

STUDIES ON DISTRIBUTION AND FUNCTION OF
ECTOMYCORRHIZAE IN UPTAKE AND TRANSLOCATION
OF LABELLED COMPOUNDS (^{32}P AND ^{14}C)
IN PINE SEEDLINGS

By

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C E R T I F I C A T E

We certify that the thesis entitled "Studies on distribution and function of ectomycorrhizae in uptake and translocation of labelled compounds (^{32}P and ^{14}C) in pine seedlings", submitted by Miss Manju Chauhan for the degree of Doctor of Philosophy of the North-Eastern Hill University, Shillong, embodies the record of original investigation carried out by her under our supervision. She has been duly registered and the thesis presented 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.

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CONTENTS

CHAPTERS	PAGE
General Introduction	1 - 5
Review of Literature	6 - 18
1. Movement of ^{14}C from shoot of pine seedlings to mycorrhizal root.	19 - 29
2. Uptake and translocation of ^{32}P from mycorrhiza to pine seedlings.	30 - 40
3. Effect of soil factors on the uptake and translocation of ^{32}P and ^{14}C in pine seedlings.	41 - 52
4. Role of mycorrhiza in short circuiting of ^{15}N nutrient.	53 - 61
5. Effect of root leachates on the germination of basidiospores and subsequent development of mycorrhiza with pine roots.	62 - 71
General Discussion	72 - 77
Summary	78 - 82
References	83 - 101

The most widespread symbiotic association amongst plants is that between the root and fungi which is known as "mycorrhizae" (Frank, 1890). It is highly specialized cooperation between roots and fungi. Nearly all plant species except few are associated with such symbiosis. The pine (*Pinus resinosa*), a timber yielding conifer grows luxuriantly at an early stage of soil tree species in the higher altitudes (1000 - 2000 m) in the foot hills of Appalachians in Virginia. Under natural conditions, the roots of pine form mycorrhizae. The mycorrhizal fungi and the pine plants mutually benefit in uptake of water and mineral nutrients from the soil. Fungal hyphae penetrate the root cells and extend into soil. Fungal hyphae are branched and bear vesicles, hyphae, and spores. The mycorrhizal fungus is called "vesicular-arbuscular mycorrhizal fungus" (VAMF) (Gerdemann, 1974; Trappe, 1982).

General Introduction

For mycorrhizae are difficult to observe. They are surrounded by a protective cuticle that contains an intricate apparatus of organelles. They are characterized by an extensive fungal sheath, associated extracellular mycelium and an intricate system of cell-to-cell contact. Fungal hyphae are the site of exchange of nutrient between fungi and host plants.

The extracellular mycelium extends and functions as a link between the root system and soil. This net work greatly increases the ability of many terrestrial plants to absorb nutrients and moisture from the soil. Mycorrhizal roots are usually larger in diameter than the aerial roots and are often branched thus enlarging the total absorptive surface (Harley, 1968; cited in Trappe, 1973). As a result, they are particularly important in uptake of nutrients that are either found in low concentration in the soil solution or are relatively immobile such as phosphorus (Olsen & Kepper, 1969; Trappe, 1973; Rex, 1981). The degree of exchange of nutrient between the cell of the host root and fungal symbiont

The most widespread symbiotic association amongst plants is found between the root and fungi which is known as "Mycorrhiza" (Frank, 1885). It is highly specialised association between roots and fungi. Nearly all plant species except few are encountered with such association. Khasi pine (*Pinus kesiya*), a timber yielding species grows luxuriantly as an early successional tree species at the higher altitudes (1000 - 3000 msl) on the foot hills of North-Eastern Himalayas. Under natural conditions, the roots of pine form mycorrhizal association. The mycorrhizal fungi aid the host plants primarily in uptake of water and mineral nutrients in return of carbon. Pines have ecto or ect-endomycorrhizal association formed mainly by basidiomycetes, hypogeous ascomycetes, phycomycetes and deuteromycetes (Gerdemann, 1974; Trappe, 1962, 1977).

Ectomycorrhizae are structurally modified rootlets surrounded by mycobiont and they function as an nutrient absorbing organs. They are characterized by an exterior fungal sheath, associated extramatrical mycelia and an internal structure called a Hartig net. Hartig net is the site of exchange of nutrient between fungi and host plant.

The extramatrical phase extends and function a link between the root system and soil. This net work greatly increases the ability of ectomycorrhizal plants to absorb nutrients and moisture from the soil. Ectomycorrhizal roots are usually larger in diameter than non mycorrhizal ones and are often branched thus enlarging the total absorptive surface (Harley, 1969; Bowen, 1973). As a result, they are particularly important in uptake of nutrients that are either found in low concentrations in the soil solution or are relatively immobile such as phosphorus (Olsen & Kemper, 1968; Bielecki, 1973; Nye, 1979). The degree of exchange of nutrient between the cell of the host root and fungal symbiont

depends largely on the amount of exchange surface and efficiency of fungal symbiont in acquiring nutrients, especially phosphorus. It is important therefore, to quantify the amount of fungal symbiont in root system.

Ectomycorrhiza benefit their host primarily by improvement in mineral nutrition although increased drought and pathogen resistance unquestionably can occur. The evidences for the nutritional benefit are however, incomplete. It appears that ectomycorrhiza can stimulate nitrogen uptake by utilizing organic nitrogen available to nonmycorrhizal roots, but the condition in which this is accomplished is not defined (Abuzinadah & Read, 1986; Jones *et al*, 1990). Increase in total content of host phosphate has been shown following infection (Heinrich *et al*, 1988). This increase has not been correlated to time and amount of ectomycorrhizae formation.

Earliest evidence for a role of ectomycorrhiza in phosphorus uptake includes observations that excised ectomycorrhiza absorb ^{32}P from solution more rapidly than do mantle has cores of root tissue (Harley, 1969). Ectomycorrhiza can also increase the uptake of phosphorus from less soluble forms such as phytate (Heinrich *et al*, 1988). In spite of several observations on structure of ectomycorrhiza and their role in phosphorus uptake work on relationship between quantification of ectomycorrhiza (i.e. fungal partner) and movement of C and P is not available.

Ectomycorrhizal fungi can form network, connecting plants of the same or different species. Recent researches have shown that both carbon and phosphorus transport occur through these mycelial networks. Carbon can move from the plant towards the advancing mycelia from and between different host plants (Reid

& Wood, 1969; Finlay & Read, 1986a; Finlay *et al*, 1986). As a result of their increased, surface area ectomycorrhizal seedlings on low soil fertility generally have higher mineral nutrient content per unit seedling dry weight than nonmycorrhizal ones. The ectomycorrhizae themselves are the site of intense accumulation of phosphorus, other mineral nutrients and carbohydrates play important role in their storage (Harley & McCready, 1950, 1952a, 1952b). Ectomycorrhizal plants also take up significantly more nitrogen than nonmycorrhizal ones (Alexander & Fairley, 1986). Ectomycorrhizal fungi are able to use various nitrogen sources like, amino acids and proteins in pure culture.

The establishment of the mycorrhizae is dependent upon the availability of carbon in and around the host roots. If carbon compounds exuded from the roots of hosts plant support metabolic activities of mycorrhizal fungi on young lateral roots, then colonization will be directly related to the internal carbohydrate status of the young lateral roots (Bjorkman, 1942; HacsKaylo, 1965). This means that light intensity, photoperiod, temperature, availability of nutrients and translocation rates and other factors affecting internal composition of carbon compounds within the roots will have a direct influence upon establishment and maintenance of mycorrhizae.

The ectomycorrhizal symbiosis of forest trees has been shown to be affected by some climatic and soil factors (Slankis, 1974; Alexander & Fairley, 1983; Rajkumar, 1990). Some information has been acquired on inter and intra specific growth variation of ectomycorrhizal fungi in response to different temperatures (Moser, 1956) and light intensity (Aston, 1979). The principal soil factors affecting mycorrhiza formation have been identified as a substrate pH (Slankis, 1974; Smith, 1974; Marx & Cordell, 1987; Metzler & Oberwinkler, 1987) soil type (Mason *et*

al, 1985), liming (Theodorou & Bowen, 1969; Erland *et al* 1989), fertility level (Alexander & Fairley, 1983), and soil microbial activity (Linderman, 1987; Marx & Cordell, 1987). These effects have been evaluated in terms of change in frequency and types of mycorrhizal infection. Growth rate, infection potential, efficiency of mineral uptake, carbon requirement and competitive ability in colonizing root and spread of mycorrhizal fungi in soil are parameters which are likely to be affected by these factors. Slankis (1974) have shown that ectomycorrhizal fungi exhibit growth optima between pH 3 and 5, when grown *in vitro*. The effect of soil liming include not only an increase of soil pH but also a complex change in total nutrient availability of the soil (Erland & Soderstrom, 1990).

Since long, it has been a great problem to germinate the basidiospores of ectomycorrhiza forming homobasidiomycetes under controlled external condition. The factors regulating the spore germination of many species are still unknown. It has been investigated that the presence of other living organisms near the fungal spore on an agar plate or any other appropriate substrate may induce germination (Fries 1976, 1978, 1979; Fries & Birraux, 1980). Generally this effect is assigned to either exudates from various microorganisms or to root of plant (Theodorou & Bowen, 1969, 1971; Fries & Birraux, 1980; Marx, 1976). Fries and Birraux (1980) have tried to investigate both physical and chemical factors which affects the spore germination of soil inhabiting mushrooms but very few positive results have been reported. Therefore, there is a great need to study the spore germination of ectomycorrhiza forming basidiomycetes, which can be manipulated experimentally.

Mycorrhizae are not only more efficient in

utilization of available nutrient from soil but are also involved in transfer of nutrients from components of soil minerals and organic residues to solution. They play an important role in nutrient cycling in an ecosystem. Therefore, their potential use no longer be ignored because of the future challenges in utilization of their mycobiont to restore soil fertility and increase forest production on either previously marginal land or at reduced fertilizer and energy inputs. Though mycorrhizal association is a natural means for improving mineral nutrition in plants the significance of the association is often ignored in both forestry and agricultural studies. During last two decades studies on ectomycorrhiza were confined to understand attributes like resistance to drought and tolerance to pathogen but the factors affecting indirectly the uptake and translocation were not taken into consideration seriously. Therefore, it was planned to investigate the role of ectomycorrhiza in nutrient uptake and translocation of labelled nutrients like ^{32}P , ^{14}C and ^{15}N . The present study has been divided into the following headings:

1. Movement of ^{14}C from shoot of pine seedling (*Pinus kesiya* Royle Ex Gordon) to mycorrhizal root.
2. Uptake and translocation of ^{32}P from mycorrhiza to pine seedling (*Pinus kesiya* Royle Ex Gordon).
3. Effect of soil factors (soil type, moisture and pH) on the movement of ^{32}P and ^{14}C in pine seedlings.
4. Role of mycorrhiza on the short circuiting of ^{15}N nutrients.
5. Effect of root leachates of the pine seedlings on the germination of spores of basidiomycetes and subsequent development of mycorrhiza with pine roots.