). At higher levels the growth diferences between the two were reduced. Higher status of nutrients had depressing effect on mycorrhiza formation (Baylis, 1967; Mosse, 1973; Menge *et.al.*, 1978) and therefore, both inoculated and uninoculated seedlings behaved alike. Kormanik *et.al.* (1977) had shown that endomycorrhizal seedlings of sweetgum when given additional supply of fertilizers grew better than endomycorrhizal seedlings grown without fertilizers.

This is here should too. explanation

The results have practical implications that endomycorrhizae alongwith controlled low supply of fertilizers can be employed for the production ~~or equality~~ of quality seedlings of *Exbucklandia populnea* in nurseries.

The survivability of VA mycorrhiza inoculated seedlings was higher at first and second levels and decreased considerably at third level of NPK. The mortality of seedlings at fourth level could be perhaps due to supra optimal concentration of salts. Bryan and Kormanik (1977) had also shown improvement in the survivability of sweetgum seedlings when inoculated with VA endomycorrhiza.

? Chap 4 ?

The results showed that mycorrhizal seedlings had greater dry weight production as compared to non-mycorrhizal seedlings of *Exbucklandia populnea*. With sweetgum, Schultz *et.al.* (1981) had shown dry weight increase of seedlings when inoculated with VA endomycorrhiza. Mycorrhizal induced growth resulted in greater shoot weight increase and lesser root weight increase. Hardie and Leyton (1981) obtained similar results with clover.

In the second experiment additional phosphates were added to the phosphorus deficient pot soil in the form of bone meal or sodium hydrogen phosphate. The results showed that VAM inoculated seedlings supplied with bone meal grew better than non-mycorrhizal ones at all levels of bone meal. Daft and Nicolson (1966) also observed better growth of tomato plants when supplied with bone meal and inoculated with VA endophyte.

With other forms of unavailable phosphorus viz. rock phosphates, Vaidyanath et.al.(1979) found growth stimulation by VA endomycorrhizae. Mosse (1977) had shown better growth of VA mycorrhizal maize plant with addition of rock phosphate.

When sodium hydrogen phosphate was added, mycorrhizal seedlings grew better than non-mycorrhizal seedlings at lower levels of applied phosphate except 17.33g sodium hydrogen phosphate per pot. At this concentration both non-mycorrhizal and mycorrhizal seedlings died, perhaps due to supra optimal concentration of salts. The mycorrhiza induced growth response was maximum at lower concentration of phosphate. Several workers have observed growth stimulation of plants by addition of phosphates at low concentrations to the phosphorus deficient soils (Baylis, 1967; Howler et.al., 1982). Abbott and Robson (1977b) found marked increase in growth of VAM inoculated clover plants at intermediate levels of superphosphate additions to soil. Owusu-Bennoah and Mosse (1979) also concluded that lucerne and onion benefitted from VA inoculation with most available phosphorus.

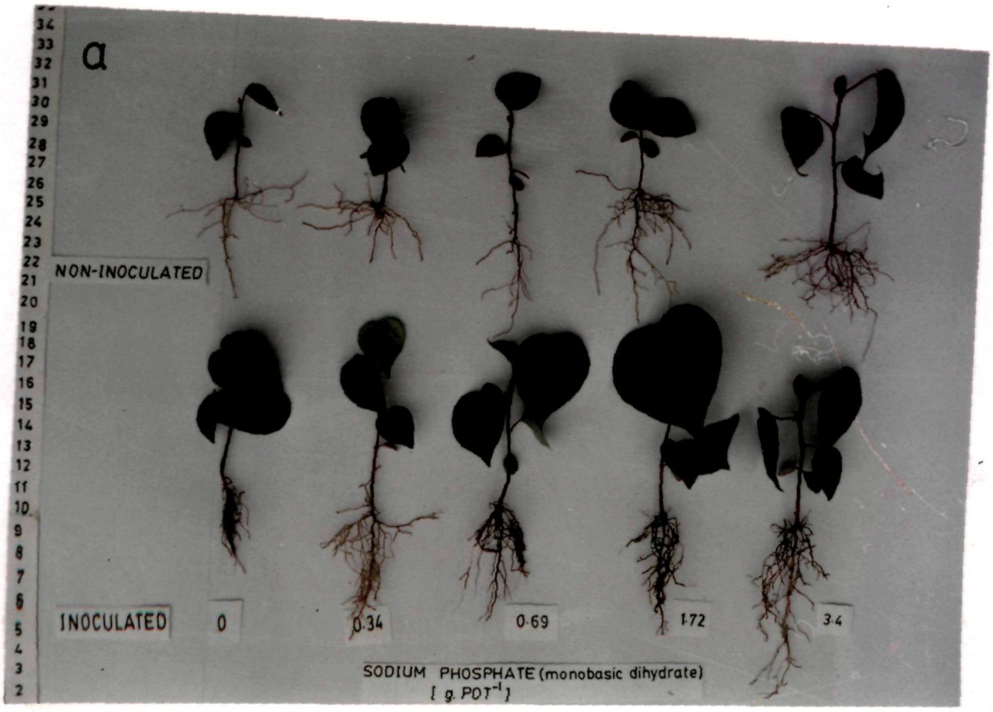
It was observed that mycorrhizal infection of seedlings decreased with increase in the presence of available phosphates in the soil. Daft and Nicolson (1969), Ratnaike et.al., (1978) and Menge et.al.(1978) had shown decrease in root infections by VA endophyte when soluble phosphates were added to the soil.

R/S ratio of inoculated plants was less than that of inoculated plants. Such results were obtained due to rapid shoot growth in comparison to root growth. Several workers have observed decrease in R/S ratio due to mycorrhizal inoculation (Hardie and Leyton, 1981; Hayman and Mosse, 1971; Crush, 1974).

Plate 3.1

Effect of different levels of (a) sodium hydrogen phosphate (b) NPK on the growth of seedlings of *Exbucklandia populnea*.

PLATE - 3.1


a
NON-INOCULATED
INOCULATED
0
0.34
0.69
1.72
3.4
SODIUM PHOSPHATE (monobasic dihydrate)
[g. POT^{-1}]

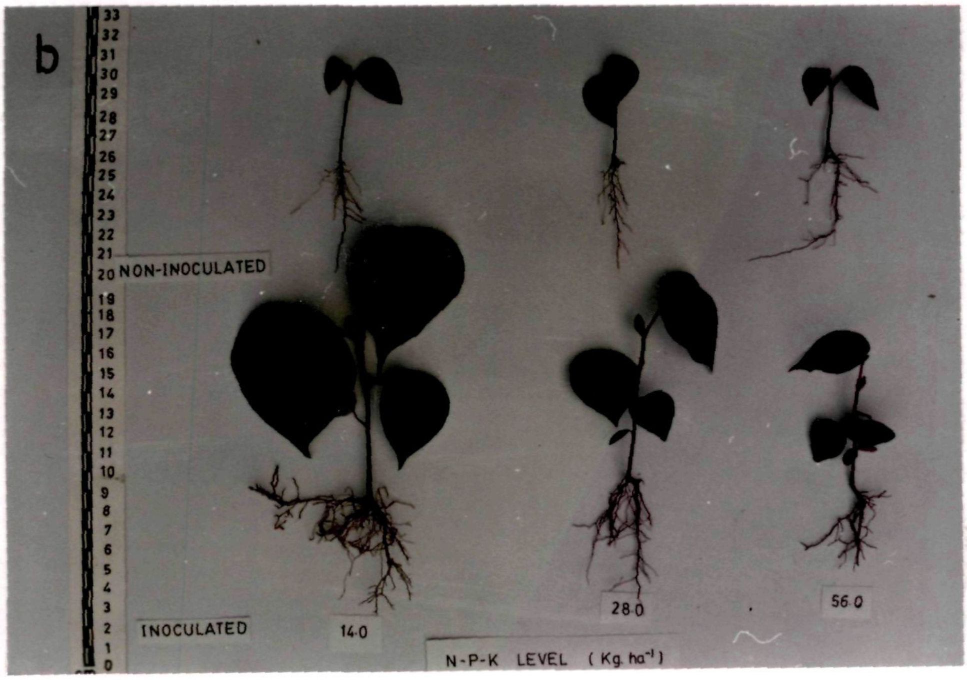
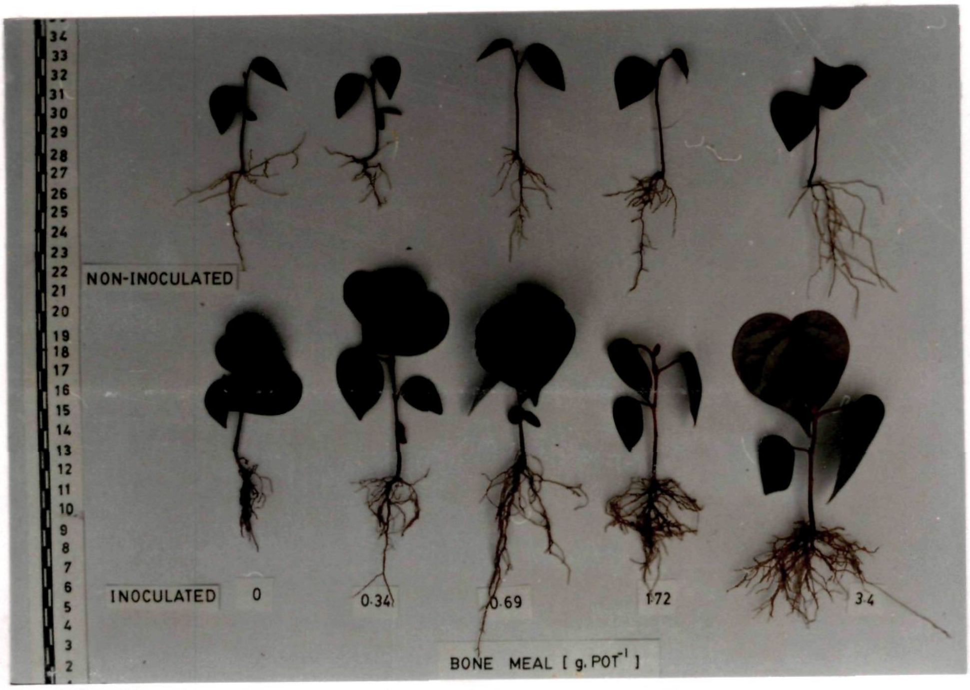

b
NON-INOCULATED
INOCULATED
14.0
28.0
56.0
N-P-K LEVEL (Kg. ha^{-1})

Plate 3.2

Effect of different levels of bone meal on the growth of seedlings of *Exbucklandia populnea*.

PLATE -3.2


NON-INOCULATED
INOCULATED
0
0·34
0·69
1·72
3·4
BONE MEAL [g. POT^{-1}]


NON-INOCULATED
INOCULATED
0
17·2
25·8
34·4
51·6
BONE MEAL [g. POT^{-1}]

CHAPTER - IV

EFFECT OF VA-MYCORRHIZA ON NUTRIENT UPTAKE

INTRODUCTION

Vesicular-arbuscular mycorrhiza enhances growth of plants growing in soils of moderate fertility (Gerdemann 1968; Mosse, 1973a). The growth enhancement is the direct result of increased nutrient uptake helped by VA endophyte (Rhodes and Gerdemann, 1980). Mycorrhizal plants absorb more phosphorus from phosphorus deficient soil than non-mycorrhizal plants as the concentration of phosphorus in the former is more than in the latter (Abbott and Robson, 1977b; Baylis, 1967; Daft and Nicolson, 1969a; Powell and Daniel, 1978). Other nutrients like nitrogen, potassium calcium, zinc, copper and magnesium have also been reported to be higher in concentration in mycorrhizal plants but the results are inconsistent. Menge et.al.(1978) found lower concentration of K, Ca and Mg in the leaves of mycorrhizal seedlings of *Citrus*. Mosse (1973) reported that concentration of potassium in the mycorrhizal plants was higher than in non-mycorrhizal plants grown in phosphorus deficient soils.

Phosphorus nutrition of endomycorrhizal plants have also been studied in presence of soluble phosphorus (Smith, 1982; Barrow, et.al.,1977; Howeler et.al.,1982) and insoluble phosphorus (Vaidyanath et.al.,1979; Powell and Daniel, 1978; Mosse et.al.,1976). Phosphorus uptake is also improved by the addition of insoluble sources of phosphorus.

The present study was undertaken to evaluate the role of VA mycorrhiza in nutrition of seedlings of Exbucklandia populnea from soils of different fertility levels and effect of mycorrhiza in phosphate uptake from different sources of phosphorus.

MATERIALS AND METHODS

Seeds of Exbucklandia populnea were grown in plastic pots. The formation of VA mycorrhiza and its effect on uptake of nutrients was observed. Two separate experiments were carried out. In the first experiment the effect of vesicular-arbuscular mycorrhiza formation on uptake of N,P,K and Ca of seedlings of Exbucklandia populnea was studied at five different levels of soil fertility. These levels were 0, 14, 28, 56 and 112 kg ha^{-1} of NPK fertilizers. For each level of soil fertility a set each of mycorrhizal and non-mycorrhizal seedlings was kept separately.

In the second experiment the effect of addition of phosphorus on the formation of vesicular-arbuscular mycorrhiza and their effect on phosphorus uptake of seedling was studied. Two sources of phosphorus were used viz. bone meal and sodium hydrogen phosphate. Eight different levels of bone meal and five levels of sodium hydrogen phosphate as stated in chapter III were used. Methods of preparation of pots, application of nutrients, inoculation of endophyte, seedling plantation and growth conditions were the same as described in chapter III. After six months of growth the

the seedlings were harvested, oven dried at 65°C and analysed for various nutrients.

Plant material analysis : Root, stem and leaves were powdered separately in small grinder and sieved through 0.2mm sieve From powdered material the total nitrogen was estimated by semi-microjeldahl method. Potassium, phosphorus and calcium were extracted by wet digestion method as suggested by Allen (1974). Phosphorus was estimated by molybdenum-blue method. Potassium was estimated by flame photometer and calcium was estimated by EDTA titration method. The methods suggested by Allen (1974) were followed. The details of these methods are given in Chapter I except that plant digest was used instead of soil extract.

RESULTS

I. Effect of infection as influenced by soil fertility level:

The concentration of phosphorus, nitrogen, potassium and calcium was determined in the leaves, roots and stems of mycorrhizal and non-mycorrhizal seedlings of *Exbucklandia populnea.*

Phosphorus : The results of phosphorus concentration in the leaves, stem and root are presented in fig.4.1. It was observed that the concentration of phosphorus in the leaves and stem of mycorrhizal seedlings was significantly ($p< 0.05$) higher than those of non-mycorrhizal seedlings at 0 and I level of NPK, whereas, in the case of root the difference between them was found to be significant ($p < 0.05$) only at 0 level of NPK. However, at higher levels of soil fertility, viz; III and IVth levels of NPK, no significant difference in the concentration of phosphorus between mycorrhizal and non-mycorrhizal seedlings was observed. In the case of VAM inoculated seedlings, the leaves had the maximum phosphorus concentration at I level, whereas, stem and root had at II level. On the other hand, in non-mycorrhizal seedlings the maximum concentration in the leaves and stem was found at II level and in roots at III level of NPK.

Nitrogen : The concentration of nitrogen in the leaves was found to be higher in comparison to that of stem and root. Nitrogen concentration increased with increase in soil fertility level. Nitrogen concentration in the leaf ? stem of mycorrhizal seedlings was significantly higher ($p < .05$) than that of non-mycorrhizal seedlings at I and II levels of NPK and in the case of root at 0 level of NPK (Fig.4.2).

Potassium : The concentration of potassium in the leaves and stem of non-mycorrhizal seedlings was higher in comparison to mycorrhizal seedlings. Significant differences ($p < .05$) were found at IV level of NPK for the leaves and at 0 and I level of NPK for the stem (Fig.4.3).

Calcium : The concentration of calcium in the leaves of mycorrhizal seedling was significantly ($p < .05$) less than that of non-mycorrhizal ones at IV level of NPK. Whereas,

Fig.4.1 The allocation of phosphate in the leaf, stem and root of mycorrhizal and non-mycorrhizal plants grown at different fertility levels of soil.

[illegible]
Variation
Sample

Fig. 4.1

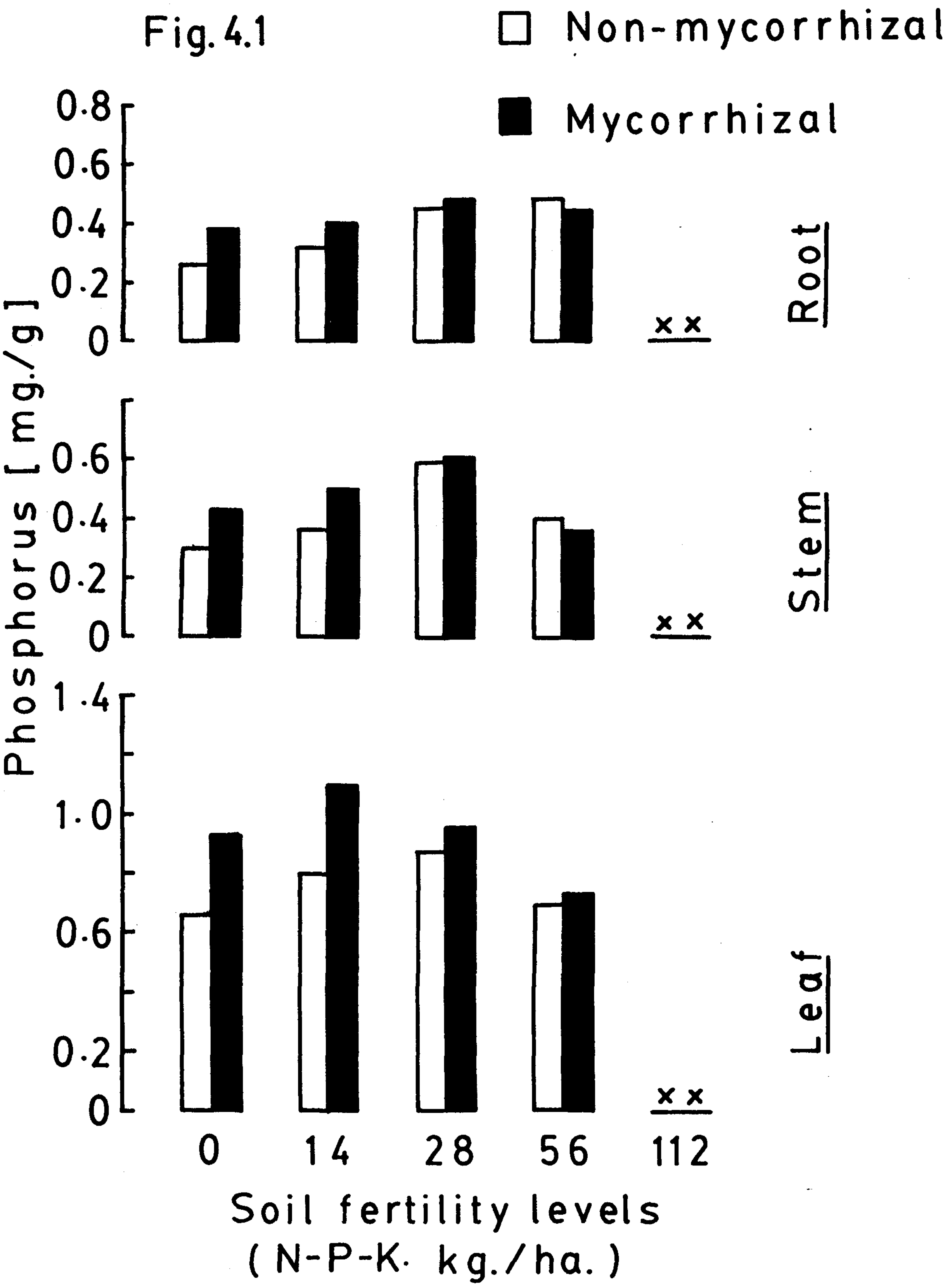

□ Non-mycorrhizal
■ Mycorrhizal
Phosphorus [mg./g]
0.8
0.6
0.4
0.2
0
Root
x x
0.6
0.4
0.2
0
Stem
x x
1.4
1.0
0.6
0.2
0
Leaf
x x
0
14
28
56
112
Soil fertility levels
(N-P-K· kg./ha.)

Fig.4.2 The allocation of nitrogen in the leaf, stem and root of the mycorrhizal and non-mycorrhizal plants grown at different fertility levels of soil.

Fig. 4.2

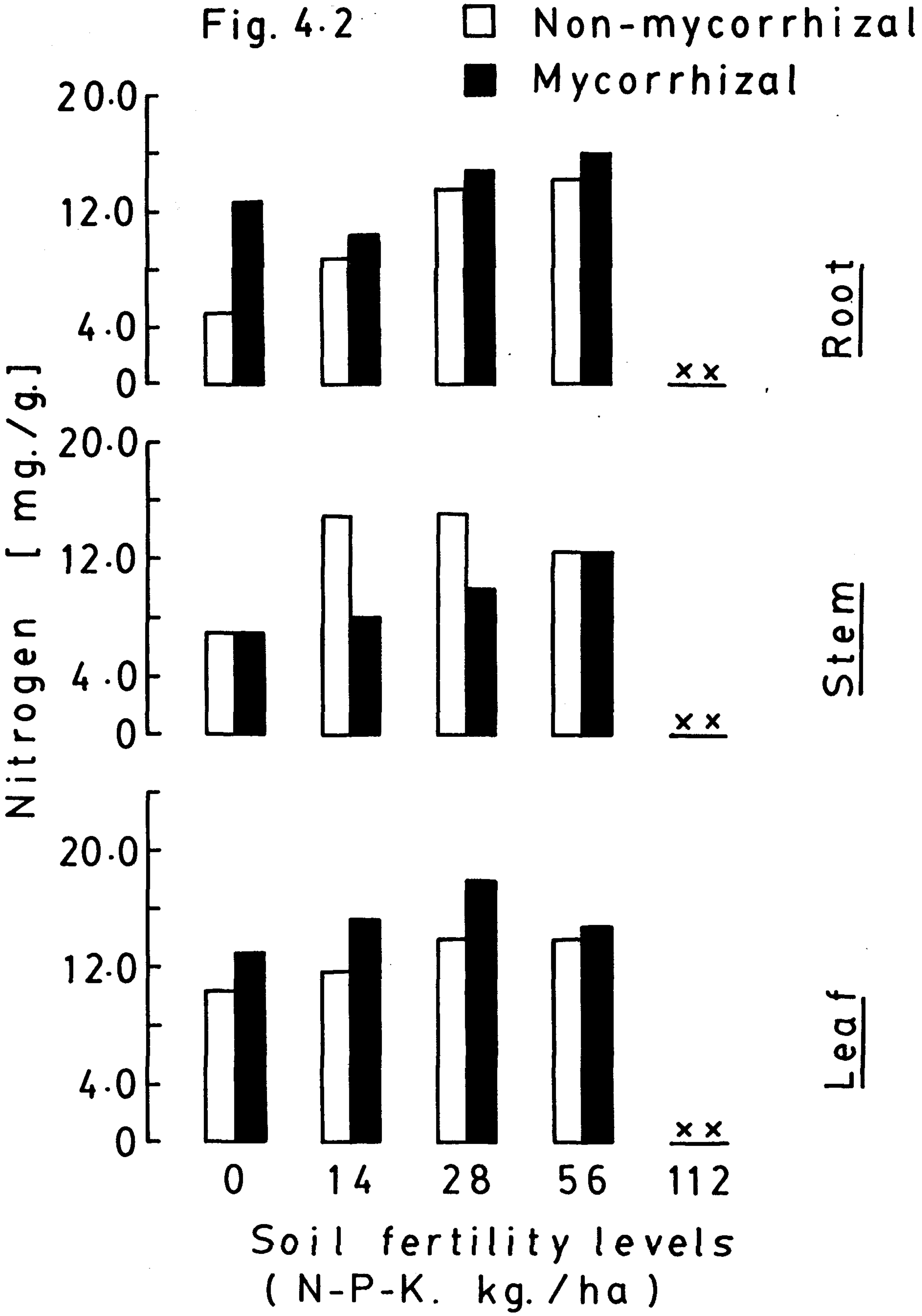

□ Non-mycorrhizal
■ Mycorrhizal
Nitrogen [mg./g.]
20.0
12.0
4.0
0
Root
Stem
Leaf
xx
0
14
28
56
112
Soil fertility levels
(N-P-K. kg./ha)

Fig.4.3 The allocation of potassium in the leaf, stem and root of the mycorrhizal and non-mycorrhizal plants grown at different fertility levels of soil.

Fig. 4·3

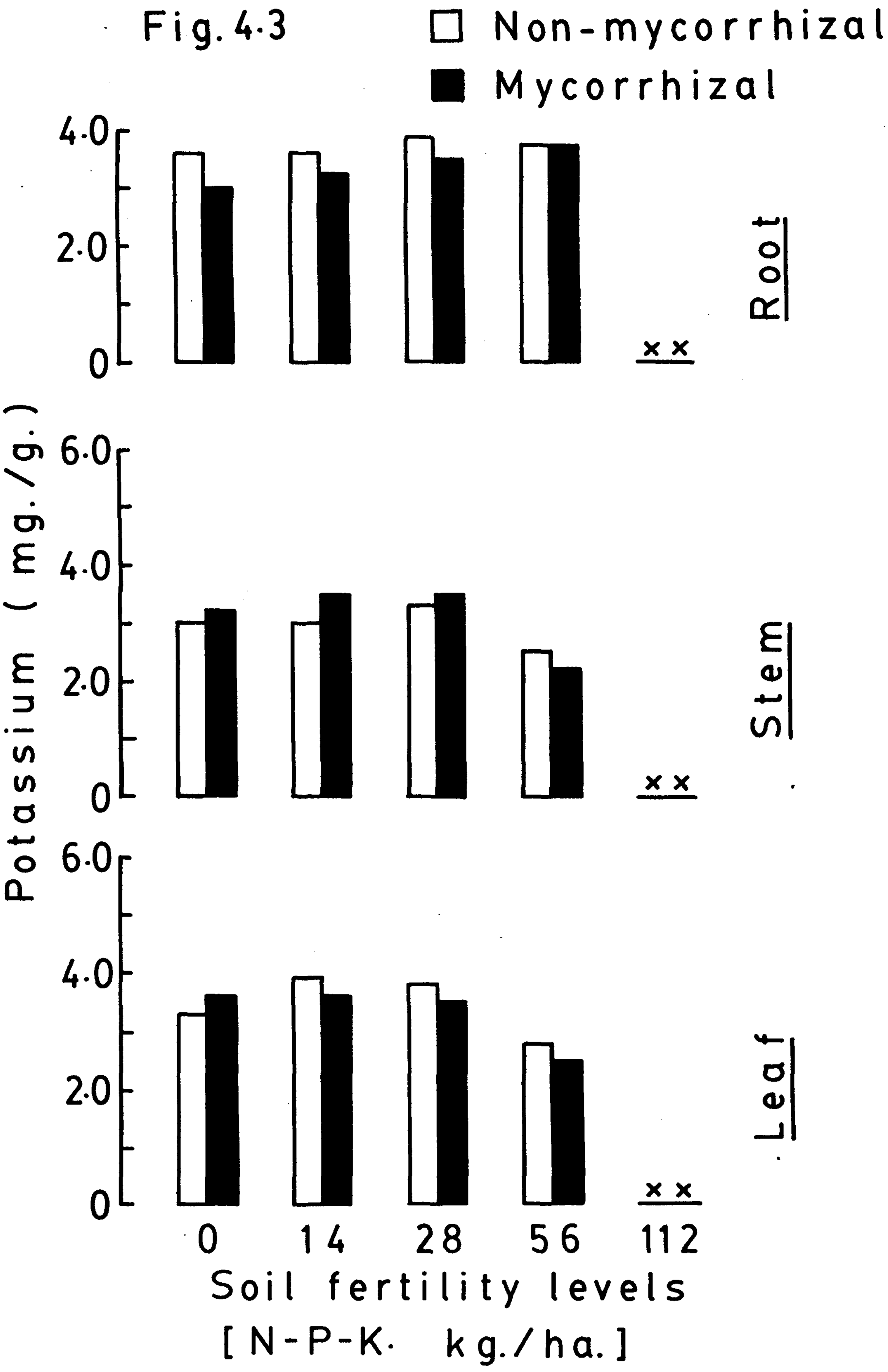
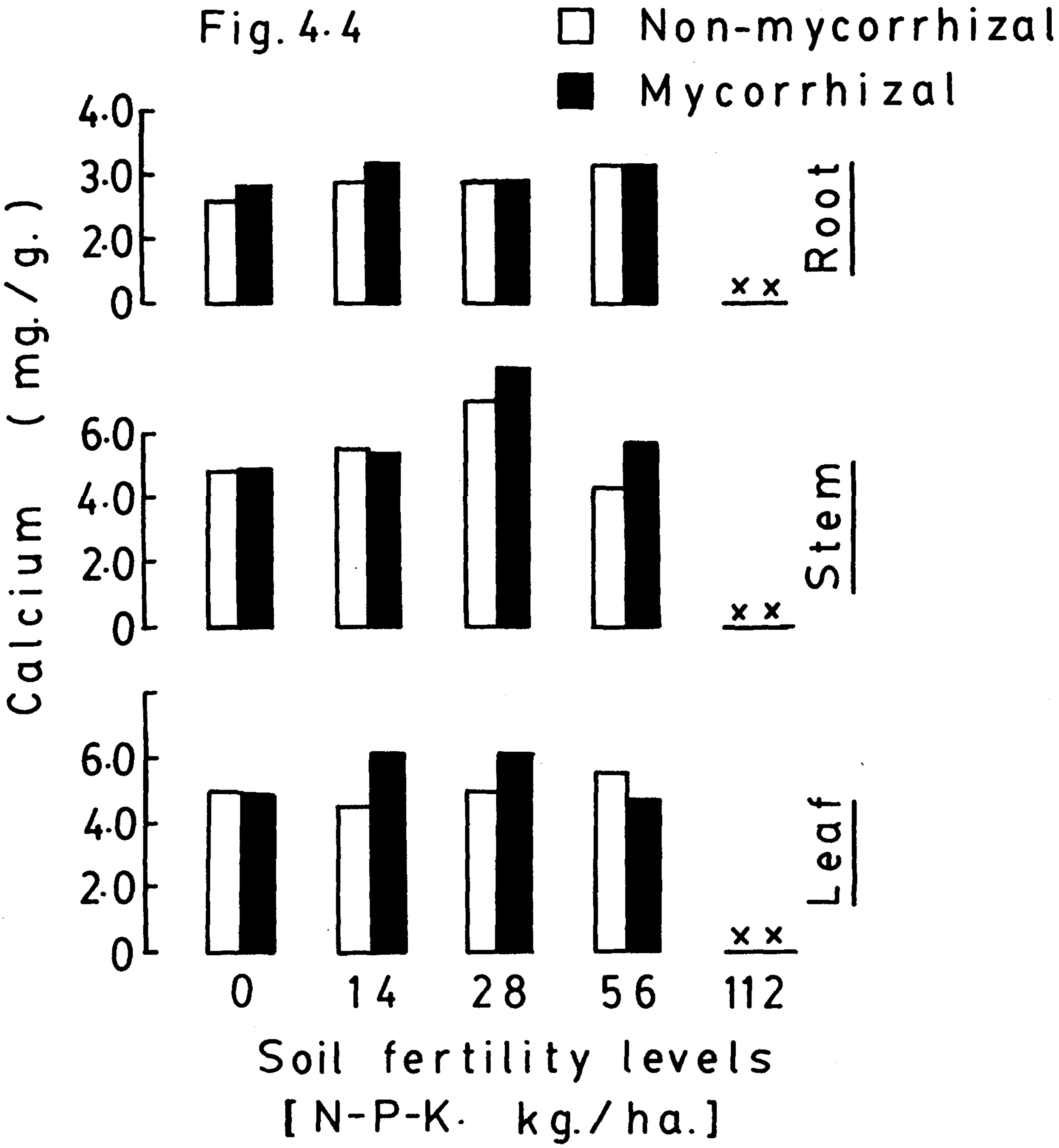

☐ Non-mycorrhizal
■ Mycorrhizal
Potassium (mg. /g.)
4·0
2·0
0
Root
× ×
6·0
4·0
2·0
0
Stem
× ×
6·0
4·0
2·0
0
Leaf
× ×
0
14
28
56
112
Soil fertility levels
[N-P-K· kg./ha.]

Fig.4.4 The allocation of calcium in the leaf, stem and root of the mycorrhizal and non-mycorrhizal plants grown at different fertility levels of soil.

Fig. 4·4



☐ Non-mycorrhizal
■ Mycorrhizal
Calcium (mg./g.)
4·0
3·0
2·0
0
Root
x x
6·0
4·0
2·0
0
Stem
x x
6·0
4·0
2·0
0
Leaf
x x
0
14
28
56
112
Soil fertility levels
[N-P-K· kg./ha.]

no significant differences were observed in the concentration of calcium in the root and stem of mycorrhizal and non-mycorrhizal seedlings (Fig.4.4).

II. Effect of Infection as influenced by bone meal as phosphate source :

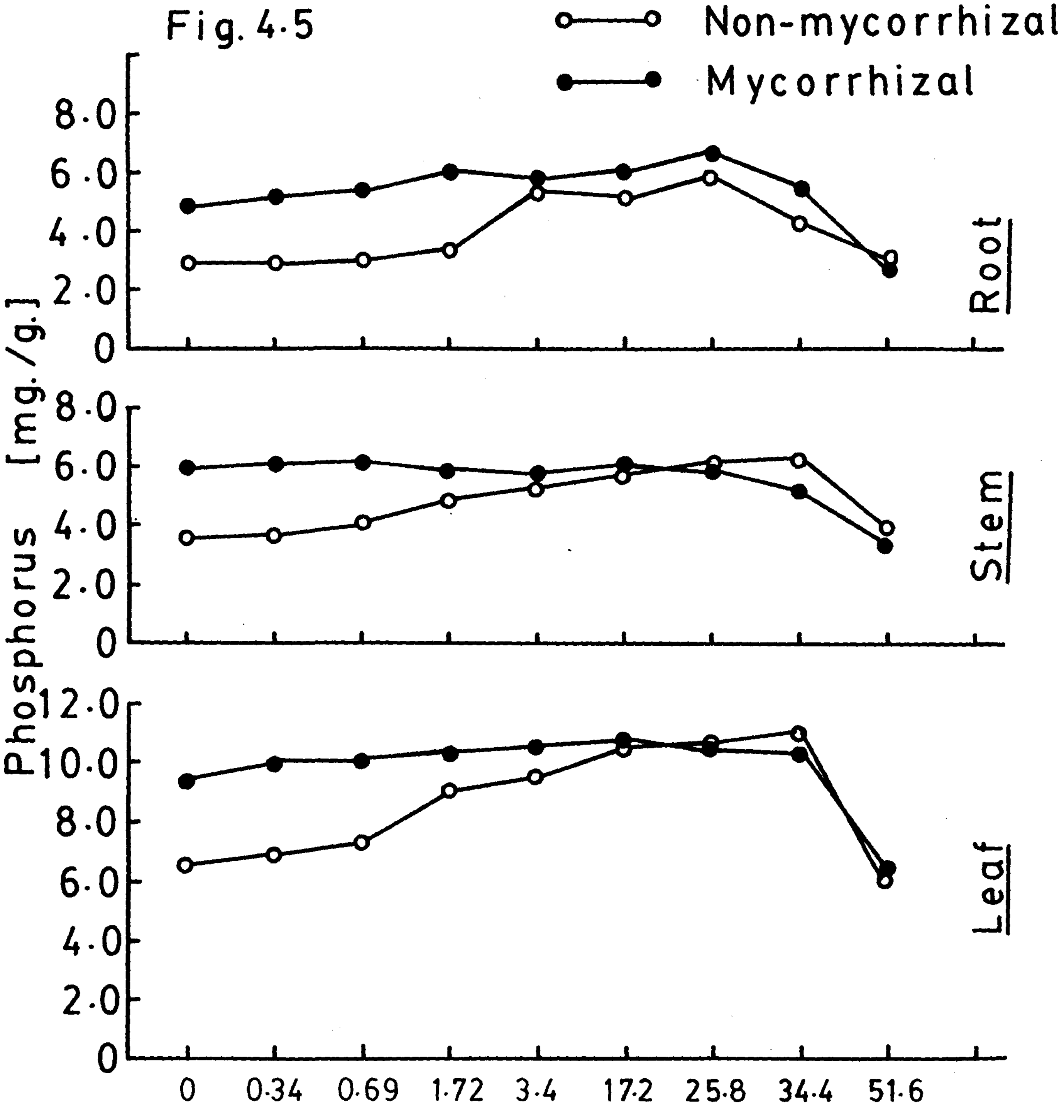
It was observed that the concentration of phosphorus in the leaves was higher in comparison to that of stem and roots in both the mycorrhizal and the non-mycorrhizal seedlings. In general it was observed that concentration of phosphorus in the leaves, stem and roots of mycorrhizal seedlings was higher than that of non-mycorrhizal plants at lower and intermediate levels of bone meal. Whereas, at higher levels of bone meal, concentration of phosphorus did not differ between mycorrhizal and non-mycorrhizal seedlings and at 51.0g bone meal level, both the mycorrhizal and non-mycorrhizal plants had low phosphorus concentration. The results are presented in Fig.4.5.

Phosphorus concentration in leaves : The foliar concentration of phosphorus of mycorrhizal plants was significantly higher ($p < .05$) at 0.34 and 0.69g application of bone meal. Whereas, at other levels there was no significant difference between mycorrhizal and non-mycorrhizal seedlings. However, the foliar concentration of both decreased at 51.6g bone meal level.

Phosphorus concentration in stem : The concentration of phosphorus in the stem of mycorrhizal plants was signifi-

Fig.4.5 The allocation of phosphate in the leaf, stem and root of the mycorrhizal and non-mycorrhizal plants grown in a range of additions of bone meal in the soil.

Fig. 4·5


Non-mycorrhizal
Mycorrhizal
Phosphorus [mg./g.]
8·0
6·0
4·0
2·0
0
Root
8·0
6·0
4·0
2·0
0
Stem
12·0
10·0
8·0
6·0
4·0
2·0
0
Leaf
0
0·34
0·69
1·72
3·4
17·2
25·8
34·4
51·6
Bone meal levels in soil [g./pot]

cantly higher ($p < .05$) at 0 and 0.34g levels of bone meal whereas, at other concentrations of bone meal no significant difference in phosphorus concentration was observed in the stem of both mycorrhizal and non-mycorrhizal seedlings.

Phosphorus concentration in root : The concentration of phosphorus in the roots of mycorrhizal seedlings was significantly higher ($p < .05$) than those of non-mycorrhizal ones at 0, 0.34, 0.69, 1.72g bone meal levels, whereas, at higher levels of bone meal no significant difference was found in phosphorus concentration between mycorrhizal and non-mycorrhizal seedlings. Also the concentration of phosphorus decreased at 34.4g and 51.6g bone meal levels in both mycorrhizal and non-mycorrhizal seedlings.

III. Effect of infection in presence of available phosphorus (Sodium hydrogen phosphate) :

The concentration of phosphorus in the leaves, roots and stem of mycorrhizal and non-mycorrhizal seedlings of *Exbucklandia populnea* grown in the presence of six different concentrations of sodium hydrogen phosphate was determined and the results are presented in fig.4.6.

Phosphorus concentration in leaves : The foliar concentration of phosphorus was higher than that of root and stem. Also the concentration of P in the leaves of mycorrhizal seedlings was significantly ($p < .05$) higher than that of non-mycorrhizal seedlings at 0 and 0.34g of sodium hydrogen phosphate application, but at higher levels of phosphate no significant difference between the two was observed. With the enhanced levels of phosphate fertilization the foliar concentration of phosphorus was found to increase except at 51.6g phosphate level when it decreased for both mycorrhizal and non-mycorrhizal seedlings.

Fig.4.6 The allocation of phosphate in the leaf, stem and root of the mycorrhizal and non-mycorrhizal plants grown in a range of additions of sodium hydrogen phosphate in the soil.

Fig. 4·6

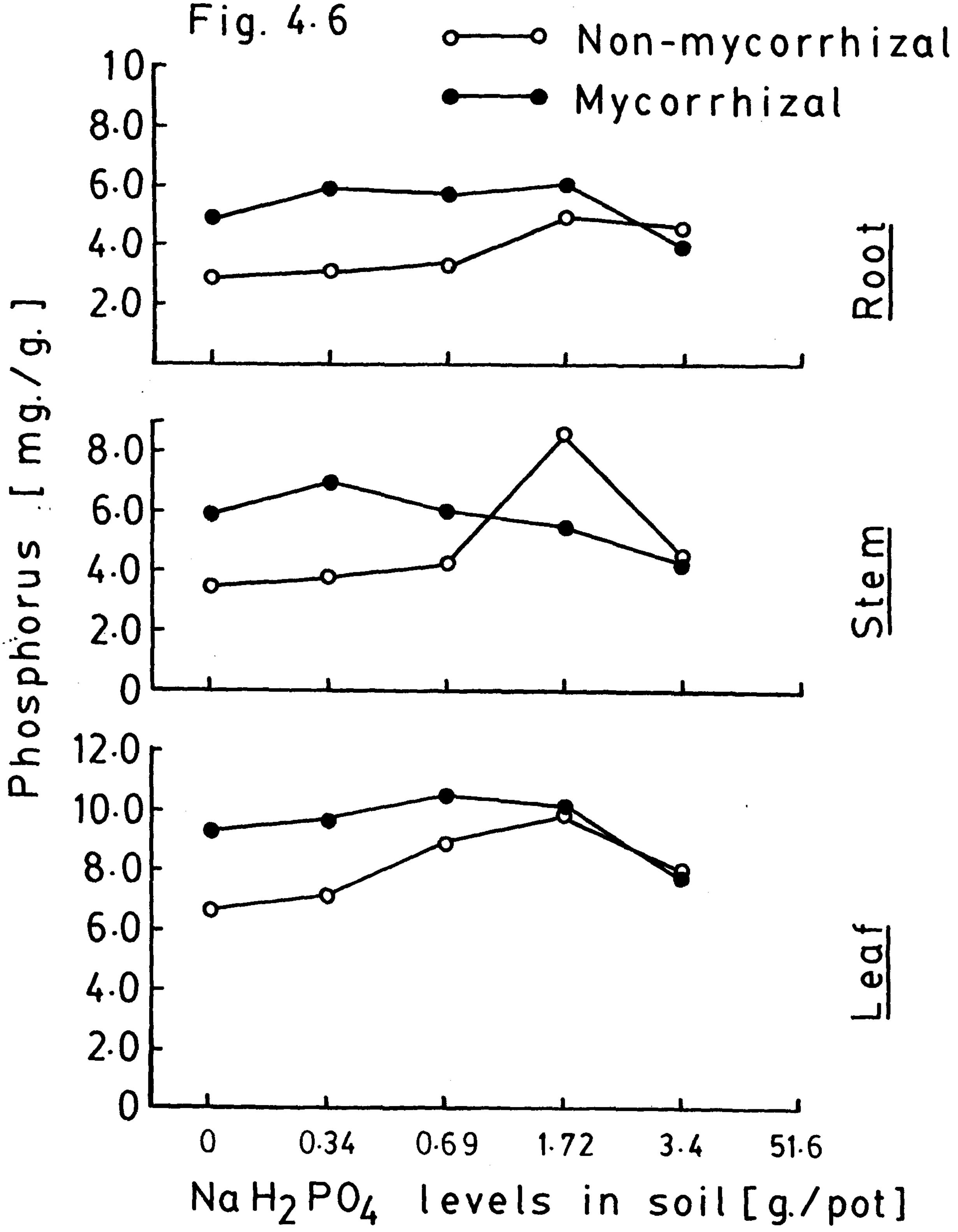
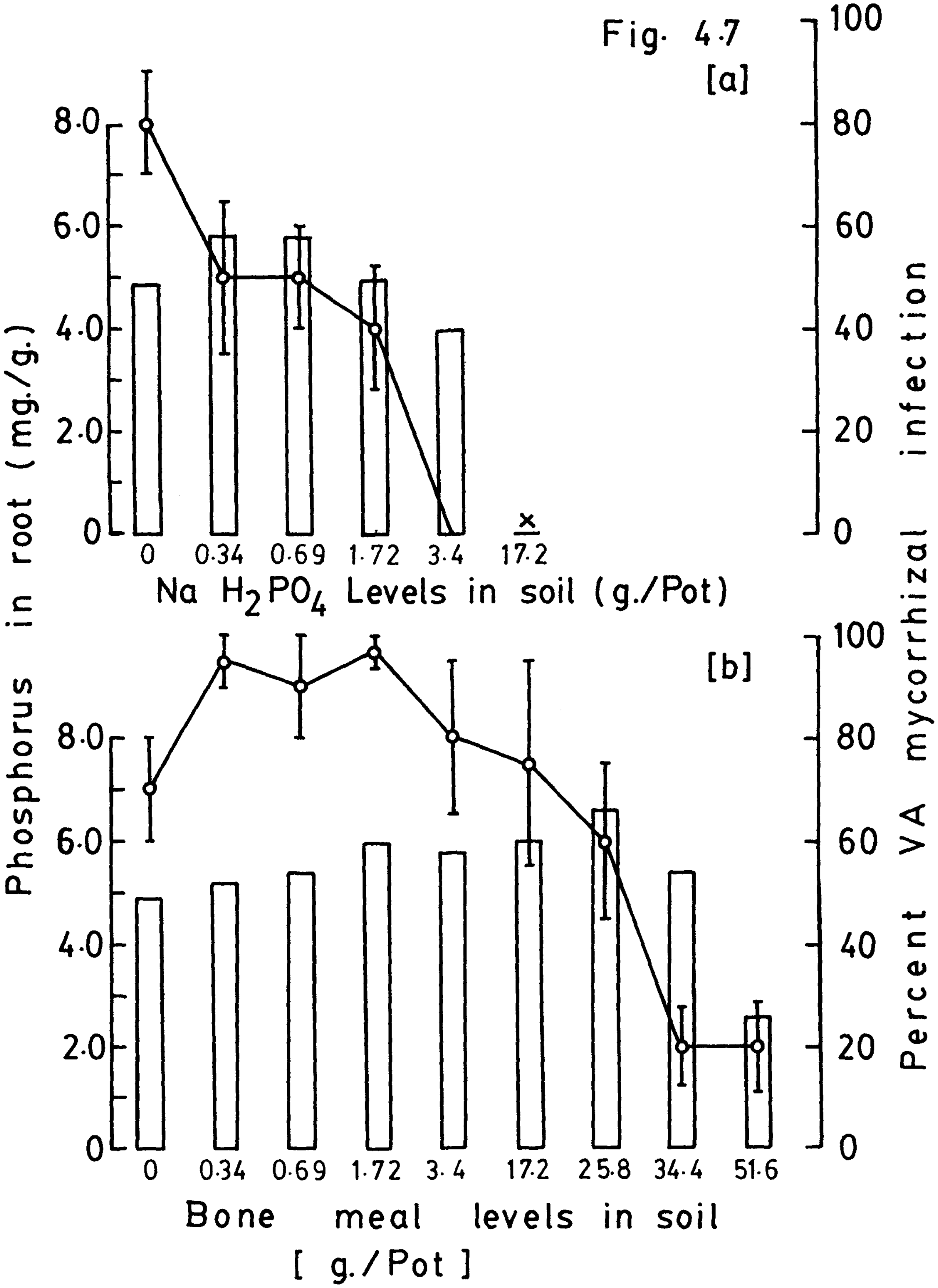

Non-mycorrhizal
Mycorrhizal
Phosphorus [mg./g.]
10
8·0
6·0
4·0
2·0
Root
8·0
6·0
4·0
2·0
0
Stem
12·0
10·0
8·0
6·0
4·0
2·0
0
Leaf
0
0·34
0·69
1·72
3·4
51·6
NaH_2PO_4 levels in soil [g./pot]

Fig. 4.7. The relationship between the concentration of phosphorus in the roots and percent VA mycorrhizal infection of seedlings of *Exbucklandia populnea* grown in a range of (a) sodium hydrogen phosphate (b) bone meal levels in soil.

Which is [illegible]

Fig. 4.7


[a]
8.0
6.0
4.0
2.0
0
100
80
60
40
20
0
0
0.34
0.69
1.72
3.4
x
17.2
Na H_2PO_4 Levels in soil (g./Pot)
[b]
8.0
6.0
4.0
2.0
0
100
80
60
40
20
0
0
0.34
0.69
1.72
3.4
17.2
25.8
34.4
51.6
Bone meal levels in soil
[g./Pot]
Phosphorus in root (mg./g.)
Percent VA mycorrhizal infection

Concentration of phosphorus in stem : The phosphorus concentration in the stem of mycorrhizal seedlings was significantly higher ($p < .05$) than that of non-mycorrhizal ones at 0 and 0.34g phosphate application. Phosphorus concentration in the stem of non-mycorrhizal seedling was found to be the maximum at 1.72g phosphate level, whereas, it was maximum at lower phosphate level i.e. 0.34g phosphate in the case of mycorrhizal seedlings.

Concentration of phosphorus in roots : Phosphorus concentration in the roots of non-mycorrhizal seedlings increased to a maximum at 1.72g phosphate level and decreased subsequently at higher levels of phosphate. The concentration of phosphorus in the roots of mycorrhizal seedlings was significantly higher ($p < .05$) than that of non-mycorrhizal seedlings at 0.34 and 0.69g application of phosphate. Whereas, at higher levels of phosphate there was no significant difference between the two.

DISCUSSION

It has been demonstrated that mycorrhizal inoculation stimulates the absorption of phosphorus in plants (Rhodes and Gerdemann, 1980). From the results of both the experiments it is evident that the higher concentration of phosphorus in the tissues of mycorrhizal seedlings at lower level of nutrient status of soil and phosphorus status of soil is probably due to improved phosphorus uptake by the mycorrhizal roots. Powell (1980) observed higher phosphorus uptake by mycorrhizal plants than the non-mycorrhizal ones at lowest fertilization level. The difference, however, was not marked at highest phosphorus fertilization. Abbott and Robson (1978) also observed higher concentration of phosphorus in mycorrhizal clover plants grown in phosphorus deficient soils. Thus, the growth enhancement due to mycorrhizal inoculation could be related to increase in the concentration of phosphorus of mycorrhizal seedlings. Not necessarily true

Many workers have reported inconsistent results of concentration of other nutrients viz., N, K and Ca in the mycorrhizal plants (Menge <u>et</u>.<u>al</u>.,1978; Pope, 1980). In some cases these elements are reported to be present in greater concentration in mycorrhizal plants than in non-mycorrhizal ones. Whereas, in other cases the concentration of these elements, are higher in non-mycorrhizal plants and sometimes no significant differences in concentration between the two groups of plants were observed. In the present investigation also the concentration of these nutrient was either low, high or unchanged by mycorrhizal inoculations at different levels of soil fertility. Therefore, it appears that enhanced absorption of these nutrient may not be a rule and better absorption of a given mineral nutrient by mycorrhizal plant is related to the plant requirement for such nutrients. Mycorrhiza is involved in the nitrogen nutrition of legumes. Ross and Harper (1970) found higher concentration of nitrogen in the tissue of the leaves which suggests that nitrogen uptake is enhanced by mycorrhiza. A perusal of the results of the present investigation and nitrogen concentration of other non-leguminous plants may suggest the absence of direct effect of mycorrhiza in uptake of nitrogen.

The results of the second experiment showed that mycorrhizal inoculation could improve phosphorus concentration of plants grown with addition of low amounts of soluble phosphates (sodium hydrogen phosphate) and relatively insoluble sources of phosphates .(bone meal). This suggest that mycorrhiza helps in better utilization of phosphorus. Abbott and Robson (1977) also found increased phosphorus uptake by mycorrhizal plants when supplied with intermediate levels of superphosphate. Higher levels of phosphorus depressed the development of mycorrhiza (Mosse and Phillips, 1971; Mosse, 1973). Therefore, at higher concentration of phosphates both inoculated and uninoculated plants behaved alike and there was no significant difference in the concentration of phospharus in the tissues of mycorrhial and non-mycorrhizal plants. Murdoch et.al.(1967) also found similar results with mycorrhizal corn grown in pots using different sources of phosphorus fertilizers of varying availability. In all cases they found that mycorrhizal plants took up more phosphorus than non-mycorrhizal plants from the soil that received relatively unavailable form of added phosphorus (rock phosphate or tricalcium phosphate). Similar results were also obtained by Daft and Nicolson (1966), Powell and Daniel (1978).

Mycorrhizal infection decreased with increased level of phosphorus in soil. Daft and Nicolson (1969; 1972). Mosse and Phillips (1971) also observed depressing effect of excessive phosphate in the soil on mycorrhiza development. Available source of phosphorus was more effective than unavailable form as mycorrhizal infection persisted even at higher concentration of bone meal though it was greatly reduced. Whereas, VA infection became absent at intermediate and higher concentration of sodium hydrogen phosphate. Sanders (1975) found that foliar application of phosphorus to onion plant result in decreased mycorrhizal infection. Similar result was also obtained by Menge et.al.(1978) who used a split root system technique. The results of the present investigation showed that the relationship between the root colonization and the concentration of phosphorus in the roots were negatively correlated. This support the hypothesis that the concentration of phosphorus in the tissues of the host plant might regulate the development of mycorrhizal infection in the roots.

CHAPTER - V

ENZYMATIC STUDIES ON VA MYCORRHIZAL SEEDLINGS OF *Exbucklandia populnea*

INTRODUCTION

Vesicular-arbuscular mycorrhiza enhances growth and improves phosphorus nutrition of plants (Mosse, 1973a). The improved phosphorus nutrition results from an increased efficiency in phosphorus uptake from the soil (Sanders and Tinker, 1971). It has been demonstrated that the external hyphae of VA mycorrhizal fungi translocate phosphate from the soil to the host root which involves active processes (Pearson and Tinker, 1975; Cooper and Tinker, 1978). Transport of phosphate takes place most probably in the form of polyphosphate granules present in vacuoles by cytoplasmic streaming (Cox et.al.,1975; Cox et.al.,1980).

In VA mycorrhizal system, little is known of the physiological processes involved in mycorrhizal infection and the mechanisms involved in the fungal transport of phosphorus (Pearson and Tinker, 1975) and its subsequent transfer from the fungal hypha into the host cell (Cox and Tinker, 1976). Alkaline phosphatase enzyme specific to VA mycorrhiza has been reported in onion and tobacco (Gianinazzi-Pearson and Gianinazzi, 1976), which could play a role in the assimilation of phosphorus by mycorrhizal roots (Gianinazzi-Pearson and Gianinazzi, 1978). Ultra-structural studies have indicated that the vacuoles which contain polyphosphate granules are characterised by intense alkaline phosphatase activity (Gianinazzi et.al.,1979). Alkaline phosphatase activity may be linked to the phosphate transport mechanism of VA fungi (Gianinazzi-Pearson and Gianinazzi,1978) Surface activity of roots may also contribute to the process of phosphorus absorption by the roots (Bieleski, 1973).

In the present investigation, the changes in the activity of phosphatase enzyme were studied in the mycorrhizal and non-mycorrhizal roots of Exbucklandia seedlings associated with the formation of VA mycorrhiza which could explain the mechanism of phosphorus uptake by mycorrhizal plants.

MATERIALS AND METHODS

The experiment consisted of mycorrhizal and non-mycorrhizal seedlings of Exbucklandia populnea grown and supplied with four different levels of single-superphosphate. The activity of phosphatase in the roots of seedlings was assayed. The details of methods are as follows.

Preparation of pots : Plastic pots of 2L capacity were used for growing seedlings of Exbucklandia populnea. Local garden soil was autoclaved twice and 2kg of the soil was taken to each pot. The soil was treated with four different levels of single superphosphate (SSP) viz: 0, 0.1, 0.5 and 5.0 g per pot. For each level of SSP five replicate pots were maintained. The fertilizer (SSP) was thoroughly hand mixed with the pot soil.

Inoculation of VA endophyte : The inoculum consisted of soil infested with VA endophyte Glomus spp. which was pot cultured and maintained on maize plant. For each level of SSP two sets of mycorrhizal treatments were given. One designated as mycorrhizal and the other as non-mycorrhizal. Mycorrhizal set received the inoculum of VA endophyte (Glomus spp),whereas, same amount of autoclaved inoculum along with a filtrate of inoculum washing was added to the non-mycorrhizal set. The methods of inoculation were the same as described in Chapter II.

Seedling preparation and plantation : The seedlings of Exbucklandia populnea were raised from the seeds in petri plates. The method of seedling preparation and their plantation in the pots were similar to those described in Chapter II.

Growth conditions and harvesting : The experiment was set up in the month of August,1985 and was carried out in a net house situated at the University campus under natural climatic conditions. The pots were watered weekly with tap water. The seedlings were grown for a period of four months after which they were harvested. The complete seedlings with intact root system were carefully excavated and brought to the laboratory for the assay of phosphatase activity, measurement of growth characteristics and assessment of VA mycorrhizal infection in the roots.

Preparation of cell free extracts : The fresh roots were detached from the seedlings and washed under running tap water and finally with the distilled water. The roots were chopped into pieces and chilled in refrigerator. 2g of the chilled roots were macerated in a pestle and mortar at 4°C using chilled 0.1M borate buffer at pH 8.8. The macerate was centrifuged at 3000 rpm for 20 minutes. The supernatent was collected and the activities of soluble acid phosphatase and alkaline phosphatase were determined in the supernatent.

Quantitative assay for soluble acid phosphatase activity : The substrate used was p-nitrophenyl phosphate. The substrate solution was prepared by dissolving 50mg of p-nitrophenyl phosphate in 10ml distilled water. 0.5ml of the substrate solution along with 0.5ml of 0.1M acetate buffer pH 4.0 was taken in test tubes and to it was added 0.1 ml of enzyme extract and the mixture was inoculated in water bath at 35°C for a period of 30 minutes. The reaction was terminated by adding 3.4ml of 0.1 N NaOH and the OD of the mixture was recorded at 410nm with spectrophotometer.

Quantitative assay for alkaline phosphatase activity : 0.5ml of substrate solution prepared above was taken in test tubes alongwith 0.5 ml of tris-citric acid buffer pH 8.5. To the mixture was added 0.1ml of enzyme extract and inoculated at 35°C for 30 minutes. The enzyme reaction was stopped by adding 3.4ml of 0.1N NaOH and OD of the mixture solution recorded at 410nm.

The O.D. recorded for both the enzymes was converted to UM of p-nitrophenol by referring to standard p-nitrophenol curve. Standard p-nitro phenol curve was prepared by taking known concentrations of p-nitrophenol solution and adding 3.4ml 0.1N NaOH solution and reading OD at 410nm. The enzyme activity was expressed as UM PNP released/min./g fresh weight of roots.

Growth measurement and assessment of mycorrhizal infection :

The fresh weight of shoot of the harvested seedlings was recorded. The roots were fixed in FAA. 25 root segments (1cm) were randomnly selected from the sample, cleared and stained by the method of Phillips and Hayman (1970). The percent VA infection was assessed by the microscopic method of Allen *et.al*. (1982).

RESULTS

Plant growth and mycorrhizal infection: Average fresh weight is presented in Table 5.1. It was observed that shoot fresh weight of mycorrhizal seedlings was significantly ($p < .05$) higher than those of non-mycorrhizal ones at 0.1 and 0.5g SSP/pot. The shoot fresh weight of both mycorrhizal and non-mycorrhizal seedlings increased with the increase in the level of single superphosphate (SSP), however, at 5.0g SSP/pot level there was little difference between the two. The level of VA mycorrhizal infection was maximum (60%) at

Table 5.1 Fresh weight of mycorrhizal and non-mycorrhizal <u>Exbucklandia populnea</u> seedlings grown in a range of superphosphate level on per plant basis.

Treatment (g.SSP/pot)	Fresh weight of shoot/plant† (g) Mycorrhizal	 Non-mycorrhizal
0.0	.203±.038	.139±.033
0.2	.324±.025*	.153±.027
0.5	.462±.036*	.175±.023
5.0	.403±.044	.390±.049

† : Values are mean of 10 replicates with standard error.

* : Significantly different ($p < 0.05$) from non-mycorrhizal.

Table 5.2 Infection level of mycorrhizal seedlings of Exbucklandia populnea grown for 3 months in autoclaved soil in a range of single superphosphate levels.

Treatment (g.SSP/pot)	Number of infected root segments (%)
0	60
0.2	60
0.5	20
5.0	0

0g and 0.1g SSP/pot and it decreased greatly at 0.5g SSP/pot and at higher level of 5.0g SSP/pot VA-mycorrhizal infection was absent (Table 5.2).

Soluble acid phosphatase activity : The observation of soluble acid phosphatase activity in the roots of *Exbucklandia populnea* seedlings is presented in Table 5.3. It was observed that the activity of acid phosphatase in the roots of mycorrhizal and non-mycorrhizal seedlings did not differ significantly. However, in mycorrhizal plants it was slightly higher in comparison to non-mycorrhizal ones at 0, 0.1 and 0.5g SSP/pot. Soluble acid phosphatase activity decreased slightly with the increase in the concentration of single superphosphate (SSP).

Soluble alkaline phosphatase activity : The activity of soluble alkaline phosphatase was low (Table 5.3). There was no significant difference in the activity of alkaline phosphatase between mycorrhizal and non-mycorrhizal plants. However, with the increasing concentration of single superphosphate the activity of soluble alkaline phosphatase in both mycorrhizal and non-mycorrhizal seedlings greatly decreased in comparison to the activity at 0g. SSP/pot.

DISCUSSION

In higher plants activity of acid phosphatase normally varied between 0.3 to 1.5 e.u/g fresh weight of

Table 5.3 Soluble acid and alkaline phosphatase enzymes activities (μM PNP released/ min/g. fresh weight of roots of 3 months old seedlings of *Exbucklandia populnea.*

Treatment (g.SSP/pot)	Soluble acid phosphatase enzyme activity		Soluble alkaline phosphatase enzyme activity	
	+M	-M	+M	-M
0.0	.309±.021	.282±.037	.032±.003	.039±.004
0.2	.337±.043	.278±.025	.034±.002	.038±.001
0.5	.306±.048	.232±.031	.022±.007	.024±.005
5.0	.198±.033	.202±.038	.015±.003*	.017±.001*

* : Significantly different from that of 0g. SSP/pot at 5% level.

tissues and deficiency of phosphorus may cause an increase in the activity of acid phosphatase (Bieleski, 1973). The results of the present experiment show that the activity of acid phosphatase in the roots of seedlings of Exbucklandia populnea was almost close to the lowest range of acid phosphatase reported by Bieleski (1973) in higher plants. It was observed that inoculation of VAM and or addition of superphosphate to the soil did not affect the activity of acid phosphatase as no significant differences could be observed in the activity of this enzyme with respect to mycorrhizal treatment or phosphate treatment of soil. However, Woolhouse (1969) reported a significant stimulation of acid phosphatase activity in the roots of Agrostis tenuis grown in solution culture under phosphorus deficient conditions. The differences in the results of Woolhouse (1969) and that of present investigation could be due to the differences in the species of plants and also due to the conditions of plant culture.

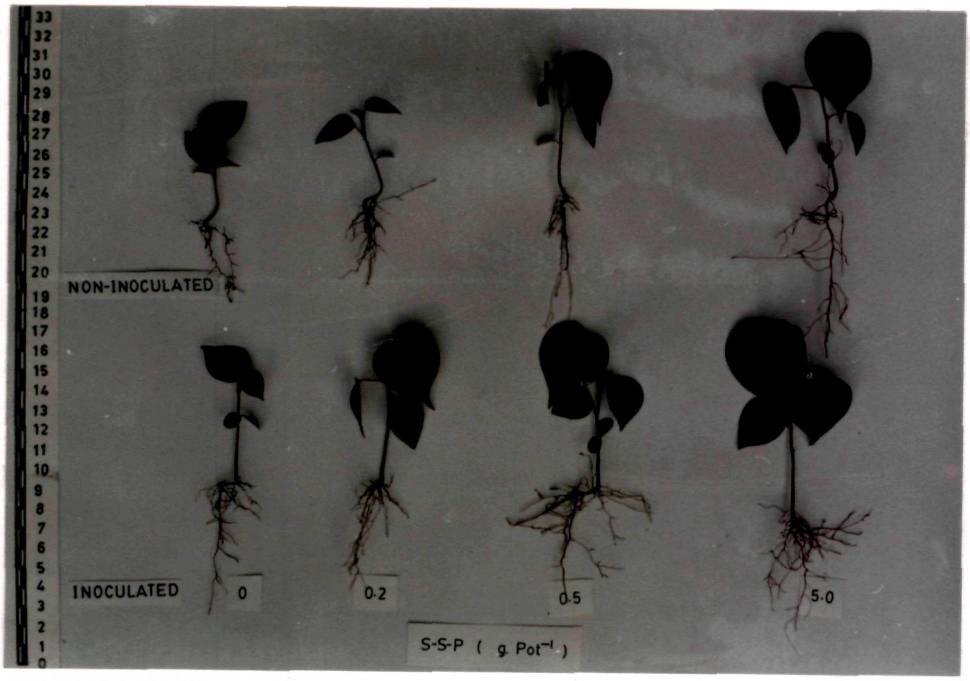
The results of quantitative assay of alkaline phosphatase activity showed that there was no significant difference between soluble alkaline phosphatase activity of mycorrhizal and non-mycorrhizal plants supplied with no phosphorus in the soil. However, activity of alkaline phosphatase significantly decreased in the presence of 5.0g SSP/pot. Gianinazzi-Pearson and Gianinazzi (1978) had also reported large differences in the soluble alkaline phosphatase activity of roots between the non-mycorrhizal plants which were supplied with high level of exogenous soluble phosphate and the mycorrhizal plants growing under the phosphorus deficient conditions of the soil. The non-mycorrhizal plants got phosphorus directly from the soil whereas, in phosphorus deficient conditions phosphorus was made available to the plants by mycorrhiza. The results, therefore, support the hypothesis that metabolism of the phosphorus between the mycorrhizal and non-mycorrhizal plants may be different (Gianinazzi-Pearson and Gianinazzi, 1978).

Activity of phosphatase enzymes in the root could also be involved in the establishment of VA mycorrhizal infection (Woolhouse, 1975). Mycorrhiza specific phosphatase enzyme activity specific to mycorrhizal infection in the roots of onion have been demonstrated by Gianinazzi, Gianinazzi-Pearson and Dexheimer (1979) in ultrastructural studies.

Plate 5.1

Effect of diferent levels of single super phosphate on the growth of seedlings of **Exbucklandia populnea.**

PLATE-5.1



GENERAL DISCUSSION

All the twenty six important tree species examined from this region were found to be heavily colonised by mycorrhizal fungi (Table 1.1). Whatever ecological benefits they may derive from the mycorrhizal fungi in their roots, the higher level of infection indicates their strong dependence on mycorrhiza. Nutrient deficient soils are the sites of greater mycorrhizal activity. The fungal hyphae spread into soil and serve as an extensions of the root system. The extensions are both physiologically and geom~~a~~etrically more effective for nutrient absorption than the roots themselves (Trappe and Fogel, 1977). The functions of mycorrhiza have mainly been associated with the phosphorus uptake by plants in the soils which are deficient in this nutrient (Mosse, 1973a). The analysis of rhizosphere soil of all the tree species revealed a low availability of phosphorus (Table 1.2). The phosphorus of less than 20ppm is regarded as of its poor availability for the growth of plants (Allen, 1974). Therefore, poor phosphorus status of the soil could be one of the conducive factors for prevalence of mycorrhizal association in the tree species of the region. Root samples were collected from the top 15 cms layer of the soil which was rich in humus as evident from the analysis of organic matter content of the soil (Fig.1.5). Therefore, humus seems to be an important source of inorganic nutrition for the plants. Mycorrhiza formation could, therefore, be an adaptive strategy of the tree species for efficient use of indigeneous sources of nutrients (St.John, 1980).

Vesicular-arbuscular mycorrhiza was found in the majority of the tree species examined from this region (Table 1.1). From other tropical locations several tree species have been reported to be endomycorrhizal with VA type of mycorrhizal infections (Baylis, 1967; Redhead, 1968; St.John, 1980). The results extend support to the report of tropical dominance of VA endomycorrhizae (Redhead, 1968).

In the case of annual plant species the level of mycorrhizal infection could be related to the age of the host plant (Hayman, 1970; Verma, 1982). According to them infection level increased with the age of the host plant. However, the study of seasonal variability in the level of VA mycorrhizal infection performed with eleven tree species (Fig.1.2a,b) under field conditions indicated that in perennial plants the factors of seasonal variations could be many and more complex. However, the seasonal study of soil factors made during two year cycle could not explain completely the seasonal variations in the level of mycorrhizal infections as no uniform pattern of significant correlations between percent mycorrhizal infection and various edaphic factors could be observed. The physiological and developmental conditions of the host could be involved. In spring the infection was high, which could probably be due to renewed root growth where rapid development of infection may take place.

Endogonaceous spore population was high in comparison to a much lower population counts made in studies of other natural vegetations (Mosse and Bowen, 1968; Cursh, 1975). However, in the study of regional weeds a similar higher population of Endogone spores was reported (Verma, 1982). This suggest that spores could be one of the major constituents of propagules for future infections of roots of tree species.

The occurrence of mixed infections in field could be expected. Tree species harboured spores of more than one species of VA fungi in their rhizosphere. Mixed infections have been reported in various woodland species by Daft (1983). Also the occurrence of similar spore types in diverse tree species suggests their host non-specificity (Sanders *et.al.*, 1977). Spores of *Glomus* spp were dominant both in spore population, host range and season coverage. Whereas, *Gigaspora* spp was observed in warmer months (Table 1.3), which suggests that higher temperature of the environment might favour its activity. Schenck and Hinson (1971) also observed frequent occurrence of *Gigaspora* spp. from the warmer regions of Florida.

Ten species of VAM fungi were identified from the forest soil of the region. Two species of *Acaulospora* namely, *A. laevis* and *A. scrobiculata* were identified which constitute their first description from Indian soils (Sharma *et.al.*, 1986). In the sporocarpic genus *Sclerocystis* three species namely, *S. coremioides*, *S. rubiformis* and

S. microcarpus were identified. The first two species have been reported by Bakshi (1974) from woodland and by Bhattacherjee *et.al.*(1980) from agricultural soils. Three species of *Gigaspora* were also identified namely, *G. calospora*, *G. gigantea* and *G. gregaria*.The species have also been described from woodland by Bakshi (1974). Besides, two species of *Glomus* were also identified namely, *G. macrocarpus* and *G. mosseae* which have also been reported from agricultural land by Bhattacherjee *et.al.*(1980).

The mycorrhizal infection was low at 0 level of NPK and increased at I level (14kg NPK ha^{-1}) but was again reduced at higher fertility levels of soil (Table 3.1). Hayman (1970, 1975), Porter and Beute (1972), Mosse (1973) and Kruckelmann (1975) suggested that excessive high or extremely poor status of nutrients in soil are inhibitory to mycorrhiza formation. Ames and Linderman (1978) also reported more mycorrhizal infections in low fertilizer treatment than in high or no fertilizer treatments. With additional phosphate supplied to mycorrhizal seedlings, the infection decreased with available source (sodium hydrogen phosphate). Daft and Nicolson (1969a), Ratnaike *et.al.* (1978) and Menge *et.al.* (1978) had shown decrease in VA mycorrhizal infection when soluble phosphates were added to the soil. However, with bone meal as the phosphate source, VA mycorrhizal infection increased at lower concentration and was greatly reduced at higher concentration of bone meal in the soil (Fig.3.5). Pairunan *et.al.*(1980) also observed increase in percent mycorrhizal roots when rock phosphates were applied to the mycorrhizal clover plants at intermediate levels whereas, higher concentration of rock phosphate decreased mycorrhizal infection.

The phosphorus content in the root was negatively correlated with percent root infection (Fig.4.7). This observation is similar to the findings of Sanders (1975) and Menge et.al.(1978) who found that infection and colonization of VAM fungi was regulated by high phosphorus concentration in the root system and not by phosphorus concentration of the soil. Hall (1977) and Jasper et.al.(1979) also found that phosphorus application to the soil depressed mycorrhizal infection by increasing the phosphorus status in plants.

The general growth superiority of mycorrhizal plants over non-mycorrhizal ones seemed to be the direct effect of mycorrhiza induced increased growth. However, the growth of mycorrhizal and non-mycorrhizal plants was almost similar at higher fertility levels of soil or phosphorus status of soil. Daft and Nicolson (1966) and Pairunan et.al.(1980) also reported a reduction in the mycorrhizal induced growth when phosphorus in the soil was no longer a limiting factor. The lesser root/shoot ratio in the mycorrhizal plants compared to the non-mycorrhizal ones has been observed in the present study (Table 3.2 and Table 3.3). Similar results were reported by Hayman and Mosse (1971), Becker and Gerdemann (1977).

Analysis of nutrients in seedlings indicated that phosphorus uptake was greater in mycorrhizal seedlings than the uninoculated ones. The enhanced growth is generally correlated with increased phosphorus uptake (Gerdemann, 1968; Mosse, 1973a). Substantial evidence favours that mycelium of mycorrhiza is responsible for increased phosphate uptake and translocation to the host plant (Sanders and Tinker, 1971; Hayman & Mosse, 1972a,b; Pearson and Tinker 1975). Increased uptake of phosphorus has been reported in many plants (Daft and Nicolson, 1969; Mosse <u>et</u>.<u>al</u>.,1976; Pearson and Read,1973; Mosse <u>et</u>.<u>al</u>.,1976; Heap and Newman, 1980).

The results of uptake of other nutrients viz., N, Ca, K were inconsistent and it appears that enhanced absorption of these nutrients may not be a rule and better absorption of a given mineral nutrient by mycorrhizal plant is related to the plant requirement for such nutrients. Similar inconsistency in the uptake of these nutrients has also been reported by Menge <u>et</u>.<u>al</u>.(1978) and Pope (1980).

The mechanism of phosphate uptake has not yet been understood properly. Transport of phosphate takes place in the form of polyphosphate granules by cytoplasmic streaming inside the hyphae of VA mycorrhizal fungi (Cox <u>et</u>.<u>al</u>.,1975; Cox <u>et</u>.<u>al</u>.,1980). The results of quantitative assay of alkaline phosphatase activity showed that the activity of this enzyme differed significantly between non-mycorrhizal seedlings supplied with 5.0g single super-phosphate and VA inoculated seedlings grown under phosphorus deficient conditions. Non-mycorrhizal plants got their phosphorus directly from the soil, whereas, in mycorrhizal plants phosphorus was made available to the plants by mycorrhiza. The results support the hypothesis that metabolism of phosphorus between mycorrhizal and non-mycorrhizal plants may be different (Gianinazzi-Pearson and Gianinazzi, 1978). The studies of (Gianinazzi-Pearson and Gianinazzi, 1978) had shown that new bands of alkaline phosphatase appeared in mycorrhizal roots and these specific bands accounted for 22-32% alkaline phosphatase activity. Based on their observation they (1979) further speculated that this MSP (mycorrhiza specific phosphatase) is of fungal origin. In ultra structural studies greater phosphatase activity has been reported on the tips of arbuscules but not in either host cell or senescent fungal hypha (Scannerini _et.al_.,1975) and the greater alkaline phosphatase activity was correlated with MSP and also with number of arbuscules (Gianinazzi-Pearson and Gianinazzi, 1979). The actual mechanism involved in phosphate uptake needs further investigations.

SUMMARY

North-Eastern region of India is endowed with rich natural vegetation and forest resources. The survey of 26 important timber tree species from a natural mountain forest ecosystem of the region indicated their highly mycotrophic nature. Six tree species were ectomycorrhizal and twenty others were endomycorrhizal. In the endomycorrhizal group two species had ericoid and remaining eighteen had vesicular-arbuscular type of endomycorrhizal association.The rhizospheric soil of all the tree species was acidic (pH:4.5-6.0), rich in soil moisture (17-40%) and organic matter (5-15%). However, the soil was poor in available phosphorus (1-10ppm).

Cryptomeria japonica, Exbucklandia populnea, Acacia dealbata, Schima khasiana, Machilus kingii, Cinnamomum tamala, Manglietia insignis, Lindera latifolia and Casearia verica exhibited seasonal variation in the level of VA mycorrhizal infection. The percent VA mycorrhizal infection increased during spring and rainy seasons and declined towards winter or late winter depending on the tree species, in both the years viz. 1983-84 and 1984-85. In Daphniphyllum himalayanse and Mallotus nepalensis, VA mycorrhizal infection was high ? in all the seasons. Tree species exhibited higher than what ? population of endogonaceous spores in their rhizospheric soil and it ? varied among various tree species and in different seasons. Cryptomeria japonica, Exbucklandia populnea, Cinnamomum tamala and Lindera latifolia displayed maximum endogonaceous spore population in rhizospheric soil in rainy season, whereas, in *Daphniphyllum himalayanse* and *Machilus kingi* it was maximum in winter. *Acacia dealbata, Schima khasiana Mallotus nepalensis, Manglietia insignis* and *Casearia verica* did not show seasonal variation in endogonaceous spore population in the rhizospheric soil.

Seasonal variation in soil pH was low. Moisture content, organic matter, total nitrogen, available phosphorus, exchangeable potassium and exchangeable calcium of the soil exhibited greater seasonal variation.

Spores of *Glomus* spp, *Acaulospora* spp, *Gigaspora* spp and *Sclerocystis* spp were observed in the rhizospheric soil of the tree species. Spores of different types representing more than one gen~~era~~us of endogonaceous fungi were present in the rhizospheric soil of tree species. Spores of *Glomus* spp and *Acaulospora* spp were abundant. Former was found in all the seasons, whereas, latter was common during winter season. Spore population of *Gigaspora* spp and *Sclerocystis* spp was low and were observed during warmer months of April and June.

Ten species of endogonaceous fungi were identified from the soil samples, viz; two species of *Acaulospora* namely *A. laevis* and *A. scrobiculata*. three species of *Sclerocystis* namely *S. rubiformis, S. coremioides* and *S. microcarpus*; two species of *Glomus* namely *G. mosseae* and *G. macrocarpus* var. *geosporus*; three species of *Gigaspora* namely *G. calospora*, *G. gregaria* and *G. gigantea*.

Seedlings of *Exbucklandia populnea* developed typical vesicular-arbuscular mycorrhizal infection with *Glomus* spp in pot culture using nutrient deficient soil. Mycorrhizal seedlings grew better than the non-mycorrhizal ones. Growth response of plants to mycorrhiza and VA mycorrhizal development depended on fertility level of soil. Mycorrhizal inoculation supplemented with low fertilization (14 kg ha^{-1}NPK) of soil resulted in better growth of seedlings than all other mycorrhizal and fertilizer treatments both in combination or alone. At higher soil fertility (56 kg ha^{-1}NPK) both mycorrhizal and non-mycorrhizal plants exhibited similar growth responses. Increase in level of soil fertility suppressed VA mycorrhizal infection. Higher levels of NPK (56 kg ha^{-1}) added to the soil inhibited VA mycorrhizal infection of plants.

Growth characteristics of mycorrhizal plants of *Exbucklandia populnea* were improved when soluble phosphate (sodium hydrogen phosphate) at low levels (0.34g per pot) and bone meal at relatively higher levels were added to the phosphorus deficient soil. In the presence of soluble phosphate VA mycorrhizal infection was reduced and was inhibited at higher levels (3.47g per pot and above). VA mycorrhizal infection was high in presence of bone meal upto intermediate levels of 17.2g per pot but was reduced to 20% at higher levels of bone meal (34.4g per pot). The concentration of phosphorus in the root was negatively co-related to percent VA mycorrhizal infection. The results suggested that phosphorus status of the host may regulate mycorrhizal infection of root.

Root/shoot ratio of mycorrhizal plants was lower than that of non-mycorrhizal plants at lower nutrient status (NPK levels) and phosphorus status of soil.

Effect of VA mycorrhiza on the mineral nutrition of the seedlings of <u>Exbucklandia populnea</u> was studied at different fertility regimes and phosphorus amendments of soil. Mycorrhizal infection improved phosphorus nutrition of seedlings. Mycorrhizal plants had higher concentration of phosphorus in comparison to non-mycorrhizal plants at lower fertility levels of soil (0,14kg ha^{-1}NPK). Mycorrhizal plants had higher concentration of phosphorus than non-mycorrhizal plants grown in both sources of phosphorus, but the difference in concentration between mycorrhizal and non-mycorrhizal was observed with relatively higher levels of bone meal and only at lower levels of sodium hydrogen phosphate (0.34, 0.69g/pot). The concentration of nitrogen in the leaves did not differ between mycorrhizal and non-mycorrhizal plants of <u>Exbucklandia populnea</u> but, stem and roots of mycorrhizal plants had higher concentration of nitrogen than non-mycorrhizal plants at lower levels of soil fertility (O, I, II levels of NPK). Concentration of potassium in the

increased [illegible]

not shown fig 4

leaves and stem of non-mycorrhizal plants was higher than that of mycorrhizal plants at lower levels (O,I) of NPK added to the soil. No significant differences were observed in the concentration of calcium between mycorrhizal and non-mycorrhizal plants except for leaves which differed at higher levels of NPK. The growth improvement of mycorrhizal plants could be due to phosphorus nutrition.

The activity of acid phosphatase did not differ between the mycorrhizal and non-mycorrhizal roots of seedlings of Exbucklandia populnea.and there was no consistent effect of addition of single super-phosphate to the soil on the activity of this enzyme. Mycorrhizal infection did not affect soluble alkaline phosphatase activity at 'O' level of single superphosphate. Soluble alkaline phosphatase activity was decreased by addition of single superphosphate at 5.0g per pot level in both mycorrhizal and non-mycorrhizal plants. The results suggest that pathway of phosphorus metabolism in mycorrhizal plants may be different from that of non-mycorrhizal plants.

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