CHAPTER II
REVIEW OF LITERATURE
The surface of plant leaves and stems generally serve as deposition sites of airborne microorganisms, pollen grains and inert particles (Barnes, 1968; Fokkema, 1971a, 1971b; Warren, 1972; Chou and Preece, 1968). Throughout the life of plants, fungal spores are continuously deposited on their surfaces by wind impaction, sedimentation and rain wash-out from the atmosphere and splash-dispersal (Dickinson, 1976; Dix and Webster, 1995).

The Phylloplane:

The first systematic study of the phylloplane biology was by Potter (1910). The term phyllosphere, meaning the external surface of the leaf, was first put to use by Last (1955). The term phylloplane was distinguished by Leben (1965) who also recognized two types of phylloplane microflora, the casuals and the residents. The casuals, consisted of organisms that are firmly lodged on the surface of the leaf but not in a position to germinate on or colonise the plant surface. The residents were those that are more acclimatized to the phylloplane where they thrive as saprophytes. The inability of the casuals to grow on the leaf surface was attributed to factors such as, surface texture, lack of essential nutrients, host specificity and competition between the resident organisms (Barnes, 1969; Ruinen, 1966; Last and Deighton, 1965).

Dickinson (1976) classified the phylloplane fungi into three categories: non-pathogenic, pathogenic and exochthonous. The non-pathogenic fungi consisted of those able to grow and sporulate in favourable and unfavourable conditions but triggered to grow only at the onset of senescence. The pathogenic fungi are wholly or partially restricted to the phylloplane and could survive long periods on the phylloplane prior to penetration. The phylloplane forms an essential link in the life
cycle of exochthonous fungi though the fungi do not derive any advantage from the habitat.

**The nature of phylloplane mycoflora:**

The leaf surface forms a host to diverse microbial population which mainly includes fungi and bacteria. The most abundant of the fungi on the surfaces of leaves are yeasts which included members of the Ascomycotina, Basidiomycotina and the Fungi Imperfecti (Last and Deighton, 1965). The genera such as *Candida*, *Cryptococcus*, *Rhodotorula*, *Sporobolomyces*, *Tilletiopsis* and *Torulopsis* were regularly encountered on the surface of the leaves (Dickinson, 1986).

The leaf surface has been looked upon as being home to a large number of filamentous fungi. Taxa belonging to filamentous Ascomycetes, the Zygomycetes, Basidiomycetes and Deuteromycetes have been recorded on the phylloplane of different plants (Dickinson, 1976). Amongst the filamentous fungi recorded on aerial plant surfaces, the sooty molds stand out as producing spectacular colonies. Epiphytic fungi such as species of *Erysiphe* which grow extensively on leaves and other surfaces develop physiological connections with the underlying host tissues. The biology of these pathogens is of interest to microbial ecologists as they influence the aerial plant surface ecosystems (Dix and Webster, 1995).

Several studies have been carried out on the phylloplane mycoflora. Hog and Hudson (1966) described the succession of fungi on leaves of *Fagus sylvatica*. Hog (1966) elucidated the factors determining the natural succession of fungi on beech leaves. Studies on the phylloplane mycoflora so far made included the plants such as *Halimone portulacoides* (Dickinson, 1965), *Pisum sativum* (Dickinson, 1967), *Cassia tora* (Mishra and Tewari, 1969), *Echinocloa crusgalli* (Mishra and Srivastava, 1971),
Northofagus truncata (Ruscoe, 1971), Typha latifolia (Pugh and Mulder, 1971),
Triticum aestivum (Mishra and Srivastava, 1974), tomato (Mishra and Kanaujia, 1974),
rye (Fokkema et al., 1975), barley (Dickinson and Skidmore, 1976), Brassica
oleracea (Gingell et al., 1976), Hippophae rhamnoides (Lindsey and Pugh, 1976a,b),
Hordeum vulgare (Mishra and Tewari, 1976), maize (Warren, 1976), Panicum
coloratum (Eicker, 1976), Picea abies (Collins and Hayes, 1976), potato (Kumar and
Gupta, 1976), poplar and plum (McKenzie and Hudson, 1976), larch (McBride and
Hayes, 1977), Quercus robur (Cox and Hall, 1978), Populus tremuloides (Wildman
and Parkinson, 1979), Ilex aquifolium (Mishra and Dickinson, 1981), Eucalyptus
vaminalis (Cabral, 1985), muskmelon (Singh, 1995), Shorea robusta (Baruah and
Bora, 1995), Citrus (Kalita et al., 1996), Quercus robur (Newsham et al., 1997) and
Myristica fatua var. magnifica and M. malabarica (Bhat and Kaveriappa, 1999).

Two broad kinds of techniques have been employed to study the microfungi
on leaf surfaces. Direct techniques include the impression films and surface stripping
(Beech and Davenport, 1971; Lindsey and Pugh, 1976a), staining (Schimdt, 1973;
Warren, 1972a), leaf clearing (McBride and Hayes, 1977), scanning electron
microscopy (Lindsey and Pugh, 1976b), infra-red photography (Purnell and Farell,
1969) and autoradiography (Waid et al., 1973). Indirect or cultural techniques include
use of selective media (Beech and Davenport, 1971), impression plates (Apinis et al.,
1972), thin agar film (Parkinson et al., 1971), dilution plate and leaf washing (Warren,
1976; Davenport, 1976; McBride and Hayes, 1977), spore-fall method (Lindsey and
Pugh, 1976b), incubation in humidity chamber (Lindsey and Pugh, 1976b) and
measurement of fungal products (Frankland et al., 1978). The methods employed to
study the colonization of aerial organs of plants have been reviewed by Lindsey
(1976), Beech and Davenport (1971) and Macauley and Waid (1981). The pros and
Cons of the direct and indirect techniques are enumerated by Dix and Webster (1995) and they concluded that for gathering comprehensive data on the fungal colonization of plant tissues, different investigative techniques must be employed.

Last and Deighton (1965) pointed out that the season, age of the plant and nutritional status of leaf, control the nature of phylloplane mycoflora. Dickinson (1976) attributed the presence of phylloplane fungi to the availability of fungal inocula, nature of plant surface, factors such as temperature, rain, dew, humidity, wind, physiology and health status of the plant and the nature of the plant community. Macauley and Waid (1981) listed out the factors such as nutrients, the ability of the organism to survive in an exposed environment depending on their resistance to the extremes such as starvation, drought, high and low temperatures, UV radiation, presence of fungicides, the grazing population, mycolytic organisms living in association with the phylloplane fungi and the response of the host plant to the presence of fungi or the production of fungal metabolites are responsible for colonization of fungi on leaf surface. Dix and Webster (1995) attributed the factors such as cell leakage, competition, the pollen effect, interspecific competition, plant inhibitors and climatic factors as influencing the growth of microorganisms on plant surfaces.

**Role of fungi in the decomposition of plant litter:**

In terrestrial ecosystems, much of the energy fixed by photosynthesis finds its way to the soil in the form of dead organic matter which is decomposed by a host of microorganisms. The rates of breakdown of forest litter influences the nutrient uptake and the standing state of nutrients in the forest floor. Because of its role in nutrient
cycling and in supporting the saprophagic component of the ecosystem, the process of decomposition has received growing attention in recent years.

Decomposition is the process of separation of any substrate into its constituent elements. This would signify the mechanical disintegration of dead plant structure from the stage where it is still attached to the living plant, to the humus stage where the gross cell structure is no longer recognisable. Decomposition is essential for recycling the forest canopy and in determining the plant and animal communities that thrive on the forest floor (Dix and Webster, 1995).

Work done at Pine Lake Preserve, had some interesting observations on forest litter decomposition. The study showed that, the speed of decomposition of litter varied from forest to forest. It was slowest in the hemlock, fastest in the mixed hardwood, and proceeded at an intermediate rate in the beech and red pine forests. Tiny bags of litter were prepared from each forest and placed in each of the other forests and how fast the litter decomposed was measured. Hemlock litter didn't decompose faster than in the mixed hardwood forest. In fact the guest litter, no matter which forest it was from, decomposed pretty the way it would have at home. Although there was some interaction between the litter and the type of forest it was in, the decomposition rate seemed to depend on the litter itself.

In conclusion, forest litter is the basis for an elaborate detritus food web. Bacteria and fungi feed on the litter, and they, in turn, are eaten by small invertebrates such as springtails, mites, and nematodes. These are devoured by larger invertebrates, namely the earthworms, eurychaed worms and insects, which in turn are eaten by vertebrates like the red-backed salamander, the top carnivore of the detritus food web in the temperate forest. It was also found that the decomposition process seemed to be sensitive to acidic conditions. More the acidic the litter and forest soil, the slower the decomposition
litter decomposed. The hemlock forest was the most acidic of the four forests on the Preserve, the hardwood forest the least. The study also showed that the decomposer bacteria were more sensitive to acidic conditions than the fungi.

The role of the fungi in the decomposition process is well studied and documented (Hayes, 1965; Hering, 1965; Hudson and Webster, 1958; Hogg and Hudson, 1966; Kendrick and Burges, 1962; Macauley and Thrower, 1966; Minderman and Daniels, 1967). Fungal floristics has been the object of many of the studies, though in some there were attempts to relate the occurrence and succession of fungi to changes in the nutritional status of the leaf (Hering, 1967; Hudson, 1971). The ecology of fungi colonizing senescent and fallen leaves has also been the subject of some of these investigations (Hudson, 1971).

The layer of dead plant material not attached to a living plant and may be present on the soil surface is generally considered as litter. The making of litter however commences with senescence of leaves. Abscission of a leaf base follows the senescence when much of the mineral content is withdrawn to the stem and the phylloplane fungi already commenced the decomposition of available carbohydrates.

On young green leaf, yeasts and yeast like imperfect fungi such as *Auriobasidium pullulans* and *Cladosporium* sp. are prominent (Dickinson, 1973, 1976; Godfrey, 1974; Leben, 1965; Ruinen, 1963; Dickinson and Wallace, 1976; Last and Warren, 1972). The presence of filamentous fungi appeared to be less frequent on young green leaves than on older ones (Dickinson, 1976). About 100 genera of phylloplane fungi have been reported so far on over 35 different higher plants studied. Most species were known to occur infrequently. As the leaf matured hyphal development increased rapidly (Dickinson, 1976; Ruscoe, 1971; Pugh and Mulder, 1971; McBride and Hayes, 1977) to a point where at abscission, the leaf was
extensively colonized. These species were the primary colonizers of the dead tissue of the leaf (Hudson, 1968; Dickinson, 1976). Last and Deighton (1965) found out that the phylloplane fungi frequent the leaves more often than in the soil suggesting that they are well adapted to the micro-environment of the leaf and possession of pigments in sooty moulds is to survive the high light intensity at the leaf surface. It has been observed that the mycoflora changed as senescence occurred and to a certain extent mycoflora affected the rate of senescence (Dickinson and Wallace, 1976). At the stage of abscission, primary saprophytic species belonging to the genera such as *Ascochyta*, *Leptosphaeria*, *Pleospora* and *Phoma* along with other parasitic fungi which may or may not be host specific, inhabit the moribund leaf (Dickinson, 1976). After abscission, the role of these fungi changed to bring about the breakdown of organic matter and to prevent the accumulation of toxic substances to levels harmful to primary colonizers and leading to mineralization of essential elements out of the organic debris in order to maintain fertility and the productivity of the ecosystem (Witkamp, 1973).

Cabral (1985) made a detailed study of the phylloplane mycoflora of *Eucalyptus viminalis*. He recognised two groups of fungi, phylloplane or epiphytic species and endophytes or endophyllic species. Phylloplane fungi colonised the interior of the leaf only occasionally and did not displace the endophytes. He also observed that ascomycetes and coelomycetes were better represented as endophytes than hyphomycetes. *Alternaria alternata*, *Cladosporium cladosporioides*, *Epicoccum nigrum* and *Microsphaeropsis callista* were phylloplane fungi isolated in high frequency, while *Coccomyces maritiniae*, *Coniothyrium* sp., *Macrophoma smilacina* and *Zolleneria eucalypti* were the common endophytes. A distinct seasonal pattern was observed for the phylloplane fungi wherein the maximum number was seen in
autumn-winter and minimum in summer in proportion to humidity and temperature. The endophytes were appeared to rely more on the age/or physiological conditions of the leaf. He also made an attempt to ecologically classify the phylloplane fungi into the ruderals, residents and primary saprophytes. Ruderals occurred sporadically in low frequencies and as inactive propagules. Residents were those that were more persistent and appeared in high frequencies. Primary saprophytes were those that disappeared before the leaf died. Residents were further subdivided into, 'specific' that did not actively indulge in the degradation of the substrate when the leaf died and 'unspecific' that participated actively in the primary degradation and did not diminish when the leaf ultimately died.

Several studies have dealt with the decomposition of plant matrices in different ecosystems and the changes in fungal saprophytic communities in the litter layers in time (Bills and Polishook, 1994; Chasseur and Beguin, 1990; Kjoller and Struwe, 1990; Sieber-Canavesi and Sieber, 1993; Aoki, et al., 1990, 1995; Tokumasu et al., 1994). Some studies were concerned with the composition of and seasonal variation in fungal species colonising the leaf litter of single plant species (Vardavakis, 1988; Marakis and Diamantoglou, 1990; Mulas et al., 1990, 1995) whereas others were with mixed litter (Lunghini, 1993, 1994; Zucconi et al., 1997).

Time-related changes in community structure are the so-called fungal successions (Dix and Webster, 1995). Many of the factors which influence successional changes have been identified and the sequence of events involved is now fairly understood. Colonisation of a dead organism leads to the immediate struggle amongst potential saprophytic colonists for establishment, what is called as the ‘prior colonisation effect’ (Barton, 1960, 1961; Bruehl and Lai, 1966). Weak parasites like Pythium and Fusarium species and harmless or mutualistic non-obligate endophytes
usually form prominent members of the pioneer communities, with their ability to germinate rapidly and grow fast. The reasons for the loss of pioneer colonisers as the community matures during succession is no longer attributed to nutritional hypothesis, wherein pioneer colonisers dependant upon simple organic sources, disappeared from communities when supplies of these became exhausted. The development of antagonistic phenomenon or the accumulation of staling and antibiotic toxins in the substratum could stop the growth of the coloniser.

Dix and Webster (1995) observed that the successional changes can be accepted if based on the presence or absence of actively growing mycelia since the appearance of sporulating structures bear little relationship in time to the appearance or disappearance of the mycelium. Actively growing mycelia may be present; but may never sporulate or the sporulation be delayed for a long period. The tendency for the climax of successions to become dominated by one or two highly antagonistic species may also result in changes in the rate of decomposition as the succession develops. Rich species diversity at the beginning of succession corresponds to highest rates of decomposition. Eventually, the rate of decomposition at the climax of succession becomes that of the most vigorous competitor. These have slow growth rates with lower metabolic activity and hence the rate of decomposition also becomes slow (Dix and Webster, 1995).

Phylloplane fungi persist on the fallen leaves of angiosperms and species of Cladosporium, Aureobasidium and others were isolated from the leaf litter for many months after the leaf fall (Hogg and Hudson, 1966); several also known to produce their sexual stages there (De-Boois, 1976). More enduring leaf litters typically develop a secondary flora of litter microfungi, the sporulating structures of which usually appear about a year after leaf fall (Hogg and Hudson, 1966). Once in the litter,
leaves become colonised by species of typical soil-inhabiting fungal genera such as *Doratomyces, Humicola, Fusarium, Gliocladium, Penicillium, Trichoderma*, etc. and as time passes these become dominant as leaves are buried and get into the deeper layers of the litter (Dix and Webster, 1995). Some of the common autochthonous soil fungi associated with tree leaf litter appeared to play only a minor role in its direct decomposition (De-Boois, 1976).


The first detailed study of fungal succession on coniferous litter was by Kendrick and Burges (1962) who followed the colonisation of leaf litter of *Pinus sylvestris* by fungi and found out that in litter, fermentation and humus layers, of the pathogens present on living needles, viz. *Lophodermium pinastri, Coniosporium* sp. and *Fusicoccum bacillare, Coniosporium* sp. did not survive even on the litter; *Lophodermium pinastri* remained active and sporulated extensively up to 6 months after needle fall and then disappeared; *Fusicoccum bacillare* showed an extensive
development from the time of death and showed another heavy production of spores 3-5 months after leaf fall. After the needle fall, the *Verticicladium* stage of *Desmazierella acicola* was the common coloniser. *Aureobasidium pullulans* was replaced on the surface by *Helicoma monospora* and *Symphodiella acicola* which appeared on the needles even in the litter layer. In the fermentation layer the external colonisers were *Trichoderma viride* and *Penicillium* sp. and the internal colonisers were basidiomycetes and a sterile dematiaceous fungus.

Succession of fungi which occurs as the leaf ages could also be correlated to the changing nutrient status of the leaf (Macauley and Waid, 1981). The initial colonizers, the yeasts and yeast like fungi utilize simple carbon compounds or leachates which are exuded from the living leaf onto the leaf surface. As the leaf ages, the frequency of filamentous fungi increases in correlation with the increasing amount of exudate. At the stage where these exudates are exhausted, fungi subsist on other available substrates such as the cellulose that persist even on the dead tissue.

For conifers, the pattern of development of the fungus flora on needles in litter had some general features in common with the mycoflora developing on the leaf litter of angiosperm trees (Dix and Webster, 1995). One similarity was that the leaf-inhabiting fungi of the phylloplane persisted on the needles in the litter for several months after needle fall and some went on to produce their sexual stages. Among these were a small group of needle-inhabiting fungi which appeared first on living needles in very low numbers but became more abundant as the needles reached the litter. In the litter, the decaying needles were invaded by litter-inhabiting fungi which completed the decomposition of the needles.

Dilly and Irmler (1998) studied the functional structure within the biota during the decomposition of leaf litter in a black alder forest in northern Germany. The
succession of the food web was analysed at a dry and wet site close to a lake with eight, four, and seven functional groups of bacteria, fungi and fauna. The decomposition process was divided into two phases separated by the summer dryness. During the first phase cellulolytic bacteria, omnipotent and minor potent fungi were present together with mycetophagous, saprophagous and humiphagous soil animals.

Derived from trophic relationships between the functional groups, a food path was suggested by Dilly and Irmler (1998) for the first phase from litter via cellulolytic bacteria to microphagous and saprophagous soil fauna and their predators. In addition, food paths led from litter via different fungal groups to mycetophagous soil fauna and their predators. During the second phase of decomposition the number of food paths was reduced. Only fungi without lignolytic potential persisted and saprophagous animals predominated. A retarded occurrence of nitrifying bacteria was observed which suggests increasing ammonium and nitrite concentration during decomposition. High correlation was found between general bacteria and proteolytic bacteria referring to an internal protein flux within these functional groups. The number of trophic links was higher during the first phase.

Rauni et al. (1999) studied the microbial composition in a primary successional sequence on the forefront of Lyman Glacier, Washington, United States. They sampled microbial communities in soil from nonvegetated areas and under the canopies of mycorrhizal and nonmycorrhizal plants from 20 to 80 year old zones. Three independent measures of microbial biomass were used: substrate-induced respiration (SIR), phospholipid fatty acid analysis (PLFA), and direct microscopic counts. All methods indicated that biomass increased over successional time in the nonvegetated soil. The PLFA analysis indicated that the microbial biomass was greater under the plant canopies than in the nonvegetated soils; the microbial
community composition was clearly different between these two types of soils. Over the successional gradient, the microbial community shifted from bacteria-dominated to fungi-dominated set up. Microbial respiration increased while specific activity (respiration per unit biomass) decreased in nonvegetated soils over the successional gradient. The maximal respiration rate and the total C released from the sample decreased sharply over the successional gradient. They proposed and recommended new parameters for estimating the carbon use efficiency of the soil microbial community. The study suggested that during the early stages of succession, the microbial community cannot incorporate all the added substrate into its biomass though rapidly increased its respiration.

Pennanen et al. (1999) studied the structure, biomass and activity of the microbial community in the humus layer of boreal coniferous forest stands of different fertility. The Scots pine dominated Calluna vulgaris type (CT) represented the lowest fertility, while Vaccinium vitis-idaea type (VT), Vaccinium myrtillus type (MT), and Oxalis acetocella-Vaccinium myrtillus type (OMT) following this order, were more fertile types. The microbial community was studied more closely by sampling a succession gradient at the MT site. The phospholipid fatty acid analysis (PLFA) revealed a gradual shift in the structure of the microbial community along the fertility gradient even though the total microbial biomass and respiration rate remained unchanged. The relative abundance of fungi decreased and that of bacteria increased with increasing fertility. The spatial variation in the structure of the microbial community was studied at a MT site. Semivariograms indicated that the bacterial biomass, the ratio between the fungal and bacterial biomasses, and the relative amount of PLFA were spatially autocorrelated within distances around 3 to 4 m. The total microbial and fungal biomasses were autocorrelated only up to 1 m. The spatial
distribution of the humus microbial community was correlated mainly with the
location of the trees, and consequently with the forest floor vegetation.

The succession in physiological capabilities of bacterial and fungal
communities was studied during leaf litter decomposition within the first 12 months at
a drier and a wet site in a black alder forest (Dilly et al., 1998). Eutrophic and
proteolytic bacteria were positively and cellulolytic and lipolytic bacteria negatively
correlated. In many cases, densities of bacterial populations were positively correlated
with fungal enzymatic potentials indicating a concerted action of bacterial and fungal
communities during degradation of litter constituents. Cellulolytic bacterial numbers
were positively linked with polygalacturonic and lignolytic potential of the fungi
indicating a fine-tuned mineralization. However, lipolytic bacterial numbers and the
respective potential of fungi were negatively correlated which suggests shifting
importance of bacteria and fungi for lipid degradation. The fungal communities seem
to play a predominant role in the litter breakdown at early stages whereas bacteria
succeeded later in order to complete the process of mineralization. The data were
related to microbial carbon content, activities and abiotic properties.

The dynamics of fungal and bacterial potentials in the decomposition of leaf,
branch and bark litter along a gap size gradient in a subtropical forest was determined
using substrate-induced respiration (SIR) with antibiotics selective for fungi and
bacteria, respectively (Zhang and Zak, 1998). Fungi had higher SIR than bacteria for
each type of litter in any size of gaps. Decomposing leaf litter exhibited higher fungal
and bacterial SIRs than branch and bark. Correlation analysis indicated that fungal
SIR was a reliable index of decomposition rates. Fungal SIR was positively correlated
with soil moisture whereas bacteria was not. The relationships among microclimatic
factors, fungal and bacterial physiological activities and rates of plant litter
decomposition suggested that in the subtropical ecosystems, fungal activities were strongly and directly regulated by the environmental heterogeneity within gaps and are important regulators of rates of plant litter decomposition.

There have been a few detailed studies of the fungal successions on lower plants. Kilbertus (1968) studied the moss, *Pseudoscleropodium purum*, and Frankland (1966, 1969) and Godfrey (1974) investigated *Pteridium aquilinum*. Dix and Webster (1995) indicated that there are differences in the mycoflora of the litter of lower plants. Fronds decayed more slowly than angiosperm leaves and were invaded early in fungal succession by basidiomycetes and deuteromycetes which become dominant by the end of the second year. This kind of succession resembled the decay of wood, probably due to similarities in the presence of low nitrogen level and high lignin content.

**Herbaceous litter - Monocots:**

Very few detailed comparative studies on herbaceous plant litter are known and in this respect, the early investigations by Webster (1956, 1957) on *Dactylis glomerata* are exemplary. His studies revealed several distinct observations. On upright stems, from the upper to lower internodes, four groups of fungi were recognised. Group I were the primary saprophytes of the leaves and stems, consisting of *Alternaria tenuis, Cladosporium herbarum, Epicoccum purpurascens, Leptosphaeria microscopica* and *Pleospora vagans*. Sporulation of primary saprophytes was first recorded in low frequency on leaves at lower internodes in May and progressed upwards as the season advanced. They persisted at the upper internodes for about 15 months until the stem collapsed in the second winter. Primary saprophytes that were recorded immediately after the flowering at lower internodes
which did not spread to upper internodes as senescence progressed made up group II, as typified by *Acrothecium sp.* Group III consisted of *Mollisia palustris* and *Tetraploa aristata* which appeared at the lower internodes. The sporulating structures of these secondary saprophytes were not recorded until the following spring. This was followed by the appearance of group IV consisting of *Helminthosporium hyalospernum* and *Tetraploa aristata* which fruited in the following summer.

Hudson and Webster (1958) studying *Agropyron repens* revealed a remarkably similar pattern of colonization which differed only in some qualitative aspects of the mycoflora. *Agropyron repens* showed major differences in the distribution of species at different levels on the stems and this suggested that moisture content or the nutritional status of stems are more important regulators of fungal growth than atmospheric humidity. The differences in fungal colonization of the upper and lower internodes were attributed to factors such as water content, nutritional status, host resistance and competition with the organisms.

Pugh (1958) discussed the distribution of fungi on *Carex paniculata* by studying the leaves from previous years and recently dead leaves. He found that the older litter harboured less number of fungal species than the recently dead leaves.

Webster and Dix (1960) worked on the culms of *Dactylis glomerata* to analyze the nutritional status of the upper and lower internodes and their ability to support fungal growth. They also looked into some of the factors controlling the pattern of colonization which was an extension of the experiments conducted by Hudson and Webster (1958). They found that upper internodes had a higher nutritional status in the early periods of colonization. Primary colonizers were capable of rapid colonization, the spores germinated rapidly and the mycelium spread at lower relative humidities than secondary colonizers.
Similar patterns of succession have been observed on plants growing in other climates. In warmer regions, there are differences in the species composition with a tendency for the species diversity to increase. Studying fungal succession on the leaves of sugarcane, Hudson (1962) observed much a pattern irrespective of the position of the leaves on the stem. He recognised three groups of fungi. Very early colonisers of green leaves (group I), viz. *Guignardia citricarpa*, *Leptosphaeria sacchari* and other parasites. These were joined by *Alternaria tenuis*, *Cladosporium herbarum*, *Curvularia lunata* and *Nigrospora sphaerica* as the leaves senesced and all of them first appeared on basal leaves and then spread upward. The early colonisers were followed 2-3 months later by group II fungi consisting of *Lacelliniopsis sacchari*, *Periconiella echinochloae* and *Pithomyces maydicus*. These were in turn followed by group III of fungi which included *Anthostomella minima*, *Apiospora camptospora*, *Didymosphaeria* sp., *Entosordaria deightonii*, *Lacellina graminicola*, *Lophodermium arundinaceum*, *Metasphaeria* sp., *Pleospora vagans*, *Spegazzinia tessartha* and *Tetraploa aristata*.

Meredith (1962) studied the mycoflora on collapsed and decaying banana (*Musa sapientum*) midrib, petiole and lamina. The primary colonisers consisted of *Deightoniella torulosa*, *Gloeosporium musarum*, *Nigrospora* sp., *Pyricularia musae* and *Verticillium theobromae*. *Verticillium theobromae* and *Deightoniella torulosa* were most prominent on petioles and midribs. As the leaves dried, the primary colonisers were replaced by species of *Acremonium*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Paecilomyces*, *Penicillium* and several others.

Khanna (1964) studied the succession of fungi on three decaying grasses, viz. *Bothriochloa pertusa*, *Cynodon dactylon* and *Dichanthium annulatum*. Fungal succession on decaying leaves of *Saccharum munja* was studied by Rai (1973) for
over two years and observed a similar pattern of colonisation like that of Hudson (1968) on *S. officinarum*. Sharma and Dwivedi (1972) recorded the mycoflora colonising different portions of the shoot system of fodder grass, *Setaria glauca*, from early senescence onwards. Fungal flora of the air overlapped with a majority of the fungi isolated from the shoots of the grass. The number of fungal species recorded from stem segments was lesser than that on blades and sheaths. This they attributed to several morphological and anatomical features of different plant parts and ecological factors such as moisture content of the substrates, temperature and relative humidity of air and competition between colonisers.

Rai (1974-75) suggested a general scheme for fungal succession on decaying grasses of the tropics. All grasses that had been studied commonly showed the presence of dominant members, though they differed in the frequency of occurrence on different substrates. Deuteromycetes and a few ascomycetes were the prime colonisers of grasses. Phycomycetes and Basidiomycetes were not recorded on any of the grasses subjected to study.

**Herbaceous litter - Dicots:**

The green and moribund leaves of *Halimone portulacoides* was studied by Dickinson (1965). Three groups of phylloplane fungi were recognised: transient, lying on the surface of the leaf, consisted of the first group; fungi such as species of *Cladosporium* thriving and sporulating at ease form the second group; the third group consisted of species such as *Ascochyta obionis* that form pycnidium on moribund leaves. Though there was similarity between the mycoflora of *Halimone* and *Dactylis* (Webster, 1957), the frequency of occurrence of *Ascochyta obionis* made a prominent difference. Dickinson (1965) also stated that abundance of air-borne spores
such as *Aspergillus* sp. and *Penicillium* sp. dictated their presence on the leaves. Kerling (1964) found a similar trend in fungal colonisation on strawberry and rye litter.

Herbaceous plants of *Calluna vulgaris, Festuca* sp., *Melandrium* sp. and *Vaccinium myrtillus* were assessed for fungal colonisation at different stages of decomposition (Mangenot, 1966). Species of *Cladosporium, Mucor* and *Rhizopus* were dominant at the early stages while those of *Chaetomium, Fusarium* and *Trichoderma* were frequent during the later stages on *Calluna* and *Vaccinium* litter. Species of *Penicillium* such as *P. aurantio-candidum, P. janthinellum* and *P. frequentans* were also present in large numbers. Species of *Fusarium* were the dominant colonisers throughout the decomposition of *Melandrium* litter. *Chaetomium globosum, C.indicum, Cladosporium* sp., *Mucor* sp. and *Rhizopus* sp. were major colonisers on *Festuca* litter.

Yadav (1966) recognised five groups of fungi based on the frequency of their occurrence on decaying stems of *Heracleum sphondylium*. With senescence, *Alternaria tenuis, Cladosporium herbarum, Botrytis cinerea, Coniothecium* sp., *Epicocum nigrum* and *Phomopsis astericus* were first observed on the leaves and leaf sheaths. The lower internodes were then attacked by *Acremonium* sp., *Cladosporium herbarum, Dendryphion comosum, Epicoccum nigrum, Hormiscium* sp., *Periconia cookei, Phoma complanata, Stachybotrys atra* and *Torula herbarum* without any localised pattern of colonisation. He concluded that the primary mycoflora, which appeared on the stem in the year of their growth were possibly deposited there by wind. The secondary mycoflora which appeared in the winter following summer, characteristic of lower internodes, probably arrived from the soil and gradually spread upwards. His findings were parallel to those reported on *Dactylis* by webster (1957).
Dickinson (1967) working on leaves of *Pisum sativum*, leaves found that the major fungi on senescent and dead leaves were *Alternaria* sp., *Aureobasidium* sp, *Cladosporium* sp and *Stemphylium* sp. On stems of *Urtica dioica* Yadav and Madelin (1968) found out that *Alternaria tenuis*, *Botrytis cinerea*, *Cladosporium herbarum* and *Epicoccum nigrum* were the primary colonisers while the lower portions were colonised by these along with *Acremoniella atra*, *Alternaria tenuis*, *Cladosporium herbarum*, *C. sphaerospermum*, and *Phoma acuta*.

Sharma and Mukerji (1972) reported the results of taxo-ecological investigations on the mycoflora of leaves of *Gossypium hirsutum* L. at different stages of senescence, while still attached to the mother plant and after abscission. The effect of seasonal variations in temperature, relative humidity, soil pH and moisture content on the quality and quantity of the mycoflora were established. *Candida albicans* and *Phoma spp.* showed remarkable fluctuations in the number of propagules per gram dry weight of leaves when correlated with seasonal variations in temperature and relative humidity.

Vittal (1973) made a detailed study of the fungi colonising leaves and litter of two dicotyledonous plants, *Atlantia monophylla* and *Gymnosporia emarginata* collected from Vandalur, Madras, over a two year period. The fungi of leaves and litter of both plants were studied in the first year. In the following year, litter from each plant was graded into three on the basis of the extent of decomposition and the fungi on each grade of litter were analysed into five groups, viz. dominant, common, frequent, occasional and rare. The number of fungal species recorded were greater on *Atlantia* than on *Gymnosporia*. Deuteromycetes were the dominant members on both the plants; in addition, myxomycetes, phycomycetes and ascomycetes were also observed on *Atlantia*. *Beltaniella portoricensis*, *Sesquicillium setosum* and
Ophiognomonia sp. were dominant on *Atlantia*, while *Beltrania rhombica*, *Idriella vandalurensis* and *Pestalotia theae* were dominant on *Gymnosporia* litter. Quite a number of fungal species were common to both the plants although the frequency and percentage occurrence differed for both plant litter types.

Many species were common to all three grades of litter and some restricted to only a single grade of litter. Percentage occurrence of each species was considered to be an index of activity on litter. Among the species common to all grades of litter, some differences in activity of the species on each grade were found. For example, *Beltaniella portoricensis*, *Sesquicillium setosum*, *Cladosporium herbarum*, *Volutina concentrca* and *Ophiognomonia* sp. were the most active in that order, on grade 1 litter of *Atlantia*; *Beltaniella portoricensis*, *Sesquicillium setosum*, *Gyrothrix circinata* and *Ophiognomonia* sp. were the most active, in that order on grade 2; *Pyrenochaeta* sp., *Sesquicillium setosum*, *Stachybotrys chartarum*, *Beltaniella portoricensis* and *Ophiognomonia* sp. were in that order the most active on grade 3 litter of *Atlantia*. Similarly, *Pestalotia theae*, *Idriella vandalurensis*, *Gyrothrix circinata* and *Cladosporium herbarum* were the most active in that order on grade 1 litter of *Gymnosporia* and *Idriella vandalurensis*, *Gyrothrix circinata*, *Pestalotia theae* and *Stachybotrys chartarum* were the most active, in that order, on grades 2 and 3 litter of *Gymnosporia*.

Vittal (1973) also compared the fungi isolated on *Atlantia* and *Gymnosporia* litter collected from different localities. This highlighted the qualitative similarity in the mycoflora of litter of both plants. It was observed that *Beltaniella portoricensis* restricted to *Atlantia* litter at Vandalur was recorded on *Gymnosporia* litter from Kambakkam, and likewise, *Beltrania rhombica* restricted to *Gymnosporia* litter at Vandalur was recorded on *Atlantia* litter from Kambakkam.
Phylloplane mycoflora of *Atlantia* and *Gymnosporia* were also studied by Vittal (1973). *Drechslera hawaiiensis, Nigrospora oryzae* and *Pestalotia theae* were common to the phylloplane of both plant species. *Rhinocladiella* sp. was found to selectively colonise green and yellowed leaves of *Gymnosporia* but not *Atlantia*. For both plant species, over 50% of the phylloplane fungi continued to be frequent on grades 1 and 2 of the litter, but on grade 3 litter, their percentage was very much lesser. A survey of the air mycoflora showed an abundance of propagules of species found on the phylloplane of *Atlantia* and *Gymnosporia*.

Sudha (1978) studied *Glycosmis cochinchinensis* and *Ixora parviflora* from a scrub jungle at Thambaram, Madras, to obtain information on the nature of the mycoflora active during different phases of decomposition of litter. Litter samples were collected once a month for a period of two years. Each monthly sample was sorted out into 3 grades on the basis of extent of decomposition. Grade 1 being the freshly fallen leaves which have undergone little decomposition; grade 2 represented litter more decomposed than grade 1 litter and grade 3 represented highly decomposed litter. Mycoflora of each grade of litter of the 2 plant species was studied by moist chamber incubation and dilution plating techniques. Besides, senescent leaves of each plant species collected every month were allowed to undergo decomposition in a separate experimental set up constructed to simulate conditions found in nature, in which yellowed senescent leaves still attached to the plant were collected every month and were placed in layers one above the other separated by a nylon mesh, which maintained physical continuity between the 12 layers. The different layers were then assessed for mycoflora at the end of 1 year. In all, 118 species in 83 genera were recorded on both plants. This included myxomycetes (2 species), mucorales (6 species), ascomycetes (7 species), coelomycetes (11 species) and hyphomycetes (92
species). As a result of the study she obtained 57 fungal species on *Glycosmis* and 70 on *Ixora* litter. *Colletotrichum dematium*, *Linospora* sp, *Sesquicillium setosum* and *Volutina concentrica* were found exclusively on *Glycosmis* litter, whereas *Endophragnia alternata*, *Weisneiriomyces javanicus* and *Zygosporium masonii* were confined to *Ixora* litter. *Beltrania rhombica*, *Beltraniella portoricensis*, *Scolecobasidium constrictum* and *Trichoderma harzianum* were common colonisers on both substrates. *Endophragnia alternata*, *Helicosporium vegetum* and *Rhinocladia sp.* occurred exclusively on grade 3 litter of *Ixora*. Similarity indices of the mycoflora of litter of the 2 plant species from the different layers of experimental set up showed that mycoflora on litter from adjacent layers had the greatest similarity, the similarity decreasing with increasing distance of the layers.

On the basis of the frequency and the colonising efficiency of the different species, Sudha (1978) proposed that for both plant species, the first colonisers on litter were predominantly a few weak parasites on living or senescent leaves, followed in succession by true litter fungi which were replaced in the final stages of decomposition by soil inhabiting fungi.

Dorai (1988), worked on the taxonomic and ecological aspects of the fungi colonising the leaf litter of *Eucalyptus* species in India. The examination of the leaf litter of 13 species of *Eucalyptus* resulted in the isolation of 264 species belonging to 170 genera. The majority of species belonged to the Deuteromycotina (84%), though members of myxomycetes, Zygomycetes, Ascomycetes and Basidiomycetes were also represented. Of the 264 species, 22, constituting 8.3% were undescribed species. Some fungi were specific to a particular host. Seven species were specific to *E. citriodors*; 13 to *E. deglupta*; 44 to *E. globulus*; 1 to *E. grandis*; 14 to *E. longifolia*; 1 to *E. maculata*; 53 to *E. tereticornis* and 4 to *E. torelliana*. No host specific species
were recorded from *E. eugenioides, E. ficifolia, E. macrorhyncha, E. regnans* and *E. saligna*. The number of host specific species recorded on *E. globulus* and *E. tereticornis* were greater when compared to the remaining 11 species of *Eucalyptus*. Dorai attributed this to the distribution of the different host species in South India; *E. globulus* was most commonly grown in the hilly tracts of Andhra Pradesh, Kerala and Tamil Nadu while *E. tereticornis* was grown in the plains of Karnataka, Kerala and Tamil Nadu. Hundred and four species were common to different species of *Eucalyptus*. *Corynespora cassicola* was recorded only from *E. globulus* and *E. tereticornis*, while *Weisneiriomyces javanicus* was recorded from 10 species. *Cryptophiale kakombensis, Cryptocoryneum rilstonii, Haplographium helicocehalum, Hyphodiscosia jaipurensis, Parasympodiella laxa* and *Pseudopetrakia kambakkamensis* were reported for the first time from *Eucalyptus* litter. Comparing the microfungi from the studies of Vittal (1973) and Sudha (1978) on *Atlantia monophyla, Gymnosporia emarginata, Glycosmis cochinchinensis* and *Ixora parviflora*, Dorai (1988) listed the following fungi common to all these plants. *Beltrania rhombica, Beltraniella portoricensis, Corynespora cassicola, Curvularia eragrostidis, C. tuberculata, Cylindrocladium parvm, Gyrothrix circinata, Memnoniella echinata, Periconia hispidula, Periconia cookei, Weisneiriomyces javanicus, Kramasamuha sibika, Zanclospora indica*. The last two fungi were a new genus and new species recorded from Vandalur. He concluded that the similarity in the mycoflora associated with leaf litter of plants belonging to unrelated but growing in the same locality suggests that while the nature of substrate is an important factor, the geographical location of the sampling area and its biogeoclimate also plays a major role in deciding the nature of the mycoflora of that particular area.
Dorai (1988) also made ecological investigations on the fungi colonising leaves and litter of *Eucalyptus tereticornis* collected at bimonthly intervals from a 17-year old plantation at Vandalur over a period of two years. The objective of this study was to know the nature of the fungus flora of the phylloplane and to understand the sequence of fungal colonisation of the living leaves, senescent and dead leaves by using moist chamber incubation technique and dilution plating. Fungi were grouped as 'most frequent', 'common', 'occasional', and 'rare' depending on their periodicity of occurrence. A total number of 119 species belonging to 88 genera were isolated from all the three layers of litter, the greater number being isolated from F₁ layer than in L and F₂ layer. *Arthrinium phaeospermum*, *Beltrania malaiensis*, *Chlamydomyces palmarum*, *Corynespora cassiicola*, *Monodictys castaneae* and *Torula herbarum* were recorded exclusively from L layer. *Cercosperma longispora*, *Coniella castaneicola*, *Hansfordia ovalispora*, *Harknessia ventricosa*, *Microdochium caespitosum*, *Mycotypha microspora* and *Rhinocladiella mansonii* were specific to F₁ layer. *Choanephora cucurbitarum*, *Chaetomium turgidopilosum*, *Dactylaria purpurella*, *Polyscytalum* sp., *Scolecosidiella tropicalis*, *Spadicoides aggregata*, *Stachybotrys kampalensis* and *Stemonitis virginiiensis* were recorded from F₂ layer.

A clear pattern of fungal colonisation of leaves and litter of *E. tereticornis* was observed by Dorai (1988). The phylloplane mycoflora consisting of *Alternaria alternata*, *Aspergillus niger*, *Cladosporium cladosporioides*, *Coxysporum*, *Curvularia* sp. and *Penicillium funiculosum* common to airflora were recorded. As the leaves became senescent and shed, these foliicolous fungi began sporulating on freshly fallen leaves represented by L layer. True litter fungi, *Gyrothrix circinata*, *Parasympodiella laxa*, *Phragmocephala* sp. and *Weisneiriumyces javanicus* appeared afresh in L layer and continued to be active in F₁, where more true litter fungi such as
Cercospora longispora, Harknessia ventricosa, Helicoubisia coronata, Hyphodiscosia jaipurensis, Kramasamuha sibika, Pleurotheciopsis tax. sp., and Zanclospora indica were observed. Some of these fungi continued to appear in F2 layer which consisted of litter in advanced stage of decomposition.

Bhat and Kaveriappa (1999) studied the phylloplane and surface mycoflora of aerial parts such as shoot bud, flower bud, flower and fruit of Myristica fatua var. magnifica and M. malabarica, two endangered tree species of evergreen forests of the Western Ghats in Uttara Kanada, in Karnataka, using serial dilution and blotter methods. A total of 83 species belonging to 48 genera were isolated. Among these, 61 species were recorded on M. malabarica and 72 species on M. fatua var. magnifica. Maximum number of species were recorded on mature leaves and minimum on flower buds. Alternaria alternata, Aspergillus aculeatus, A. niger, Cladosporium oxysporum, Fusarium oxysporum, Penicillium chrysogenum and Trichoderma viride were found on all parts of both plant species. Maximum number of fungal species were recorded on mature leaves and shoot buds during summer months (Dec-March), while minimum number of species were recorded during rainy season (June-Sept.)

Roberts et al. (1986) studied the fungi occurring in the achenes of Helianthus annus. About 28,000 samples of achenes from several production areas in the United states were subjected for analysis. Ninety-eight species in 38 genera were identified from graded samples of achenes stored at 20°C, and 63, 83 and 93% relative humidities. Sixty four fungal taxa were reported from sunflower aches for the first time. Forty-five potentially mycotoxigenic, five thermophilic and five new records of species of Microascus were isolated.
Litter in semi-aquatic habitats:

Fungi colonising aerial stems, leaves and roots of Salsola kali were categorised into three groups by Pugh and Williams (1968). Group I consisting of Acremonium sp. and Fusarium sp. were commonly associated with aerial parts, although these were frequently isolated from the roots. Group II such as Acremoniella atra, Alternaria tenuis, Botrytis cinerea, Camarosporium sp., Cladosporium herbarum, Epicoccum nigrum and Stemphylium sp. were more prominent on the aerial parts than on the roots. Group III had only a Chaetomium sp. that was isolated from buried stems and leaves. Pugh and Mulder (1971) traced the succession of fungi colonising Typha latifolia right from the time of its appearance, senescence and to its final decay. In the early stages, the leaf was colonised by typical leaf surface fungi, in the secondary stages pyrenomycetes dominated. In the final stages soil fungi were replaced by predacious fungi.

The mycoflora of submerged leaves of Phragmites communis in various stages of development, i.e. senescent, dead and decaying leaves from various habitats in England, were compared (Apinis et al., 1972, 1975). A total of 49 species were recorded, Acremonium sp., Alternaria tenuis, Cladosporium herbarum, Dasycyphus controversus, Diplosporium sp. and Oidiodendron fuscum were common to all the 6 habitats studied. Young culms harboured few fungi while the number of species on the nodes and internodes increased with age.

Van-Maanen and Gourbiere (1997) have studied the host and geographical distribution of Verticicladium trifidum, Thysanophora penicilloides and similar fungi on decaying coniferous needles and conclude that the coexistence of these dematious hyphomycetes on some samples and the colonisation of some Pinus litter by
Thysanophora penicilloides suggests that these distributions result from competition rather than strict host specificity.

The ecological mechanisms by which plant biodiversity and species composition are regulated and maintained are not well understood. Marcel et al, (1998) made an attempt to show that below ground diversity of arbuscular mycorrhizal fungi is a major factor contributing to the maintenance of plant biodiversity and to ecosystem functioning. It also shows that conservation of the fungal gene pool is likely to be prerequisite for maintenance of floristic diversity in grasslands, as well as in other ecosystems such as boreal forests, where the fungal web is known to influence allocation of resources between plant species (Read, 1998).

Raviraja et al. (1998) studied the fungal colonisation and processing of eucalyptus and banyan leaves in organically enriched reaches of the river Nethravathi in coastal Karnataka in southern India. They found that conidial production and species numbers of aquatic hyphomycetes were very low. Comparisons with their earlier studies (Raviraja et al., 1996) that is with geographically close but clean streams showed that pollution was the determining factor.

**Abundance and diversity of microfungi in tropical litter:**

Knowledge on plant-inhabiting tropical microfungi has been based on collections of fungi sporulating on their natural substrata in situ or those sporulating on plant debris incubated in moist chambers. Such collections have been studied in order to document fungal floristics, and to provide basic data for taxonomic monographs. These studies have revealed that tropical plant debris exhibits an enormous diversity of fungi and further provided countless descriptions of species with which workers can identify the microfungi of their region. Only few
investigators have so far attempted to measure the abundance of diversity of microfungal species that inhabit tropical litter (Heredia, 1993; Bills and Polishook, 1994).

Based on particle-filtration technique, Bills and Polishook (1994) estimated how many species of saprobic microfungi could be expected on decaying leaves of a single plant species, *Heliconia mariae* in the lowlands of south-eastern Costa Rica. Pulverized decayed leaves were separated into fine particles and repeatedly washed. When 0.1 ml particle suspensions were plated onto 4 petri plates of two selective media, a total of 1676 isolates were recovered, ranging from 310 to 599 isolates/plant. The number of species/plant ranged from 56 to 98.

Bills and Polishook (1994) devised the particle filtration technique based on the conventional soil washing method, to determine if the characteristic fungal flora of leaf litter could be preferentially isolated while minimising recovery of soil and common saprobic fungi. They also made preliminary measurements of the magnitude of fungal species richness in tropical forest litter. Rarefaction curves based on the number of species expected in random subsamples were used to compare species richness among samples. From their study many uncommon genera of litter fungi belonging to coelomycetes, sterile strains, endophytes and phytopathogens were recovered. Typical soil fungi were a relatively minor component of the total isolates. Species abundance distribution showed that there were few abundant species and a high proportion of rare species. Species present in all samples belong to genera *Cylindrosympodiella, Glomerella, Lasiodiplodia* and *Pestalotiopsis*. Hyphomycetes and coelomycetes were the most abundant type of fungi. Some of the true genera of litter fungi isolated included *Beltrania, Chalara Chloridium, Cordana, Cryptophiale,*
Dendrosporium, Dichtyochaeta, Gyrothrix, Kutilakesopsis, Leptodiscella, Speiropsis, Tetractadium, Thozetella, Trinacrium, Volutella and Zygosporium.

Limited sampling of endophytes from leaves and stems of trees at the same locations of litter samples by Bills and Polishook (1994) indicated that some of the endophytic species appeared in the litter layer as well. For example, Cylindrocarpon sp., Fusarium decemcellulare, F. solani, Glomerella cingulata, Lasiodiplodia theobromae, Nodulisporium sp., Phomopsis sp., Tubercularia lateritia and Xylaria sp. and many species of coelomycetes. This observation was similar to the studies made by Boddy and Griffith, (1989), Parkinson and Kendrick (1960) and Wildman and Parkinson (1979) wherein they found that endophytic and phytopathogenic fungi are commonly recovered from the upper layers of forest litter and are associated with litter decomposition.

Fungus flora of the air over a wheat field was studied by Misra and Tewari (1975). Air was sampled at heights of 15, 30 and 45 cm, by exposing nutrient plates; simultaneously the wheat leaf samples were also collected from the corresponding heights for assessment of leaf surface mycoflora. They found that the number of spores on the leaf surface was nearly proportional to the number of spores in the air. The fungal population was highest at 15cm and decreased with increasing height. The population also increased from November to March in both the cases. Seventy percent fungal species were trapped from both the environments whereas only 3.5 and 26.4% were restricted to air and the leaf surface respectively.
The Endophytes:

Detailed study of the fungal endophytes commenced only in the middle of the 1970's (Berstein and Carroll, 1977; Carroll and Carroll, 1978), although their presence was first discovered by Sampson in 1935 within the plant tissues of Festuca rubra (Petrini1991) microorganisms are now known to interact with surfaces as well as interior tissues of plants. All living plants so far investigated have been shown to harbour fungi inside their tissues (Petrini1991).

The term endophytes was originally used by De Bary (1866) to refer to any organism occurring within plant tissues, distinct from the epiphytes that live on plant surfaces. Microbes living within the interior tissues of healthy plants, without causing any disease symptoms, are called endophytes (Wilson, 1993). It is now known that the fungi in grasses and trees living asymptptomatically within the host plant give the host acquired resistance against herbivores (Carroll, 1988; Clay, 1988; Isaac, 1992). Some of these fungi are considered to be mutualistic, because they afford host plants a degree of protection from herbivory. Although, the term endophyte has been used to describe mycorrhizal fungi (O'Dell and Trappe, 1992), because of their characteristic external hyphae extending into the soil surrounding the infected root tips and such fungi necessarily residing only partly inside plant tissues, the taxonomic limit to the definition of an endophyte now remains an ongoing biological debate. In addition, several bacterial endophytes have also been recognized (Chanway, 1996).

Petrini 1991 consideres all organisms inhabiting internal tissues of plant organs at some time in their life without causing apparent harm as endophytes. This consideration also includes latent pathogens which are found as endophytes during stages of their life cycles. This definition obscured the boundaries between epiphyte, endophyte and latent pathogen. Some fungi persist within the plant as endophytes and
in order to facilitate the infection of other plants, release its spores into the air. During this stage, the fungus might be seen as an epiphyte living on the surface of the plant leaves, where spore dispersal into the air may be achieved. In this form, an endophyte with external structures can be seen as an epiphyte with hyphae growing into the plant (Clay, 1991). Endophytes may also be weak plant pathogens, for example some smuts which are systemic and inhibit host growth (Clay, 1991). A species of Rhabdocline, a weak pathogen of Douglas fir leaves, can cause no signs of infection for up to two years and, according to Carroll (1988), during this latent period, the fungus may be referred an endophyte.

Chapela (1989) used the term 'xylotropic endophytes' for fungi that were found within host trees and have the tendency of growing into secondary xylem upon drying of the wood, thereby emphasizing their relatedness to endophytic fungi.

**Grass endophytes:**

Taxonomy and biology of fungal endophytes of grasses (Poaceae) and sedges (Cyperaceae and Juncaceae) have been the subject of extensive studies, mainly because of the impact fungi on the ecology of grass populations. Diehl (1950) investigated the Balansiae (Clavicipitaceae), a group of fungi that parasitize grasses and sedges, both taxonomically and ecologically. A high degree of host specificity has been shown by *Balansia strangulans* which was found in a given site almost invariably on only one host, although other grasses known as hosts may be growing in the immediate vicinity of the infected plant. Other species of the Balansiae are also host-specific atleast at the tribe level, and infect only closely related host plants.

The first report of endophytes of grasses was published in 1924 by Lewis (Petrini, 1991). There have since been extensive reviews of fungi inhabiting terrestrial
grasses (Clay, 1991; Carroll, 1986). Species of grasses previously known as poisonous are now known to be endophytically infected by fungi (Clay, 1991). Fescue toxicosis, caused by the consumption of the ergot toxin ergovaline by grazers of tall fescue grass, was found to be correlative of the ergosterol content of grass seeds to the endophyte content of the seeds. The endophyte impact of fine fescue seed samples was confirmed from the ergosterol and microscopic analysis. The ergosterol analysis can now be used in both diagnostc and research applications to predict endophyte content in samples (Richardson and Logendra, 1997).

Endophytic fungus, *Acremonium* spp., infections were detected from wild populations of *Lolium* spp., examined from 15 of 20 European countries. Of the 523 populations studied, 38% contained no infection, 48% contained 1-50% infection and 14% contained 51-100% infection. Significant correlations were obtained between the level of infection and 5 climatic variables, the highest being with evapo-transpiration and water supply deficit. Groups of *Lolium* populations with a high level of infection were located mostly in Mediterranean regions, where stress from summer drought is common (Lewis *et al.*, 1997). Clement *et al.*, (1997) underscored the potential of endophytic fungi in conferring insect resistance in wild barley. They conducted experiments to compare the expression of *Diurophis noxia* (Homoptera: aphididae) resistance in four plant lines of wild barley (*Hordeum* sp.) infected with different species of endophytic fungi [tribe Balansieae, family Clavicipitaceae, *Neotyphodium* gen. nov. (formerly *Acremonium* sp.)]. Aphid densities were significantly lower on endophyte-infected plants (*H. bogdani* and *H. brevisubulatum*), compared with densities on endophyte-free plants of both species in population growth experiments. This endophyte-associated resistance was attributed to antibiosis effects or starvation. Allelopathy of endophytic fungi, *Fusarium* sp. and *Colletotrichum* sp., was evaluated
as factors affecting the biological control of marsh reed grass, a weed of boreal reforestation areas in a study carried out by Winder (1997).

It is now known that endophytic fungi living within some grasses have contributed to the increase in resistance of their host plants to insect herbivores. Boning and Bultman (1996) have proved that endophytes mediate induced resistance by a grass to a herbivorous insect. Tall fescue, both infected and uninfected with an endophyte, *Acremonium coenophialum*, was artificially damaged by clipping a tiller from each plant four weeks after germination. They observed that eight day old Fall armyworm (*Spodoptera frugiperda*) larvae weighed less and took longer duration to develop into adults when fed endophyte-infected vs. endophyte-free plant material. In contrast, pupae weighed more when fed infected vs. endophyte-free plant material and the interaction between infection status and damage had a marginally significant effect on pupal mass. Pupae reared from damaged infected plants weighed less than those reared from undamaged infected plants. No pattern with damage was apparent for insects reared on endophyte-free plants. The results suggested that the clipping damage could have resulted in an induced response in plants infected with the fungal endophyte.

Cheplick (1997) tried to examine whether endophytic fungi influence plastic responses of host genotypes to variable soil nutrients and whether or not endophyte infection and host genotype interact to determine the extent of this plasticity. He observed that responses to nutrient conditions in relation to fungicide treatment were genotype specific. High levels of endophytic fungi appeared to reduce plasticity. The potential for microscopic symbionts to affect phenotypic plasticity in genetically variable populations has not often been recognized. However, the clandestine effects of symbionts on the plasticity of host genotypes could impact microevolutionary
processes occurring within plant populations that occupy heterogeneous environments.

Differences in species composition, infection frequencies and fungal colonization were observed in asymptomatic leaves and culms of an annual and three perennial *Juncus* species in western Oregon by Cabral et al., (1993). They observed that infections limited to a single host epidermal cell were characteristic of *Drechslera* sp., *Stagonospora innumerosa* and an unidentified endophyte of *Juncus bufonius*. Infections originated in the substomatal cavity followed by limited intercellular colonization of the mesophyll. *Alternaria alternata* and *Cladosporium cladosporioides* were isolated in low frequencies and further found restricted to substomatal chambers. Marks and Clay (1996) attempted to analyse the physiology underlying the enhanced growth rates of several grass species infected by fungal endophytes. Carbon exchange rates (CER) and leaf conductances of 13 genotypes of tall fescue infected by the fungal endophyte *Acremonium coenophialum* were measured. At leaf temperatures above 35°C, infected tall fescue plants photosynthetized at a significantly greater rate (20-25%) than uninfected plants. This resulted from a decrease in the CER of uninfected plants, not an increase in the rate of infected plants, at high temperature. There were also significant infection and genotype interactions, indicating that the response to infection was specific to a given genotype. The results indicated that physiological responses of host plants to fungal endophyte infection depended both on the physical environment and the genotype of the plants.
Diversity of endophytic fungi:

Several authors have suggested that evolution of land plants has been intimately related to that of their endophytes (Bernard, 1916; Pirozynski and Malloch, 1975; Boullard, 1979; Atsatt, 1988). According to Chapela (1989), floristic differences in the xylotrophic endophytes of separate plant families might provide information on their phylogeny. Hawksworth (1991) estimated that there are 1.5M species of fungi and of which 70,000 fungal species have been described worldwide. However, this may be a vast underestimate, especially in light of the large numbers of new fungal endophyte species recovered from almost every plant species sampled. Fungi may turn out as one of the most undescribed group of organisms on earth. The major difference between the two is that most of the world's undescribed insects are believed to reside only in the tropics whereas world's endophytic fungi still await discovery almost in every climatic zone within the leaves and stems of both common and rare plants (Wilson et al., 1997).

Fisher et al. (1992) isolated fungal endophytes from five Thymus species collected in the mountains of Austria and in Spain. The frequency of colonisation for stems and leaves was approximately the same in the alpine samples, in contrast to the Mediterranean species, where the leaves showed low colonisation frequencies when compared to the stems. A total of 30 species had been isolated. The dominant fungi in the stems of four species of Thymus sampled in the Mediterranean were Alternaria alternata.

Fungal endophytes have been isolated from a wide range of evergreen and deciduous plants. (Carroll et al., 1977; Fisher et al., 1986; Petrini, 1986; Petrini and Fisher, 1986). Of the examined, 21 evergreen plants from Ishigaki and Irimote islands in Okinawa, some of the endophytic fungi were found in all the plants examined;
Xylariaceous fungi and *Phyllosticta* spp. were isolated from about half of the plants tested, *Pestalotiopsis* from 7, *Phomopsis* spp. and *Colletotrichum gloeosporioides* from 6 plants each. *Acremonium* spp., *Alternaria alternata*, *Cladosporium cladosporioides*, *Coccomyces* sp., *Curvularia* sp., *Gliocladium roseum*, *Nigrospora oryzae* and *Phoma* sp. were also isolated from several plants (Okane *et al.*, 1997). Bayman *et al.*, (1997) isolated fungal endophytes from roots and leaves of seven species of epiphytic and lithophytic *Lepanthes* from rainforests in Puerto Rico. *Xylaria* spp. and *Rhizoctonia*-like fungi were the most dominant, though their differences in frequency were negligible in the root and leaf. However, differences in number and types of endophytes among orchid species were distinct. Heterogeneity of endophytes in single plants and plant organs was greater than differences between species. Many *Lepanthes* species are restricted in distribution and knowledge of their interactions with endophytes is said to be helpful in species management.

Assessment of diversity, species richness and intraspecific variation within a habitat was often difficult when morphological criteria were used for identification and classification of isolates. Michael and Hallaksela (1998) have showed how combined fatty acid and sterol profiles (FAST-profiles) can be used for classification of fungal isolates into FAST-groups (i.e. operational chemotaxonomic units) according to a defined upper variation limit. They used endophytic fungi of Norway spruce needles as a model system. The endophytic fungi of *Eucalyptus viminalis* phyllosphere was studied by Bertoni and Cabral (1988). They observed that the highest level of infection is in the blade and the basal half of the leaf, followed by the midrib and petiole, and the upper half of the leaf. The more frequently isolated species recorded were *Coniothyrium* sp., *Coccomyces martinae* and *Mycosphaerella* sp. and less frequent ones were *Macrophoma smilacina* and *Nigrospora* sp. The
distributions showed that the infections probably developed from deposited propagules rather than systemic infection.

Petrini and Fisher (1988) aimed to evaluate host specificity of fungal endophytes in a mixed stand of two distantly related *Fagus sylvatica* and *Pinus sylvestris* and assess specificity of the endophytes with respect to whole stem and xylem. Cluster analysis showed that *Pinus* tissues can be separated from that of *Fagus* on the basis of their endophyte populations, and a K-means cluster analysis revealed that eleven of the isolated fungi were mainly responsible for this separation.

Twelve species of endophytic fungi were isolated from the leaves and stems of *Suaeda fruticosa*, a Mediterranean plant from England, by Fisher and Petrini (1987). They found out that *Colletotrichum phyllachoroides* was entirely confined to the leaves. Two species of *Camarosporium* were mainly isolated from the stems and a higher incidence of colonization was found for complete stems as compared with xylem. They also made a qualitative comparison of the epiphytic fungi growing on unsterilized host species with the endophytic population of complete stems and xylem. Their study showed that the most frequently occurring endophytes were not present among the epiphytes, and correspondingly, epiphytes were uncommon among the endophytic fungi.

Endophytic fungi isolated from five species of broad-leaved evergreen shrubs from 16 sites in western Oregon by Petrini *et al.* (1982) showed different rates of infection in these plants. A pattern of species dominance was with the most common endophyte of a given host when isolated less frequently from other hosts; less commonly isolated endophytes appeared to be less host specific. Site and climate related differences in the endophytic fungal assemblages of leaves, xylem and bark of *Eucalyptus nitens* from Australia and England were analyzed by Fisher *et al.* (1993).
Sixty-four fungal taxa were isolated with a relative importance of more than 5% in any of the tissues examined. Australian and British samples were clearly separated according to their geographic origin.

Fungi inhabiting healthy stems and branches of American beech and aspen were induced to respond to drying of wood. The two tree species were similar in that the water content of the wood strongly determined fungal development, with a high water content preventing fungal growth for at least 25 weeks, fast drying resulting in poor development and slow drying inducing very fast growth of fungi within the wood. The fungi, dominated by ascomycetes and coelomycetes, were clearly different for tree species, even though samples of each were obtained from the same site and the experimental conditions were identical for both. *Hypoxylon fragiforme* was most frequently and abundantly isolated from beech (Chapela, 1989). Xylem and bark from stems of *Alnus glutinosa* and whole stems of *A. incana* and *A. viridis* from England and Switzerland were screened for endophytic fungi (Fisher and Petrini, 1990). Multiple colonisation frequency was comparatively higher for bark and xylem but colonization of segments by more than two fungi were rare. Fungal communities mainly composed of a small number of dominant species accompanied by a cohort of rare isolates. Cluster analysis showed that plant organs and tissues can be separated on the basis of their endophytic fungi.

Suryanarayanan and Rajagopal (1998) studied fungal endophytes from the leaves of some South Indian trees, viz; *Acacia malanoxylon, A. dealbata, A. decurrens, Dalbergia latifolia, Grewia tiliaefolia, Michelia champaca, M. nilgirica, Pterocarpus marsupium* and *Rhododendron arboreum* and *Eucalyptus globulus* from two places in the Nilgiri Biosphere Reserve, Tamil nadu. A total of 60 different species of endophytic fungi were isolated from the lamina and petiole of ten trees. Of
these, 37 were sterile forms, 17 belonged to Hyphomycetes, 4 were Ascomycetes and 2 belonged to Coelomycetes. *Acacia melanoxylon* and *Rhododendron arboreum* harbored more number of endophytic fungi. In all the trees a larger number of endophytes were isolated from the petiole than the lamina. Nine endophytes were unique to the lamina, 24 to the petiole and 27 occurred both in petiole and lamina tissues. *Alternaria alternata* was isolated from all the ten trees while *Curvularia lunata* occurred in nine trees. *Chaetomium indicum*, *C. globosum*, *Pestalotiopsis* sp., *Phoma* sp. and *Phyllosticta* sp. were also isolated, but their CF was low. They also found that the ethylacetate extract of the culture filtrates of five of the endophytic fungi increased the mitotic index of onion root considerably.

Andrews et al. (1985) used leaves to study the species dynamics of microbial epiphytes on apple. They suggested that leaves represent an ideal model system to examine the macroecological principles such as the theory of island biogeography because leaves were easily quantifiable units, well defined in time and space, easily replicable, subject to frequent immigration and emigration through wind and rain, and the entire population of microbes can be sampled. Observation of lists of endophytes suggests that each species of vascular plant is infected by at least two to four endophyte species that are specific to that plant species (Bills, 1996). According to Dreyfuss (1989) endophytic fungi represent one of the largest reservoirs of undescribed fungal species.

**Ecology of endophytes:**

Recent extensive surveys in a wide variety of plants indicate that endophytes are apparently ubiquitous, at least within plants growing in humid or mesic conditions (Petrini, 1986). Many of them have a rather reduced host range which in some cases
may be confined to a single plant species (Carroll and Carroll, 1978; Bacon et al., 1986) whereas others are widespread (Petrini, 1986). The ecological roles of endophytic fungi are varied. They may be dormant saprobes (Chapela and Boddy, 1988), latent pathogens (Verhoeff, 1974; Carroll, 1986), mutualists (Clay et al., 1985), antagonizing plant enemies (Latch et al., 1985) or inducers of growth and competitive ability (Bose, 1956; Clay, 1986).

Results from a study on the species composition of endophytic fungi in healthy needles of Austrian pine (Pinus nigra Am.) investigated at eight locations in Slovenia showed that ecological factors have the most pronounced effects on species composition and on frequency of colonisation (Jurc et al., 1996). Eighty species of microfungi isolated from October 1994 and January 1995 when compared with analyses of macronutrients, sulphur and lead content of the needles, showed frequency of isolated fungi the lowest in the site with the highest amount of lead in needles. Similar study was carried out in poor growth/polluted and good growth/unpolluted stands of symptomless green needles of Sitka spruce and its infection level by endophytes Lophodermium piceae and Rhizosphaera kalkhoffii by Magan (1996). In general, both the endophytes increased with the age of the needles but a higher isolation frequency of R. kalkhoffii was obtained from the polluted site rather than the unpolluted site. Complementary in vitro studies showed that R. kalkhoffii was more tolerant of elevated sulphur dioxide, lowered water availability and had a lower temperature optimum than Lophodermium piceae. Thus Magan (1996) tried to highlight the potential of endophytic fungi as possible bioindicators of tree vitality in polluted areas.

Several mutualistic roles of endophytic fungi have been demonstrated (Bacon et al., 1986; Carroll, 1988; Clay, 1991; Rowan, 1993; Wilson, 1993; Gange, 1995).
However, the exact nature of the interaction and the strength of the proposed mutualism, still remain an enigma, because testing hypotheses on the ecological role of these fungi is difficult as manipulating micro-organisms in the field or greenhouse is not easy. Wilson (1996) described a method which involves placing bags (composed of clear PVC tops and polyester netting bottoms and sides) over branches to protect newly emerging leaves from infective propagules. Leaves can then be infected with target fungi by repeated spraying of spore suspensions onto the leaves.

Although the role played by individual endophytes are well speculated, the significance of endophytic communities in plant ecology has been assessed very little. Espinosa-Garcia and Langenheim (1990), isolated leaf endophytic fungi from 1 to 12 year old leaves of mature trees and basal sprouts of coastal redwood *Sequoia sempervirens* in a redwood forest in Central California. The two most frequent species were *Pleuroplaconema* sp. and *Cryptosporiopsis abietina*. Species composition in leaves of progressing age in single branches revealed a patchy pattern of leaf colonisation without an obvious sequence of succession. This kind of patchiness may be important for plant interactions with herbivores and pathogens and could result from factors like microclimate, previous infections and changes in the host chemistry. The endophytic communities from leaves of trees and sprouts were generally similar, but differed in species richness and in the distribution of *Pleuroplaconema* sp. and *Pestalotiopsis funerea*. Principal component analysis based on endophytic frequency indicated closeness of trees and sprouts as groups, but clearly separated each tree from its sprout. Thus, the distribution patterns within and among plants, as well as possible consequences of their presence, reinforces the idea that not only single endophyte species but whole endophytic communities may be important for the plants that harbour them.
**Endophytes as mutualists:**

Most endophytes show limited growth within host tissues, in many cases such growth limitation probably results from activation of the same localized host defense mechanisms. Reserves of fixed C and nutrients in plants fluctuate seasonally. In perennial herbaceous plants and in trees, leaves represent a significant fraction of the plants accessible nutrient capital. Typically a portion of these reserves are mobilized and recovered prior to leaf abscission (Chapin and Kedrowski, 1983). Fungal domains in senescing deciduous leaves appear as green spots against a background of red, yellow or brown. These green islands develop through the elaboration of cytokinins and other metabolites by the asymptomatic endophytic fungi, metabolites which locally retard senescence and impede the mobilization of fixed carbon and other nutrients (Goodman *et al.*, 1986).

A number of endophytes have now been shown to function as antagonists to plant diseases and insect pests (Carroll, 1990). The protective effects of endophytes are apparently not confined to plant shoots and their targets may be broader than insect pests. Grass endophytes may be active against soil nematodes (Pederson *et al.*, 1988). A number of leaf and stem pathogens of crops have been reported to persist as endophytes in weeds (Hartman *et al.*, 1986; McClean and Roy, 1988). While such fungi cause little damage to their weed hosts, they may debilitate the commercially important hosts which are in competition with the weeds. Endophytic fungi are diverse and abundant in woody plants and are thought to increase resistance of host trees to invertebrate and vertebrate herbivores (Faeth and Hammon, 1997a).

Though endophytic fungi have their advantages, they may introduce genetic level changes on host plants. It is speculated that the DNA isolated and amplified from higher plants may originate from symbiotic microbes occupying the plant
tissues. A recent report on the phylogeny of *Picea* contained sequence data that on later analysis proved to originate from filamentous ascomycetes (Camacho *et al.*, 1997). Isolates of endophytic fungi from *Picea* foliage collected from the same location, when examined to identify the source of the contaminating DNA, showed a DNA sequence originally attributed to *Picea engelmannii* as that of *Hormonema dematioides*, an ubiquitous foliar endophyte of conifers.

**Economic importance of endophytic fungi:**

Following the discovery of taxol from the endophytic fungus, *Taxomyces andreanae*, originally isolated from *Taxus brevifolia*, other fungi are also screened for potential drugs. In a recent study Pulici *et al.*, (1997) found that two strains of *Pestalotiopsis* spp., endophytic fungi of *Taxus brevifolia*, produced several new sesquiterpenes including three caryophyllenes, and pestalotiopsin A, B and C.

Substrate utilization studies conducted with fungal endophytes by Carroll and Petrini (1983), Sieber-Canavesi *et al.* (1991) and White *et al.* (1991) have conclusively demonstrated that most endophytes are able to utilize, at least *in vitro*, most substrates present on the surfaces or in the cell wall of the host. Most of the endophytes investigated are able to utilize xylan and pectin and produce non-specific peroxidases and laccases. Production of extracellular cellulosases and hemicellulases other than xylanases are widespread but usually limited to organisms derived from selected hosts or even host tissues. However the utilization of starch is limited to small number of endophytes (Petrini *et al.*, 1991). Production of both pectin and polygalacturonic acid degrading enzymes, responsible for the degradation of cell wall middle layer is also extremely widespread among endophytes (Petrini *et al.*, 1992b).
Isolates of a given species derived from the same host were generally more homogenous with respect to their enzymatic activities (Leuchtmann et al., 1992).

The production of enzymes and secondary metabolites in endophytes is closely related to their ecological significance. The secretion of extracellular enzymes needed for cell wall degradation supports the hypothesis that fungal endophytes represent a group of organisms specialized to live within plant tissues (Carroll, 1988). The general tolerance of endophytes to phenolic compounds (Carroll and Petrini, 1983) and the differential reactions shown by certain redwood endophytes against terpenoids produced by their host (Espinosa-Garcia and Langenheim, 1991a, 1991b) suggests the potential ability of host-specific endophytic fungi to cope with compounds produced by the pathogens.

Production of secondary metabolites by endophytes:

Carroll (1986) presumed that conifer needle endophytes might produce toxins that effect defoliating insects. Larry et al., (1992) screened fungal strains for metabolites toxic to spruce budworm larvae. Five percent of the strains produced extractable compounds that resulted in mortality or reduced rate of development in the larvae. Fermentations of three strains yielded relatively potent extracts. This is said to be the first report of the identification of toxins from fungal endophytes of woody plants. Polishook et al., (1993) reported the isolation and antibiotic activity of Preussomerin D from the endophytic fungus Hormonema dematioides recovered from living plant tissue of a coniferous tree.
Endophytes in other plants and plant parts:

Fungal endophytes were isolated from the pinnules, leaf vein, rachis and rhizome of spring and autumn plants of *Pteridium aquilinum* in Devon, U.K. (Petrini *et al.*, 1992a). Barrow *et al.* (1997) tried to determine the nature and incidence of root endophytes on fourwing saltbrush, *Atriplex canescens*. They found that the root cortex cells in arid rangelands of Southwestern United States were regularly colonized with three types of endophytic fungi: The widespread occurrence of these non-destructive fungal associations with plants implied that they have an important role in plant survival in arid environments. Fisher and Petrini (1989, 1990) studied the fungi that inhabit the bark and the xylem of mature roots of *Alnus glutinosa* and found that alder roots are colonised by a comparatively large and diverse community of fungal endophytes. They isolated nearly 40 species of endophytic fungi from long roots of mature trees of *Pinus sylvestris*. Most species were predominantly bark colonisers, some able to penetrate deep into the host tissue. They suggested that root endophyte communities may not be host specific and are probably influenced by the environment. Aquatic hyphomycetes were isolated from living root tissues of spruce, birch and maple in a woodland stream of Nova Scotia (Sridhar and Barlocher, 1992). The results suggested that roots of different species may be colonized by different fungal species.

Enzyme activity of fungi:

In order to grow, fungi require sources of C, N, a supply of energy and certain essential nutrients such as potassium and phosphorus. Supplies of nitrogen may be obtained from proteins and other organic sources or from simple inorganic substances such as nitrates and ammonium salts. Energy and most of the carbon required for
growth, however, are obtained by fungi directly from living organisms or indirectly from their waste and dead tissue. The latter two provide a great range of different substrata for the growth of saprophytic fungi and include such diverse resources as animal faeces, cast-off skins, hooves, fur, feathers, nails, and horns of vertebrates, the exoskeletons of arthropods, dead bodies of animals, plant litter and the mycelia and fruit-bodies of other fungi (Kendrick, 1992).

Plant litter is composed of six main categories of chemical constituents: cellulose, hemicellulose, lignin, water-soluble sugars, amino acids and aliphatic acids, ether- and alcohol-soluble constituents including fats, oils, waxes, resins and many pigments and proteins. The break down of these constituents is effected as a sequence of specific reactions with the enzyme systems of specific organisms.

The decomposition of leaf litter follows an enymatic degradative sequence as follows. Initially, the phylloplane fungi attack the easily decomposable sugars exuded from the leaf surface or released by aphids and other insects from sub-cuticular tissues. Melezitose, glucose and fructose were identified as the main organic constituents in the through fall from the canopy of a Quercus woodland. As the leaf becomes senescent, the phylloplane fungi which individually or in combination possessing cutinase, pectinase and cellulase, penetrate the cuticle, attack the middle lamella and degrade the cell walls (Dickinson and Pugh, 1974). When species diversity is richest, this is likely to correspond with the higher rate of decomposition, greater genetic diversity and greater the enzyme diversity (Dix and Webster, 1995).

Ecological adaptation is achieved through a range of enzymes produced and the multiple forms of individual enzymes. The full range of these and hence the substrates that can be utilized depends upon species. Extracellular enzymes are
extremely stable glycoproteins that operate in the fluids of the substratum. Enzymes may diffuse through the substratum but, if pore sizes are limiting, enzymes will not be able to move into the substratum and reactions will then be restricted to interfaces between the substratum and the penetrating hyphae (Dix and Webster, 1995). Enzymes have applications in many fields, including organic synthesis, clinical analysis, pharmaceuticals, detergents, food production and fermentation. The application of enzymes to organic synthesis is currently attracting more and more attention. The discovery of new microbial enzymes through extensive and persistent screening will open new, simple routes for synthetic processes and consequently, new ways to solve human problems (Ogawa and Shimizu, 1999).

**Hydrolysis of starch:**

Starch is the commonest of food reserves in plants, and fungi, with the notable exception of most yeasts, produce amylases which catalyse starch hydrolysis. Chemically starch is made up of two polymers of glucose: amylose and amylopectin. These are present in varying proportions according to plant species but invariably amylopectin is in the greater amount, and is usually about 75-85% of most starches. Both polymers consist of chains of glucose molecules linked by $\alpha$-1-4 glucosidic linkages but an important difference is that amylopectin is highly branched and carries side-chains which are linked to the main chain through $\alpha$-1-6 bonding.

The starch-hydrolysing enzymes and their distribution in microorganisms have been described by Fogarty and Kelly (1979). $\alpha$-amylase is the commonest starch-hydrolysing extracellular enzymes found in fungi. Fungi also produce extracellular amyloglucosidase (glucoamylase), an enzyme which seems to be exclusive to fungi. $\alpha$-amylase hydrolyses both amylopectin and amylose to maltose and higher molecular
weight fractions, by-passing α-1-6 linkages and randomly cleaving chains in the fashion of an endo-enzyme. Amyloglucosidase hydrolyses α-1-4 and α-1-6 glucose residues to glucose, working on the ends of chains in the manner of an exoenzyme and is also capable of hydrolysing amylopectin, amylase and glycogen almost completely to glucose. Since α-amylase cannot hydrolyse α-1-6 linkages it cannot attack the branch points in amylopectin; thus in fungi which produce no amyloglucosidase, high molecular weight dextrins tend to accumulate when starch is hydrolysed (Fogarty and Kelly, 1979). All the maltose produced by the hydrolysis of starch is finally split into two glucose molecules by the catalytic action of intracellular α-glucosidase.

Degradation of cellulose:

Cellulose is the most abundant substance in plant litter and as a major constituent of all the layers of plant cellwalls it forms about 30-40% of the dry weight of wood and can be as high as 45% of the dry weight of cereal straw.

Cellulose is a straight-chain β-1-4 glucan polymer containing as many as 10,000 glucose molecules linked together by the removal of water from two hydroxyl groups. Glucan chains join to form microfibrils, bundles of which run in the matrix of the plant cell wall as strengthening components. Each microfibril consists of about 40-100 glucan chains linked together by hydrogen bonding between adjacent hydroxyl groups.

In parts of the microfibril the glucan chains are regularly arranged in a parallel fashion forming cellulose with crystalline characteristics. Crystalline cellulose is the more resistant to decay, possibly because the close packing of the molecules prevents the penetration of microbial enzymes. Hydrolysis of cellulose is catalysed by an
enzyme complex called cellulase that consists of a number of extracellular \( \beta \)-1-4 glucanases, some of which are endohydrolases randomly disrupting linkages throughout \( \beta \)-1-4 glucan chains, producing glucose, cellobiose and high molecular weight fractions, while others are exohydrolases or \( \beta \)-1-4 cellobiohydrolases, which act only on the ends of \( \beta \)-1-4 glucan chains releasing the disaccharide cellobiose (Halliwell, 1979). Glycohydrolases that release single glucose units from glucan chains are also part of the cellulase complex of some microorganisms. The decomposition of cellulose is finally completed by the transformation of trisaccharides and disaccharides to glucose by the action of \( \beta \)-1-4 glucosidases within the hyphae.

All wood-rotting fungi degrade cellulose as do apparently many microfungi from soil and litter as measured by their ability to hydrolyse carboxymethyl-cellulose and pure cellulose in the laboratory (Domsch and Gams, 1969; Flanagan, 1981). However, in nature cellulolytic activity depends upon a number of substratum-related factors, notably pH and mineral composition. The ability to hydrolyse cellulose is very variable. Some fungi have very low rates of utilization and others are unable to degrade cellulose at all.

The ability to decompose cellulose (or other plant polymers) has been used to classify fungi into several substrate-related ecological groups (Garrett, 1966). Theodorou et al. (1980) observed cellulase and \( \beta \)-glucosidase activities 50 h after inoculation of *Trichoderma reesei* in an artificially structured ecosystem, simulating soil conditions like leaching and the attachment of microorganisms to a solid substrate and the activity was still present in the effluent collected 300 h later. An extensive review of the ecology of microbial cellulose degradation has been produced by Ljungdahl and Eriksson (1985).
Hydrolysis of Pectin:

Pectin is a polyuronide of plant origin and is of variable composition depending on the source. Pectin occurs chiefly in the middle lamella (intracellular layer) of plant tissue and may be looked on as the cementing material lending rigidity to the tissue. Many fungi, including well known plant pathogens, secrete enzymes which solubilize by hydrolysis the pectin in situ causing the softening characteristics of rotting. In the plant, pectin exists in the form of a labile combination either with cellulose, hemicellulose or other material known as protopectin. The enzyme complex besides protopectinase also consists of pectase and pectinase. Pectase, is an esterase (pectinesterase) which hydrolyses the methoxy groups off from the esterified carboxyl groups of the galacturonic acid residues in the soluble pectin molecule. Methyl alcohol results and in the presence of calcium ion, the soluble pectin is converted into a gel. Pectase action is a necessary prerequisite for pectinase action, for only deesterified pectin is attacked by the latter enzyme. Though these two enzymes are distinct and separable, in virtually every case they are produced together by fungi attacking pectin. Pectinase is the enzyme responsible for the complete rupture of the polymerized pectin molecule into its structural components. This enzyme is extremely widespread in fungi both, parasites and saprophytes (Foster, 1949; Osagie and Obuekwe, 1991).

Pectinases have applications in the food industry; they also play an important role in the degradation of cell wall material by plant pathogens and have been associated with fruit development, ripening and cell wall extention (Fogarty and Kelly, 1993; Ward and Moo-Young, 1989). Aguilar & Huitron, (1993), found that intact conidia of Aspergillus sp. were able to degrade pectin in vitro even when
protein synthesis was inhibited, thus indicating the presence of cell bound pectinases. They also found an exo-pectinase, present in the mycelium.

**Hydrolysis of Protein:**

Proteins are the most abundant nitrogen-containing constituent of living organisms. Soluble proteins, of about 30 amino acids or less in chain length, can pass through hyphal walls; insoluble proteins are hydrolysed externally before utilized by fungi.

Most fungi have extracellular proteolytic activity against proteins over a range of environmental conditions. Peptide endohydrolases (proteases) cleave internal peptide bonds, releasing soluble peptides. These when taken into the hyphae can be degraded to their component amino acids by a range of different peptidases. Four broad classes of proteinases have been detected in fungal cultures, serine; aspartic; cysteine and metallo-proteinases. Multiple forms of serine and aspartic proteinases appear to be the proteinases most widely produced by fungi (North, 1982). Fungal proteinases have a low substrate specificity and are very durable under extreme environmental conditions.

**Degradation of Lignin:**

A significant proportion of the carbon in plants is in the form of complex aromatic polymers, such as tannin, lignin and related phenolics. Lignin is most abundant in woody plants where it accounts for up to about 30% of the carbon content, providing rigidity and resistance to biological attack. Microorganisms in soil ultimately oxidize these compounds to carbon dioxide and water. The essence of this
process is that in the final stages of degradation, fission of the benzene ring must occur to produce straight-chain aliphatic substances which can be completely respired.

Certain fungi, mostly basidiomycetes, are able to extensively biodegrade the lignin; white-rot fungi can mineralize lignin, whereas brown-rot fungi merely modify lignin while removing the carbohydrates in wood. Several oxidative and reductive extracellular enzymes (lignin peroxidase, manganese peroxidase, laccase, and cellobiose: quinone oxidoreductase) have been isolated from lignolytic fungi; the role of these enzymes in lignin biodegradation is being intensively studied (Reid, 1995; Zhao et al., 1996). The dissimilation of lignin by fungi can be conveniently thought of as occurring by three mechanisms: (i) depolymerization by cleavage of bonds within the polymer; (ii) removal and modification of side-chains with substitution on benzene rings; and (iii) fission by ring-splitting enzymes to convert aromatic nuclei into respirable aliphatic compounds. About 15 separate enzymes are required for the complete oxidation of lignin polymers (Tuor et al., 1995).

The economic consequences of lignin biodegradation include wood decay and the biogeochemical cycling of woody biomass, degrade a variety of pollutants in wastewaters and soils, to increase the digestibility of lignocellulosics, and possibly to bioconvert lignins to higher value products (Reid, 1995, Youn et al., 1995; Raghukumar et al., 1999).

The enzyme laccase is a copper-containing oxidase; it does not require peroxide. Like Mn peroxidase, it normally oxidises only those lignin compounds with a free phenolic group, forming phenoxy radicals. However, in the presence of the artificial substrate 2,2'-azinobis (3-ethylbenzthiazoline-5-sulphonate) (ABTS), laccase can also oxidise certain non-phenolic compounds, probably by hydrogen abstraction from benzyl carbons. ABTS also enhances the ability of laccase to degrade the
residual lignin in Kraft pulps; other synthetic mediators reportedly have a similar effect. Laccase is not produced by all white-rot fungi (Kirk and Kelman, 1965; Setliff and Eudy, 1980) and many microfungi from soil and litter that cannot degrade lignin produce abundant laccase (Dix, 1979).

Fungal laccases have been implicated in sporulation, rhizomorph formation, pathogenesis and formation of fruity bodies and lignin degradation (Thurston, 1994; Bourbonnais and Paice, 1990; 1992; Yaropolov et al., 1994; Heinfling et al., 1998). Thus laccases appear to have a significant role in fungal biology (Dittmer et al., 1997) and is widely distributed in fungi found on decaying lignocellulosic materials in the marine environment (Raghukumar et al., 1994). Li et al. (1999), compared the ability of four different fungal laccases for the oxidation of lignin model compounds in a laccase mediator system. They have also suggested the criteria for better laccase utility and more effective laccase-mediator systems for pulp bleaching.

**Hydrolysis of xylan:**

D-xylans are the major components found in the hemicellulosic fraction in the cell walls of higher plants (Monti et al., 1991). Natural xylans are heterogenous polysaccharides consisting of a backbone chain of β-1,4-linked D-xylopyranosyl residues and side chains of different substituents. The complete hydrolysis requires the action of several enzymes, probably analogous to the synergistic enzyme action involved in crystalline cellulose degradation (Kluepfel et al., 1990). Xylanases are produced by hemicellulose-degrading fungi (Dekker and Richards, 1976). Common microfungi may degrade xylan more actively than carboxymethyl-cellulose or pectin (Domsch and Gams, 1969). The xylanase complex is known to consist of four endohydrolases, two capable of attacking branch points and branches, reducing the
size of side-chains, and two that only reduce the size of the main chain (Reilly, 1981). Endoxylanases randomly cleave the β-1,4 bonds in the polyxylose backbone, yielding oligosaccharides of varied chain lengths. β-Xylosidase activity generates D-xylose from both short chain oligosaccharides and xylobiose (Bachmann and McCarthy, 1989; Huang et al, 1991; Alconada and Martinez, 1994). The action of exoxylanases is less frequent (Kluepfel et al., 1990) although it is not clear whether this exoenzyme is a separate entity from the β-xylosidase (Hayashida et al., 1988; Puls and Poutanen, 1989).